Advisory Action Before the Filing of an Appeal Brief	**Application No.** 14/451,876	**Applicant(s)** KARTALOV ET AL.	
	Examiner Russell S. Negin	**Art Unit** 1631	**AIA (First Inventor to File) Status** No

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 11 August 2017 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

NO NOTICE OF APPEAL FILED

1. ☒ The reply was filed after a final rejection. No Notice of Appeal has been filed. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114 if this is a utility or plant application. Note that RCEs are not permitted in design applications. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action; or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
   c) ☐ A prior Advisory Action was mailed more than 3 months after the mailing date of the final rejection in response to a first after-final reply filed within 2 months of the mailing date of the final rejection. The current period for reply expires months from the mailing date of *the prior Advisory Action* or SIX MONTHS from the mailing date of the final rejection, whichever is earlier.
   Examiner Note: If box 1 is checked, check either box (a), (b) or (c). ONLY CHECK BOX (b) WHEN THIS ADVISORY ACTION IS THE FIRST RESPONSE TO APPLICANT'S FIRST AFTER-FINAL REPLY WHICH WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. ONLY CHECK BOX (c) IN THE LIMITED SITUATION SET FORTH UNDER BOX (c). See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) or (c) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☒ The proposed amendments filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   a) ☒ They raise new issues that would require further consideration and/or search (see NOTE below);
   b) ☐ They raise the issue of new matter (see NOTE below);
   c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: *See Continuation Sheet*. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☒ For purposes of appeal, the proposed amendment(s): (a) ☒ will not be entered, or (b) ☐ will be entered, and an explanation of how the new or amended claims would be rejected is provided below or appended.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
9. ☐ The affidavit or other evidence filed after final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
10. ☐ The affidavit or other evidence filed after the date of filing the Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing of good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
11. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

12. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See attached document.
13. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
14. ☒ Other: PTO-2323 form is attached.

STATUS OF CLAIMS

15. The status of the claim(s) is (or will be) as follows:
    Claim(s) allowed: .
    Claim(s) objected to: .
    Claim(s) rejected: 49,51,53,55,56,75-81,83-88 and 90-92.
    Claim(s) withdrawn from consideration: 1,2,4,5,9,13,17-19,22,27-29,34,35,37-39,42,43,45,47 and 82.

/Russell S. Negin/
Primary Examiner, Art Unit 1631

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2397

Continuation of 3. NOTE: The limitations regarding intensities and/or ranges of intensities would require further search and/or consideration by the Examiner.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2398

Electronically Filed On: August 11, 2017

PATENT
Attorney Docket No.: 38075-716.301

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application	
Inventors: Emil KARTALOV, *et al.*	Confirmation No.: 1065
Application No.: 14/451,876	Art Unit: 1631
Filed: August 5, 2014	Examiner: Russell Scott NEGIN
Title: **SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS**	Customer No. 021971

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DO NOT ENTER
/RSN/ 8/20/2017

RESPONSE TO FINAL OFFICE ACTION AND REQUEST FOR CONSIDERATION UNDER AFCP 2.0

Dear Sir:

This paper responds to the Final Office Action mailed June 6, 2017, setting an initial due date of September 6, 2017. Applicants have replied within the shortened statutory period for reply. Accordingly, this response is timely filed, and no fees are believed to be due.

Amendments to the Claims begins on page **2** of this paper.
Remarks begin on page **8** of this paper.
Conclusion begin on page **21** of this paper.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2399

AFCP 2.0 Decision	Application No.	Applicant(s)
	14/451,876	KARTALOV ET AL.
	Examiner	Art Unit
	Russell S. Negin	1631

This is in response to the After Final Consideration Pilot request filed 11 August 2017.

1. **Improper Request** – The AFCP 2.0 request is improper for the following reason(s) and the after final amendment submitted with the request will be treated under pre-pilot procedure.

   ☐ An AFCP 2.0 request form PTO/SB/434 (or equivalent document) was not submitted.

   ☐ A non-broadening amendment to at least one independent claim was not submitted.

   ☐ A proper AFCP 2.0 request was submitted in response to the most recent final rejection.

   ☐ Other:

2. **Proper Request**

   A. After final amendment submitted with the request will not be treated under AFCP 2.0.
   The after final amendment cannot be reviewed and a search conducted within the guidelines of the pilot program.

   ☒ The after final amendment will be treated under pre-pilot procedure.

   B. Updated search and/or completed additional consideration.
   The examiner performed an updated search and/or completed additional consideration of the after final amendment within the time authorized for the pilot program. The result(s) of the updated search and/or completed additional consideration are:

   ☐ 1. All of the rejections in the most recent final Office action are overcome and a Notice of Allowance is issued herewith.

   ☐ 2. The after final amendment would not overcome all of the rejections in the most recent final Office action. See attached interview summary for further details.

   ☐ 3. The after final amendment was reviewed, and it raises a new issue(s). See attached interview summary for further details.

   ☐ 4. The after final amendment raises new issues, but would overcome all of the rejections in the most recent final Office action. A decision on determining allowability could not be made within the guidelines of the pilot. See attached interview summary for further details, including any newly discovered prior art.

   ☐ 5. Other:

   Examiner Note: Please attach an interview summary when necessary as described above.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2400

Applicant-Initiated Interview Summary	Application No. 14/451,876	Applicant(s) Kartalov et al.		
	Examiner RUSSELL S NEGIN	Art Unit 1631	AIA (First Inventor to File) Status No	Page 1 of 2

All participants (applicant, applicants representative, PTO personnel):

1. RUSSELL S NEGIN(Examiner); Telephonic
2. Lee Johnson(Attorney); Telephonic
3. Adrianna Zhang(Attorney); Telephonic

Date of Interview: 19 September 2017

Exhibit shown or demonstration conducted (If so, please describe): An agenda was discussed.

Claims Discussed: Claim 49.

Prior Art Discussed: Lin et al.

Amendment proposed: The proposed amendments not entered from the previous communication were discussed.

Brief Description of the main topic(s) of discussion: Strategies for overcoming the prior art and 101 rejections were discussed.

Issues Discussed:

Item(s) under 35 U.S.C. 101:
The fact pattern of the instant set of claims was compared to BASCOM. This argument is not persuasive because the judicial exceptions were not required to be executed on a computer. No agreement was reached at this time on patent eligibility.

Item(s) under 35 U.S.C. 103:
The proposed amendments overcome Lin et al. In view of paragraph 100 of Lin et al., applicants argue that Lin et al. used immobilization and degeneracy to detect analytes. Out of the 27 combinations listed in Figure 2C of Lin et al., only seven combinations have to be non-degenerate.

Attachment(s): Agenda,

/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1631	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

U.S. Patent and Trademark Office
PTOL-413/413b (Rev. 01/01/2015) **Interview Summary** Paper No. 20170919

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

Wilson Sonsini Goodrich & Rosati
PROFESSIONAL CORPORATION

Date: 2017-09-17 12:23:35 PDT

Fax: 15712731083

From: Johnson, Lee

Austin
900 South Capital of Texas Hwy
Las Cimas IV, Fifth Floor
Austin, TX 78746-5546
Ph 512-338-5400
Fx 512-338-5499

Beijing
Unit 2901, 29F, Tower C
Beijing Yintai Centre
No. 2 Jianguomenwai Avenue
Chaoyang District, Beijing 100022
People's Republic of China
Ph +86 10 6529 8300
Fx +86 10 6529 8399

Brussels
Rue Montoyer 47
1000 Brussels, Belgium
Ph +32 (0)2 274 57 00
Fx +32 (0)2 274 57 99

Georgetown, DE
Eight West Laurel Street
Georgetown, DE 19947
Ph 302-856-4235
Fx 866-974-7329

Hong Kong
Unit 1001
10/F Henley Building
5 Queen's Road Central
Hong Kong
Ph +852 3972 4988
Fx +852 3972 4999

New York
1301 Avenue of the Americas
40th Floor
New York, NY 10019-6022
Ph 212-999-5800
Fx 212-999-5899

Palo Alto
650 Page Mill Road
Palo Alto, CA 94304-1050
Ph 650-493-9300
Fx 650-493-6811

San Diego
12235 El Camino Real
Suite 200
San Diego, CA 92130-3002
Ph 858-350-2300
Fx 858-350-2399

San Francisco
One Market Plaza
Spear Tower, Suite 3300
San Francisco, CA 94105-1126
Ph 415-947-2000
Fx 415-947-2099

Seattle
701 Fifth Avenue
Suite 5100
Seattle, WA 98104-7036
Ph 206-883-2500
Fx 206-883-2699

Shanghai
Jin Mao Tower
38F, Unit 01
88 Century Boulevard
Pudong, Shanghai 200121
People's Republic of China
Ph +86 21 6165 1700
Fx +86 21 6165 1799

Washington, DC
1700 K Street NW, Fifth Floor
Washington, DC 20006-3817
Ph 202-973-8800
Fx 202-973-8899

This fax may contain confidential and privileged material for the sole use of the intended recipient. Any review or distribution by others is strictly prohibited.
If you are not the intended recipient please contact the sender and destroy all copies.

"Entire Transmission Copyright © 2013 Wilson Sonsini Goodrich & Rosati. All Rights Reserved."

467889614.1.docx (761)

PAGE 1/4 * RCVD AT 9/17/2017 3:24:25 PM [Eastern Daylight Time] * SVR:W-PTOFAX-003/7 * DNIS:2731083 * CSID:Wilson Sonsini Goodr * DURATION (mm-ss):01-44

WSGR Fax Cover Sheet (continued)
Page 2

Subject: Proposed Interview Agenda US Pat Appn 14/451,876

Dear Examiner Negin:

Please find attached a proposed agenda for our interview scheduled for Tuesday, September 19, at 2pm ET regarding the above-captioned application.

Yours,

-Lee A. Johnson

Lee A. Johnson, PhD, Esq
Associate
+1 858.350.2344
ljohnson@wsgr.com

Wilson Sonsini Goodrich & Rosati
12235 El Camino Real
Suite 200
San Diego, CA 92130

This email and any attachments thereto may contain private, confidential, and privileged material for the sole use of the intended recipient. Any review, copying, or distribution of this email (or any attachments thereto) by others is strictly prohibited. If you are not the intended recipient, please contact the sender immediately and permanently delete the original and any copies of this email and any attachments thereto.

This fax may contain confidential and privileged material for the sole use of the intended recipient. Any review or distribution by others is strictly prohibited.
If you are not the intended recipient please contact the sender and destroy all copies.

Entire Transmission Copyright © 2013 Wilson Sonsini Goodrich & Rosati. All Rights Reserved.

Wilson Sonsini Goodrich & Rosati
PROFESSIONAL CORPORATION

12235 El Camino Real, Suite 200
San Diego, California 92130
FAX (858) 350-2399

www.wsgr.com

MEMORANDUM

To: Examiner Russell Scott Negin

From: Lee Alan Johnson

Date: September 18, 2017

Re: US Patent Application Serial No. 14/451,876

WSGR reference no. 38075-716.301

Proposed Agenda for Interview scheduled for September 19, 2017

(Lee Johnson to dial 571-272-1083)

Proposed Agenda:

1. Discuss potential claim amendments and the rejections raised in the Final Office Action mailed June 6th, 2017 and the Advisory Action mailed August 23rd, 2017.

   a. L. Johnson to provide detailed explanations to Examiner Negin as to the non-obviousness of the pending claims over the cited Nie, Lin, and Orpana references. In particular, L. Johnson to provide detailed explanations as to the failure of the combination of cited references to teach each and every feature of independent claim 49, as pending.

   b. L. Johnson to provide arguments as to the patent eligibility of the claimed methods. L. Johnson to solicit input from Examiner Negin as to potential arguments and/or claim amendments.

Sincerely,

WILSON SONSINI GOODRICH & ROSATI
Professional Corporation

/Lee A. Johnson/

Lee A. Johnson, PhD, Esq.
Reg # 75,671
(858) 350-2344

PAGE 3/4 * RCVD AT 9/17/2017 3:24:25 PM [Eastern Daylight Time] * SVR:W-PTOFAX-003/7 * DNIS:2731083 * CSID:Wilson Sonsini Goodr * DURATION (mm-ss):01-44

PAGE 4/4 * RCVD AT 9/17/2017 3:24:25 PM [Eastern Daylight Time] * SVR:W-PTOFAX-003/7 * DNIS:2731083 * CSID:Wilson Sonsini Goodr * DURATION (mm-ss):01-44

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2406

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/451,876	08/05/2014	Emil Kartalov	38075-716.301	1065

21971 7590 09/22/2017

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER	
NEGIN, RUSSELL SCOTT	

ART UNIT	PAPER NUMBER
1631	

NOTIFICATION DATE	DELIVERY MODE
09/22/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

Large Entity Declaration

PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number: 14/451,876 **Current Date:** September 21, 2017

Filing Date: August 5, 2017

Title: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

CHANGE OF ENTITY STATUS PURSUANT TO 37 C.F.R. §1.27 (g)(2)

Commissioner for Patents
Mail Stop M Correspondence
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

This communication hereby notifies the United States Patent and Trademark Office that small entity status is no longer applicable for the above-identified patent.

Respectfully submitted,

COMPANY or FIRM

NAME AND ADDRESS:

Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 021971

/Lee A. Johnson/
Signature
Lee Johnson, Reg. No.: 75,671

9442385-1.DOC

Electronically Filed: September 22, 2017

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Patent of:		Group Art Unit:	1631
Inventors:	Emil KARTALOV, et al.	Confirmation No.:	1065
Serial No.	14/451876	Docket No.:	38075-716.301
Filed:	August 5, 2014	Customer No.:	021971
Title: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS			

SUPPLIMENTAL NOTIFICATION OF ERROR IN PAYMENT OF FEES AS A SMALL ENTITY
UNDER 37 C.F.R. 1.28(c) AND NOTICE OF CHANGE IN ENTITY STATUS
UNDER 37 C.F.R. 1.27(g)(2)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

In addition to the notification of change in entity filed September 22, 2017, please accept the enclosed payment or deduct the fee deficiency amount indicated in Table I from the account listed on file for this application which was inadvertently left of the previous submission.

TABLE I

Fee Erroneously Paid as Small Entity	**Fees Actually Paid as Small Entity**	**Deficiency Owed (Based on Fees at the time of original filing)**
Submission of IDS	$90	$90
Extension of Fees in 3rd Mo.	$300	$300
	Total Deficiency Owed	$390

The Commissioner is authorized to charge the amount of $390.00 and any additional fees which may be required, including petition fees, or credit any overpayment to Deposit Account No. 23-2415 (Docket No. 38075-716.301).

Respectfully submitted,
WILSON SONSINI GOODRICH & ROSATI

Dated: September 22, 2017

/Lee A. Johnson/
Lee A. Johnson
Reg. No. 75,671

Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
650-493-9300
Customer Number: 21971

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2410

Electronic Acknowledgement Receipt	
EFS ID:	30445158
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	Vernon A. Norviel/Katherine Lopez
Filer Authorized By:	Vernon A. Norviel
Attorney Docket Number:	38075-716.301
Receipt Date:	22-SEP-2017
Filing Date:	05-AUG-2014
Time Stamp:	12:13:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	CIT_38075_716_301_Entity_Change.pdf	76348 e6c31e5f1831858a5892c50ec667374734ddb844	no	1
Warnings:					

Information:					
2	Miscellaneous Incoming Letter	CIT_38075_716_301_Pymt.pdf	95413 7a6e802f7833b635348d437de1847215337bf0b4	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	171761		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 11/30/2014. OMB 0651-0051
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
14/451,876	August 5, 2014

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 021971

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Fred Farina/	Date (Optional)	December 10, 2014
Name	Fred Farina		
Title	Chief Innovation and Corporate Partnerships Officer, California Institute of Technology		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ Total of forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	30617054
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	Vernon A. Norviel/Katherine Lopez
Filer Authorized By:	Vernon A. Norviel
Attorney Docket Number:	38075-716.301
Receipt Date:	10-OCT-2017
Filing Date:	05-AUG-2014
Time Stamp:	18:08:45
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Assignee showing of ownership per 37 CFR 3.73	CIT_38075_716_301_Signed_3_73_C_Statement.pdf	137953 5e5a23329022a004e65707b3a3848a3b9db47189	no	2

Warnings:

Information:					
2	Power of Attorney	CIT_38075_716_301_Specific_POA.pdf	1153127 976a61846a71b5204268349cc455f14e1fa5cdda	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	1291080	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: California Institute of Technology

Application No./Patent No.: 14/451,876 Filed/Issue Date: August 5, 2014

Titled: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

California Institute of Technology , a university

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☒ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

☐ The extent (by percentage) of its ownership interest is ___%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel **033577**, Frame **0148**, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at Reel ___, Frame ___, or for which a COPY thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at Reel ___, Frame ___, or for which a copy thereof is attached.

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel ___, Frame ___, or for which a COPY thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel ___, Frame ___, or for which a COPY thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel ___, Frame ___, or for which a COPY thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel ___, Frame ___, or for which a COPY thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Lee A. Johnson/	October 10, 2017
Signature	Date
Lee A. Johnson	75,671
Printed or Typed Name	Title or Registration Number

CIT_38075_716_301_Signed_3_73_C_Statement.pdf.doc

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/451,876	08/05/2014	Emil Kartalov	38075-716.301

CONFIRMATION NO. 1065

21971
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

POA ACCEPTANCE LETTER

OC000000094690092

Date Mailed: 10/13/2017

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/10/2017.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/atesfai/

Applicant-Initiated Interview Summary	Application No. 14/451,876	Applicant(s) Kartalov et al.		
	Examiner RUSSELL S NEGIN	Art Unit 1631	AIA (First Inventor to File) Status No	Page 1 of 2

All participants (applicant, applicants representative, PTO personnel):

1. RUSSELL S NEGIN(Examiner); Telephonic
2. Marjorie A. Moran(TC 1600 SME); Telephonic
3. Lee A. Johnson(Attorney); Telephonic
4. Adrianna Zhang(Attorney); Telephonic

Date of Interview: 17 October 2017

Exhibit shown or demonstration conducted (If so, please describe): An agenda with proposed amendments was discussed.

Claims Discussed: Claim 49.

Amendment proposed: The proposed amendments in the attachment were discussed.

Brief Description of the main topic(s) of discussion: Strategies for overcoming the 35 USC 101 rejection were discussed. There is a potential that the amended claim reads on a technique similar to FACS analysis.

Issues Discussed:

Item(s) under 35 U.S.C. 101:
The proposed amendments were not viewed favorably in terms of overcoming the rejection. Eliminating the judicial exceptions from the claims would overcome the 101 rejection, but would lead to prior art rejections. The steps of the claim not encompassing the judicial exception were discussed with regard to being routine and conventional. No agreement has been reached.

Attachment(s): Agenda, Proposed Amendments,

/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1631	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

U.S. Patent and Trademark Office
PTOL-413/413b (Rev. 01/01/2015) **Interview Summary** Paper No. 20171017

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/451,876	08/05/2014	Emil Kartalov	38075-716.301	1065

21971 7590 10/20/2017

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER	
NEGIN, RUSSELL SCOTT	

ART UNIT	PAPER NUMBER
1631	

NOTIFICATION DATE	DELIVERY MODE
10/20/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

Wilson Sonsini Goodrich & Rosati
PROFESSIONAL CORPORATION

12235 El Camino Real, Suite 200
San Diego, California 92130
FAX (858) 350-2399

www.wsgr.com

MEMORANDUM

To: Examiner Russell Scott Negin

From: Lee Alan Johnson

Date: October 16, 2017

Re: US Patent Application Serial No. 14/451,876

WSGR reference no. 38075-716.301

Proposed Agenda for Interview scheduled for October 17, 2017

(Lee Johnson to dial 571-272-1083)

Proposed Agenda:

1. Discuss potential claim amendments and the rejections raised in the Final Office Action mailed June 6th, 2017 and the Advisory Action mailed August 23rd, 2017.

   a. L. Johnson to provide arguments as to the patent eligibility of the claimed methods. L. Johnson to solicit input from Examiner Negin as to potential arguments and/or claim amendments. In particular, L. Johnson to discuss potential patentability of the proposed independent claim 49 presented on Schedule A to this Memorandum.

Sincerely,

WILSON SONSINI GOODRICH & ROSATI
Professional Corporation

/Lee A. Johnson/

Lee A. Johnson, PhD, Esq.
Reg # 75,671
(858) 350-2344

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2423

SCHEDULE A

Proposed amendments to independent claim 49 of US Pat. App. Ser. No. 14/451,876

49. A computer implemented method of detecting the presence or absence of each analyte of a plurality of analytes in a sample volume, comprising:

a)[[a.]] generating, with a computer, a coding scheme wherein (i) ~~encoding~~ each of said analytes is encoded as [[a]]at least one first value in a first component of an electromagnetic signal~~, thereby generating a coding scheme, wherein~~ (ii) each of said analytes is represented ~~in said coding scheme~~ by one of said first values, and (iii) said first value is an intensity or range of intensities, and storing said coding scheme on a computer readable medium;~~whereinsaid encoding is performed in a manner that reduces or eliminates degeneracy~~;

b)[[b.]] providing a sample volume, the sample volume comprising, or potentially comprising, at least one of said analytes;

c)[[c.]] providing a set ~~contacting said sample with~~ of analyte-specific reagents that generate said first values~~, as represented in said coding scheme,~~ when excited and when each of said analytes is present;

d) contacting said sample with said set of analyte-specific reagents and exciting said reagents, thereby generating aid electromagnetic signal;

e) receiving and detecting said electromagnetic signal with a photodetector operatively coupled to a computer;

f)[[d.]] cumulatively measuring said first values of said electromagnetic signal within said sample, thereby providing a cumulative measurement; and

g)[[e.]] ~~determining,~~comparing, using a computer executing software containing said coding scheme, said cumulative measurement and said coding scheme, and making a determination as to whether each of said analytes is present or absent in said sample ~~based on said cumulative measurement and said coding scheme~~;

wherein said method non-degenerately detects the presence or absence of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization of said analytes, physical separation of said analytes, or mass ~~spectometry~~spectrometry.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2424

PTO/SB/30 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	14/451,876
Filing Date	August 5, 2014
First Named Inventor	Emil KARTALOV
Art Unit	1631
Examiner Name	Russell Scott NEGIN
Attorney Docket Number	38075-716.301

This is a Request for Continued Examination (RCE) under 37 CFR 1 .114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

   a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

      i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

      ii. ☐ Other _____

   b. ☒ Enclosed

      i. ☒ Amendment/Reply

      ii. ☐ Affidavit(s)/ Declaration(s)

      iii. ☐ Information Disclosure Statement (IDS)

      iv. ☐ Other _____

2. Miscellaneous

   Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a

   a. ☐ period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

   b. ☐ Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   The Director is hereby authorized to charge the following fees any underpayment of fees or credit any overpayments to

   a. ☒ Deposit Account No. 23-2415 .

      i. ☒ RCE fee required under 37 CFR 1.17(e)

      ii ☒ Extension of time fee (37 CFR 1.136 and 1.17)

      iii ☐ Other _____

   b. ☐ Check in the amount of $ _____ enclosed

   c. ☐ Payment by credit card (Form PTO-2038 enclosed)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	/Lee A. Johnson/	Date	November 6, 2017
Name (Print/Type)	Lee A. Johnson	Registration No.	75,671

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below.

Signature			
Name (Print/Type)		Date	

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

9535419_1.doc

PTO/SB/30 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	14/451,876
Filing Date	August 5, 2014
First Named Inventor	Emil KARTALOV
Art Unit	1631
Examiner Name	Russell Scott NEGIN
Attorney Docket Number	38075-716.301

This is a Request for Continued Examination (RCE) under 37 CFR 1 .114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

   a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

      i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

      ii. ☐ Other _____

   b. ☒ Enclosed

      i. ☒ Amendment/Reply

      ii. ☐ Affidavit(s)/ Declaration(s)

      iii. ☐ Information Disclosure Statement (IDS)

      iv. ☐ Other _____

2. Miscellaneous

   Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a

   a. ☐ period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

   b. ☐ Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   The Director is hereby authorized to charge the following fees any underpayment of fees or credit any overpayments to

   a. ☒ Deposit Account No. 23-2415 .

      i. ☒ RCE fee required under 37 CFR 1.17(e)

      ii ☒ Extension of time fee (37 CFR 1.136 and 1.17)

      iii ☐ Other _____

   b. ☐ Check in the amount of $ _____ enclosed

   c. ☐ Payment by credit card (Form PTO-2038 enclosed)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	/Lee A. Johnson/	Date	November 6, 2017
Name (Print/Type)	Lee A. Johnson	Registration No.	75,671

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below.

Signature			
Name (Print/Type)		Date	

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

9535419_1.doc

Electronic Patent Application Fee Transmittal

Application Number:	14451876
Filing Date:	05-Aug-2014
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Filer:	Vernon A. Norviel/Katherine Lopez/LJ6
Attorney Docket Number:	38075-716.301

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 2 months with $0 paid	1252	1	600	600
Miscellaneous:				
RCE- 1st Request	1801	1	1200	1200
		Total in USD ($)		**1800**

Electronic Acknowledgement Receipt	
EFS ID:	30868150
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	Vernon A. Norviel/Katherine Lopez/LJ6
Filer Authorized By:	Vernon A. Norviel
Attorney Docket Number:	38075-716.301
Receipt Date:	06-NOV-2017
Filing Date:	05-AUG-2014
Time Stamp:	17:18:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$1800
RAM confirmation Number	110717INTEFSW00004851232415
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		CIT_38075_716_301_Resp_FINAL_OA.pdf	206901 dfe94422b6e2346428d482cc8369de91695d7b56	yes	14
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Applicant Arguments/Remarks Made in an Amendment		8	14	
	Claims		2	7	
	Response After Final Action		1	1	
Warnings:					
Information:					
2	Request for Continued Examination (RCE)	CIT_38075_716_301_Signed_RCE.pdf	139419 9eacf73484cb12ceeb743ac1457684af616bd19a	no	1
Warnings:					
This is not a USPTO supplied RCE SB30 form.					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	32815 c3b4b481a31b634b0626097dc5500da21b835dbe	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	379135		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

REMARKS

The following remarks are in response to the Examiner's Office Action mailed on June 6, 2017 and Advisory Action mailed August 23, 2017.

I. Examiner Interview

Applicant thanks Examiners Russell Scott Negin and Marjorie Moran for the courtesy extended during the telephonic interviews conducted on September 17, October 16, and November 1, 2017.

Discussions were held with the Examiners regarding strategies for overcoming the rejections raised under 35 USC § 101, and a proposal to amend the claims along the lines of the amendments presented herein.

II. Status of the Claims

Claims 49, 81, 83, 87, and 88 are pending. Claims 3, 6-8, 10-12, 14-16, 20-21, 23-26, 30-33, 36, 40-41, 44, 46, 48-80, 84-86, and 89-92 are canceled without prejudice. Claims 1-2, 4-5, 9, 13, 17-19, 22, 27-29, 34-35, 37-39, 42-43, 45, 47, and 82 are withdrawn. Claims 49, 81, 83, 87, and 88 are amended herein. New claims 93-95 are added herein.

Claim Support

Support for the amended and new claims is found in the original claims and throughout the specification. In particular, support for the step of "measuring the cumulative intensity signal(s), thereby generating a cumulative signal measurement" as recited at step c) of independent claim 49, as amended, is provided at least at paragraphs [0134], [0135], and [0154] of the application as published.

Further, support for amended claim 81, which depends from claim 49 and recites "said hybridization probes comprise an identical fluorophore… [and] wherein said plurality of hybridization probes are specific for different polynucleotide analytes" is provided at least at section "E. Encoding Methods Using One Color and One Intensity Per Analyte but Different Intensities

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2432

Among Analytes" at paragraphs [0101]-[0105]. In particular, at paragraph [0104], the third sentence provides an exemplary description in how a single colored fluorophore and different intensities are utilized to distinguish up to 16 different analytes.

No new matter is introduced by any amendment made herein.

Upon entry of the amended claims, claims 49, 81, 83, 87, and 93-95 will be under examination. Favorable consideration of these claims is respectfully requested in light of the present amendments and the following remarks.

III. Claim Rejections Under 35 U.S.C. §101

The Office had maintained the rejection of former claims 49, 51, 53, 55, 56, 75-81, 83-88, and 90-92 under 35 U.S.C. § 101, asserting that these claims were directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more. *See* Office Action at pp. 3-4. This rejection is respectfully traversed in view of the present amendments to sole pending independent claim 49, for at least the following reasons.

The 2014 USPTO interim guidance on patent subject matter eligibility, *Federal Register*, vol. 79, no. 241 ("2014 IEG") sets out a two part test to be applied when evaluating a claim for patent eligibility. Step 1 asks whether the claim is directed to a process, machine, manufacture, or composition of matter. Independent claim 49, as amended, is directed to a "method of detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume". Applicant submits that this claim, as amended, is directed to a process, and is therefore satisfies Step 1 of the test set out in the 2014 IEG. Step 2A of the test asks whether the claim is directed to a law of nature, a natural phenomenon, or an abstract idea. Applicant respectfully submits that claim 49, as amended, does not recite any of these judicial exceptions, and that Step 2A of the 2014 IEG test is satisfied. Accordingly, Applicant submits that independent claim 49, as amended, is patent eligible under 35 U.S.C. § 101.

Even if the Office asserts that amended claim 49 is directed to an abstract idea, which Applicant would strongly disagree with, the claims recite "significantly more" than an abstract idea itself. As noted in the previous response filed on May 2, 2017, the Nov. 2016 Eligibility Memo

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2433

further provides guidance on how examiners should apply the second step. Specifically, the Nov. 2016 Eligibility Memo highlights that regarding *BASCOM*, "examiners should consider the additional elements in **combination,** as well as individually, when determining whether a claim as a whole amounts to significantly more, as this may be found in the nonconventional and non-generic arrangement of known, conventional elements." (Emphasis in Original) Furthermore, the Nov. 2016 Eligibility Memo notes that "the district court erred by failing to recognize that when combined, an inventive concept may be found in the non-conventional and non-generic arrangement of the additional elements, *i.e.,* the installation of a filtering tool at a specific location, remote from the end-users, with customizable filtering features specific to each end user (note that the term 'inventive concept' is often used by the courts to describe additional element(s) that amount to significantly more than a judicial exception)."

The Instant Invention Does Not Seek to Monopolize

Lastly, Applicant does not seek to preempt others from all possible uses of analyte detection by a coding scheme. The Federal Circuit, in *McRO, Inc. v. Bandai Namco Games Am. Inc.*, opined that "[t]he preemption concern arises when the claims are not directed to a specific invention and instead improperly monopolize 'the basic tools of scientific and technological work.'" *McRO, Inc. v. Bandai Namco Games Am. Inc.*, 837 F.3d 1314 (Fed. Cir. 2016) (quoting *Alice*, 134 S. Ct. at 2354, in turn quoting *Myriad*, 133 S. Ct. at 2116). The *McRO* court further explains that the claims at issue were valid under § 101 because:

> The specific structure of the claimed rules would prevent broad preemption of all rules-based means of automating lip synchronization unless the limits of the rules themselves are broad enough to cover all possible approaches. … By incorporating the specific features of the rules as claim limitations, claim 1 is limited to a specific process for automatically animating characters using particular information and techniques and does not preempt approaches that use rules of a different structure or different techniques.
>
> *Id.* at 1315-16.

Similarly, the *BASCOM* court notes that "the claims of the '606 patent do not preempt the use of the abstract idea of filtering content on the Internet or on generic computer components performing conventional activities. The claims carve out a specific location for the filtering system

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2434

(a remote ISP server) and require the filtering system to give users the ability to customize filtering for their individual network accounts." *BASCOM Global Internet Services v. AT&T Mobility LLC,* 827 F .3d 1350 (Fed. Cir. 2016).

Here, as with the claims in *McRO* and *BASCOM*, the instant claims do not preempt means of detecting target analytes. Instead, the instant claims are limited to a specific process of detecting the presence or absence of each analyte of a plurality of polynucleotide analytes in a single sample volume without immobilization, physical separation, or mass spectrometry.

Based on the above, Applicant respectfully submits that the instant claims are directed to patent eligible subject matter. Withdrawal of the rejection under 35 U.S.C. § 101 is respectfully requested.

IV. Claim Rejections Under 35 U.S.C. §§102 and 103

The Office had maintained the rejection to previously submitted claims 49, 51, 53, 55-56, 75-78, 80-81, 84-88, and 90-92 under pre-AIA 35 U.S.C. 102, citing a new reference, namely Lin et al. (US2013/0261019A1) (hereinafter Lin), to support the rejection. In particular, the Office asserted that Lin teaches "nondegenerate combinations of barcodes" and that the methods of Lin "do not involve immobilization of analytes, physical separation of analytes, or mass spectrometry." *See* Office Action at pp. 4-7.

As set out in the MPEP, [a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. (MPEP 2131, quoting *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). "[U]nless a reference discloses within the four corners of the document not only all of the limitations claimed but also all of the limitations arranged or combined in the same way as recited in the claim, it cannot be said to prove prior invention of the thing claimed and, thus, cannot anticipate under 35 U.S.C. § 102." *Net MoneyIn, Inc. v. VeriSign, Inc.*, No. 2007-1565 slip op. at 18-19 (Fed. Cir. Oct. 20, 2008).

As discussed in the interview held September 19, 2017, and as reflected in the Interview Summary dated September 22, 2017, the present amendments to sole independent claim 49, which has been amended to recite a step of measuring a cumulative intensity signal, overcome the rejections based on Lin. Lin does not teach a method for indicating the presence or absence of at

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2435

least seven polynucleotide analytes based on measurement of intensity. Instead, the crux of Lin's invention is the use of color-coded patterns deposited on a nanorod barcode probe to detect a target of interest. The instant invention utilizes intensity to detect targets of interest. Therefore, the instant invention is not anticipated by Lin for at least this reason.

Further, Applicant maintains the position set out in the previous Office Action response that the claims, as amended, are not anticipated by Lin, since Lin teaches a method which is inherently redundant, and fails to non-degenerately indicate the presence or absence of at least seven polynucleotide analytes, as recited by the presently amended independent claim 49.

In light of the above, Applicant respectfully submits that independent claims 49 and their respective dependent claims are not anticipated by Lin. Withdrawal of the rejection under pre-AIA 35 U.S.C. §102(e) is respectfully requested.

V. Claim Rejections Under 35 U.S.C. §103

The Office had rejected former claims 79 and 83 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Lin in further view of Nie et al. (US 2003/0148544 A1) (claim 79) and in view of Orpana et. Al (WO 2010/128206) (claim 83). Without admission of the propriety of this rejection and solely in the interest of expediting prosecution, Applicant has cancelled claim 79 herein, rendering the rejection of claim 79 moot.

As to the rejection of claim 83, which depends from independent claim 49, Applicant refers to the deficiencies in Lin discussed at § IV, *supra*. Applicant respectfully submits that for at least these reasons, Lin fails to teach or suggest all of the limitations of dependent claim 83. Further, the teachings of Nie and Orpana fail to remedy the deficiencies of Lin. Accordingly, Applicant submits that claim 83, as amended, is not obvious over Lin, Nie, and Orpana, either alone or in combination. Withdrawal of the rejection under pre-AIA 35 U.S.C. §103 is respectfully requested.

VI. Double Patenting

Former claims 49, 51, 53, 55, and 56 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 20-24, respectively, of U.S. Patent No. 8,838,394.

Applicant respectfully traverses the rejection at least in view of the present amendments to sole independent claim 49. Claims 20-24 of U.S. Patent No. 8,838,394 fail to teach or suggest the

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2436

elements of claim 49, as amended. In particular, the cited claims at least fail to teach or suggest steps b) and c) of the presently amended claim 49, namely:

b) contacting said sample solution volume with the plurality of hybridization probes and exciting the hybridization probes to generate the cumulative intensity signal(s) if one or more of the at least seven polynucleotide analytes is present in the sample volume; and

c) measuring the cumulative intensity signal(s), thereby generating a cumulative signal measurement, wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence…

For at least the foregoing reasons, Applicant submits that the present rejection is rendered moot by the amendments to the claims submitted herewith.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2437

CONCLUSION

In light of the remarks set forth above, Applicant believes that the pending claims are under condition for allowance. Applicant respectfully solicits the Examiner to expedite the prosecution of this patent application to issuance. Should the Examiner have any question, the Examiner is encouraged to telephone the undersigned.

The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit account No. 23-2415 (Attorney Docket No. 38075-716.301).

Respectfully submitted,

Date: November 6, 2017 By: /Lee A. Johnson/
Lee A. Johnson, Reg. No. 75,671

WILSON SONSINI GOODRICH & ROSATI
650 Page Mill Road
Palo Alto, CA 94304-1050
Phone: (650) 493-9300
Customer No. 021971

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2438

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in this application. The following amendments do not constitute an admission regarding the patentability of the amended subject matter and should not be so construed. Applicants reserve the right to pursue the subject matter of the canceled claims in this or any other appropriate patent application. Further, none of Applicants' amendments are to be construed as dedicating any such subject matter to the public, and Applicants reserve all rights to pursue any such subject matter in this or a related patent application.

Listing of claims:

1. **(Withdrawn)** An assay that is capable of unambiguously detecting the presence or absence of each of at least seven (7) analytes, in any combination of presence or absence, in a single sample volume without immobilization of said analytes, physical separation of said analytes, melting curve analysis, or mass spectrometry.
2. **(Withdrawn)** The assay of claim 1, wherein each of said analytes is encoded as a value of one component of a signal.
3. **(Canceled)**
4. **(Withdrawn)** The assay of claim 2, wherein each of said analytes is encoded as at least one first value in a first component of said signal and at least one second value in a second component of said signal.
5. **(Withdrawn)** The assay of claim 2, wherein each of said analytes is encoded as a first value in a first component of said signal at each of a plurality of second values in a second component of said signal.

6.-8. **(Canceled)**

9. **(Withdrawn)** The assay of claim 4, wherein said first value is an intensity or range of intensities and said second value is a wavelength or range of wavelengths.

10.-12. **(Canceled)**

13. **(Withdrawn)** The assay of claim 2, wherein said signal is an electromagnetic signal.

14.-16. **(Canceled)**

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2439

17. **(Withdrawn)** The assay of claim 1, wherein said detecting is performed with reagents comprising hybridization probes.

18. **(Withdrawn)** The assay of claim 1, wherein said detecting is performed with a plurality of hybridization probes, comprising an identical fluorophore, wherein said plurality of hybridization probes are specific for different analytes.

19. **(Withdrawn)** The assay of claim 1, wherein said detecting is performed with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes.

20.-21. **(Canceled)**

22. **(Withdrawn)** The assay of claim 2, wherein said signal is generated during a polymerase chain reaction.

23.-26. **(Canceled)**

27. **(Withdrawn)** The assay of claim 4, further comprising constructing a diagram.

28. **(Withdrawn)** The assay of claim 27, wherein said diagram is constructed by plotting possible combinations of said first values and said second values for positive control samples.

29. **(Withdrawn)** The assay of claim 1, wherein at least one of said analytes comprises a polynucleotide.

30.-33. **(Canceled)**

34. **(Withdrawn)** The assay of claim 1, further comprising transmitting information concerning the presence or absence of said analyte through a computer network.

35. **(Withdrawn)** The assay of claim 1, further comprising providing information concerning the presence or absence of said analyte to a health care professional, wherein the health care professional makes a clinical decision based on said information.

36. **(Canceled)**

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2440

37. **(Withdrawn)** The assay of claim 1, wherein at least one step of said assay is performed using a computer.

38. **(Withdrawn)** The assay of claim 37, wherein said computer is located on a remote server.

39. **(Withdrawn)** The assay of claim 37, wherein said computer is located on a thermal cycler.

40.-41. **(Canceled)**

42. **(Withdrawn)** The assay of claim 2, wherein said coding is innately non-degenerate.

43. **(Withdrawn)** The assay of claim 42, wherein said coding is designed to be non-degenerate by enumerating legitimate results that can be obtained from said encoding, identifying legitimate results that are degenerate, and eliminating at least one potential analyte code from said encoding to eliminate degeneracy.

44. **(Canceled)**

45. **(Withdrawn)** The assay of claim 1, wherein said assay is an end-point assay.

46. **(Canceled)**

47. **(Withdrawn)** The assay of claim 1, wherein said assay is a liquid phase assay.

48. **(Canceled)**

49. **(Currently Amended)** A method of detecting the presence or absence of at least seven polynucleotide~~each~~ analytes, in any combination of presence or absence, in a single sample solution volume ~~of a plurality of analytes~~, the method comprising:

~~a)~~[[a.]] ~~encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by one of said first values, wherein said encoding is performed in a manner that reduces or eliminates degeneracy;~~

a)[[b.]] providing a sample solution volume comprising, or potentially comprising, at least one of ~~said~~ at least seven polynucleotide analytes, and comprising at least seven hybridization probes, each hybridization probe corresponding to one of the at least seven polynucleotide analytes, wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores, and wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2441

cumulative intensity signal, further wherein each generated cumulative intensity signal comprises at least one signal generated by excitement of at least one of the four fluorophores;

b)[[c.]] contacting said sample solution volume with the plurality of hybridization probes and exciting the hybridization probes to generate the cumulative intensity signal(s) if one or more of the at least seven polynucleotide analytes is present in the sample volume~~analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present~~; and

c)[[d.]] measuring the cumulative intensity signal(s), thereby generating a cumulative signal measurement, wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence,~~cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement;~~

~~e)[[e.]] determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme~~;

wherein said method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in [[a]]said single sample solution volume, in any combination of presence or absence, without requiring any step of immobilization of said polynucleotide analytes, physical separation of said polynucleotide analytes, or mass ~~spectometry~~spectrometry.

50. **(Canceled)**

51. **(Canceled)**

52. **(Canceled)**

53. **(Canceled)**

54. **(Canceled)**

55. **(Canceled)**

56. **(Canceled)**

57.-74. **(Canceled)**

75. **(Canceled)**

76. **(Canceled)**

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2442

77. **(Canceled)**

78. **(Canceled)**

79. **(Canceled)**

80. **(Canceled)**

81. **(Currently Amended)** The method of claim 49, wherein said ~~detecting is performed with a plurality of~~ hybridization probes[[,]]comprise ~~comprising~~ an identical fluorophore, and wherein said plurality of hybridization probes are specific for different polynucleotide analytes.

82. **(Withdrawn)** The method of claim 49, wherein said detecting is performed with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes.

83. **(Currently Amended)** The method of claim 49, wherein said cumulative intensity signal(s) ~~signal is~~ are generated during a polymerase chain reaction.

84. **(Canceled)**

85. **(Canceled)**

86. **(Canceled)**

87. **(Currently Amended)** The method of claim 49, further comprising transmitting information concerning the presence or absence of said polynucleotide analytes through a computer network.

88. **(Currently Amended)** The method of claim 49, further comprising providing information concerning the presence or absence of said polynucleotide analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information.

89. **(Canceled)**

90. **(Canceled)**

91. **(Canceled)**

92. **(Canceled)**

93. **(New)** The method of claim 49, wherein at least one step of said method is performed using a computer.

94. **(New)** The method of claim 93, wherein said computer is located on a remote server.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2443

95. **(New)** The method of claim 93, wherein said computer is located on a thermal cycler.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2444

Electronically Filed On: November 6, 2017

PATENT
Attorney Docket No.: 38075-716.301

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application	
Inventors: Emil KARTALOV, *et al.*	Confirmation No.: 1065
Application No.: 14/451,876	Art Unit: 1631
Filed: August 5, 2014	Examiner: Russell Scott NEGIN
Title: **SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS**	Customer No. 021971

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO FINAL OFFICE ACTION AND REQUEST FOR CONTINUED EXAMINATION

Dear Sir:

This Amendment is submitted in response to the Final Office Action mailed June 6, 2017 and the Advisory Action mailed August 23, 2017. A Request for Continued Examination and requisite fees are submitted herewith.

A Petition for Extension of Time is requested and the fee set forth under 37 C.F.R. §1.17(a)(3) is electronically submitted herewith.

Amendments to the Claims begins on page **2** of this paper.
Remarks begin on page **8** of this paper.
Conclusion begin on page **14** of this paper.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2445

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/451,876	Filing Date 08/05/2014	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☒ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	**140**
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	**140**

APPLICATION AS AMENDED – PART II

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	11/06/2017						
	Total (37 CFR 1.16(i))	* 31	Minus	** 45	= 0	X $40 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
GLORIA J. TRAMMELL

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		None			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	WO-2013116780-A1	08-08-2013	CALIFORNIA INST OF TECHN [US], et al.		☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	001	Holland, et al., Detection of specific polymerase chain reaction product by utilizing the 5´ to 3´ exonuclease activity of Thermus aquaticus DNA polymerase. PNAS (USA) 88:7276-7280, 1991.	☐
	002	International search report and written report dated 06/22/2017 for PCT Application No. PCT/US2017/24933.	☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 3 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Lee A. Johnson/	Date (YYYY-MM-DD)	2017-11-16
Name/Print	Lee A. Johnson	Registration Number	75,671

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 August 2013 (08.08.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/116780 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2013/024509

(22) International Filing Date:
1 February 2013 (01.02.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/594,480 3 February 2012 (03.02.2012) US
61/703,093 19 September 2012 (19.09.2012) US

(71) Applicant *(for all designated States except US)*: **CALIFORNIA INSTITUTE OF TECHNOLOGY** [US/US]; 1200 East California Boulevard, Pasadena, CA 91125 (US).

(72) Inventors; and
(71) Applicants *(for US only)*: **KARTALOV, Emil, P.** [US/US]; 2133 East Mountain Street, Pasadena, CA 91104 (US). **RAJAGOPAL, Aditya** [US/US]; 27 Lee, Irvine, CA 92620 (US). **SCHERER, Axel** [DE/US]; 1661 North Road, Barnard, VT 05031 (US).

(74) Agents: **MEGEED, Zaki** et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304-1050 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) **Title**: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

(57) **Abstract**: This disclosure provides methods, systems, compositions, and kits for the multiplexed detection of a plurality of analytes in a sample. In some examples, this disclosure provides methods, systems, compositions, and kits wherein multiple analytes may be detected in a single sample volume by acquiring a cumulative measurement or measurements of at least one quantifiable component of a signal. In some cases, additional components of a signal, or additional signals (or components thereof) are also quantified. Each signal or component of a signal may be used to construct a coding scheme which can then be used to determine the presence or absence of any analyte.

WO 2013/116780 A1

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2451

SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. provisional applications 61/594,480, filed February 3, 2012 and 61/703,093, filed September 19, 2012, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Multiplexed reactions offer significant advantages over traditional uniplex reactions, including performance of parallel reactions on the same sample, use of the same chamber to perform multiple reactions, and the ability to extract rich information from a sample in a fast and efficient manner. However, to achieve these benefits, multiplexed assays generally require complex reporting mechanisms, namely spectrally resolved fluorescence or chemiluminescence (e.g., PCR, ELISA), spatially resolved signals (e.g., microarrays, gel electrophoresis), temporally resolved signals (e.g., capillary electrophoresis), or combinations thereof (e.g., Sanger sequencing). There is a need for multiplexed reactions that can be carried out in a single solution.

SUMMARY OF THE INVENTION

[0003] This disclosure provides methods, compositions, systems, and kits for the multiplexed detection of analytes. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis. In some examples, each of the analytes is encoded as a value of one (or at least one) component of a signal.

[0004] In some examples, an assay provided in this disclosure is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of one component of a signal (e.g., an intensity) when all the analytes are present.

[0005] In some cases, each analyte is encoded as at least one first value in a first component of a signal (e.g., at least one intensity or range of intensities) and at least one second value in a second component of a signal (e.g., at least one wavelength or range of wavelengths). In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2452

second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths).

[0006] In some examples, an assay provided herein is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = C * \log_2 (F + 1)$, where C is the number of the second values used to encode the analytes and F is the maximum cumulative value of the first component of the signal, for any second value, when all of the analytes are present.

[0007] In some cases, an assay provided in this disclosure is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = (P * T) + 1$, where P is the number of codes per tier in a coding scheme and T is the number of tiers. In some cases, the number of tiers $T = \log_4 (F + 1)$, and F is the maximum cumulative value of a first component of a signal, for any second value, when all of the analytes are present.

[0008] In some examples, the first value is an intensity or range of intensities. In some cases, a coding scheme comprises at least three intensities or ranges of intensities.

[0009] In some cases, the second value is a wavelength or range of wavelengths. In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths.

[0010] In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths.

[0011] In some cases, an assay provided herein is performed with reagents that are lyophilized prior to use.

[0012] In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores. In some examples, a sample is contacted with at least 18 of said hybridization probes. In some examples, a detecting step is performed with reagents comprising at least one pair of primers. In some cases, at least one pair of primers are capable of amplifying a region complementary to at least three of said hybridization probes.

[0013] In some examples, the signal that is measured is generated during a polymerase chain reaction. In some cases, the polymerase chain reaction is selected from the group consisting of an end-point polymerase chain reaction, a real-time polymerase chain reaction, a digital polymerase chain reaction, and combinations thereof.

[0014] In some examples, at least one cumulative measurement is performed on a solution.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2453

[0015] In some examples, at least one analyte is encoded by at least one additional value (i.e., at least two values together), wherein the at least one additional value is selected from the group consisting of a value from at least one additional component of a signal, a value from at least one component of a different signal, and combinations thereof. For example, the at least one additional value may be selected from the group consisting of a fluorescence emission intensity, a fluorescence emission wavelength, a Förster resonance energy transfer (FRET) emission intensity, a FRET emission wavelength, an electrochemical signal, a chemiluminescence wavelength, a chemiluminescence intensity, a fluorescence bleaching rate, a chemiluminescence bleaching rate, and combinations thereof.

[0016] In some cases, a chromatogram is constructed. The chromatogram may be constructed by plotting all possible combinations of first values and second values for positive control samples for each analyte.

[0017] In some examples, at least one of the analytes comprises a polynucleotide. In some cases, the polynucleotide is from a source selected from the group consisting of an animal, a plant, a bacteria, a fungus, a parasite, and a virus. In some examples, the polynucleotide is from a source selected from the group consisting of human immunodeficiency virus, herpes simplex virus, human papilloma virus, Plasmodium, Mycobacterium, dengue virus, hepatitis virus, and influenza virus. In some cases, the polynucleotide is selected from the group consisting of human immunodeficiency virus polyprotease, human immunodeficiency virus p17, human papilloma virus E6, and human papilloma virus E7.

[0018] In some cases, a sample is selected from the group consisting of a clinical sample, a food sample, an environmental sample, a pharmaceutical sample, and a sample from a consumer product.

[0019] In some examples, information concerning the presence or absence of an analyte is transmitted through a computer network.

[0020] In some cases, information concerning the presence or absence of an analyte is provided to a physician. In some examples, a clinical decision is made based on such information.

[0021] In some examples, at least one step of a method provided herein is performed using instructions on a computer readable medium. In some cases, the instructions are located on a remote server. In some examples, the instructions are located on a thermal cycler. In some cases, the instructions are located on a computer in communication with a thermal cycler.

[0022] In some cases, at least one of the analytes is a positive control analyte.

[0023] In some examples, the coding scheme is non-degenerate. In some cases, the coding scheme is designed to be non-degenerate. In some examples, the coding scheme is made non-degenerate by enumerating every legitimate result that can be obtained from the coding scheme, identifying each

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2454

legitimate result that is degenerate, and eliminating at least one potential analyte code from the coding scheme to eliminate degeneracy.

[0024] In some cases, an assay provided herein is an end-point assay. In some examples an assay provided in this disclosure is ended at a threshold number of cycles set by the limit of detection of an instrument.

[0025] In certain examples, an assay provided herein is a liquid phase assay.

[0026] In some cases, an assay provided herein is quantitative.

[0027] In some cases, this disclosure provides a method of detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme.

[0028] In some examples, the method described in the preceding paragraph is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of the first values when all of the analytes are present. In some cases, the first value is an intensity or range of intensities. In some examples, the first value has a minimum value in the coding scheme that is selected from the group consisting of at least 1, at least 2, at least 4, at least 8, at least 16, at least 32, and at least 64. In some cases, the first value is incremented in the coding scheme by an amount equal to the cumulative maximum of said preceding first values plus one. In other cases, the first value is incremented in the coding scheme by an amount greater than the cumulative maximum of the preceding first values plus one.

[0029] In some cases, this disclosure provides a method of detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (b) providing a sample comprising, or potentially comprising,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2455

at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said at least one first value and at least one said second value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme, wherein, when degeneracy is eliminated, said method is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

[0030] In some cases, each of the analytes of the method described in the preceding paragraph is encoded as a first value in a first component of the signal at each of a plurality of second values in a second component of the signal. In some cases, when degeneracy is eliminated, this method is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single volume using four second values. In some examples, when degeneracy is eliminated, the coding scheme comprises T non-degenerate tiers, wherein $T = \log_4 (F + 1)$ and F is the maximum cumulative value of the first component of the signal, for any second value, when all of the analytes are present. In some cases, when degeneracy is eliminated, the method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = (P * T) + 1$ and P is the number of codes per tier. In some examples, when degeneracy is eliminated, the method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = C * \log_2 (F + 1)$, C is the number of second values in the coding scheme, and F is the maximum cumulative value of the first component of the signal, for any second value, when all of the analytes are present.

[0031] This disclosure also provides non-degenerate coding schemes capable of unambiguously encoding the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis.

[0032] In some examples, a non-degenerate coding scheme of this disclosure is generated by a method comprising: (a) generating a code for each potential analyte, wherein each potential analyte is encoded by at least one value of at least one component of a signal; (b) enumerating every legitimate cumulative result for all possible combinations of presence or absence of each analyte; (c) identifying each legitimate result that is degenerate; and (d) eliminating at least one code to eliminate degeneracy. In some cases, the coding scheme may be expanded by adding additional codes that are at least one unit greater than the sum of all previous codes in the at least one component of said

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2456

signal. In some cases a coding scheme is generated by a method of mathematical iteration that guarantees non-degeneracy by construction.

[0033] In some cases, this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme.

[0034] In some cases, this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme, wherein, when degeneracy is eliminated, said system is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

[0035] In some cases, this disclosure provides methods of detecting the presence or absence of each analyte of a plurality of analytes for a third party, comprising: (a) obtaining the identity of each of said analytes from a party; (b) encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy; (c) providing said party with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present, said party: (i) contacting a sample

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2457

comprising, or potentially comprising, at least one of said analytes with said reagents; and (ii) cumulatively measuring said first values within said sample, thereby providing a cumulative measurement; and (d) obtaining said cumulative measurement from said party; (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme; and (f) providing said party with information about the presence or absence of each of said analytes.

[0036] In some cases, this disclosure provides methods of detecting the presence or absence of each analyte of a plurality of analytes for a third party, comprising: (a) obtaining the identity of each of said analytes from a party; (b) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (c) providing said party with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present, said party: (i) contacting a sample comprising, or potentially comprising, at least one of said analytes with said reagents; and (ii) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and (d) obtaining said cumulative measurement from said party; (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme; and (f) providing said party with information about the presence or absence of each of said analytes.

[0037] In some cases, this disclosure provides compositions for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, each reagent generating a signal comprising a first value, wherein said reagents are capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, mass spectrometry, or melting curve analysis.

[0038] In some cases, this disclosure provides compositions for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, each reagent generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said reagents are capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2458

[0039] In some cases, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, packaging, and instructions, each reagent generating a signal comprising a first value, wherein said kit is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, mass spectrometry, or melting curve analysis.

[0040] In some examples, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, packaging, and instructions, each reagent generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said kit is capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

[0041] In some cases, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, comprising a kit body (601), clamping slots arranged in the kit body for placing bottles, a bottle comprising probes for the detection of an analyte (602), a bottle comprising primers for amplification (603), and a bottle comprising reagents for amplification (604), wherein said kit is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, mass spectrometry, or melting curve analysis.

[0042] In some examples, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, a kit body (601), clamping slots arranged in the kit body for placing bottles, a bottle comprising probes for the detection of an analyte (602), a bottle comprising primers for amplification (603), and a bottle comprising reagents for amplification (604), each probe generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said kit is capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said probes generates only one second value.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2459

INCORPORATION BY REFERENCE

[0043] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0045] **Fig. 1** shows a comparison between a traditional encoding method of detecting four analytes with four colors and an encoding method of the invention able to detect 16 or more sequences with four colors. Colors are indicated by B, G, Y, and R, which indicate blue, green, yellow, and red, respectively.

[0046] **Fig. 2** shows a schematic representation of detection of six analytes (including control) with four colors. The analytes are detected using hybridization probes attached to a fluorophore (B, G, Y, R; blue, green, yellow, and red, respectively) and a quencher (oval with "X"). The control sequence and the five other sequences (Dengue Fever = dengue virus; Tuberculosis = *Mycobacterium tuberculosis*; P17 = HIV p17; Malaria = *Plasmodium falciparum;* and Herpes = herpes simplex virus 2) are all detected using a probe labeled with a blue fluorophore. The non-control analytes are each detected using 1-3 additional probes. For example, the dengue virus analyte is detected using three additional probes with green, yellow, and red fluorophores; the herpes simplex virus 2 analyte is detected using one additional probe with a red fluorophore; and so on.

[0047] **Fig. 3** shows chromatograms of experimental results obtained as described in Example 2.

[0048] **Fig. 4** shows an exemplary embodiment of the invention in which a computer is used to perform one or more steps of the methods provided herein.

[0049] **Fig. 5** shows a schematic of two different methods of detecting wavelength and intensity of a light signal, such as a fluorescence emission signal.

[0050] **Fig. 6** shows a schematic of exemplary kits.

[0051] **Fig. 7** shows chromatograms of experimental results obtained as described in Example 3.

[0052] **Fig. 8** shows a schematic of a legitimate result (top) and an illegitimate result (bottom).

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2460

DETAILED DESCRIPTION OF THE INVENTION

[0053] Fluorescence detection has been a preferred technique for multiplexed assays because of several desirable features, including compatibility with biochemical assays, the relatively small size of fluorescent labels, simple means of conjugation to molecules of interest, affordability, low toxicity, stability, robustness, detectability with inexpensive optics, and an ability to be combined with spatial arrays. However, standard fluorophores have wide emission spectra. Therefore, in order to avoid spectral overlap (i.e., to preserve spectral resolution) only a relatively small number of colors (e.g., 4 to 6) are typically used simultaneously in multiplexed fluorescent assays.

[0054] The traditional encoding method for multiplexed fluorescent assays has been to encode each analyte with a single color; i.e., M=N, where M is the number of analytes that can be detected and N is the number of spectrally resolved fluorescent probes. Whenever higher factors of multiplexing are required (i.e., M>N), fluorescence is generally combined with other techniques, such as aliquoting, spatial arraying, or sequential processing. These additional processing steps are labor-intensive and frequently require relatively expensive and complex optical and mechanical systems (e.g., spectrometers, mechanized microscopy stages, microfluidics, droplet generators, scanners, and the like). Such systems are often impractical to deploy in certain settings, particularly point-of care and low-resource settings. Thus, there is a significant need for multiplexed encoding and decoding methods that can provide an inexpensive means of multiplexing while avoiding the use of expensive additional processing steps.

[0055] This disclosure provides methods, systems, compositions, and kits for the detection of multiple analytes in a sample. Analytes are detected based on the encoding, analysis, and decoding methods presented herein. In some examples, each analyte to be detected is encoded as a value of a signal (e.g., intensity), where the values are assigned so that the results of the assay unambiguously indicate the presence or absence of the analytes being assayed. In other examples, each analyte to be detected is encoded as a value in each of at least two components of a signal (e.g., intensity and wavelength). The at least two components of a signal may be orthogonal. Similarly, as described more fully elsewhere in this disclosure, multiple orthogonal signals may be used, such as a combination of a fluorescent signal and an electrochemical signal. The analyte may be any suitable analyte, such as a polynucleotide, a protein, a small molecule, a lipid, a carbohydrate, or mixtures thereof. The signal may be any suitable signal such as an electromagnetic signal, a light signal, a fluorescence emission signal, an electrochemical signal, a chemiluminescent signal, and combinations thereof. The at least two components of the signal may be any suitable two components, such as an amplitude and a frequency or an intensity and a wavelength.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2461

[0056] After encoding of the analytes, a sample is provided wherein the sample comprises or may comprise at least one of the encoded analytes. The sample is contacted with an analyte-specific reagent or reagents that generate a particular signal, as specified for each analyte in the coding scheme, in the presence of an analyte. A reagent may be any suitable reagent that is capable of generating such a signal in the presence of its corresponding analyte, for example, an oligonucleotide probe attached to a fluorophore and a quencher (e.g., a TAQMAN probe). If the reagent is an oligonucleotide probe attached to a fluorophore and a quencher, a nucleic acid amplification may be performed to generate the signal.

[0057] After addition of the reagent(s), the signal is quantified. In some cases, this quantification is performed by measuring one component of the signal (e.g., fluorescence intensity) and determining the presence and absence of certain analytes based on the values used to encode the presence of each analyte and the cumulative value of the signal.

[0058] In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths. The presence or absence of an analyte may then be determined based on the values of each of the at least two components of the signal and the values used to encode the presence of the analyte (i.e., those values in the coding scheme).

[0059] The encoding may be performed in a manner that reduces or eliminates the number of possible degenerate (e.g., ambiguous) results that can be obtained by the method. As described elsewhere in this specification, the full coding capability of a particular coding scheme may be enumerated, and certain potential analyte codes may be eliminated from the coding scheme to reduce or eliminate degeneracy. Similarly, a coding scheme may be designed to be non-degenerate, so that a reduction or elimination of degeneracy is not necessary. A decoding matrix may be constructed to translate cumulative signal measurements (e.g., intensities or intensities at particular wavelengths) into the presence or absence of certain analytes, corresponding to the constituent signals of the cumulative signal measurement.

I. Definitions

[0060] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0061] As used herein, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including," "includes," "having," "has," "with," "such as," or variants thereof, are used in

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2462

either the specification and/or the claims, such terms are not limiting and are intended to be inclusive in a manner similar to the term "comprising".

[0062] The term "about," as used herein, generally refers to a range that is 15% greater than or less than a stated numerical value within the context of the particular usage. For example, "about 10" would include a range from 8.5 to 11.5.

[0063] The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component).

[0064] The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes.

[0065] The term "encoding," as used herein, generally refers to the process of representing an analyte using a code comprising values of a signal, such as intensity, or values in each of at least two components of a signal or signals, such as wavelength and intensity.

[0066] The terms "oligonucleotide probe attached to a fluorophore and a quencher" and "TAQMAN probe" generally refer to hydrolysis probes used to detect the presence of an analyte in a polynucleotide amplification assay. These probes comprise an oligonucleotide probe attached to a fluorophore and a quencher. So long as the quencher and the fluorophore are in proximity, the quencher quenches the fluorescence emitted by the fluorophore upon excitation by a light source. The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte. Hybridization of the oligonucleotide probe is performed in a nucleic acid amplification reaction comprising primers (e.g., a polymerase chain reaction). Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present. If no analyte is present, the probe will not hybridize to an analyte, and the fluorophore and quencher will remain in close proximity. Little or no signal will be produced.

[0067] The term "orthogonal," as used herein, generally refers to at least two components of a signal (e.g., wavelength and intensity), or at least two different signals (e.g., fluorescence emission and electrochemical signal), that can be varied independently or approximately independently. For

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2463

example, wavelength and intensity are considered orthogonal or approximately orthogonal when fluorescent molecules are used. Among other factors, the wavelength of fluorescence emission will depend on the composition of the fluorescent molecule and the intensity of the fluorescence will depend on the amount of molecule present. Although wavelength and intensity are examples of two components of a signal that can be varied approximately independently, the methods described herein are not limited to components that can be varied independently or approximately independently. Components of a signal, or signals, that vary non-independently may also be used, so long as the components or signals are characterized well enough to enable encoding, measurement, and decoding. For example, if the variance in one component or signal affects the variance in another component or signal, the two components or signals may still be used so long as the relationship between the variances is understood.

[0068] The terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides. Exemplary polynucleotides include deoxyribonucleic acids, ribonucleic acids, and synthetic analogues thereof, including peptide nucleic acids.

[0069] The term "probe," as used herein, generally refers to a reagent capable of generating a signal in the presence of a particular analyte. A probe generally has at least two portions: a portion capable of specifically recognizing an analyte, or a portion thereof, and a portion capable of generating a signal in the presence of an analyte, or a portion thereof. A probe may be an oligonucleotide probe attached to a fluorophore and a quencher, as described above and elsewhere in this disclosure. A probe may also be any reagent that generates a signal in the presence of an analyte, such as an antibody that detects an analyte, with a fluorescent label that emits or is quenched upon binding of the antibody to an analyte. Any suitable probe may be used with the methods presented in this disclosure, so long as the probe generates a quantifiable signal in the presence of an analyte. For example, the analyte-specific portion of a probe may be coupled to an enzyme that, in the presence of an analyte, converts an uncharged substrate into a charged product, thereby increasing the electrical conductivity in the medium over time. In this case, different analytes may be encoded by coupling the analyte-specific portion of the probe (e.g., hybridization probe or antibody) to an enzyme at different ratios. The resulting rate of increased conductivity in the medium will be cumulative for all analytes present in the medium. Encoding analytes according to the methods provided herein enables conversion of the conductivity measurements into unambiguous (i.e., non-degenerate) results providing information about the presence or absence of particular analytes. Similarly, a probe may comprise an enzyme producing a chemiluminescent product from a substrate. The amount of chemiluminescence may then be used to encode the presence of particular analytes.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2464

II. Encoding and Decoding Methods

A. Traditional Fluorescent Encoding and Decoding Method

[0070] A commonly used method of determining the presence of an analyte uses four spectrally resolved fluorescent molecules to indicate the presence or absence of four analytes. An example of this method is presented on the left-hand side of **Fig. 1**. The left-hand side of **Fig. 1** shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively). The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher. In the system shown on the left-hand side of **Fig. 1**, there are four different oligonucleotide probes, each comprising a single fluorophore (blue, green, yellow, or red) and a quencher. The presence or absence of an analyte is determined based on the presence or absence of a signal in a particular color.

[0071] The chart on the left-hand side of **Fig. 1** shows intensity versus color for a hypothetical sample containing two analytes: Seq 1 and Seq 3. The presence of these analytes is determined based on the measurement of a blue signal (corresponding to Seq 1) and a yellow signal (corresponding to Seq 3). The absence of Seq 2 and Seq 4 is indicated by the absence of a blue and red signal.

[0072] Table 1 shows a translation of this coding scheme into a binary format. Each analyte is encoded as a value in each of two components of a fluorescent signal: (1) color (also known as wavelength; or range of wavelengths) and (2) intensity (indicated by the numbers within the table). For example, A (e.g., Seq 1) has a color of blue and an intensity of 1; B (e.g., Seq 2) has a color of green and an intensity of 1; and so on. The intensity of the signal within each color range may be quantified as described herein, for example by measuring the intensity of the signal within a particular wavelength range determined by a band pass filter. A result of 1000 indicates that only analyte A is present; a result of 1100 indicates that analytes A and B are present; and so on.

Table 1. Traditional encoding of four analytes with four probes, each probe having a single color, and one probe per analyte.

	Blue	Green	Yellow	Red
A	1	0	0	0
B	0	1	0	0
C	0	0	1	0
D	0	0	0	1

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2465

B. Encoding Methods Using More Than One Color Per Analyte

[0073] The traditional method described above suffers from the fact that it is limited by the number of spectrally resolvable fluorophores. More specifically, the number of detectable analytes is equal to the number of spectrally resolvable fluorophores. Therefore, the number of analytes may only be increased by increasing the number of spectrally resolvable fluorophores.

[0074] This disclosure provides methods that overcome this limitation. More specifically, in some cases, by utilizing at least two components of a signal during encoding, the methods described herein may be used to detect more than one analyte per fluorophore. For example, using the method provided herein 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes may be detected per fluorophore. In some cases, the methods provided herein may be used to detect at least 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes may be detected per fluorophore. In some cases, the methods provided herein may be used to detect 1.5-2, 2-4, 1.5-4, 4-6, 2-6, 6-10 analytes per fluorophore.

[0075] In some cases, the methods provided in this disclosure may include the use of a control color. The control color may be attached to one or more probes binding a positive control analyte, and each analyte to be detected, in a sample. If the same sequence occurs in the positive control analyte and each analyte to be detected, a single control probe may be used. If the same sequence does not occur in the positive control analyte and each analyte to be detected, different probes may be used, but each probe may still be attached to the control color.

[0076] For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method.

Table 2. Encoding of four analytes, including control, with four colors and up to two colors per analyte.

	Blue	Green	Yellow	Red
Ctrl. (p)	1	0	0	0
A	1	0	0	1
B	1	0	1	0
C	1	1	0	0

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2466

[0077] In Table 2, the presence of the control (p) is indicated by a result of 1000. The presence of the control and analyte A is indicated by a result of 2001. The presence of the control and all three other analytes is indicated by a result of 4111. The control color (blue) provides an indication that the assay is functioning properly. The intensity of the blue color reports the number of analytes that are present in a test sample. Of course, any color may be used as the control color. One of skill in the art will recognize that certain practical considerations might make it preferable to use one color over another as the control color. For example, if one color is better detected in a particular optical system (or system of fluorophores), it might be practically preferable to use that color as a control color.

[0078] The coding scheme shown in Table 2 encodes each analyte using one control color and one additional color (up to 2 probes per analyte). However, as shown below, the number of analytes that can be encoded increases when up to 4 colors are used per analyte. Table 3 shows an exemplary coding scheme where each analyte is encoded by up to 4 colors. In the scheme shown in Table 3, the control color (blue) is generated in the presence of the control sequence and each of the other seven analytes (A-G). The other seven analytes are each encoded by the presence of one to three additional colors. The colors may be contained on different probes (e.g., oligonucleotide probes attached to a fluorophore and a quencher) or on the same probe (which can have multiple fluorophores and multiple quenchers).

Table 3. Encoding of eight analytes (including control) with four colors and up to four colors per analyte.

	Blue	Green	Yellow	Red
Ctrl. (p)	1	0	0	0
A	1	0	0	1
B	1	0	1	0
C	1	1	0	0
D	1	0	1	1
E	1	1	0	1
F	1	1	1	0
G	1	1	1	1

[0079] In Table 3, the presence of the control and all seven other analytes is indicated by a result of 8444. Three analytes (A, B, and C) are encoded by two colors. Three analytes (D, E, and F) are encoded by three colors, and one analyte (G) is encoded by four colors. The presence of the control (p) is indicated by a result of 1000. The presence of the control and analyte A is indicated by a result of 2001. The presence of the control and analyte G is indicated by a result of 2111. These results express the cumulative intensity of the signal in each color. For example, the result 2111 has a 2X

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2467

signal intensity in the blue channel, while the result 1000 has only a 1X signal intensity in the blue channel.

[0080] Using Table 3, each of the possible cumulative assay results can be enumerated, in terms of color (blue, green, yellow, red) and intensity (0-8). Table 4 shows a "decoding matrix" generated by enumerating each of the possible cumulative assay results based on the encoding method presented in Table 3 and providing the corresponding decoded result of each assay in terms of the analyte(s) present in the sample. A similar decoding matrix may be generated for any coding scheme described herein, by enumerating each of the possible cumulative assay results based on the coding scheme and providing the corresponding decoded result of each assay in terms of the analyte(s) present in (or absent from) the sample. In some cases, as described below, one or more analytes may be removed from the coding scheme in order to reduce or eliminate degeneracy.

Table 4. Decoding matrix for encoding method presented in Table 3.

Cumulative Assay Result				
Blue	**Green**	**Yellow**	**Red**	**Analyte(s) Present**
1	0	0	0	p
2	0	0	1	pA
2	0	1	0	pB
2	1	0	0	pC
2	0	1	1	pD
2	1	0	1	pE
2	1	1	0	pF
2	1	1	1	pG
3	0	1	1	pAB
3	1	0	1	pAC
3	0	1	2	pAD
3	1	0	2	pAE
3	1	1	1	pAF, pCD, pBE
3	1	1	2	pAG, pDE
3	1	1	0	pBC
3	0	2	1	pBD
3	1	2	0	pBF
3	1	2	1	pBG, pDF
3	2	0	1	pCE
3	2	1	0	pCF
3	2	1	1	pCG, pEF
3	1	2	2	pDG
3	2	1	2	pEG
3	2	2	1	pFG
4	1	1	1	pACB
4	0	2	2	pABD
4	2	2	0	pBCF
4	2	0	2	pACE
4	1	1	2	pABE, pACD

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2468

4	1	2	1	pABF, pBCD
4	2	1	1	pACF, pBCE
4	1	2	2	pADF, pABG, pBDE
4	2	1	2	pACG, pCDE, pAEF
4	2	2	1	pBCG, pBEF, pCDF
4	2	2	2	pCDG, pAFG, pBEG, pDEF
4	1	1	3	pADE
4	1	3	1	pBDF
4	3	1	1	pCEF
4	1	2	3	pADG
4	3	2	1	pCFG
4	2	1	3	pAEG
4	2	3	1	pBFG
4	1	3	2	pBDG
4	3	1	2	pCEG
4	2	2	3	pDEG
4	3	2	2	pEFG
5	1	2	2	pABCD
5	2	1	2	pABCE
5	2	2	1	pABCF
5	2	2	2	pABCG, pABEF, pBCDE, pACDF
5	1	2	3	pABDE
5	2	1	3	pACDE
5	1	3	2	pABDF
5	2	1	3	pBCDF
5	3	1	2	pACEF
5	3	2	1	pBCEF
5	2	2	3	pABEG, pACDG, pADEF
5	2	3	2	pABFG, pBCDG, pBDEF
5	3	2	2	pACFG, pBCEG, pCDEF
5	1	3	3	pABDG
5	3	1	3	pACEG
5	3	3	1	pBCFG
5	2	3	3	pADFG, pBDEG
5	3	3	2	pBEFG, pDEFG
5	3	2	3	pCDEG, pAEFG
5	2	2	4	pADEG
5	2	4	2	pBDFG
5	4	2	2	pCEFG
5	3	3	3	pDEFG
6	2	2	3	pABCDE
6	2	3	2	pABCDF
6	3	2	2	pABCEF
6	2	3	3	pABCDG, pABDEF
6	3	2	3	pABCEG, pACDEF
6	3	3	2	pABCFG, pBCDEF
6	4	3	2	pBCEFG
6	4	2	3	pACEFG
6	3	3	3	pABEFG, pACDFG, pBCDEG

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2469

6	3	4	2	pBCDFG
6	2	4	3	pABDFG
6	3	2	4	pACDEG
6	2	3	4	pABDEG
6	4	3	3	pCDEFG
6	3	4	3	pBDEFG
6	3	3	4	pADEFG
7	4	4	3	pBCDEFG
7	4	3	4	pACDEFG
7	3	4	4	pABDEFG
7	4	3	3	pABCEFG
7	3	4	3	pABCDFG
7	3	3	4	pABCDEG
7	3	3	3	pABCDEF
8	4	4	4	pABCDEFG

[0081] The decoding matrix provided in Table 4 is constructed using two assumptions. First, the decoding matrix assumes that the positive control (p) always produces a positive outcome. Second, the decoding matrix assumes that, within each color, the intensity is additive and scales in the same way with changing probe concentration, regardless of which probe the signal may come from. This essentially means that the signals are additive and digital. The conditions underlying these assumptions may be met by properly preparing the assay. If a fluorescent signal is used, the intensity need only be approximately additive and digital, as demonstrated in the Examples provided herein.

[0082] Table 4 allows the conversion of a cumulative measurement of intensity in four ranges of fluorescent wavelengths (i.e., signal intensity within each color range), into the corresponding analytes present in the sample. For example, a result of 4321 indicates that p, C, F, and G are present and the other analytes are not present. This result is referred to as a "legitimate" result, because it is present in the decoding matrix. By contrast, a result of 4000, while possible to measure, does not occur in the decoding matrix. More specifically, the result of 4000 cannot be achieved by adding any combination of control and analyte codes from Table 3. This result is referred to as an "illegitimate" result. An illegitimate result may indicate that the assay malfunctioned. Thus, the control (p) and the decoding matrix provide a means of verifying that the assay is functioning properly.

[0083] Table 4 is exhaustive for any combination of fluorophores generating four resolvable emission spectra (e.g., colors). The term "rank" is used to describe the number of detected analytes, including the control. In the example provided above, if the assay functions properly, the rank is equal to the value of the blue signal. For example, a rank of 8 indicates that the control and all seven other analytes (A-G) are present. A rank of 2 indicates that the control and only one analyte are

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2470

present. The lowest rank is a rank of 1, which indicates that only the control is present. The number of possibilities at each rank can be enumerated. For example, continuing to refer to the encoding and decoding method described in Tables 3-4, there are 7 possibilities for rank 2. The number of possibilities at rank 3 can be calculated as a combination of 7 take 2, or 7!/(5! * 2!) = 21 possibilities. More generally, the number of possibilities for a combination of N take K is N!/((N-K)! * K!). Analogously, at ranks 4, 5, 6, 7, and 8, the number of possibilities is 35, 35, 21, 7, and 1, respectively. Referring to Table 4 shows that the table agrees with the theoretical prediction. Thus, Table 4 is an exhaustive decoding matrix for the encoding method provided in Table 3.

C. Reducing or Eliminating Degeneracy

[0084] The terms "degenerate" and "degeneracy," as used herein, generally describe a situation where a legitimate result is not definitive, because it can indicate more than one possibility in terms of the presence or absence of an analyte. For example, with reference to Table 4, result 5233 is degenerate because it can be decoded as either pADFG or pBDEG. Similarly, result 4222 is degenerate because it can be decoded as any of the following: pCDG, pAFG, pBEG, pDEF. By contrast, result 3110 can only indicate pBC and thus is not degenerate.

[0085] This disclosure provides methods of reducing or eliminating degeneracy, thereby increasing the confidence with which an analyte is detected. In one embodiment, degeneracy is eliminated by a method comprising (i) encoding each potential analyte to be detected as a value of a signal and, optionally, as a value in each of at least two components of a signal; (ii) enumerating every legitimate result that can be obtained from the coding scheme; (iii) identifying each legitimate result that is degenerate; and (iv) eliminating at least one potential analyte (or potential analyte code) from the coding scheme, wherein eliminating the at least one potential analyte reduces or eliminates degeneracy. For example, with reference to the coding scheme described in Table 3 and the decoding matrix described in Table 4 (enumerating every legitimate result), eliminating any two of analytes D, E, and F eliminates the degeneracy. Eliminating any one of analytes D, E, and F would not eliminate the degeneracy, but would reduce it.

[0086] With continued reference to the coding scheme described in Table 3, eliminating any two of analytes D, E, and F from the coding scheme results in a scheme where six analytes (including control) can be analyzed, with no degeneracy, using only 4 colors. By contrast, conventional methods of multiplexing would allow for only the reporting of 3 analytes and 1 control using 4 colors. Therefore, the number of analytes that can be analyzed is nearly doubled by using the methods provided herein.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2471

[0087] **Fig. 2** shows an exemplary embodiment of the invention in which 4 colors are used to detect five analytes and a control. The analytes are nucleic acids from dengue virus ("Dengue Fever"), *Mycobacterium tuberculosis* ("Tuberculosis"), human immunodeficiency virus (HIV) p17 (P17), *Plasmodium* ("Malaria"), and herpes simplex ("Herpes"). The "colors" in this example are fluorophores attached to oligonucleotide probes that also comprise a quencher. The oligonucleotide probes will generally be different for different analytes, whereas the color is the same. For example, all probes designated as Probe 1 have a blue color but generally will have a different oligonucleotide sequence. Of course, probes designated as Probe 1 could also have the same oligonucleotides sequence, if the complementary sequence was present in each of the analytes. The mechanism of detection with these probes is described elsewhere in this disclosure. Probe 1 (blue) hybridizes to all six analytes, including control. Probe 2 (green) hybridizes to analytes from dengue virus, *Mycobacterium*, and HIV p17. Probe 3 (yellow) hybridizes to analytes from dengue fever and *Plasmodium*. Probe 4 hybridizes to analytes from dengue virus, *Mycobacterium*, and herpes simplex. The coding scheme defined by these probes is illustrated in Table 5. With reference to Table 3, potential analytes D (1011) and F (1110) have been eliminated from the coding scheme. Therefore, the coding scheme presented in Table 5 is non-degenerate and each legitimate result from the assay corresponds to the presence or absence of a unique combination of analytes in a sample.

Table 5. Coding scheme for detection of dengue virus, *Mycobacterium*, HIV, *Plasmodium*, and herpes simplex, as exemplified in Fig. 2.

	Blue	Green	Yellow	Red
Ctrl. (p)	1	0	0	0
Herpes Simplex	1	0	0	1
Plasmodium	1	0	1	0
HIV	1	1	0	0
Mycobacterium	1	1	0	1
Dengue Virus	1	1	1	1

[0088] The methods for encoding and decoding presented above, including the methods for eliminating degeneracy, are all equally applicable to encoding methods using additional colors. For example, Table 3 could be extended by including additional colors and additional intensities (described further below). The decoding matrix is then generated as described above, enumerating every legitimate result that can be obtained from the coding scheme. A decoding matrix analogous to the decoding matrix provided in Table 4 may be constructed for any coding scheme described in this disclosure. The legitimate results that are degenerate are then identified and at least one

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2472

potential analyte code is eliminated from the coding scheme to reduce or eliminate degeneracy. The method of eliminating degeneracy may be carried out using software on a computer readable medium, or hardware configured to carry out the method (e.g., a microchip).

[0089] Although degeneracy can be reduced or eliminated by the methods described above, and elsewhere in this disclosure, this disclosure also provides coding schemes that are non-degenerate by design. For example, after elimination of degeneracy in the coding scheme described in Table 3, the coding scheme may be extended indefinitely in a non-degenerate manner where the non-degeneracy is by design (see, e.g., the coding scheme exemplified in Table 6, described more fully below). Similarly, this disclosure provides coding schemes that are completely non-degenerate by design and therefore do not require any reduction or elimination of degeneracy (see, e.g., the coding scheme exemplified in Table 8, described more fully below). Thus, the coding schemes provided in this disclosure may have reduced or eliminated degeneracy, or be non-degenerate by design.

D. Encoding Methods Using More Than One Color and More Than One Intensity

[0090] The encoding method described above (e.g., Tables 3-5) may be further extended by allowing analytes to be encoded by an intensity greater than 1. For example, each of the analytes encoded in the coding scheme provided in Table 5 is encoded by a fluorescence intensity of either 1 or 0. Allowing higher values for the signal intensity in at least one color further increases the number of analytes that can be encoded by any of the methods provided in this disclosure. In some examples, these higher intensity values may be assigned any color except for the control color, in order to maintain the analyte counting capability of the control color.

[0091] Table 6 shows the first three tiers of an exemplary coding scheme that utilizes four colors and multiple intensities. Tier 1 of Table 6 is a reproduction of Table 3, showing the encoding of seven analytes and a control with four colors. As described above, any two of potential analytes D, E, and F may be eliminated from the coding scheme in order to produce a non-degenerate coding scheme. Tier 1 of Table 6 indicates that potential analytes D (1011) and F (1110) have been eliminated from the coding scheme to eliminate degeneracy. Therefore, the coding scheme presented in Tier 1 of Table 6 is capable of determining the presence of five analytes and one control using four colors, as described above.

[0092] The coding scheme of Tier 1 of Table 6 may be expanded to a second tier (Tier 2) by allowing the intensity in any of the colors to increase. As described above, the intensity of the control color may be maintained at 1, in order to preserve the sequence counting capability of the control. Increasing the intensity of any of the remaining three colors will yield codes 100Y, 10Y0, 1Y00, 10YY, 1Y0Y, 1YY0, and 1YYY, where $Y > 1$. The minimal value of Y for a new tier of

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2473

encoding is equal to the cumulative maximum value from the prior tier(s) plus 1. As described below, a value greater than 1 could also be used, to maximize the differences between the intensities.

[0093] Thus, in this context, the term "tier" is generally used to describe a set of codes that fully utilize the coding capability provided by a particular number of first values (e.g., intensities) and second values (e.g., colors), without degeneracy. For example, Tier 1 of Table 6 fully utilizes the coding capability provided by four colors with up to one intensity in each color, without degeneracy. As shown in Table 6, Tier 1, this results in six encoded analytes, including the control. To introduce a second tier, the minimum value of Y (described above) may be incremented to equal the cumulative maximum result from the prior tier(s) plus one (or more than one). In the example provided in Table 6, the intensity of the blue (control) color is maintained as one, to preserve the sequence counting capability in this color. Thus, Tier 2 consists of five non-degenerate encoding possibilities obtained by incrementing the intensities in the green, yellow, and red channels to equal the cumulative maximum results in each of these channels from Tier 1, plus one. All possibilities of these codes may then be enumerated for Tier 2, and codes resulting in degeneracy may be eliminated, or Tier 2 may be made non-degenerate by design, using the information used to eliminate the corresponding codes from Tier 1. Further coding capacity may then be achieved by adding a third tier, or further tiers, which are constructed according to analogous methods. A coding scheme may have an infinite number of tiers, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100 or more tiers.

[0094] More specifically, with reference to Table 6, Tier 2, analyte H is encoded by 1004. The value of the control color is maintained as 1. The value of the red color is equal to the cumulative maximum result from the prior tier (3) plus 1, or 4. Similarly, analytes I and J are encoded by 1030 and 1400, respectively. Combinations of these codes are used to encode the remaining four analytes (K-N), as was done for analytes D-G in Tier 1. This completes Tier 2. In Tier 2, the inclusion of potential analytes K (1034) and M (1430) results in degeneracy. Therefore, these analytes have been eliminated from the coding scheme, to eliminate degeneracy.

[0095] Continuing to refer to Table 6, a third tier (Tier 3) is constructed using the same principles described above. Analyte O is encoded by 1-0-0-16. The value of the control color is still maintained as 1. The value of the red color is equal to the cumulative maximum result from the prior tiers (15) plus 1, or 16. Similarly, analytes P and Q are encoded by 1-0-9-0 and 1-16-0-0, respectively. Combinations of these codes are used to encode the remaining four analytes (R-U), as was done for analytes D-G in Tier 1 and analytes K-N in Tier 2. In Tier 3, the inclusion of potential

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2474

analytes R (1-0-9-16) and T (1-16-9-0) results in degeneracy. Therefore, these analytes have been eliminated from the coding scheme, to eliminate degeneracy.

[0096] The three-tier coding scheme shown in Table 6 shows the encoding of 15 analytes and one control using four colors. This coding scheme may be indefinitely extended, by adding more intensities to generate additional tiers and/or adding more colors, to generate additional coding capability within the tiers. The methods of reducing or eliminating degeneracy, as described in this disclosure, may be used with the addition of each intensity and/or color, to reduce or eliminate degenerate results.

[0097] More generally, the coding scheme depicted in Table 6 is a non-degenerate, infinite extension of the coding scheme depicted in Tables 3-5. The maximum intensity of the cumulative measurement at the first tier is 6. The maximum intensity of the cumulative measurement at the second tier is 15. The maximum intensity of the cumulative measurement at the third tier is 63, and so on. Given a maximum cumulative intensity value (F), the maximum number of tiers (T) available in this coding scheme is $T = \log_4 (F + 1)$. The coding scheme depicted in Table 6 provides five non-degenerate codes per tier (P). Thus, the maximum number of codes $M = 5 * \log_4 (F + 1)$, or $M = P * T$, where P is the number of non-degenerate codes per tier and T is the number of tiers. For example, given $F = 63$, the maximum number of codes (i.e., analytes) is 15 for 5 non-degenerate codes per tier. This formula does not include the 1000 code, which is reserved for a positive control in Table 6. To include the control in the total number of analytes, one would simply add one, to provide the formula $M = (P * T) + 1$.

[0098] The methods provided in this disclosure may be used to expand this coding scheme infinitely. For example, by utilizing combinations of different intensities (i.e., first values) and colors (i.e., second values) one can encode any number of analytes (M) by varying the number of non-degenerate codes per tier (P) and the number of tiers (T). For example, the number of non-degenerate codes per tier is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more. Similarly, the number of tiers may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more.

Table 6. Encoding of 15 analytes and one control using four colors and multiple intensities.

Tier	Analyte	B	G	Y	R	Comments
1	Ctrl. (p)	1	0	0	0	
	A	1	0	0	1	
	B	1	0	1	0	

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2475

	C	1	1	0	0	
	D	1	0	1	1	Eliminated from coding scheme to eliminate degeneracy.
	E	1	1	0	1	
	F	1	1	1	0	Eliminated from coding scheme to eliminate degeneracy.
	G	1	1	1	1	
Cumulative Maximum Result		*6*	*3*	*2*	*3*	
2	**H**	1	0	0	4	
	I	1	0	3	0	
	J	1	4	0	0	
	K	1	0	3	4	Eliminated from coding scheme to eliminate degeneracy.
	L	1	4	0	4	
	M	1	4	3	0	Eliminated from coding scheme to eliminate degeneracy.
	N	1	4	3	4	
Cumulative Maximum Result		*11*	*15*	*8*	*15*	
3	**O**	1	0	0	16	
	P	1	0	9	0	
	Q	1	16	0	0	
	R	1	0	9	16	Eliminated from coding scheme to eliminate degeneracy.
	S	1	16	0	16	
	T	1	16	9	0	Eliminated from coding scheme to eliminate degeneracy.
	U	1	16	9	16	
Cumulative Maximum Result						
Cumulative Maximum Result for Three Non-Degenerate Tiers		***16***	***63***	***26***	***63***	

[0099] The right-hand side of **Fig. 1** shows one exemplary embodiment of the method described above. More specifically, the right-hand side of **Fig. 1** shows an encoding method where nine or more analytes (Seq 1 - Seq 9, etc.) are each encoded by at least two colors, with varying intensities within each color. The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher. The system can be designed so that each probe is labeled with single

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2476

fluorophore, or each probe is labeled with more than one fluorophore. For example, the code for analyte H, in Table 6, is 1004. The intensity of 4 in the red channel may be achieved by either using an H-specific probe comprising 4 red fluorophores, or by using 4 H-specific probes each comprising a single red fluorophore. Of course, any combination of probes and fluorophores producing a result of 4 in the red channel would be equally appropriate, such as 2 probes with 2 red fluorophores each, and 1 probe with 1 red fluorophore and 1 probe with 3 red fluorophores, or simply one probe with one red fluorophore but present at 4x amount in the reaction mixture.

[00100] The coding scheme depicted on the right-hand side of **Fig. 1** may be represented as in Table 7. The result of the analysis shown on the right-hand side of **Fig. 1** is 4112, or an intensity of 4 in the blue channel, 1 in the green channel, 1 in the yellow channel, and 2 in the red channel. Using a decoding matrix constructed as described herein, this result is decoded to indicate the presence of Seq 1, Seq 3, Seq 4, and Seq 5.

Table 7. Coding scheme illustrated on the right-hand side of Fig. 1.

Tier	Analyte	B	G	Y	R	Comments
1	**Seq 1**	1	0	0	0	
	Seq 2	1	1	0	0	
	Seq 3	1	0	1	0	
	Seq 4	1	0	0	1	
	Seq 5	1	1	0	1	
	Seq 6	1	1	1	1	
	~~Eliminated Potential Seq~~	~~1~~	~~0~~	~~1~~	~~1~~	Eliminated from coding scheme to eliminate degeneracy.
	~~Eliminated Potential Seq~~	~~1~~	~~1~~	~~1~~	~~0~~	Eliminated from coding scheme to eliminate degeneracy.
Cumulative Maximum Result		*6*	*3*	*2*	*3*	
2	**Seq 7**	1	4	0	0	
	Seq 8	1	0	3	0	
	Seq 9	1	0	0	4	
Etc. - i.e. Continue as provided in Table 6.						

E. Encoding Methods Using One Color and One Intensity Per Analyte but Different Intensities Among Analytes

[00101] In some methods provided herein, each analyte is encoded by a single color and intensity combination. For example, in a four color system, the first four analytes may be encoded

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2477

by 1000, 2000, 4000, and 8000. The next four analytes may be encoded by 0100, 0200, 0400, and 0800. Analytes 9-12 and 13-16 would be assigned analogously, as shown in Table 8.

[00102] Like the encoding method described in Table 6, this coding scheme is theoretically infinite, non-degenerate by construction, and only limited by the bandwidth of the instrument used to measure the signal. However, this coding scheme enables more analytes to be quantified per unit of bandwidth than the encoding method described in Table 6. The reason is that the available multiplicity of signal is used with maximal efficiency, as each level of intensity is utilized in the coding (i.e. there are no gaps in the chromatogram; see below for description of chromatograms). Table 8 shows one embodiment of this method, illustrating four tiers of encoding based on four colors and intensities 1, 2, 4, and 8. The coding scheme is non-degenerate, and the result if all 16 analytes are present is 15-15-15-15. This encoding method is more efficient, in terms of bandwidth utilization, than the encoding methods presented above. However, this method does not have the proofreading capability of the first scheme, as all the results decode to legitimate outcomes in the absence of gaps in the chromatogram.

Table 8. Example of encoding method using one color and one intensity per analyte, but different intensities among analytes.

Tier	Analyte	B	G	Y	R
1	**A**	1	0	0	0
	B	2	0	0	0
	C	4	0	0	0
	D	8	0	0	0
2	**E**	0	1	0	0
	F	0	2	0	0
	G	0	4	0	0
	H	0	8	0	0
3	**I**	0	0	1	0
	J	0	0	2	0
	K	0	0	4	0
	L	0	0	8	0
4	**M**	0	0	0	1
	N	0	0	0	2
	O	0	0	0	4
	P	0	0	0	8
		15	***15***	***15***	***15***

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2478

[00103] The method presented above, and exemplified in Table 8, may be extended by introducing additional colors and/or intensities. For example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more colors may be used. Additional intensity levels, such as 16, 32, 64, 128, 255, 512, 1024, 2048, 4096, 8192, 16384, 32768, 65536, and so on may also be used. The method is generalizable and may be used to encode an infinite number of analytes. Each analyte is represented by a code in a single color, wherein the value of the code in that single color equal to the sum of all previous values plus one. For example, if the first code contains a 1 in a particular color, the next codes are 2, 4, 8, 16, 32, 64, 128 and so on. Other progressions are possible, but will not be as efficient in terms of usage of the bandwidth. However, one of ordinary skill in the art will recognize that less efficient use of bandwidth may be desirable when one wishes to maximize separation between values. Similarly, the value 1 could be excluded from the coding scheme, for example to maximize the difference in the intensity between the first encoded value and instrumental noise. For example, in some cases a coding scheme may begin with a value of 2, which would provide a progression of 2, 4, 8, 16, 32, 64, 128, and so on for the analyte codes, while the progression of cumulative results would be 2, 4, 6, 8, 10, 12, 14, 16, and so on. This approach generates uniform gaps in the possible cumulative results, allowing for higher tolerance to noise in comparison to an analyte code progression of 1, 2, 4, 8, 16, 32, and so on, and its corresponding cumulative result progression of 1, 2, 3, 4, 5, 6, 7, 8, and so on. In this example, the increased robustness in the measurement comes at a cost of a decrease in the number of available codes within a fixed bandwidth of multiplicity. For example, if only 63 multiplicity states can be measured reliably, the coding scheme starting with 1 offers 7 codes per color whereas the coding scheme starting with 2 offers 6 codes per color. If 4 colors are available, the former will offer 28 codes, while the latter will offer 24 codes. In summary, both the starting signal intensity and the progression (i.e., difference between intensity values) may be scaled in order to maximize the ability to distinguish over instrumental noise and maximize the differences between the intensity values, thereby enhancing the ability to distinguish between distinct experimental outcomes.

[00104] The coding scheme illustrated in Table 8 is non-degenerate by design. Although the coding scheme illustrated in Table 8 uses both intensity and color to encode each of the 16 analytes, each of the analytes could also be encoded by simply utilizing intensity. For example, given the 16 analytes provided in Table 8 (A-P), a single color coding scheme encoding all 16 analytes may assign the values 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192, and 16384 to analytes A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, and P, respectively. Such a coding scheme is non-degenerate by design and the cumulative intensity result can be unambiguously decoded to indicate

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2479

the presence or absence of analytes A-P. This coding scheme is capable of detecting the presence or absence of M analytes, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of a signal (e.g., signal intensity). By adding a second component to this signal (e.g., color, as depicted in Table 8), the capacity of this coding scheme can be increased to $M = C * \log_2 (F + 1)$, where C is the number of colors used in said coding scheme.

[00105] As described throughout this specification, any suitable value may be used for C or F. For example, C may be at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more. F may be at least about 2, 4, 8, 16, 32, 64, 128, 255, 512, 1024, 2048, 4096, 8192, 16384, 32768, 65536, 131072, 262144, 524288, 1048576, and so on. As described elsewhere in this disclosure, both the initial starting value of F and the steps in its progression may also be varied.

III. Analytes

[00106] An analyte may be any suitable analyte that may be analyzed using the methods and compositions of the invention, where the analyte is capable of interacting with a reagent in order to generate a signal with at least two components that can be measured. An analyte may be naturally-occurring or synthetic. An analyte may be present in a sample obtained using any methods known in the art. In some cases, a sample may be processed before analyzing it for an analyte. The methods and compositions presented in this disclosure may be used in solution phase assays, without the need for particles (such as beads) or a solid support.

[00107] In some cases, an analyte may be a polynucleotide, such as DNA, RNA, peptide nucleic acids, and any hybrid thereof, where the polynucleotide contains any combination of deoxyribo- and/or ribo-nucleotides. Polynucleotides may be single stranded or double stranded, or contain portions of both double stranded or single stranded sequence. Polynucleotides may contain any combination of nucleotides or bases, including, for example, uracil, adenine, thymine, cytosine, guanine, inosine, xanthine, hypoxanthine, isocytosine, isoguanine and any nucleotide derivative thereof. As used herein, the term "nucleotide" may include nucleotides and nucleosides, as well as nucleoside and nucleotide analogs, and modified nucleotides, including both synthetic and naturally occurring species. Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to cDNA, mitochondrial DNA (mtDNA), messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), nuclear RNA (nRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), small Cajal body-specific RNA (scaRNA), microRNA (miRNA), double

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2480

stranded (dsRNA), ribozyme, riboswitch or viral RNA. Polynucleotides may be contained within any suitable vector, such as a plasmid, cosmid, fragment, chromosome, or genome.

[00108] Genomic DNA may be obtained from naturally occurring or genetically modified organisms or from artificially or synthetically created genomes. Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art. For example, genomic DNA may be isolated with or without amplification. Amplification may include PCR amplification, multiple displacement amplification (MDA), rolling circle amplification and other amplification methods. Genomic DNA may also be obtained by cloning or recombinant methods, such as those involving plasmids and artificial chromosomes or other conventional methods (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*., cited *supra*.) Polynucleotides may be isolated using other methods known in the art, for example as disclosed in *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV) or *Molecular Cloning: A Laboratory Manual*. If the isolated polynucleotide is an mRNA, it may be reverse transcribed into cDNA using conventional techniques, as described in Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*., cited *supra*.

[00109] An analyte may be a protein, polypeptide, lipid, carbohydrate, sugar, small molecule, or any other suitable molecule that can be detected with the methods and compositions provided herein. An analyte may be an enzyme or other protein. An analyte may be a drug or metabolite (e.g., anti-cancer drug, chemotherapeutic drug, anti-viral drug, antibiotic drug, or biologic). An analyte may be any molecule, such as a co-factor, receptor, receptor ligand, hormone, cytokine, blood factor, antigen, steroid, or antibody.

[00110] An analyte may be any molecule from any pathogen, such as a virus, bacteria, parasite, fungus, or prion (e.g., PrP^{Sc}). Examples of viruses include those from the families Adenoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Orthomyxoviridae, Papovaviridae, Paramyxoviridae, Picornaviridae, Polyomavirus, Retroviridae, Rhabdoviridae, and Togaviridae. Specific examples of viruses include adenovirus, astrovirus, bocavirus, BK virus, coxsackievirus, cytomegalovirus, dengue virus, Ebola virus, enterovirus, Epstein-Barr virus, feline leukemia virus, hepatitis virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, herpes simplex virus (HSV), HSV type 1, HSV type 2, human immunodeficiency virus (HIV), HIV type 1, HIV type 2, human papilloma virus (HPV), HPV type 1, HPV type 2, HPV type 3, HPV type 4, HPV type 6, HPV type 10, HPV type 11, HPV type 16, HPV type 18, HPV type 26, HPV type 27, HPV type 28, HPV type 29, HPV type 30, HPV type 31, HPV type 33, HPV type 34, HPV type 35, HPV type 39, HPV type 40, HPV type 41, HPV type 42, HPV type 43, HPV type 44, HPV type 45, HPV type 49, HPV type 51, HPV type 52, HPV type 54, HPV type 55, HPV type 56, HPV type 57, HPV type 58, HPV type 59, HPV type 68, HPV type 69, influenza virus, JC virus, Marburg

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2481

virus, measles virus, mumps virus, Norwalk virus, parovirus, polio virus, rabies virus, respiratory syncytial virus, retrovirus, rhinovirus, rotavirus, Rubella virus, smallpox virus, vaccinia virus, West Nile virus, and yellow fever virus.

[00111] Examples of bacteria include those from the genuses *Bordetella, Borrelia, Brucella, Campylobacter*, *Chlamydia, Clostridium, Corynebacterium, Enterococcus, Escherichia, Francisella, Haemophilus, Helicobacter, Legionella, Leptospira, Listeria, Mycobacterium*, *Mycoplasma, Neisseria, Pseudomonas*, *Rickettsia, Salmonella*, *Shigella*, *Staphylococcus, Streptococcus*, *Treponema, Vibrio, and Yersinia.* Specific examples of bacteria include *Bordetella par apertussis, Bordetella pertussis, Borrelia burgdorferi, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia psittaci, Chlamydia trachomatix, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium diphtheriae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli*, *Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Leptospira interrogans, Listeria monocytogenes, Mycobacterium leprae, Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella choleraesuis, Salmonella dublin, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Vibrio cholerae, Yersinia pestis, and Yersinia enterocolitica.*

[00112] Examples of parasites include those from the genuses *Acanthamoeba, Babesia, Balamuthia, Balantidium, Blasocystis, Cryptosporidium, Dientamoeba, Entamoeba, Giardia, Isospora, Leishmania, Naegleria, Pediculus, Plasmodium, Rhinosporidium, Sarcocystis, Schistosoma, Toxoplasma, Trichomonas,* and *Trypanosoma.* Specific examples of parasites include *Babesia divergens, Babesia bigemina, Babesia equi, Babesia microfti, Babesia duncani, Balamuthia mandrillaris, Balantidium coli, Dientamoeba fragilis, Entamoeba histolytica, Giardia lamblia, Isospora belli, Naegleria fowleri, Pediculus humanus, Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax, Rhinosporidium seeberi, Sarcocystis bovihominis, Sarcocystis suihominis, Schistosoma mansoni, Toxoplasma gondii, Trichomonas vaginalis, Trypanosoma brucei,* and *Trypansoma cruzi.*

[00113] Examples of fungi include those from the genuses *Apophysomyces, Aspergillus, Blastomyces, Candida, Cladosporium, Coddidioides, Cryptococcos, Exserohilum, Fusarium, Histoplasma, Pichia, Pneumocystis, Saccharomyces, Sporothrix, Stachybotrys,* and *Trichophyton.* Specific examples of fungi include *Aspergillus fumigatus, Aspergillus flavus, Aspergillus clavatus,*

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2482

Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Crytptococcus neoformans, Exserohilum rostratum, Fusarium verticillioides, Histoplasma capsulatum, Pneumocystis jirovecii, Sporothrix schenckii, Stachybotrys chartarum, and *Trichophyton mentagrophytes.*

[00114] In some cases, the methods provided in this disclosure may be used to detect any one of the analytes described above, or elsewhere in the specification. In some cases the methods provided in this disclosure may be used to detect panels of the analytes described above, or elsewhere in the specification. For example, a panel may comprise an analyte selected from the group consisting of any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100 or more analytes described above or elsewhere in the specification.

[00115] An analyte may be obtained from any suitable location, including from organisms, whole cells, cell preparations and cell-free compositions from any organism, tissue, cell, or environment. Analytes may be obtained from environmental samples, biopsies, aspirates, formalin fixed embedded tissues, air, agricultural samples, soil samples, petroleum samples, water samples, or dust samples. In some instances, an analyte may be obtained from bodily fluids which may include blood, urine, feces, serum, lymph, saliva, mucosal secretions, perspiration, central nervous system fluid, vaginal fluid, or semen. Analytes may also be obtained from manufactured products, such as cosmetics, foods, personal care products, and the like. Analytes may be the products of experimental manipulation including, recombinant cloning, polynucleotide amplification, polymerase chain reaction (PCR) amplification, purification methods (such as purification of genomic DNA or RNA), and synthesis reactions.

[00116] More than one type of analyte may be detected in each multiplexed assay. For example, a polynucleotide, a protein, a polypeptide, a lipid, a carbohydrate, a sugar, a small molecule, or any other suitable molecule may be detected simultaneously in the same multiplexed assay with the use of suitable reagents. Any combination of analytes may be detected at the same time.

[00117] Detection of an analyte may be useful for any suitable application, including research, clinical, diagnostic, prognostic, forensic, and monitoring applications. Exemplary applications include detection of hereditary diseases, identification of genetic fingerprints, diagnosis of infectious diseases, cloning of genes, paternity testing, criminal identification, phylogeny, anti-bioterrorism, environmental surveillance, and DNA computing. For example, an analyte may be indicative of a disease or condition. An analyte may be used to make a treatment decision, or to assess the state of a disease. The presence of an analyte may indicate an infection with a particular pathogen, or any other disease, such as cancer, autoimmune disease, cardiorespiratory disease, liver disease, digestive

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2483

disease, and so on. The methods provided herein may thus be used to make a diagnosis and to make a clinical decision based on that diagnosis. For example, a result that indicates the presence of a bacterial polynucleotide in a sample taken from a subject may lead to the treatment of the subject with an antibiotic.

[00118] In some cases the methods and compositions of the invention may be used to detect at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes. In some cases the methods and compositions of the invention may be used to detect 7-50, 8-40, 9-30, 10-20, 10-15, 8-12, or 7-12 analytes.

[00119] In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis.

IV. Signals

[00120] The methods presented in this disclosure may be used with any quantifiable signal. In some cases, this disclosure provides coding schemes and methods to encode an infinite number of targets without degeneracy, using a single component of a signal (e.g., intensity). For example, as

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2484

described above and in Example 3, a coding scheme may rely on a multiplicity of signal intensity without consideration of color. Although fluorescent probes have been used to illustrate this principle, the coding scheme is equally applicable to any other method providing a quantifiable signal, including an electrochemical signal and a chemiluminescent signal, as described elsewhere in this disclosure.

[00121] The methods presented in this disclosure may also utilize the measurement of a signal in at least two dimensions, also referred to as the measurement of at least two components of a signal. In comparison to the coding scheme described in the paragraph above, which relies on, for example, signal intensity to differentiate between analytes, utilization of at least two components of a signal (e.g., color and intensity) allows the generation of more unique codes per unit of signal intensity bandwidth. When at least two components of a signal are utilized, a cumulative measurement of the at least two components may be obtained for a single sample volume. For example in the coding scheme described in Table 5, the presence of each analyte results in a particular intensity (one component of the signal) in each of the four colors (a second component of a signal). The combination of these constituent signals leads to a cumulative signal that may be measured by measuring an intensity at each wavelength or range of wavelengths. The corresponding coding scheme or decoding matrix may then be used to convert the cumulative measurement into a determination of the presence or absence of an analyte.

[00122] In some cases, a quantifiable signal comprises a waveform that has both a frequency (wavelength) and an amplitude (intensity). A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal. In some cases, an electromagnetic signal may be a fluorescent signal, for example a fluorescence emission spectrum that may be characterized in terms of wavelength and intensity.

[00123] In certain portions of this disclosure, the signal is described and exemplified in terms of a fluorescent signal. This is not meant to be limiting, and one of ordinary skill in the art will readily recognize that the principles applicable to the measurement of a fluorescent signal are also applicable to other signals. For example, like fluorescent signals, any of the electromagnetic signals described above may also be characterized in terms of a wavelength and an intensity. The wavelength of a fluorescent signal may also be described in terms of color. The color may be determined based on measuring intensity at a particular wavelength or range of wavelengths, for example by determining a distribution of fluorescent intensity at different wavelengths and/or by utilizing a band pass filter to determine the fluorescence intensity within a particular range of wavelengths. Such band pass filters are commonly employed in a variety of laboratory

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2485

instrumentation, including quantitative PCR machines. Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel."

[00124] In some cases, the methods provided in this disclosure may be used with any signal where the cumulative signal scales with the constituent signals of the same color, frequency, absorption band, and so on. However, the cumulative signal need not be digital or scale linearly with the number and intensity of the constituent signals. For example, if the physical principle of measurement is absorption, the cumulative attenuation is a product of constituent attenuations while the constituent concentrations are additive, due to the exponential nature of the Beer-Lambert law. The logarithm of the cumulative attenuation will then scale linearly with constituent concentrations in each absorption band (the equivalent of color, if fluorescent detection is used). The methods of the invention are therefore applicable. The methods of the invention may also be used with chemiluminescent signals and electrochemical signals.

[00125] The number of signals, and the number of dimensions or components measured, may also be expanded beyond the numbers shown in the exemplary embodiments of the invention, leading to an expansion in multiplexing capability. The exemplary embodiments provided in this disclosure utilize coding schemes constructed utilizing a fluorescent signal with one or two components measured: wavelength and/or intensity. The number of analytes that can be encoded can be increased by increasing the number of wavelengths and/or intensities. The number of analytes that can be encoded can also be increased by increasing the number of signals, for example by combining a fluorescent signal with an electrochemical signal or a FRET signal (fluorescence resonance energy transfer).

[00126] In some cases, more than two components of a signal may be measured. For example, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, 20 or more components of a signal may be measured. At least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, or 20 components of a signal may be measured. At least 2, but fewer than 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, or 20 components of a signal may be measured. In some cases, 2-3, 2-4, 2-5, 2-6, 3-5, 3-6, 3-8, or 5-10 components of a signal may be measured. These additional components may include kinetic components, such as a rate of signal decay and rate of photobleaching.

[00127] If a fluorescent signal is employed, the number of analytes that can be encoded may be further expanded by utilizing additional fluorophores. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more fluorophores may be used. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2486

may be used. In some cases, fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores may be used. In some cases, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 fluorophores may be used.

[00128] Generally, the number of analytes that can be encoded may be further expanded by utilizing additional first values (e.g., intensities) that are values or ranges of values from a first component of a signal. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more first values that are values or ranges of values from a first component of a signal may be used. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more first values that are values or ranges of values from a first component of a signal may be used. In some cases, fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 first values that are values or ranges of values from a first component of a signal may be used. In some cases, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 first values that are values or ranges of values from a first component of a signal may be used.

[00129] In instances in which intensity is a component of a signal that is quantified, such as when a fluorescent signal is utilized, the presence of an analyte may be encoded using a variety of intensities or ranges of intensities. For example, a coding scheme may utilize 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more intensities or ranges of intensities. A coding scheme may utilize at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more intensities or ranges of intensities. A coding scheme may utilize fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 intensities or ranges of intensities. In some cases, a coding scheme may utilize 2-20, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, or 5-10 intensities or ranges of intensities.

[00130] The number of analytes that can be encoded may be further expanded by utilizing additional second values (e.g., wavelengths) that are values or ranges of values from a second component of a signal. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2487

48, 49, 50, or more second values that are values or ranges of values from a second component of a signal may be used. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more second values that are values or ranges of values from a second component of a signal may be used. In some cases, fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 second values that are values or ranges of values from a second component of a signal may be used. In some cases, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 second values that are values or ranges of values from a second component of a signal may be used.

[00131] In instances in which wavelength is a component of a signal that is quantified, such as when a fluorescent signal is utilized, the presence of an analyte may be encoded using a variety of wavelengths or ranges of wavelengths. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more wavelengths or ranges of wavelengths may be used. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more wavelengths or ranges of wavelengths may be used. In some cases, fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 wavelengths or ranges of wavelengths may be used. In some cases, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 wavelengths or ranges of wavelengths may be used.

[00132] In some cases, when degeneracy is eliminated, the methods of the invention are capable of detecting the presence or absence of at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more analytes in a single volume when each reagent used to generate a signal in the volume generates only one second value (e.g., each reagent, emits light at only one wavelength).

[00133] In other cases, when degeneracy is eliminated, the methods of the invention are capable of detecting the presence or absence of at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more analytes in a single volume using a total of four second values in that volume (e.g., a total of four wavelengths or ranges or wavelengths, which might be implemented by using four spectrally resolvable fluorophores).

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2488

[00134] As described throughout the specification, the assay provided herein utilizes cumulative measurements on a sample. A cumulative measurement may be, for example, a single measurement of intensity values, or a measurement of intensity values at one or more wavelengths or ranges of wavelengths. A plurality of cumulative measurements may be obtained. For example, an intensity may be measured at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more wavelengths or ranges of wavelengths. An intensity may be measured at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more wavelengths or ranges of wavelengths. An intensity may be measured at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 wavelengths or ranges of wavelengths.

[00135] More generally a cumulative measurement may be obtained for any quantifiable component of a signal, and for any quantifiable component of a signal at another quantifiable component of a signal. For example, at least a first component of a signal may be measured at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more second components of a signal. At lest a first component of a signal may be measured at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more second components of a signal. At least a first component of a signal may be measured at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 second components of a signal.

[00136] As is apparent from this disclosure, each analyte to be detected can be encoded as a code utilizing any number of suitable components of a signal or any number of signals. For example, each analyte to be detected can be encoded in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more components of a signal or signals. In some cases, each analyte to be detected can be encoded in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more components of a signal or signals. In some cases, each analyte to be detected can be encoded in fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 components of a signal or signals. In some cases, 1-4, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2489

5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 components of signal or signals may be used to encode each analyte. Each analyte in a coding scheme may be encoded by the same number of components of a signal, or signals, or different numbers of components of a signal, or signals.

A. Probes, Primers, Fluorophores, and Quenchers

[00137] Some of the methods provided in this disclosure utilize a reagent that generates a signal in the presence of an analyte. Any suitable reagent may be used with the invention. Generally, a reagent will have an analyte-specific component and a component that generates a signal in the presence of the analyte. In some cases, these reagents are referred to as probes. The probes may be hybridization probes. The hybridization probes may be an oligonucleotide probe attached to a fluorophore and a quencher (e.g., a TAQMAN probe).

[00138] The methods of the invention may use one or more reagents or probes to detect the presence or absence of each analyte. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more or probes may be used to detect the presence or absence of each analyte. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 probes may be used to detect the presence or absence of each analyte. In some cases, fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, , 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 probes may be used to detect the presence or absence of each analyte. In some cases, the number of probes used to detect the presence or absence of each analyte is 1-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 1-3, or 1-4.

[00139] In some cases, a sample is contacted with 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more probes to detect the presence or absence of all analytes. In some cases, a sample is contacted with at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more probes to detect the presence or absence of all analytes. In some cases, a sample is contacted with fewer than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 probes to detect the presence or absence of all analytes. In some cases, the number of probes that a sample is contacted with to detect the presence or absence of all analytes is 4-50, 4-40, 4-30, 4-20, 5-15, 5-10, or 3-10.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2490

[00140] As described above, oligonucleotide probes attached to a fluorophore and a quencher may be used to detect the presence of an analyte in a polynucleotide amplification assay. So long as the quencher and the fluorophore are in proximity, the quencher quenches the fluorescence emitted by the fluorophore upon excitation by a light source. The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte. Hybridization of the oligonucleotide probe may be performed in a nucleic acid amplification reaction comprising primers (e.g., a polymerase chain reaction). Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present. If no analyte is present, the probe will not hybridize to an analyte, and the fluorophore and quencher will remain in close proximity. Little or no signal will be produced.

[00141] Oligonucleotide probes may have one or a plurality of fluorophores and quenchers per probe. For example, in some embodiments an oligonucleotide probe may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more fluorophores. An oligonucleotide probe may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores. An oligonucleotide probe may comprise fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores.

[00142] An oligonucleotide probe may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more quenchers. An oligonucleotide probe may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 quenchers. An oligonucleotide probe may comprise fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 quenchers.

[00143] Attachment of probes and quenchers to a probe may be performed in the same reaction or in serial reactions. A series of reactions may be performed to label probes with at least

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2491

one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores.

[00144] If an oligonucleotide probe comprises two or more fluorophores, these fluorophores may be arranged to allow Förster (Fluorescence) resonance energy transfer (FRET) to occur between the fluorophores. Briefly, FRET is a mechanism of energy transfer between fluorophores. Using FRET-based probes allows one excitation source to generate two different fluorescence emission signals by excitation of a single fluorophore. For example, the methods described herein may used paired probes, where one probe in the pair is attached to a fluorophore and a quencher and a second probe is attached to two fluorophores (in close enough proximity for FRET to occur) and a quencher. Any combination of fluorophores and quenchers that provide for FRET may be used. Such an approach doubles the number of fluorescent probes that may be used with an excitation source, because a single excitation source can be used to produce two signals: one from the probe with one fluorophore and one from the probe with two fluorophores in close enough proximity to provide for FRET. For example, using the encoding method described in Table 8, the number of unambiguously detectable analytes could be increased from 16 to 32 by pairing each of the probes in Table 8 with a corresponding FRET probe.

[00145] The primers may be specific for a particular analyte and capable of amplifying a region complementary to a probe. In some cases, the number of pairs of primers used is equivalent to the number of probes. In other cases, the number of probes used may exceed the number of primer pairs used. In still other cases, the number of primer pairs used may exceed the number of probes used. In some cases a sample to be analyzed is contacted with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more pairs of primers. In some cases a sample to be analyzed is contacted with at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more pairs of primers. In some cases a sample to be analyzed is contacted with fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 pairs of primers. In some cases, the number of pairs of primers is 2-10, 3-15, 4-20, 3-10, 4-10, 5-10, 6-8, or 6-10.

[00146] The primers may amplify regions of a polynucleotide in which different numbers of hybridization probes hybridize. For example, at least one pair of primers may amplify a region complementary to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 hybridization probes. In some cases, all of said pairs of primers may amplify a region

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2492

complementary to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 hybridization probes.

[00147] In one example, a first probe may be labeled with the fluorophore 5'-fluorescein amidite (5'-FAM) and the quencher black hole quencher 1 (BHQ-1). A second probe may be labeled with 5'-FAM in close proximity (e.g., attached to) to cyanine 5.5 (Cy5.5) and the quencher black hole quencher 3 (BHQ-3). Upon digestion of the probe, via the nuclease activity of the polymerase, two fluorescent signals are generated from a single excitation wavelength (e.g., 470 nm). The fluorophore from the first probe will fluoresce at about 520 nm. The fluorophores on the second probe undergo FRET. The donor fluorophore, FAM is excited by the excitation light source (e.g., 470 nm) and transfers its energy to the acceptor fluorophore, Cy5.5. The acceptor fluorophore emits at about 705 nm. These fluorophores and quenchers are merely exemplary. Any fluorophores that can undergo FRET and any quenchers that can quench fluorescence are suitable for use with the invention. Methods for producing FRET-based probes are described in Jothikumar et al., BioTechniques, 2009, 46(7):519-524.

[00148] In some examples, a single hybridization probe may be used for each analyte. In order to utilize multi-color encoding (e.g., as shown in Table 3), each probe may be labeled with a plurality of fluorophores at pre-determined ratios. The ratio may be determined so that a positive control signal provides the same intensity as other positive control signals of the same intensity, in the same color. This approach reduces the number of probes that must be synthesized and may be less expensive to deploy than methods utilizing multiple probes. Moreover, in the case of hybridization probes, it is easier to fit one hybridization probe to an analyte sequence than multiple probes.

[00149] Although many aspects of the invention are exemplified using nucleic acid-based probes, one of ordinary skill in the art will readily recognize that other forms of probes would work equally well with the invention described in this disclosure. For example, a binding molecule specific to an analyte could be used as a probe. Non-limiting exemplary binding molecules include an antibody recognizing an analyte, and generating a signal in the presence of an analyte.

[00150] In embodiments of the invention that utilize fluorescent labels, any suitable fluorescent label may be used. Exemplary fluorescent labels suitable for use with the invention include rhodamine, rhodol, fluorescein, thiofluorescein, aminofluorescein, carboxyfluorescein, chlorofluorescein, methylfluorescein, sulfofluorescein, aminorhodol, carboxyrhodol, chlororhodol, methylrhodol, sulforhodol; aminorhodamine, carboxyrhodamine, chlororhodamine, methylrhodamine, sulforhodamine, and thiorhodamine, cyanine, indocarbocyanine, oxacarbocyanine,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2493

thiacarbocyanine, merocyanine, cyanine 2, cyanine 3, cyanine 3.5, cyanine 5, cyanine 5.5, cyanine 7, oxadiazole derivatives, pyridyloxazole, nitrobenzoxadiazole, benzoxadiazole, pyrene derivatives, cascade blue, oxazine derivatives, Nile red, Nile blue, cresyl violet, oxazine 170, acridine derivatives, proflavin, acridine orange, acridine yellow, arylmethine derivatives, auramine, crystal violet, malachite green, tetrapyrrole derivatives, porphin, phtalocyanine, and bilirubin. Exemplary quenchers include black hole quenchers, such as BHQ-0, BHQ-1, BHQ-2, BHQ-3; ATTO quenchers, such as ATTO 540Q, ATTO580Q, and ATTO612Q; and the like.

[00151] The fluorophores that may be used with the invention are not limited to any of the fluorophores described herein. For example, fluorophores with improved properties are continually developed, and these fluorophores could readily be used with the methods provided in this disclosure. Such improved fluorophores include quantum dots, which may emit energy at different wavelengths after being excited at a single wavelength. The advantage of using such fluorophores is that only a single excitation source is needed, but many different signals may be quantified, for example in terms of color and intensity. Moreover, fluorophores with narrow emission spectra would be particularly useful with the method described herein, as such fluorophores could be included in multiplex assays with minimal or no overlap between their emission spectra, thereby offering many more "colors" and boosting significantly the overall number of coded analytes.

[00152] In some cases, one or more reagents are lyophilized. Any suitable reagent may be lyophilized. For example, probes, primers, enzymes, antibodies, or any other reagent used for detection may be lyophilized. In some cases, a sample comprising an analyte may also be lyophilized. Lyophilization may be useful, for example, when distributing reagents and/or samples in developing regions where access to cold storage is expensive, not readily available, or not reliably available. In one example, lyophilized reagents comprise any probe or analyte-specific reagent described in this disclosure or otherwise suitable for use with the invention. In another example, lyophilized reagents comprise PCR primers. In yet another example, lyophilized reagents comprise reagents suitable for performing a PCR reaction.

V. Analytical Techniques and Instrumentation

[00153] The methods provided herein are suitable for use with a variety of detection methods. For example, the methods may be applied using an analytical technique that measures the wavelength and intensity of a fluorescent signal. This may be accomplished by measuring the intensity of a signal across a spectrum of wavelengths, or by using band pass filters that restrict the passage of certain wavelengths of light, thereby allowing only light of certain wavelengths to reach a photodetector. Many real-time PCR and quantitative PCR instruments comprise an excitation light

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2494

source and band pass filters that enable the detection of fluorescent signals in four colors (e.g., blue, green, yellow, and red). Therefore, the methods of the invention can be readily applied using instruments widely used in the art. Importantly, the methods and compositions provided herein may be used to detect multiple analytes by obtaining a cumulative measurement on a single solution. No separation is necessary. The invention does not require the use of beads or a solid phase. Of course, one of ordinary skill in the art would understand that the invention could be used with separation, beads, or a solid phase, if desired.

[00154] **Fig. 5** shows two examples of instrumental configurations that may be used to obtain cumulative measurements useful for the methods of the invention. With reference to **Fig. 5**, an instrument with a detector configured to detect both wavelength and intensity (e.g., a fluorometer) is shown in **501.** With reference to **501**, at least one excitation light source **502** is directed into a chamber **503** containing analytes and reagents that generate a signal in the presence of the analytes **504.** As described elsewhere, the analyte may be a nucleic acid and the reagents generating the signal may be hybridization probes comprising a fluorophore and a quencher. If the analyte is present, an emission signal **505** is generated. In the configuration shown in **501**, the wavelength and intensity of this emission signal are measured across a spectrum by a detector capable of generating a fluorescence emission spectrum **506.**

[00155] The wavelength and intensity may also be determined using a combination of a photodetector and band pass filters. This configuration is used in several thermal cyclers known in the art. With reference to **Fig. 5**, an instrument with band pass filters and a photodetector is depicted in **507.** With reference to **507**, at least one excitation light source **508** is directed into a chamber **509** containing analytes and reagents that generate a signal in the presence of the analytes **510.** As described elsewhere, the analyte may be a nucleic acid and the reagents generating the signal may be hybridization probes comprising a fluorophore and a quencher. If the analyte is present, an emission signal **511** is generated. In the configuration shown in **507**, band pass filters **512, 513,** and **514** are used to restrict the passage of light to light within certain ranges of wavelengths. For example band pass filter **512** may restrict the passage of light to light within wavelength range 1. Band pass filter **513** may restrict the passage of light to light within wavelength range 2. Band pass filter **514** may restrict the passage of light to light within wavelength range 3. Any number of band pass filters (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more) may be used with the invention. In addition, the positions of the band pass filters in **507** are not meant to be limiting. In some cases, emitted light may be passed through more than one band pass filter at any time. In other cases, the band pass filters may be configured so that all emitted light passes through only one band pass filter at a time. After passage through a band pass

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2495

filter, filtered light **515, 516,** and **517** is detected by a photodetector. For example, filtered light **515** may be light within wavelength range 1, filtered light **516** may be light within wavelength range 2, and filtered light **517** may be light within wavelength range 3. The intensity of each of the filtered lights **515, 516,** and **517** may then be quantified by a photodetector **518.**

[00156] The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, end point PCR methods, reverse transcriptase PCR, and digital PCR methods. Digital PCR methods (e.g., DROPLET DIGITAL (BIORAD) and DYNAMIC ARRAY (FLUIDIGM)) produce highly sensitive quantification of polynucleotide copy numbers. The methods provided herein can be easily integrated into these systems to significantly expand their throughput by allowing multiplexing in a droplet or a dynamic array.

[00157] Similarly, the methods described in this disclosure may be applied in a real-time PCR assay. For example, real-time data may be fully recorded as the PCR runs to completion. The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color. As a particular analyte is amplified, fluorophores are released from its corresponding probes in the same ratios as the color multiplicities in the coding of that sequence. Hence, e.g. a code of 1100 will have the same Ct in blue and green, while a code of 1400 will have its green Ct precede its blue Ct by 2 cycles. This additional data enables determination of the identity and starting quantity of each analyte.

[00158] The methods described herein may also be used directly on a tissue sample. For example, a tissue sample may be obtained and photolithographic methods may be used to build wells directly onto the tissue sample. The tissue sample may be fixed prior to building the wells. The tissue sample may then be analyzed by dispensing appropriate reagents into the wells in the tissue sample. The encoding and decoding methods described in this disclosure may be used for the multiplexed detection of analytes within the wells etched into the tissue. Each well may correspond to a single cell or a few cells, providing excellent spatial resolution when analyzing different portions of a tissue for an analyte. This method may be used to detect analytes in different areas of the same tissue.

[00159] In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum. Including additional excitation sources would allow for the excitation of a larger variety of fluorophores. Including additional band pass filters, or modifying or constructing an instrument capable of determining an entire spectrum allows

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2496

detection of a wider variety of emissions from fluorophores. These techniques can be used to increase the number of fluorophores that can be used with the methods described in this disclosure and, accordingly, to increase the number of analytes that be simultaneously detected.

[00160] In some cases, the methods described in this disclosure utilize end-point PCR methods. However, in some cases a result may be obtained before the end-point. This may be advantageous when, for example, results are needed as soon as possible. For example, in one case calibration experiments are performed in which different starting concentrations of an analyte are analyzed to determine the cycle number at which the signal becomes saturated, as a function of the starting amount of the analyte. Using this data, a computer monitoring a PCR reaction in real-time can be programmed to search for saturation up to the maximal cycle number according to the limit of detection (LOD) of the particular system. If there is no amplification of an analyte (e.g., other than a positive control, if present), by the maximal cycle number, the result for that analyte is negative. If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme. In both cases, there would be no need to run the PCR reaction beyond the number of cycles set by the LOD for that instrument. Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time.

VI. Compositions and Kits

[00161] This disclosure also provides compositions and kits for use with the methods described herein. The compositions may comprise any component, reaction mixture and/or intermediate described herein, as well as any combination. For example, the disclosure provides detection reagents for use with the methods provided herein. Any suitable detection reagents may be provided, including hybridization probes labeled with a fluorophore and a quencher and primers, as described elsewhere in the specification.

[00162] In some cases, compositions comprise reagents for the detection of at least seven analytes using four fluorophores. In some cases, compositions comprise reagents for the detection of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using four fluorophores. In some cases, compositions comprise reagents for the detection of at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using five fluorophores. In some

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2497

cases, compositions comprise reagents for the detection of at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using six fluorophores. In some cases, compositions comprise reagents for the detection of at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using seven fluorophores.

[00163] In some cases the compositions comprise primers. The compositions may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 pairs of primers.

[00164] The invention also provides kits for carrying out the methods of the invention. Accordingly, a variety of kits are provided in suitable packaging. The kits may be used for any one or more of the uses described herein, and, accordingly, may contain instructions for detecting the presence or absence of each analyte of a plurality of analytes. A kit may comprise a coding scheme, or a decoding matrix, to assist the user in converting a cumulative measurement to a result indicating the presence or absence of each of a plurality of analytes. A kit may be a diagnostic kit, for example, a diagnostic kit suitable for the detection of any analyte, including the analytes recited herein. A kit may contain any of the compositions provided in this disclosure, including those recited above.

[00165] **Fig. 6** shows schematics of exemplary kits of the invention. With reference to **Fig. 6**, a kit comprising a kit body **601** is shown that contains reagents for the detection of analytes (e.g., polynucleotide probes comprising a fluorophore and a quencher) **602** and primers for the amplification of a nucleic acid **603.** The kit may also contain any other reagents, such as reagents suitable for performing an amplification reaction **604.** In the embodiment depicted in **601**, the reagents for the detection of analytes and the primers are each contained in a single volume (e.g., tube or a bottle). However, these reagents may also be provided in separate volumes, as depicted the kit body shown in **605.** The kit body **605** contains seven separate volumes. Three volumes (**606, 607,** and **608**) contain reagents for the detection of analytes (or mixtures of such reagents) and three volumes (**609, 610,** and **611**) contain primers (or mixtures of primers). The kit may also contain reagents suitable for performing an amplification reaction **604.**

[00166] **Fig. 6** is provided for illustrative purposes only. Any number of reagents for the detection of analytes (e.g., probes) and any number of primers may be included in a single tube or bottle, as appropriate for the application. For example, in some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more reagents for the detection of analytes may be included in a single bottle. In other cases, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2498

17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more primers for amplification may be included in a single bottle or tube. In some cases, primers and probes may be provided in the same bottle or tube. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more probes may be provided per pair of primers. In other cases, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more primers may be provided per pair of probes. The kits may contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more bottles or tubes. Control analytes, and their associated detection reagents and primers, may also be included in the kit. These may be included in a separate bottle or tube or, for example, included within the bottle or tube providing reagents for an amplification reaction **604**. As described elsewhere in the specification, any reagent in the kits may be lyophilized.

VII. Systems and Software

[00167] The methods of this disclosure may also be performed as part of a system. For example, one or more steps of the methods described herein may be performed by software contained on a computer readable medium. The software may be used to encode analytes, check for degeneracy, reduce or eliminate degeneracy, and/or produce a decoding matrix. The software may also be used to convert cumulative measurements made on an instrument (e.g., a PCR machine) into information concerning the presence or absence of an analyte. For example, with reference to **Fig. 4**, a computer **401** may be connected to an input device **403**, a display **402**, and a printer **404**. The computer may execute one or more of the steps of any of the methods described in this disclosure, including, for example, encoding methods **410**, analysis methods **420**, and decoding methods **430.** A computer may reduce or eliminate degeneracy, for example as part of the encoding methods **410.** Similarly, a computer may generate a coding schme that is non-degenerate by design using, for example, mathematical iteration. A computer may also be connected to a network **405**. The network may be a local network and/or the Internet. The network may be used to perform the methods of the invention on multiple computers in different locations. For example, one computer may perform the encoding, another computer may perform the measuring, and yet another computer may perform the decoding. A computer may perform any or all of these methods. The network may be used to transmit information obtained using the methods described in this disclosure throughout the world, for example across a border.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2499

[00168] In one example, the software is embedded in the instrument making the measurement, such as a thermal cycler. In another example, the software is installed on a computer attached to the instrument making the measurement, such as a computer attached to a thermal cycler. In another example, the software is in the "cloud" -- i.e., on a computer that another computer may communicate with through a network.

[00169] Each of the elements described above may be connected to a controller that communicates with each of the elements and coordinates their actions. For example, a controller may initiate the encoding of a series of analytes into values of color and intensity. As described throughout this disclosure, the controller can execute software to reduce or eliminate degeneracy by removing one or more potential analyte codes from the encoding matrix. The controller may then automatically order probes corresponding to this code from a manufacturer of probes. Alternatively, the controller may operate an oligonucleotide synthesis instrument that can synthesize the appropriate probes. After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix. The controller may then provide these results to a user.

VIII. Services

[00170] The methods provided herein may also be performed as a service. For example, a service provider may obtain the identity of a plurality of analytes that a customer wishes to analyze. The service provider may then encode each analyte to be detected by any of the methods described herein and provide appropriate reagents to the customer for the assay. The customer may perform the assay and provide the results to the service provider for decoding. The service provider may then provide the decoded results to the customer. The customer may also encode analytes, generate probes, and/or decode results by interacting with software installed locally (at the customer's location) or remotely (e.g., on a server reachable through a network). Exemplary customers include clinical laboratories, physicians, manufacturers of food and consumer products, industrial manufacturers (e.g., petroleum companies) and the like. A customer or party may be any suitable customer or party with a need or desire to use the methods, systems, compositions, and kits of the invention.

EXAMPLES

Example 1: General Materials & Methods

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2500

1. General Reagents

[00171] TE Buffer, pH7 (LIFE TECHNOLOGIES, Carlsbad, CA); UltraPure RNAse-free Water (LIFE TECHNOLOGIES, Carlsbad, CA); Taq 5x Master Mix (FISHER SCIENTIFIC COMPANY, Tustin, CA).

2. DNA Sequences, Primers, and Probes

[00172] Five nucleic acids from organisms of clinical relevance were chosen for the exemplary study: (1) human immunodeficiency virus 1 (HIV-1); (2) *Plasmodium falciparum* (Malaria); (3) herpes simplex virus-2 (HSV-2); (4) *Mycobacterium tuberculosis* (TB); (5) and dengue virus type 3 (dengue fever).

[00173] Two regions of diagnostic relevance from the HIV-1 genome, p17 and poly-protease, were selected from the Los Alamos National Laboratory HIV-1 reference sequence. A diagnostic sequence from the *Plasmodium falciparum* ChR7 gene was obtained from the UCSC *Plasmodium falciparum* Genome Browser. A sequence for Herpes Simplex Virus-2 was synthesized from the sequence obtained from the European Molecular Biology Library. Similarly a diagnostic sequence for the rpoB gene in *Mycobacterium tuberculosis* was synthesized from a sequence obtained from the European Molecular Biology Library. A PCR diagnostic sequence for Dengue Virus Type 3 was obtained from the National Institute of Health genetic sequence database.

[00174] Oligonucleotides were synthesized by INTEGRATED DNA TECHNOLOGIES (IDT). Probes and primer pairs were chosen for each analyte using OLIGOANALYZER, from IDT. Sense probes containing a fluorophore at the 5' end and a quencher at the 3' end were synthesized for all analytes. All oligonucleotides were lyophilized and reconstituted in TE buffer before use. Tables 9 - 14 show sequence information, target sequence, primers, and probes for each of the six analytes.

Table 9. HIV-1 polyprotease sequence information.

Sequence Information	HIV-1 Poly protease 198mer synthesized from bases 2253 – 2550
Source	HIV-1 Reference Sequence, Los Alamos National Laboratory
Target sequence (3' to 5')	GGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGA GTTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTT ATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGACATAA AGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGG
Forward Primer (3' to 5')	GGAAGCTCTATTAGATACAGGAGCAG

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2501

Reverse Primer (3' to 5')	CCAATTATGTTGACAGGTGTAGGTCC
Probe 1 (3' to 5')	/**56-FAM**/TGAGTTTGCCAGGAAGATGGAAACCA/3BHQ_1/

Table 10. HIV p17 sequence information.

Sequence Name	HIV-1 P17 199mer synthesized from bases 790 – 1186
Source	HIV-1 Reference Sequence, Los Alamos National Laboratory
Target sequence (3' to 5')	CAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATA TAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAG ACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAG AAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGCAATCAGGTCA
Forward Primer (3' to 5')	CAGCTACAACCATCCCTTCAGACA
Reverse Primer (3' to 5')	TGACCTGATTGCTGTGTCCTGTGT
Probe 1 (3' to 5')	/**56-FAM**/AGCAACCCTCTATTGTGTGCATCAAAGG/3BHQ_1
Probe 2 (3' to 5')	/**5Cy3**/AAAGCACAGCAAGCAGCAGCTGA/3BHQ_2/

Table 11. *Plasmodium* ChR7 sequence information.

Sequence Name	*Plasmodium* ChR7 199mer synthesized from bases 1139138 - 1141223
Source	UCSC Plasmodium falciparum Genome Browser Gateway
Target sequence (3' to 5')	GCCTAACATGGCTATGACGGGTAACGGGGAATTAGAGTTCGATTCCGGAG AGGGAGCCTGAGAAATAGCTACCACATCTAAGGAAGGCAGCAGGCGCGTA AATTACCCAATTCTAAAGAAGAGAGGTAGTGACAAGAAATAACAATGCAA GGCCAATTTAAAACCTTCCCAGAGTAACAATTGGAGGGCAAGTCTGGTG
Forward Primer (3' to 5')	GCCTAACATGGCTATGACGGGTAA
Reverse Primer (3' to 5')	CACCAGACTTGCCCTCCAATTGTT
Probe 1 (3' to 5')	/**56-FAM**/ATTCCGGAGAGGGAGCCTGAGAAATA/3BHQ_1/
Probe 2 (3' to 5')	/**56-ROXN**/AAGGAAGGCAGCAGGCGCGTAAATTA/3BHQ_2/

Table 12. Herpes simplex virus 2 sequence information.

Sequence Name	HSV-2 193mer synthesized from HSV-2 genome
Source	EMBL Bank AJ303204

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2502

Target sequence (3' to 5')	TCAGCCCATCCTCCTTCGGCAGTATGGAGGGTGTCGCGGCGGCGAGCCGC CGTCCCCAAAGACGTGCGGGTCGTACACGTACACGTACCAGGGCGGCGGG CCTCCGACCCGGTACGCTCTCGTAAATGCTTCCCTGCTGGTGCCGATCTG GGACCGCGCCGCGGAGACATTCGAGTACCAGATCGAACTCGG
Forward Primer (3' to 5')	TCAGCCCATCCTCCTTCGGCAGTAT
Reverse Primer (3' to 5')	CCGAGTTCGATCTGGTACTCGAATGT
Probe 1 (3' to 5')	/**56-FAM**/AAAGACGTGCGGGTCGTACACGTACA/3BHQ_1/
Probe 2 (3' to 5')	/**5Cy5**/TAAATGCTTCCCTGCTGGTGCCGAT/3IAbRQSp/

Table 13. *Mycobacterium tuberculosis* rpoB sequence information

Sequence Name	Mycobacterium Tuberculosis rpoB 200mer synthesized from Mycobacterium tuberculosis rpoB genome
Source	EMBL Bank GQ395623
Target sequence (3' to 5')	GAGTGCAAAGACAAGGACATGACGTACGCGGCCCCGCTGTTCGTCACGGC CGAGTTCATCAACAACAACACCGGCGAGATCAAGAGCCAGACGGTGTTCA TGGGTGACTTCCCGATGATGACCGAGAAGGGCACCTTCATCATCAACGGC ACCGAGCGCGTCGTGGTCAGCCAGCTGGTCCGCTCGCCCGGTGTGTACTT
Forward Primer (3' to 5')	GAGTGCAAAGACAAGGACATGACG
Reverse Primer (3' to 5')	AAGTACACACCGGGCGAGC
Probe 1 (3' to 5')	/**56-FAM**/CGCTGTTCGTCACGGCCGAGTTCAT/3BHQ_1/
Probe 2 (3' to 5')	/**5Cy3**/AGATCAAGAGCCAGACGGTGTTCATG/3BHQ_2/
Probe 3 (3' to 5')	/**5Cy5**/AAGGGCACCTTCATCATCAACGGCA/3IAbRQSp/

Table 14. Dengue virus type 3 sequence information.

Sequence Name	Dengue Virus Type 3 200mer synthesized from Dengue Virus Type 3 genome
Source	GenBank M93130
Target sequence (3' to 5')	ATGCCAACTGTGATTGAGCACTTAGAAAGACTACAAAGGAAACATGGAGG AATGCTTGTGAGAAATCCACTCTCACGAAACTCCACGCACGAAATGTATT GGATATCCAATGGTACAGGCAACATCGTCTCATTCACAATGACACACAGG AGACCCACCATAGAGAAAGATGTGGATTTAGGAGCAGGAACCCGACATGT
Forward Primer (3' to 5')	ATGCCAACTGTGATTGAGCACT

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2503

Reverse Primer (3' to 5')	ACATGTCGGGTTCCTGCTCCTAAA
Probe 1 (3' to 5')	/**56-FAM**/ACAAAGGAAACATGGAGGAATGCTTGTGA/3BHQ_1/
Probe 2 (3' to 5')	/**5Cy3**/ACTCTCACGAAACTCCACGCACGAAA/3BHQ_2/
Probe 3 (3' to 5')	/**56-ROXN**/ACAATGACACACAGGAGACCCACCAT/3BHQ_2/
Probe 4 (3' to 5')	/**5Cy5**/GGATATCCAATGGTACAGGCAACATCGT/3IAbRQSp/

3. Polymerase Chain Reactions

[00175] All PCR reactions were performed in a ROCHE 480 LIGHTCYCLER instrument. Forty-five thermal cycles were performed, with a 60 second hot-start at 95°C. The cycling conditions for denaturation, annealing, and extension were 45 seconds, 50 seconds, and 60 seconds at 95°C, 65°C, and 70°C, respectively. Each experiment was run in quintuplicate, with a reaction volume of 15 μL. Fluorescence measurements were obtained in the following channels after every annealing step: 483 nm to 533 nm (FAM), 523 nm to 568 nm (Cy3), 558 nm to 610 nm (ROX), and 615 nm to 670 nm (Cy5). Fluorescence measurements were also obtained after the hot-start and the end of the 45 thermal cycles. The change in the fluorescence intensity between these two measurements (after hot-start and at the end of the 45 cycles) determined the end-point fluorescent signal.

Example 2: Simultaneous Detection of Polynucleotide Markers from Pathogens Based on an Encoding Method Using More Than One Color Per Analyte

[00176] This example describes the simultaneous detection of up to four polynucleotides selected from HIV (2 polynucleotides), HSV-2, *Mycobacterium*, *Plasmodium*, and dengue virus. Fourteen PCR reactions were assembled, using the reagents described in Table 15.

Table 15. Reagents for 14 PCR reactions.

Reaction No.	Type of Reagent	Reagent	Concentration	Volume
1		UltraPure Water	-	34 μL
		Taq 5x Master Mix	-	42 μL
	Template	Poly Protease Template	17 nM	2 μL
	Primers	Poly Protease FWD Primer	1 nM	2 μL
		Protease RVS Primer	1 nM	2 μL
	Probe	Poly Protease Probe 1	1 nM	2 μL
2		UltraPure Water	-	28 μL
		Taq 5x Master Mix	-	42 μL
	Templates	Malaria Template	15 nM	2 μL

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2504

		Herpes Template	17 nM	2 µL
		TB Template	16 nM	2 µL
		Dengue Virus Template	17 nM	2 µL
	Primers	Poly Protease FWD Primer	1 nM	2 µL
		Protease RVS Primer	1 nM	2 µL
	Probe	Poly Protease Probe 1	1 nM	2 µL
3		UltraPure Water	-	32 µL
		Taq 5x Master Mix	-	42 µL
	Template	Malaria Template	15 nM	2 µL
	Primers	Malaria FWD Primer	1 µM	2 µL
		Malaria RVS Primer	1 µM	2 µL
	Probes	Malaria Probe 1	1 µM	2 µL
		Malaria Probe 2	1 µM	2 µL
4		UltraPure Water	-	26 µL
		Taq 5x Master Mix	-	42 µL
	Templates	Poly Protease Template	17 nM	2 µL
		Herpes Template	17 nM	2 µL
		TB Template	16 nM	2 µL
		Dengue Virus Template	17 nM	2 µL
	Primers	Malaria FWD Primer	1 µM	2 µL
		Malaria RVS Primer	1 µM	2 µL
	Probes	Malaria Probe 1	1 µM	2 µL
		Malaria Probe 2	1 µM	2 µL
5		UltraPure Water	-	32 µL
		Taq 5x Master Mix	-	42 µL
	Template	Herpes Template	17 nM	2 µL
	Primers	Herpes FWD Primer	1 µM	2 µL
		Herpes RVS Primer	1 µM	2 µL
	Probes	Herpes Probe 1	1 µM	2 µL
		Herpes Probe 2	1 µM	2 µL
6		UltraPure Water	-	26 µL
		Taq 5x Master Mix	-	42 µL
	Templates	Poly Protease Template	17 nM	2 µL
		Malaria Template	15 nM	2 µL
		TB Template	16 nM	2 µL
		Dengue Virus Template	17 nM	2 µL
	Primers	Herpes FWD Primer	1 µM	2 µL
		Herpes RVS Primer	1 µM	2 µL
	Probes	Herpes Probe 1	1 µM	2 µL
		Herpes Probe 2	1 µM	2 µL
7		UltraPure Water	-	30 µL
		Taq 5x Master Mix	-	42 µL
	Template	TB Template	16 nM	2 µL
	Primers	TB FWD Primer	1 µM	2 µL
		TB RVS Primer	1 µM	2 µL
	Probes	TB Probe 1	1 µM	2 µL
		TB Probe 2	1 µM	2 µL
		TB Probe 3	1 µM	2 µL
8		UltraPure Water	-	30 µL

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2505

		Taq 5x Master Mix	-	42 µL
	Templates	Poly Protease Template	17 nM	2 µL
		Malaria Template	15 nM	2 µL
		Herpes Template	17 nM	2 µL
		Dengue Virus Template	17 nM	2 µL
	Primers	TB FWD Primer	1 µM	2 µL
		TB RVS Primer	1 µM	2 µL
	Probes	TB Probe 1	1 µM	2 µL
		TB Probe 2	1 µM	2 µL
		TB Probe 3	1 µM	2 µL
9		UltraPure Water	-	28 µL
		Taq 5x Master Mix	-	42 µL
	Template	Dengue Virus Template	17 nM	2 µL
	Primers	Dengue Virus FWD Primer	1 µM	2 µL
		Dengue Virus RVS Primer	1 µM	2 µL
	Probes	Dengue Virus Probe 1	1 µM	2 µL
		Dengue Virus Probe 2	1 µM	2 µL
		Dengue Virus Probe 3	1 µM	2 µL
		Dengue Virus Probe 4	1 µM	2 µL
10		UltraPure Water	-	22 µL
		Taq 5x Master Mix	-	42 µL
	Templates	Poly Protease Template	17 nM	2 µL
		Malaria Template	15 nM	2 µL
		Herpes Template	17 nM	2 µL
		TB Template	16 nM	2 µL
	Primers	Dengue Virus FWD Primer	1 µM	2 µL
		Dengue Virus RVS Primer	1 µM	2 µL
	Probes	Dengue Virus Probe 1	1 µM	2 µL
		Dengue Virus Probe 2	1 µM	2 µL
		Dengue Virus Probe 3	1 µM	2 µL
		Dengue Virus Probe 4	1 µM	2 µL
11		Taq 5x Master Mix	-	42 µL
	Templates	Poly Protease Template	17 nM	2 µL
		Malaria Template	15 nM	2 µL
		Herpes Template	17 nM	2 µL
		Dengue Virus Template	17 nM	2 µL
	Primers	Poly Protease FWD Primer	1 µM	2 µL
		Poly Protease RVS Primer	1 µM	2 µL
		Malaria FWD Primer	1 µM	2 µL
		Malaria RVS Primer	1 µM	2 µL
		Herpes FWD Primer	1 µM	2 µL
		Herpes RVS Primer	1 µM	2 µL
		Dengue Virus FWD Primer	1 µM	2 µL
		Dengue Virus RVS Primer	1 µM	2 µL
	Probes	Poly Protease Probe 1	1 µM	2 µL
		Malaria Probe 1	1 µM	2 µL
		Malaria Probe 2	1 µM	2 µL
		Herpes Probe 1	1 µM	2 µL
		Herpes Probe 2	1 µM	2 µL

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2506

		Dengue Virus Probe 1	1 μM	2 μL
		Dengue Virus Probe 2	1 μM	2 μL
		Dengue Virus Probe 3	1 μM	2 μL
		Dengue Virus Probe 4	1 μM	2 μL
12		UltraPure Water	-	2 μL
		Taq 5x Master Mix	-	42 μL
	Templates	Poly Protease Template	17 nM	2 μL
		Malaria Template	15 nM	2 μL
		Herpes Template	17 nM	2 μL
		TB Template	16 nM	2 μL
	Primers	Poly Protease FWD Primer	1 μM	2 μL
		Poly Protease FWD Primer	1 μM	2 μL
		Malaria FWD Primer	1 μM	2 μL
		Malaria RVS Primer	1 μM	2 μL
		Herpes FWD Primer	1 μM	2 μL
		Herpes RVS Primer	1 μM	2 μL
		TB FWD Primer	1 μM	2 μL
		TB RVS Primer	1 μM	2 μL
	Probes	Poly Protease Probe 1	1 μM	2 μL
		Malaria Probe 1	1 μM	2 μL
		Malaria Probe 2	1 μM	2 μL
		Herpes Probe 1	1 μM	2 μL
		Herpes Probe 2	1 μM	2 μL
		TB Probe 1	1 μM	2 μL
		TB Probe 2	1 μM	2 μL
		TB Probe 3	1 μM	2 μL
13		UltraPure Water	-	12 μL
		Taq 5x Master Mix	-	42 μL
	Templates	Poly Protease Template	17 nM	2 μL
		Herpes Template	17 nM	2 μL
		TB Template	16 nM	2 μL
	Primers	Poly Protease FWD Primer	1 μM	2 μL
		Poly Protease FWD Primer	1 μM	2 μL
		Herpes FWD Primer	1 μM	2 μL
		Herpes RVS Primer	1 μM	2 μL
		TB FWD Primer	1 μM	2 μL
		TB RVS Primer	1 μM	2 μL
	Probes	Poly Protease Probe 1	1 μM	2 μL
		Herpes Probe 1	1 μM	2 μL
		Herpes Probe 2	1 μM	2 μL
		TB Probe 1	1 μM	2 μL
		TB Probe 2	1 μM	2 μL
		TB Probe 3	1 μM	2 μL
14		UltraPure Water	-	10 μL
		Taq 5x Master Mix	-	42 μL
	Templates	Poly Protease Template	17 nM	2 μL
		Malaria Template	15 nM	2 μL
		Dengue Virus Template	17 nM	2 μL
	Primers	Poly Protease FWD Primer	1 μM	2 μL

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2507

		Poly Protease FWD Primer	1 μM	2 μL
		Malaria FWD Primer	1 μM	2 μL
		Malaria RVS Primer	1 μM	2 μL
		Dengue Virus FWD Primer	1 μM	2 μL
		Dengue Virus RVS Primer	1 μM	2 μL
	Probes	Poly Protease Probe 1	1 μM	2 μL
		Malaria Probe 1	1 μM	2 μL
		Malaria Probe 2	1 μM	2 μL
		Dengue Virus Probe 1	1 μM	2 μL
		Dengue Virus Probe 2	1 μM	2 μL
		Dengue Virus Probe 3	1 μM	2 μL
		Dengue Virus Probe 4	1 μM	2 μL

[00177] Referring to Table 15, reactions 1, 3, 5, 7, and 9 (containing HIV polyprotease, *Plasmodium*, HSV, *Mycobacterium*, and dengue analytes, respectively) were positive controls that provided baseline fluorescence intensity for each polynucleotide analytes alone, in the absence of other analytes. The change in fluorescence intensity in each color was used to provide the expected cumulative intensity level for each color in the chromatogram **(Fig. 3)**, as described more fully below. Reactions 2, 4, 6, 8, and 10 were negative controls used to determine the extent of non-specific amplification. In these reactions, the primers provided were not specific for the analytes provided.

[00178] Experiments 11, 12, 13, and 14 were multiplex detection experiments. Encoding was performed as indicated in Table 16. The measured fluorescence intensity for each assay, in each color, was plotted as black circles in the chromatogram of **Fig. 3.**

Table 16. Coding scheme for detection of pathogens.

	FAM	**Cy3**	**ROX**	**Cy5**
HIV poly-protease	1	0	0	0
HIV p17	1	1	0	0
Plasmodium	1	0	1	0
HSV	1	0	0	1
Mycobacterium	1	1	0	1
Dengue Virus	1	1	1	1

[00179] To construct the chromatogram, the signals of the positive controls (reactions 1, 3, 5, 7, and 9) were used to assemble every possible combination of present sequences, in each color. The cumulative signals were plotted in a diagram, called a "chromatogram" (**Fig. 3**). Cumulative signals corresponding to all possible combinations of the same rank in the same color were organized into their own "band." Doing this for all four colors produced a level and band structure, against which the experimental results of each combination (reactions 11, 12, 13, and 14) could be evaluated. **Fig.**

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2508

3 shows three replicates of a chromatogram constructed using the positive control samples, with experimental results 4112, 3121, and 3102 superimposed on the chromatogram. The experimental results are indicated by black dots.

[00180] The intensity value for each color in reactions 11-14 was measure in arbitrary fluorescence units (AFU), plotted on the chromatogram, and then assigned to a band based on two criteria. First, if an experimental result was within a band, then the result was assigned to the multiplicity of the band (e.g., the Cy3 value of 1 in the left-most chromatogram of **Fig. 3**). Second, if an experimental result was between two bands, a two-step analysis was employed. If the experimental result was between two legitimate results, the experimental result was assigned to the closest band. (e.g., the Cy5 value of 2 in the right-most chromatogram of **Fig. 3**). If the experimental result was between a legitimate result and an illegitimate result, the result was assigned to the band with the legitimate result (e.g., the FAM value of 3 in the middle chromatogram of **Fig. 3**). **Fig. 8** schematically shows a legitimate result of 4112 (top) indicating the presence of HIV polyprotease, *Plasmodium*, herpes simplex, and *Mycobacterium*; and an illegitimate result of 4110 (bottom), which cannot be decoded and indicates a malfunction in the assay.

[00181] Using these criteria, the output of Experiment 12 was designated 4112, which was the correct answer. The output of Experiment 14 could have been designated as 2121 or 3121, but 2121 is illegitimate, so the result must be 3121, which is the correct answer. The output of Experiment 13 could be designated as 3102 or 3101, both of which are legitimate, but the result is clearly closer to 2 than 1 in the Cy5 channel, so 3102, the correct answer, was designated as the result. These results provide experimental validation of the methods of the provided herein.

[00182] The results indicate a trend of slightly lower combined signal than what would be expected from a simple summation of positive-control signals. This may be because the combination of signals is not perfectly linear. This may be a result of additional effects for which the chromatogram construction does not account for at present. These effects may be related to the absolute concentration of quenchers. The higher the absolute concentration of quenchers, the larger the percentage of reaction volume they quench, so that the same percentage of released fluorophores fails to contribute as much as expected to the cumulative signal. One solution to this problem is to use low concentrations of probes, so that the percentile loss in signal is negligible, regardless of how much quencher is released.

[00183] **Fig. 3** shows that each rank containing multiple combinations has a relatively wide band. The width of the band is ultimately the difference between the highest and lowest cumulative signals for the positive control samples. If all positive controls in the particular color produced exactly the same fluorescence signal, then the width of each band would be zero. Since instead those

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2509

signals are somewhat different, the resulting bands are relatively wide. However, there is a simple means to tighten the bands. Instead of loading all probes at the same concentration, as was done for the experiment above, the concentration of each probe can be adjusted so that each of the resulting positive-control signals is the same as the others in the same color. This approach would significantly tighten the bands, making it easier to determine the multiplicity of a signal for a sample containing analytes.

Example 3: Simultaneous Detection of Polynucleotide Markers from Pathogens Based on an Encoding Method Using One Color Per Analyte and Different Intensities

[00184] This example describes the simultaneous detection of up to three polynucleotides selected from dengue virus, HIV p17, and HIV polyprotease. The coding scheme was as depicted in Table 11. As shown in Table 17, each analyte was encoded as a single value of fluorescence intensity in a single color. In this case, as described elsewhere in this disclosure, the coding scheme is non-degenerate by design. This is achieved by assigning an intensity value to each analyte that is equal to the cumulative intensity values for the prior analytes, plus one. Of course, as described elsewhere in the specification, the initial assigned value can be greater than one, for example to better distinguish over noise. Similarly, the values assigned to each analyte can be greater than the cumulative intensity values for the prior analytes plus one, for example, to increase the separation between the intensities and enable more simple assignment of intensities.

Table 17. Coding scheme for detection of dengue virus, HIV polyprotease, and HIV p17.

	FAM
Dengue Virus	1
HIV p17	2
HIV polyprotease	4

[00185] The same reagents (e.g., templates, primers, and probes) used in Example 2 were used here. The fluorescence signals generated by the respective probes in positive-control end-point PCR reactions were measured and used to calculate the probe concentrations that would produce 1x, 2x, and 4x signal intensities. Experiments were then set up to detect the seven non-null combinations of analyte occurrences. Tables 18-24 provide the PCR reaction components for each of these mixtures.

Table 18. Binary FAM Experiment 1 Cocktail: HIV Polyprotease, HIV p17, and dengue virus

Reagents	Concentration	Volume Added
UltraPure Water	-	50μL

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2510

Taq 5x Master Mix	-	25μL
Templates	Concentration	Volume Added
Poly Protease Template	17nM	4μL
P17 Template	15nM	4μL
Dengue Virus Template	17nM	4μL
Primers	Concentration	Volume Added
Poly Protease FWD Primer	1μM	4μL
Poly Protease RVS Primer	1μM	4μL
P17 FWD Primer	1μM	4μL
P17 RVS Primer	1μM	4μL
Dengue Virus FWD Primer	1μM	4μL
Dengue Virus RVS Primer	1μM	4μL
Probes	Concentration	Volume Added
Poly Protease Probe 1	800nM	4μL
P17 Probe 1	400nM	4μL
Dengue Virus Probe 1	200nM	4μL

Table 19. Binary FAM Experiment 2 Cocktail: dengue virus and HIV p17

Reagents	Concentration	Volume Added
UltraPure Water	-	54μL
Taq 5x Master Mix	-	25μL
Templates	Concentration	Volume Added
Poly Protease Template	17nM	-
P17 Template	15nM	4μL
Dengue Virus Template	17nM	4μL
Primers	Concentration	Volume Added
Poly Protease FWD Primer	1μM	4μL
Poly Protease RVS Primer	1μM	4μL
P17 FWD Primer	1μM	4μL
P17 RVS Primer	1μM	4μL
Dengue Virus FWD Primer	1μM	4μL
Dengue Virus RVS Primer	1μM	4μL
Probes	Concentration	Volume Added

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2511

Poly Protease Probe 1	800nM	4μL
P17 Probe 1	400nM	4μL
Dengue Virus Probe 1	200nM	4μL

Table 20. Binary FAM Experiment 3 Cocktail: dengue virus and HIV polyprotease

Reagents	Concentration	Volume Added
UltraPure Water	-	54μL
Taq 5x Master Mix	-	25μL
Templates	Concentration	Volume Added
Poly Protease Template	17nM	4μL
P17 Template	15nM	-
Dengue Virus Template	17nM	4μL
Primers	Concentration	Volume Added
Poly Protease FWD Primer	1μM	4μL
Poly Protease RVS Primer	1μM	4μL
P17 FWD Primer	1μM	4μL
P17 RVS Primer	1μM	4μL
Dengue Virus FWD Primer	1μM	4μL
Dengue Virus RVS Primer	1μM	4μL
Probes	Concentration	Volume Added
Poly Protease Probe 1	800nM	4μL
P17 Probe 1	400nM	4μL
Dengue Virus Probe 1	200nM	4μL

Table 21. Binary FAM Experiment 4 Cocktail: HIV polyprotease and HIV p17

Reagents	Concentration	Volume Added
UltraPure Water	-	54μL
Taq 5x Master Mix	-	25μL
Templates	Concentration	Volume Added
Poly Protease Template	17nM	4μL
P17 Template	15nM	4μL
Dengue Virus Template	17nM	-
Primers	Concentration	Volume Added

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2512

Poly Protease FWD Primer	1μM	4μL
Poly Protease RVS Primer	1μM	4μL
P17 FWD Primer	1μM	4μL
P17 RVS Primer	1μM	4μL
Dengue Virus FWD Primer	1μM	4μL
Dengue Virus RVS Primer	1μM	4μL
Probes	Concentration	Volume Added
Poly Protease Probe 1	800nM	4μL
P17 Probe 1	400nM	4μL
Dengue Virus Probe 1	200nM	4μL

Table 22. Binary FAM Experiment 5 Cocktail: dengue virus

Reagents	Concentration	Volume Added
UltraPure Water	-	58μL
Taq 5x Master Mix	-	25μL
Templates	Concentration	Volume Added
Poly Protease Template	17nM	-
P17 Template	15nM	-
Dengue Virus Template	17nM	4μL
Primers	Concentration	Volume Added
Poly Protease FWD Primer	1μM	4μL
Poly Protease RVS Primer	1μM	4μL
P17 FWD Primer	1μM	4μL
P17 RVS Primer	1μM	4μL
Dengue Virus FWD Primer	1μM	4μL
Dengue Virus RVS Primer	1μM	4μL
Probes	Concentration	Volume Added
Poly Protease Probe 1	800nM	4μL
P17 Probe 1	400nM	4μL
Dengue Virus Probe 1	200nM	4μL

Table 23. Binary FAM Experiment 6 Cocktail: HIV p17

Reagents	Concentration	Volume Added

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2513

UltraPure Water	-	58μL
Taq 5x Master Mix	-	25μL
Templates	Concentration	Volume Added
Poly Protease Template	17nM	-
P17 Template	15nM	4μL
Dengue Virus Template	17nM	-
Primers	Concentration	Volume Added
Poly Protease FWD Primer	1μM	4μL
Poly Protease RVS Primer	1μM	4μL
P17 FWD Primer	1μM	4μL
P17 RVS Primer	1μM	4μL
Dengue Virus FWD Primer	1μM	4μL
Dengue Virus RVS Primer	1μM	4μL
Probes	Concentration	Volume Added
Poly Protease Probe 1	800nM	4μL
P17 Probe 1	400nM	4μL
Dengue Virus Probe 1	200nM	4μL

Table 24. Binary FAM Experiment 7 Cocktail: HIV polyprotease

Reagents	Concentration	Volume Added
UltraPure Water	-	58μL
Taq 5x Master Mix	-	25μL
Templates	Concentration	Volume Added
Poly Protease Template	17nM	4μL
P17 Template	15nM	-
Dengue Virus Template	17nM	-
Primers	Concentration	Volume Added
Poly Protease FWD Primer	1μM	4μL
Poly Protease RVS Primer	1μM	4μL
P17 FWD Primer	1μM	4μL
P17 RVS Primer	1μM	4μL
Dengue Virus FWD Primer	1μM	4μL
Dengue Virus RVS Primer	1μM	4μL

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2514

Probes	Concentration	Volume Added
Poly Protease Probe 1	800nM	4μL
P17 Probe 1	400nM	4μL
Dengue Virus Probe 1	200nM	4μL

[00186] The total fluorescence signal of each experiment was plotted on a chromatogram **(Fig. 7)** that was constructed as described above. The width of the bands around each positive control measurement is the propagated uncertainty of the 1X measurement. That uncertainty was equated to the standard deviation of the fluorescence signals of the last five PCR cycles in saturation.

[00187] **Fig. 7** shows the results of this experiment. The three right-most results show the measured intensities for dengue virus alone (DEN), HIV p17 alone (P17), and HIV polyprotease (TPP). The assigned intensities for each of these analytes were as indicated in Table 11, and **Fig. 7** confirms that the three analytes yielded the expected intensity results when alone (see DEN, P17, and TPP). At the end of the assay, essentially all of the probes have been hydrolyzed, releasing essentially all of the fluorophores, which emit a signal. A fixed amount of a fluorophore produces a fixed and predictable amount of signal. Therefore a target that is present should generally produce a fixed amount of signal, as determined by the amount of probe added to the reaction volume.

[00188] As expected, the intensity values for the combinations of analytes are equivalent to the sum of the assigned intensities. Since this coding scheme is designed to be non-degenerate, each result can be unambiguously decoded into the presence or absence of a particular analyte. For example, starting from the left side of **Fig. 7**, a result of 7 indicates the presence of all three analytes. A result of 3 indicates the presence of dengue virus and HIV p17 analytes, and the absence of HIV polyprotease. A result of 5 indicates the presence of dengue virus and HIV polyprotease, and the absence of HIV p17. Finally a result of 6 indicates the presence of HIV p17 and HIV polyprotease, and the absence of dengue virus. This coding scheme can be extended to encode additional analytes, as described elsewhere in this disclosure.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2515

CLAIMS

WHAT IS CLAIMED IS:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis.
2. The assay of claim 1, wherein each of said analytes is encoded as a value of one component of a signal.
3. The method of claim 2, wherein said method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of said one component of said signal when all of said analytes are present.
4. The assay of claim 1, wherein each of said analytes is encoded as at least one first value in a first component of said signal and at least one second value in a second component of said signal.
5. The assay of claim 4, wherein each of said analytes is encoded as a first value in a first component of said signal at each of a plurality of second values in a second component of said signal.
6. The method of claim 4, wherein said method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = C * \log_2 (F + 1)$, where C is the number of said second values used to encode said analytes and F is the maximum cumulative value of said first component of said signal, for any second value, when all of said analytes are present.
7. The method of claim 4, wherein said method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = (P * T) + 1$, where P is the number of codes per tier in a coding scheme and T is the number of tiers.
8. The method of claim 7, wherein the number of tiers $T = \log_4 (F + 1)$, and F is the maximum cumulative value of said first component of said signal, for any second value, when all of said analytes are present.
9. The method of claim 4, wherein said first value is an intensity or range of intensities.
10. The method of claim 9, wherein said coding scheme comprises at least three intensities or ranges of intensities.
11. The method of claim 4, wherein said second value is a wavelength or range of wavelengths.
12. The method of claim 11, wherein said coding scheme comprises at least five wavelengths or ranges of wavelengths.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2516

13. The method of claim 1, wherein said signal is an electromagnetic signal.

14. The method of claim 13, wherein said electromagnetic signal is a fluorescence emission signal.

15. The method of claim 14, wherein intensity of said fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths.

16. The method of claim 1, wherein said detecting is performed with reagents that are lyophilized prior to use.

17. The method of claim 1, wherein said detecting is performed with reagents comprising hybridization probes.

18. The method of claim 17, wherein the number of said hybridization probes is greater than the number of said analytes.

19. The method of claim 17, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

20. The method of claim 17, wherein said sample is contacted with at least 18 of said hybridization probes.

21. The method of claim 17, wherein said reagents further comprise at least one pair of primers capable of amplifying a region complementary to at least three of said hybridization probes.

22. The method of claim 21, wherein said signal is generated during a polymerase chain reaction.

23. The method of claim 22, wherein said polymerase chain reaction is selected from the group consisting of an end-point polymerase chain reaction, a real-time polymerase chain reaction, and a digital polymerase chain reaction.

24. The method of claim 1, wherein said cumulative measurement is performed on a solution.

25. The method of claim 1, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.

26. The method of claim 25, wherein said at least one additional value is selected from the group consisting of a fluorescence emission intensity, a fluorescence emission wavelength, a Förster resonance energy transfer (FRET) emission intensity, a FRET emission wavelength, an electrochemical signal, a chemiluminescence wavelength, a chemiluminescence intensity, a fluorescence bleaching rate, a chemiluminescence bleaching rate, and combinations thereof.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2517

27. The method of claim 1, further comprising constructing a chromatogram.

28. The method of claim 27, wherein said chromatogram is constructed by plotting all possible combinations of said first values and said second values for positive control samples.

29. The method of claim 1, wherein at least one of said analytes comprises a polynucleotide.

30. The method of claim 29, wherein said polynucleotide is from a source selected from the group consisting of an animal, a plant, a bacteria, a fungus, a parasite, and a virus.

31. The method of claim 23, wherein said polynucleotide is from a source selected from the group consisting of human immunodeficiency virus, herpes simplex virus, human papilloma virus, Plasmodium, Mycobacterium, dengue virus, hepatitis virus, and influenza virus.

32. The method of claim 31, wherein said polynucleotide is selected from the group consisting of human immunodeficiency virus polyprotease, human immunodeficiency virus p17, human papilloma virus E6, and human papilloma virus E7.

33. The method of claim 1, wherein said sample is selected from the group consisting of a clinical sample, a food sample, an environmental sample, a pharmaceutical sample, and a sample from a consumer product.

34. The method of claim 1, further comprising transmitting information concerning the presence or absence of said analyte through a computer network.

35. The method of claim 1, further comprising providing information concerning the presence or absence of said analyte to a physician.

36. The method of claim 35, further comprising making a clinical decision based on said information.

37. The method of claim 1, wherein at least one step of said method is performed using instructions on a computer readable medium.

38. The method of claim 37, wherein said instructions are located on a remote server.

39. The method of claim 37, wherein said instructions are located on a thermal cycler.

40. The method of claim 37, wherein said instructions are located on a computer in communication with a thermal cycler.

41. The method of claim 1, wherein at least one of said analytes is a positive control analyte.

42. The method of claim 1, wherein said coding scheme is non-degenerate.

43. The method of claim 42, wherein said coding scheme is designed to be non-degenerate.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2518

44. The method of claim 42, wherein said coding scheme is made non-degenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.
45. The method of claim 1, wherein said assay is an end-point assay.
46. The method of claim 1, wherein said assay is ended at a threshold number of cycles set by the limit of detection of an instrument.
47. The method of claim 1, wherein said assay is a liquid phase assay.
48. The method of claim 1, wherein said assay is quantitative.
49. A method of detecting the presence or absence of each analyte of a plurality of analytes, comprising:
    a. encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy;
    b. providing a sample comprising, or potentially comprising, at least one of said analytes;
    c. contacting said sample with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present;
    d. cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement; and
    e. determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme.
50. The method of claim 49, wherein said method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of said first values when all of said analytes are present.
51. The method of claim 49, wherein said first value is an intensity or range of intensities.
52. The method of claim 49, wherein said first value has a minimum value in said coding scheme that is selected from the group consisting of at least 1, at least 2, at least 4, at least 8, at least 16, at least 32, and at least 64.
53. The method of claim 49, wherein said first value is incremented in said coding scheme by an amount equal to the cumulative maximum of said preceding first values plus one.
54. The method of claim 49, wherein said first value is incremented in said coding scheme by an amount greater than the cumulative maximum of said preceding first values plus one.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2519

55. A method of detecting the presence or absence of each analyte of a plurality of analytes, comprising:

    a. encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy;

    b. providing a sample comprising, or potentially comprising, at least one of said analytes;

    c. contacting said sample with analyte-specific reagents that generate said at least one first value and at least one said second value, as represented in said coding scheme, when each of said analytes is present;

    d. cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and

    e. determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme,

    wherein, when degeneracy is eliminated, said method is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

56. The method of claim 55, wherein each of said analytes is encoded as a first value in a first component of said signal at each of a plurality of second values in a second component of said signal.

57. The method of claim 55, wherein, when degeneracy is eliminated, said method is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single volume using four second values.

58. The method of claim 55, wherein, when degeneracy is eliminated, said coding scheme comprises T non-degenerate tiers, wherein $T = \log_4 (F + 1)$ and F is the maximum cumulative value of said first component of said signal, for any second value, when all of said analytes are present.

59. The method of claim 56, wherein, when degeneracy is eliminated, said method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = (P * T) + 1$ and P is the number of codes per tier.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2520

60. The method of claim 55, wherein, when degeneracy is eliminated, said method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = C * \log_2 (F + 1)$, C is the number of second values in said coding scheme, and F is the maximum cumulative value of said first component of said signal, for any second value, when all of said analytes are present.

61. A non-degenerate coding scheme capable of unambiguously encoding the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, or melting curve analysis.

62. The non-degenerate coding scheme of claim 61, wherein said coding scheme is generated by a method comprising:

    a. generating a code for each potential analyte, wherein each potential analyte is encoded by at least one value of at least one component of a signal;

    b. enumerating every legitimate cumulative result for all possible combinations of presence or absence of each analyte;

    c. identifying each legitimate result that is degenerate; and

    d. eliminating at least one code to eliminate degeneracy.

63. The method of claim 62, further comprising expanding said coding scheme by adding additional codes that are at least one unit greater than the sum of all previous codes in said at least one component of said signal.

64. The method of claim 61, wherein said coding scheme is generated by a method of mathematical iteration that guarantees non-degeneracy by construction.

65. A system for detecting the presence or absence of each analyte of a plurality of analytes, comprising:

    a. encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy;
    b. providing a sample comprising, or potentially comprising, at least one of said analytes;
    c. contacting said sample with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present;
    d. cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement; and
    e. determining whether each of said analytes is present or absent based on said cumulative

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2521

measurement and said coding scheme.

66. A system for detecting the presence or absence of each analyte of a plurality of analytes, comprising:

    a. encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy;

    b. providing a sample comprising, or potentially comprising, at least one of said analytes;

    c. contacting said sample with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present;

    d. cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and

    e. determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme,

    wherein, when degeneracy is eliminated, said system is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

67. A method of detecting the presence or absence of each analyte of a plurality of analytes, comprising:

    a. obtaining the identity of each of said analytes from a party;

    b. encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy;

    c. providing said party with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present, said party:

        i. contacting a sample comprising, or potentially comprising, at least one of said analytes with said reagents; and

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2522

ii. cumulatively measuring said first values within said sample, thereby providing a cumulative measurement; and

d. obtaining said cumulative measurement from said party;

e. determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme; and

f. providing said party with information about the presence or absence of each of said analytes.

68. A method of detecting the presence or absence of each analyte of a plurality of analytes, comprising:

g. obtaining the identity of each of said analytes from a party;

h. encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy;

i. providing said party with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present, said party:

i. contacting a sample comprising, or potentially comprising, at least one of said analytes with said reagents; and

ii. cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and

j. obtaining said cumulative measurement from said party;

k. determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme; and

l. providing said party with information about the presence or absence of each of said analytes.

69. A composition for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, each reagent generating a signal comprising a first value, wherein said reagents are capable of unambiguously detecting the presence or absence of each of

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2523

at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, or melting curve analysis.

70. A composition for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, each reagent generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said reagents are capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

71. A kit for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, packaging, and instructions, each reagent generating a signal comprising a first value, wherein said kit is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, or melting curve analysis.

72. A kit for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, packaging, and instructions, each reagent generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said kit is capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

73. A kit for detecting the presence or absence of each analyte of a plurality of analytes, comprising a kit body (601), clamping slots arranged in the kit body for placing bottles, a bottle comprising probes for the detection of an analyte (602), a bottle comprising primers for amplification (603), and a bottle comprising reagents for amplification (604), wherein said kit is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, or melting curve analysis.

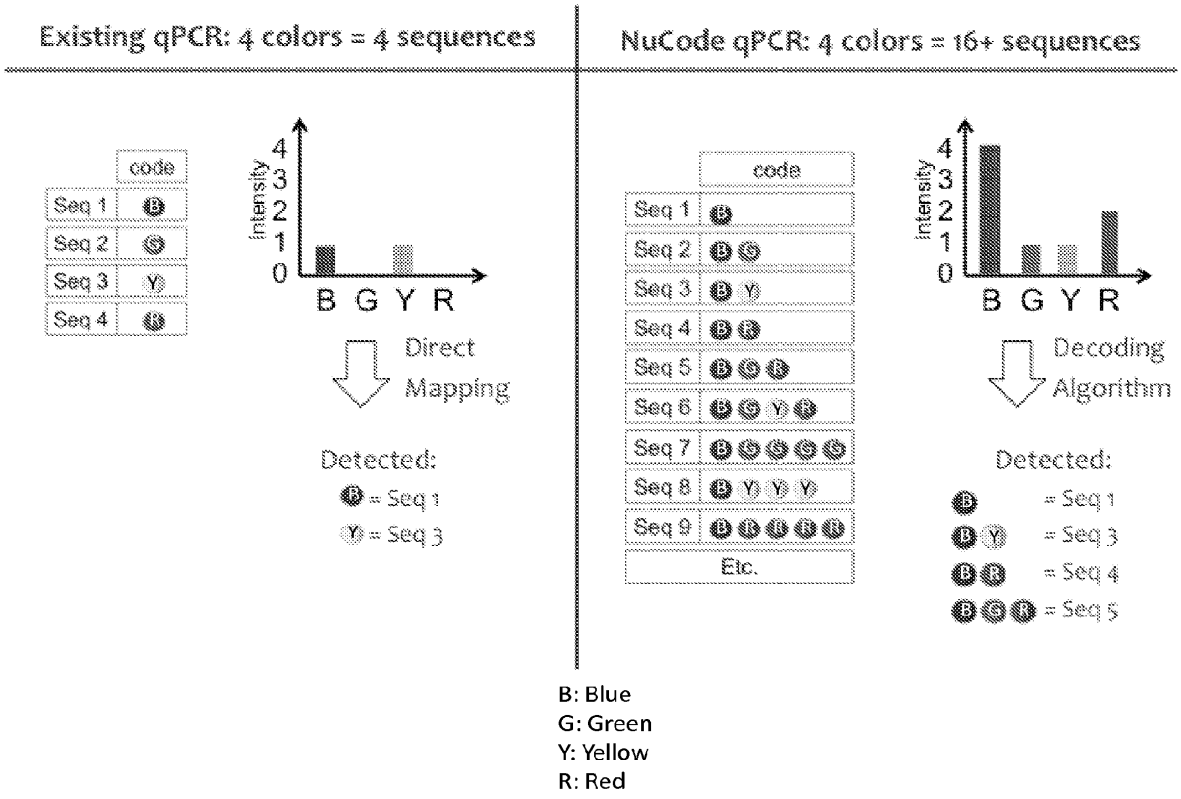
74. A kit for detecting the presence or absence of each analyte of a plurality of analytes, a kit body (601), clamping slots arranged in the kit body for placing bottles, a bottle comprising probes for the detection of an analyte (602), a bottle comprising primers for amplification (603), and a bottle comprising reagents for amplification (604), each probe generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said kit is capable of detecting

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2524

the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said probes generates only one second value.

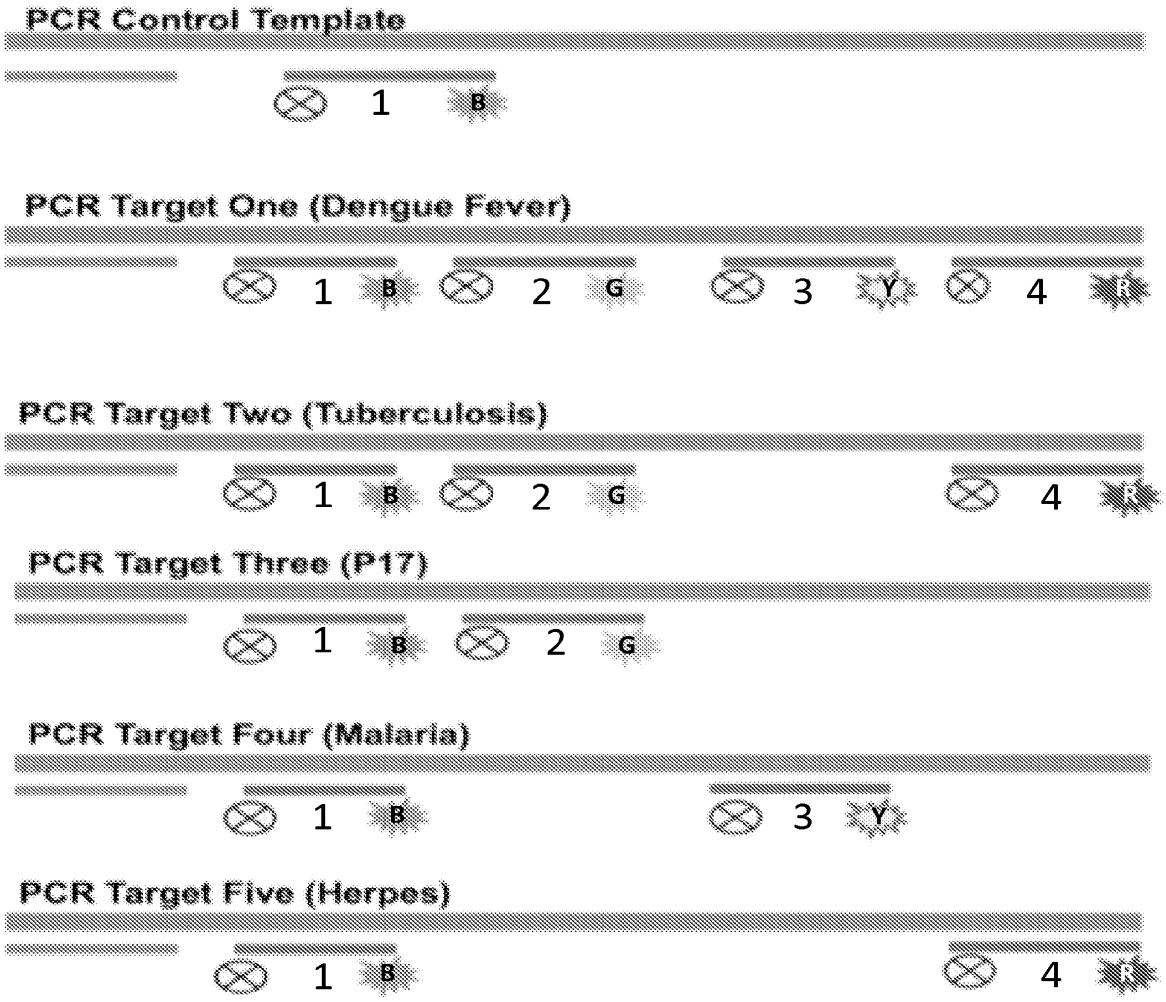
IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2525

Fig. 1



B: Blue
G: Green
Y: Yellow
R: Red

Fig. 2



B: Blue
G: Green
Y: Yellow
R: Red

Fig. 3

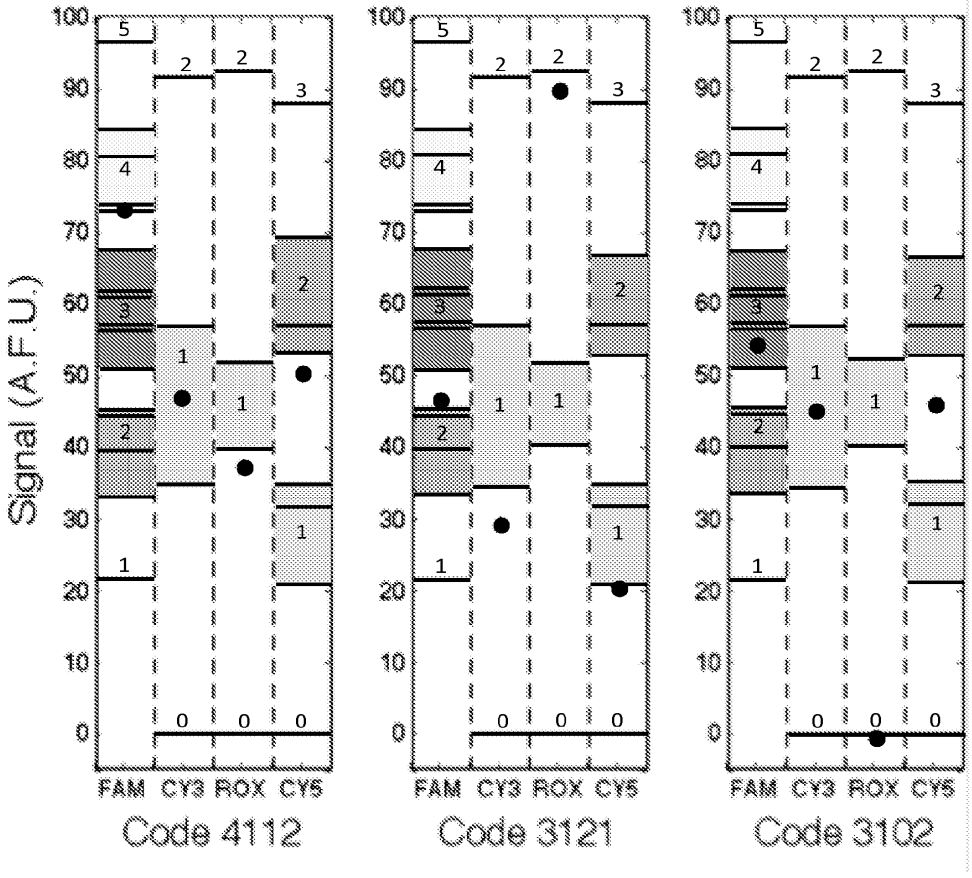


Fig. 4

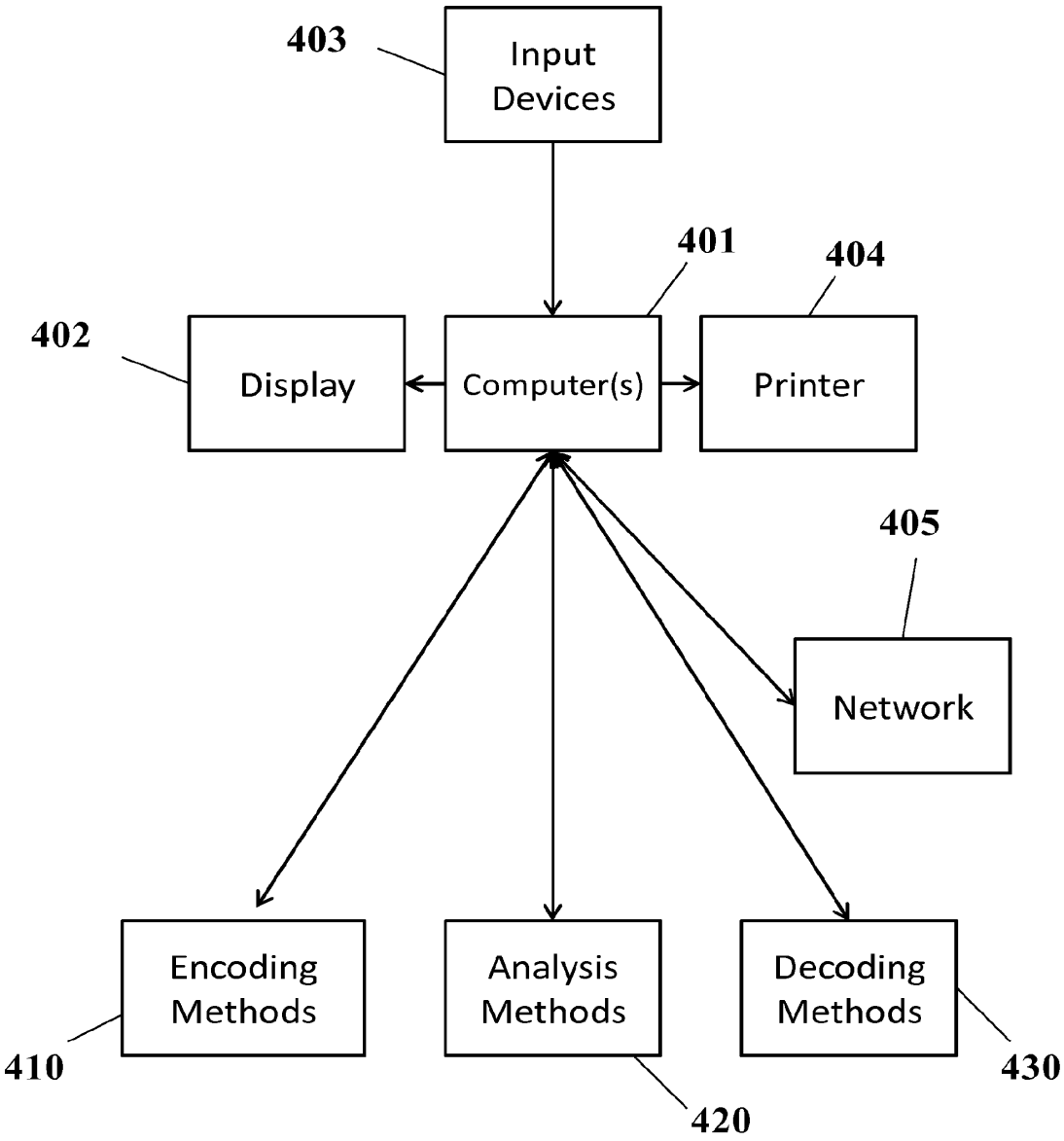

403
Input Devices
401
404
402
Display
Computer(s)
Printer
405
Network
Encoding Methods
Analysis Methods
Decoding Methods
410
420
430

Fig. 5

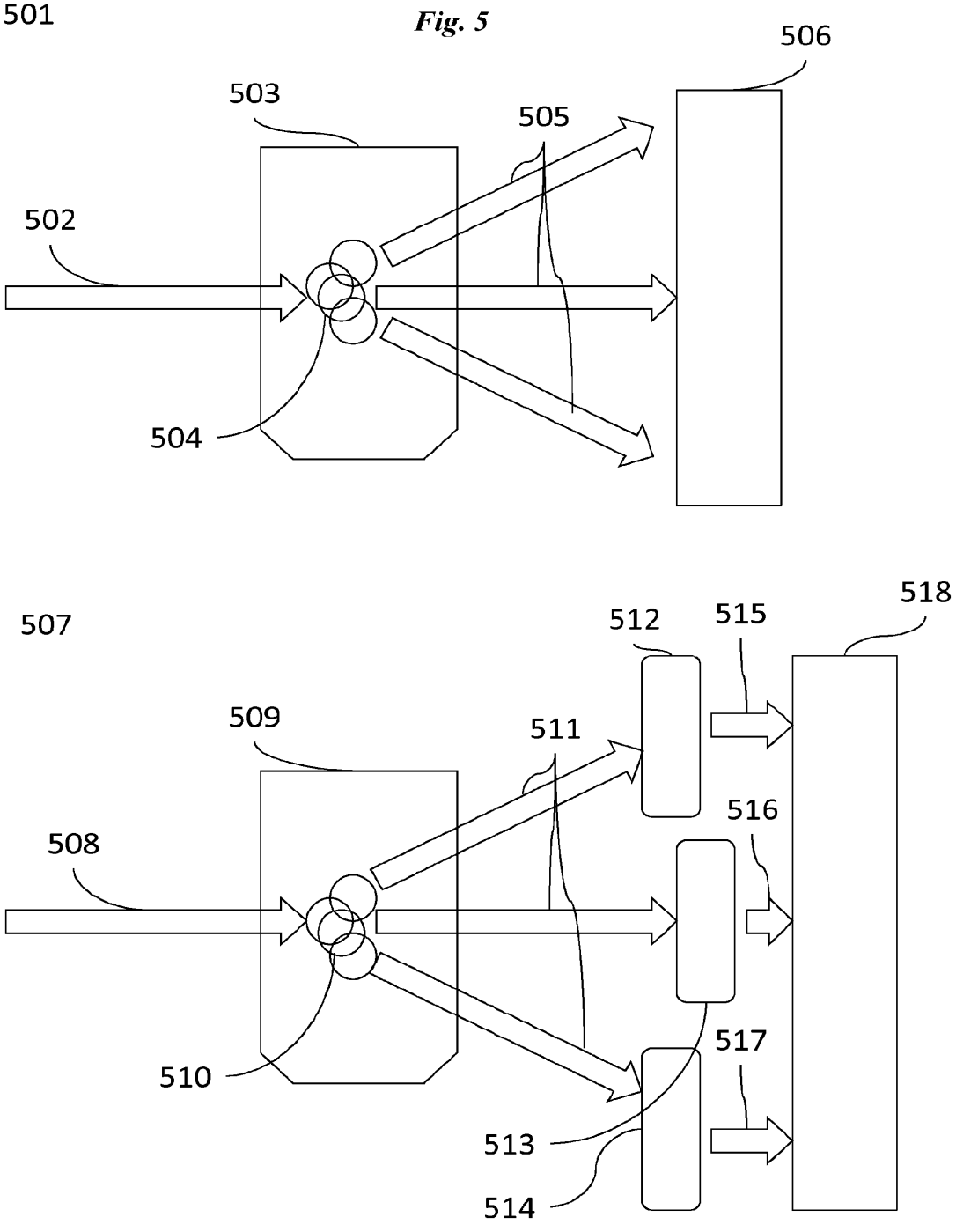
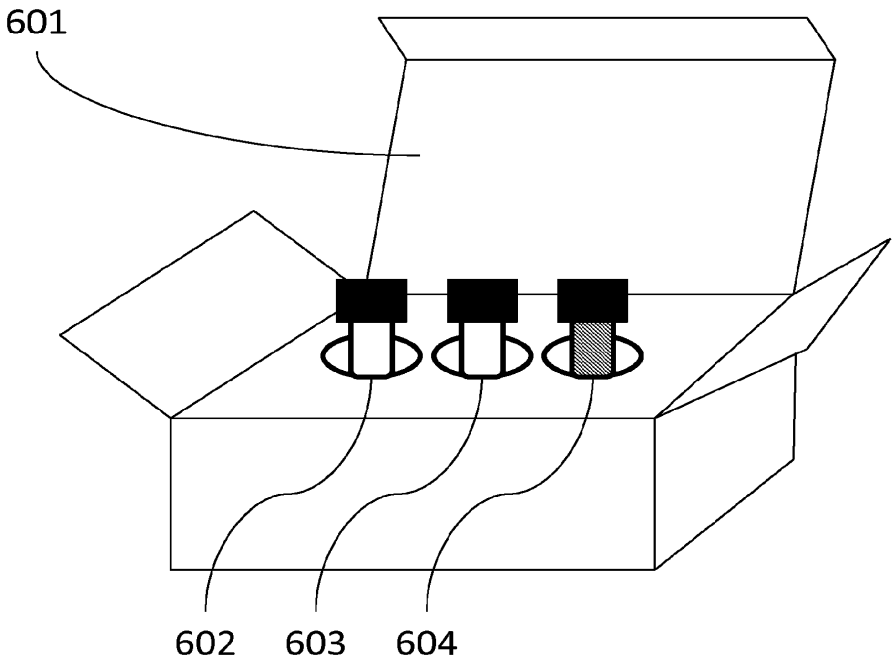

501
506
503
505
502
504
507
518
512
515
509
511
516
508
510
517
513
514

Fig. 6



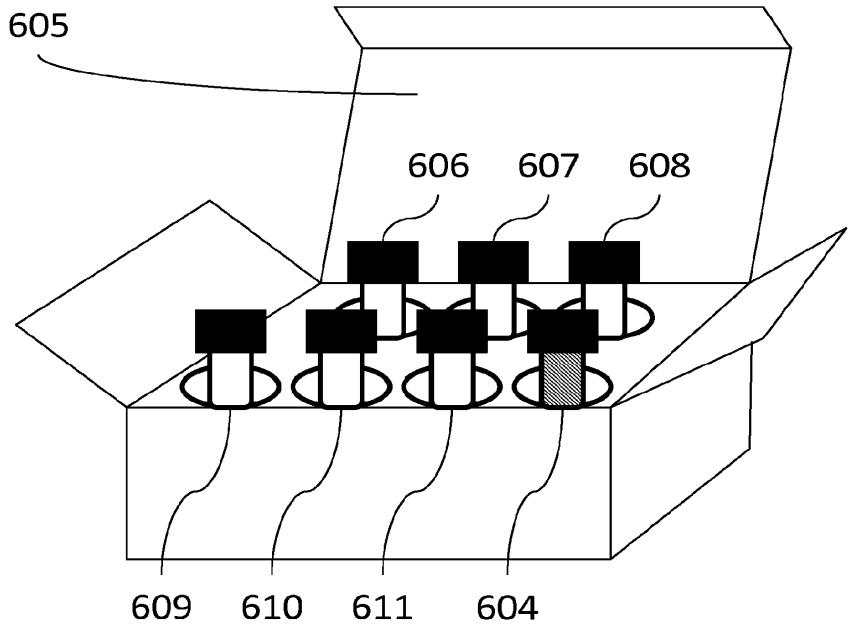


Fig. 7

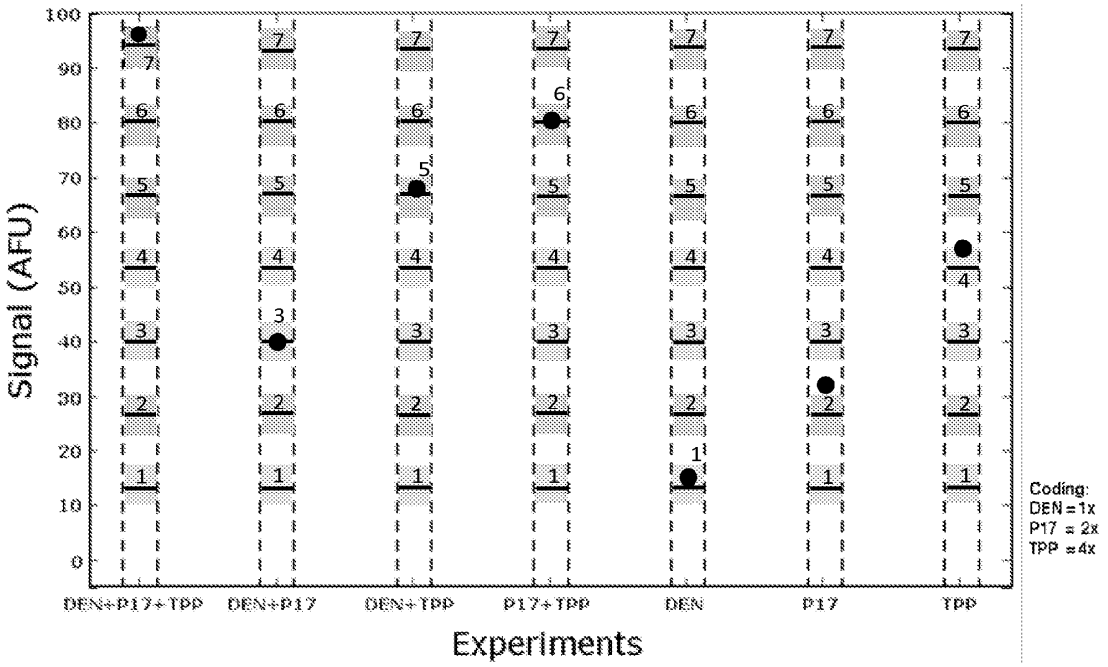
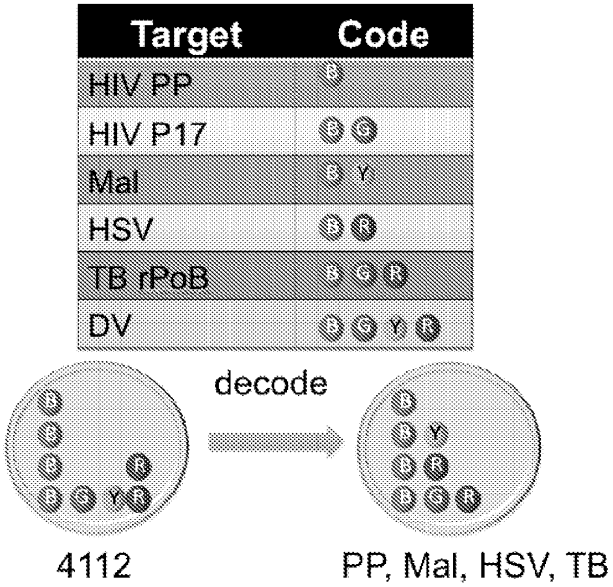
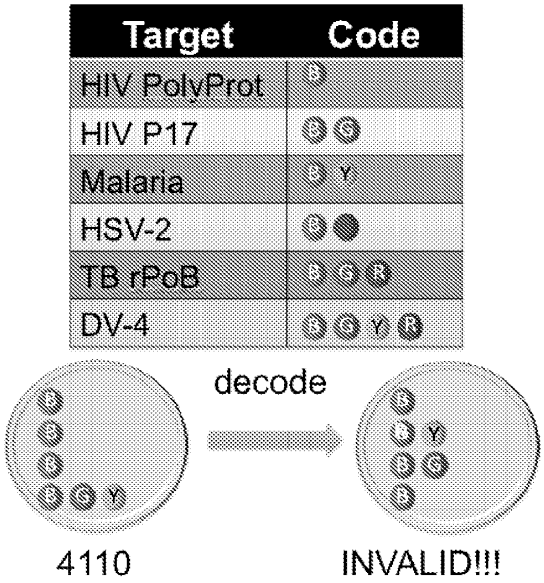

Signal (AFU)
Experiments
DEN+P17+TPP
DEN+P17
DEN+TPP
P17+TPP
DEN
P17
TPP
Coding:
DEN = 1x
P17 = 2x
TPP = 4x

Fig. 8


Target
Code
HIV PP
HIV P17
Mal
HSV
TB rPoB
DV
decode
4112
PP, Mal, HSV, TB


Target
Code
HIV PolyProt
HIV P17
Malaria
HSV-2
TB rPoB
DV-4
decode
4110
INVALID!!!

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/24509

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC 435/6.1
IPC C12Q 1/68 (2013.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC 435/6.11; 435/6.19 (Text search - see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Scholar Search Terms: signal, encoding, decoding, multiplexed, biochemical, assays

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0267064 A1 (KARTALOV et al) 21 October 2010 (21.10.2010) entire document	1-74
A	US 2005/0260640 A1 (ANDERSEN et al) 24 November 2005 (24.11.2005) entire document	1-74
A	US 2007/0231824 A1 (CHEE et al) 04 October 2007 (04.10.2007) entire document	1-74
A	US 2002/0022273 A1 (EMPEDOCLES et al) 21 February 2002 (21.02.2002) entire document	1-74
A	US 2010/0210472 A1 (EMPEDOCLES et al) 19 August 2010 (19.08.2010) entire document	1-74
A	US 2008/0069733 A1 (MALTEZOS et al) 20 March 2008 (20.03.2008) entire document	1-74
A	US 2009/0062129 A1 (McKERNAN et al) 05 March 2009 (05.03.2009) entire document	1-74
A	US 2011/0237459 A1 (NOVA et al) 29 September 2011 (29.09.2011) entire document	1-74
A	US 2005/0214753 A1 (SCHULTZ et al) 29 September 2005 (29.09.2005) entire document	1-74
A	US 2005/0164264 A1 (SHIPWASH) 28 July 2005 (28.07.2005) entire document	1-74

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2013 (22.03.2013)	12 APR 2013

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

ChromaCode / 44158-706.601
VM / KSS / DJG / CGD / LKIM / ASB / AAM

Jun 28 2017

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: Lee Alan Johnson
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference: 44158-706601	Date of mailing (day/month/year): 22 JUN 2017
International application No.: PCT/US17/24933	FOR FURTHER ACTION See paragraphs 1 and 4 below
Applicant: CHROMACODE INC.	International filing date (day/month/year): 30 March 2017 (30.03.2017)

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46).

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to: 34, chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices
   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made

4. **Reminders**

The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/US: Mail Stop PCT, Attn. ISA/US, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450	Authorized officer: Shane Thomas
Facsimile No. 571-273-8300	Telephone No. PCT Helpdesk: 571-272-4300, PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2014)

WSGR PATENT DOCKET
S: FOREIGN: ✓
DOCKETED: 6/26/17 BY:
ACTION: Search report 8/22/17
DUE DATES: Written opinion 9/1/18
No related US case
CM #

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 44158-706601	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US17/24933	International filing date *(day/month/year)* 30 March 2017 (30.03.2017)	(Earliest) Priority Date *(day/month/year)* 01 April 2016 (01.04.2016)
Applicant CHROMACODE INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2 ☒ **Certain claims were found unsearchable** (See Box No. II).

3 ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2536

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/24933

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-42 and 49-82
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2537

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/24933

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07H 21/00, 21/04; C12Q 1/68; G01N 21/64, 33/533, 33/542 (2017.01)

CPC - C07H 21/00, 21/04; C12Q 1/68, 1/686, 1/6806, 1/6851, 2561/101, 2561/113; G01N 21/64, 33/533, 33/542

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/079049 A2 (THIRD WAVE TECHNOLOGIES, INC.) 27 July 2006; page 2, lines 10-17 and 23-31; page 3, lines 13-20, page 5, lines 5-8 and 23-30; page 6, lines 18-30; page 7, lines 14-19; page 15, lines 24-31; page 16, lines 1-3 and 18-28	1-4, 5/3-4, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99
A	US 2009/0042735 A1 (BLAIR SM, et al.) 12 February 2009; paragraphs [0018], [0035], [0041]-[0043], [0072], [0074]	1-4, 5/1-3, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99
A	US 2014/0213471 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 31 July 2014; paragraphs [0003], [0010]-[0011], [0045], [0082])	1-4, 5/1-3, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99
A	US 2012/0040349 A1 (LODE PV, et al.) 16 February 2012; paragraphs [0030]-[0034], [0054]	1-4, 5/1-3, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 2017 (03.06.2017)	22 JUN 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Lee Alan Johnson
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 22 JUN 2017

Applicant's or agent's file reference	FOR FURTHER ACTION
44158-706601	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US17/24933	30 March 2017 (30.03.2017)	01 April 2016 (01.04.2016)

International Patent Classification (IPC) or both national classification and IPC
IPC - C07H 21/00, 21/04; C12Q 1/68; G01N 21/64, 33/533, 33/542 (2017.01)
CPC - C07H 21/00, 21/04; C12Q 1/68, 1/686, 1/6806, 1/6851, 2561/101, 2561/113; G01N 21/64, 33/533, 33/542

Applicant CHROMACODE INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	03 June 2017 (03.06.2017)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5 Additional comments:

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2540

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 6-42 and 49-82

because:

☐ the said international application, or the said claims Nos. ..... relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 6-42 and 49-82 are so unclear that no meaningful opinion could be formed *(specify)*:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

☐ the claims, or said claims Nos. ..... are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 6-42 and 49-82

☐ a meaningful opinion could not be formed without the sequence listing: the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2541

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1 Statement

Novelty (N)	Claims	***see box below***	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	***see box below***	YES
	Claims	NONE	NO
Industrial applicability (IA)	Claims	***see box below***	YES
	Claims	NONE	NO

2. Citations and explanations:

Novelty (N): YES; 1-4, 5/3-4, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99

Inventive step (IS): YES; 1-4, 5/3-4, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99

Industrial applicability (IA): 1-4, 5/3-4, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99
****

Claims 1-4, 5/3-4, 43-47, 48/45-47, 83-87, 88/83-87, 89/83 87, 90/83-87, 90/83-87, 91-97, 98/91-97, and 99 meet the criteria set out in PCT Article 33(2)-(3).

Claim 1 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest method of identifying a first target nucleic acid comprising, providing a sample comprising the first target nucleic acid, providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid, said first set of paired oligonucleotides comprising a first ratio of (a) first competitive oligonucleotides to (b) first signal oligonucleotides comprising a first signal tag, wherein the first competitive oligonucleotides compete with the first signal oligonucleotides for binding to the first target nucleic acid, amplifying the first target nucleic acid with the polymerase chain reaction, thereby degrading the first signal oligonucleotide and permitting generation of a first signal, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target nucleic acid.

WO 2006/079049 A2 to Third Wave Technologies, Inc. (hereinafter 'Wave') discloses method of identifying a first target nucleic acid (method of detection of a target nucleic acids (first); page 2, lines 10-14); comprising, providing a sample comprising the first target nucleic acid (providing a sample with target nucleic acids (first); page 2, lines 10-14), providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid (first and second probe oligonucleotides that occupy probe hybridization site on the target nucleic acid (first); page 2, lines 23-31), said first set of paired oligonucleotides comprising a first ratio (ratio of paired oligonucleotides, first probe oligonucleotides to second probe oligonucleotides, page 3, lines 13-20; page 6, lines 28-30) of (a) first oligonucleotides (first probe oligonucleotides; page 2, lines 23-31) to (b) first signal oligonucleotides comprising a first signal tag (first oligonucleotides have a first label; page 5, lines 5-8), wherein the first competitive oligonucleotides compete with the first signal oligonucleotides for binding to the first target nucleic acid (first probe oligonucleotides and second probe oligonucleotides are configured to bind the target nucleic acid with same affinity and identical sequences (competitive); page 15, lines 24-31; page 16, lines 6-8), amplifying the first target nucleic acid with the polymerase chain reaction (amplifying the first target nucleic acid with TAQMAN assay (PCR); page 6, lines 18-28; page 7, lines 14-19), thereby degrading the first signal oligonucleotide and permitting generation of a first signal (cleavage (degrading) of first probe oligonucleotide containing a label and allowing generation of a signal (first); page 7, lines 14-25) , generating the first signal (generating a first signal; page 18, lines 14-16), measuring intensity of the first signal (measuring the strength of the signal; page 18, lines 22-28), and thereby identifying the first target nucleic acid (identifying the target nucleic acid; page 7, lines 14-19), but Wave does not disclose (a) first competitive oligonucleotides, and correlating the intensity of the first signal to the first ratio.

US 2009/0042736 A1 to Blair, et al. (hereinafter 'Blair') discloses method of identifying a first target nucleic acid (method of identifying a target nucleic acid; paragraph [0018]) comprising, providing a sample comprising the first target nucleic acid (one target nucleic acid is present in a given sample; paragraphs [0035], [0041]), providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid (providing a set (first) of paired nucleic acid probes that can hybridize (complementarity) to target (first) nucleic acids; paragraphs [0041]-[0042]), said first set of paired oligonucleotides comprising a first ratio (set of paired probe and competitive labeled probe nucleic acids (first set), would inherently have a ratio; paragraphs [0041]-[0042]) of (a) first competitive oligonucleotides to (competitive probe nucleic acid; paragraphs [0041]-[0042]); (b) first signal oligonucleotides comprising a first signal tag (labeled competitor nucleic acids (first signal); paragraphs [0041]-[0042]), wherein the first competitive oligonucleotides compete with the first signal oligonucleotides for binding to the first target nucleic acid (probe nucleic acids (first) competes with labeled competitor nucleic acids; paragraphs [0041]-[0042]), amplifying the first target nucleic acid with the polymerase chain reaction (amplifying the first target nucleic acid with a polymerase chain reaction; paragraph [0072]), but Blair does not disclose thereby degrading the first signal oligonucleotide and permitting generation of a first signal, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target nucleic acid

-***-Continued Within the Next Supplemental Box-***-

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2542

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of

-***-Continued from Box V: Citations and Explanations-***-.
US 2014/0213471 A1 to California Institute of Technology (hereinafter 'CalTech') discloses method of identifying a first target nucleic acid (method of detecting at least one polynucleotide analyte (target); paragraph [0003]) comprising, providing a sample comprising the first target nucleic acid (providing a sample comprising a first polynucleotide analyte (target); paragraph [0003]), providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid (providing a set of first primer and a first oligonucleotide specific (complementarity) for binding a first polynucleotide analyte (target; paragraph [0003]), said first set of paired oligonucleotides comprising a first ratio (set of paired probe labeled nucleic acids (first set), would inherently have a ratio; paragraph [0003]) of (b) first signal oligonucleotides comprising a first signal tag (first primer oligonucleotide comprises a first signal tag; paragraph [0011]), amplifying the first target nucleic acid with the polymerase chain reaction (amplifying the first polynucleotide analyte using a polymerase chain reaction; paragraph [0010]), generating the first signal (generating a first signal; paragraph [0011]), measuring intensity of the first signal (measuring a first signal; paragraph [0011]), thereby identifying the first target nucleic acid (identifying the first polynucleotide analyte (target); paragraph [0011]), but Caltech does not disclose (a) first competitive oligonucleotides to, wherein the first competitive oligonucleotides compete with the first signal oligonucleotides for binding to the first target nucleic acid, thereby degrading the first signal oligonucleotide and permitting generation of a first signal, and correlating the intensity of the first signal to the first ratio.
US 2012/0040349 A1 to Lode, et al. (hereinafter 'Lode') discloses method of identifying a first target nucleic acid (method of detecting target nucleic acids; paragraphs [0031]) comprising, providing a sample comprising the first target nucleic acid (providing a sample with target nucleic acids; paragraph [0032]), providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid (providing a set (first) of paired oligonucleotides that are complementary to the target nucleic acids (first); paragraph [0032]), said first set of paired oligonucleotides comprising: a first ratio of said first set of paired oligonucleotides comprising a first ratio (set (first) of paired oligonucleotides would inherently have a ratio; paragraph [0032]) of (a) first oligonucleotides (quencher probe oligonucleotide; paragraph [0032]-[0033]) to (b) first signal oligonucleotides comprising a first signal tag (label probe oligonucleotide for target nucleic acids (first); paragraphs [0032]-[0033]), amplifying the first target nucleic acid with the polymerase chain reaction (amplifying the target nucleic acids (first) by polymerase chain reaction; paragraph [0034]), but Lode does not disclose (a) first competitive oligonucleotides, wherein the first competitive oligonucleotides compete with the first signal oligonucleotides for binding to the first target nucleic acid, thereby degrading the first signal oligonucleotide and permitting generation of a first signal, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target nucleic acid.
Wave, Blair, Caltech, and Lode, fail to disclose either solely or in combination the method of correlating the intensity of the first signal to the first ratio. Wave discloses ratios of a first probe oligonucleotide with a label and a second probe oligonucleotide with a label, pair of oligonucleotides, that are complimentary to a target nucleic acids, amplifying the target nucleic acids, detecting the cleavage (degradation) of first and second probes (page 5, lines 23-30; page 7, lines 14-19; page 15, lines 25-31; page 16, lines 1-3), but Wave does not disclose correlating the intensity of the first signal to the first ratio. Caltech discloses method of detecting a plurality of polynucleotide analytes using oligonucleotides with attached chromophore in polymerization techniques and measuring signals to detect presence or absence of analytes in samples (paragraph [0011], [0045], [0062]), but Caltech does not disclose correlating the intensity of the first signal to the first ratio. Further there is no motivation to combine the references of record without the advantage of permissible hindsight.

Claim 2-4 and 5/3-4 meet the criteria set out in PCT Article 33(2)-(3), based on their dependency on positive claim 1.

Claim 43 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a composition comprising: a first set of paired oligonucleotides with complementarity to a first target nucleic acid, said first set of paired oligonucleotides comprising a first ratio of (a) competitive oligonucleotides to (b) signal oligonucleotides comprising a signal tag, wherein the competitive oligonucleotides are configured to compete with the signal oligonucleotides for binding to the first target nucleic acid during amplification of the first target nucleic acid with the polymerase chain reaction, wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and excited by an energy source, and wherein the first intensity correlates with the first ratio.
Wave discloses a composition (composition; page 2, lines 15-17) comprising: a first set of paired oligonucleotides with complementarity to a first target nucleic acid (first and second probe oligonucleotides that occupy probe hybridization site on the target nucleic acid (first); page 2, lines 23-31), said first set of paired oligonucleotides comprising a first ratio (ratio of paired oligonucleotides, first probe oligonucleotides to second probe oligonucleotides; page 3, lines 13-20; page 6, lines 28-30) of (a) oligonucleotides (first probe oligonucleotides; page 2, lines 23-31) to (b) signal oligonucleotides comprising a signal tag (first oligonucleotides have a first label; page 5, lines 5-8), wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction (cleavage (degrading) of first probe oligonucleotide containing a label by a polymerase chain reaction and allowing generation of a signal (first); page 7, lines 14-25) and excited by an energy source (generating a first detectable signal in FRET (exited by an energy source); page 18, lines 14-16), but Wave does not disclose (a) competitive oligonucleotides, wherein the competitive oligonucleotides are configured to compete with the signal oligonucleotides for binding to the first target nucleic acid during amplification of the first target nucleic acid with the polymerase chain reaction, and wherein the first intensity correlates with the first ratio.
Blair discloses a composition (composition; paragraph [0016]) comprising: a first set of paired oligonucleotides with complementarity to a first target nucleic acid (providing a set of paired probe nucleic acids and labeled probe nucleic acids that competitive to target nucleic acids; paragraph [0041]-[0042]), said first set of paired oligonucleotides comprising a first ratio (set of paired probe and competitive labeled probe nucleic acids, would inherently be in a ratio; paragraphs [0042]-[0043]) of (a) competitive oligonucleotides to (competitive probe nucleic acid; paragraphs [0041]-[0042]) (b) signal oligonucleotides comprising a signal tag (labeled competitor nucleic acids (first signal); paragraphs [0041]-[0042]), wherein the competitive oligonucleotides are configured to compete with the signal oligonucleotides for binding to the first target nucleic acid (probe nucleic acids (first) competes with labeled competitor nucleic acids (first); paragraphs [0041]-[0042], [0072]), but Blair does not disclose during amplification of the first target nucleic acid with the polymerase chain reaction wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and excited by an energy source, and wherein the first intensity correlates with the first ratio.

-***-Continued Within the Next Supplemental Box-***-.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2543

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-
Caltech discloses a composition (compositions; paragraph [0003]) comprising: a first set of paired oligonucleotides with complementarity to a first target nucleic acid (providing a set of first primer and a first oligonucleotide specific (complementarity) for binding a first polynucleotide analyte (target; paragraph [0003]), said first set of paired oligonucleotides comprising a first ratio (set of paired probe labeled nucleic acids (first set) would inherently have a ratio; paragraph [0003]) of (b) signal oligonucleotides comprising a signal tag (first primer oligonucleotide comprises a first signal tag; paragraph [0011]), but Caltech does not disclose (a) competitive oligonucleotides to, wherein the competitive oligonucleotides are configured to compete with the signal oligonucleotides for binding to the first target nucleic acid during amplification of the first target nucleic acid with the polymerase chain reaction, wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and excited by an energy source, and wherein the first intensity correlates with the first ratio.
Lode discloses a composition (composition; paragraph [0054]) comprising: a first set of paired oligonucleotides with complementarity to a first target nucleic acid (providing a set (first) of paired oligonucleotides that are complementary to the target nucleic acids (first); paragraph [0032]), said first set of paired oligonucleotides comprising a first ratio (set (first) of paired oligonucleotides would inherently have a ratio; paragraph [0032]) of (a) oligonucleotides (quencher probe oligonucleotide, paragraph [0032]-[0033]) to (b) signal oligonucleotides comprising a signal tag (label probe oligonucleotide for target nucleic acids (first); paragraphs [0032]-[0033]), but Lode does not disclose (a) competitive oligonucleotides, wherein the competitive oligonucleotides are configured to compete with the signal oligonucleotides for binding to the first target nucleic acid during amplification of the first target nucleic acid with the polymerase chain reaction, wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and excited by an energy source, and wherein the first intensity correlates with the first ratio.
Wave, Blair, Caltech, and Lode, fail to disclose either solely or in combination a composition during amplification of the first target nucleic acid with the polymerase chain reaction wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and excited by an energy source, and wherein the first intensity correlates with the first ratio. Wave discloses ratios of a first probe oligonucleotide with a label and a second probe oligonucleotide with a label, pair of oligonucleotides, that are complimentary to a target nucleic acids, amplifying the target nucleic acids, detecting the cleavage (degradation) of first and second probes (page 5, lines 23-30; page 7, lines 14-19, page 15, lines 25-31; page 16, lines 1-3), but Wave does not disclose amplification of the first target nucleic acid with the polymerase chain reaction wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and correlating the intensity of the first signal to the first ratio. Caltech discloses method of detecting a plurality of polynucleotide analytes using oligonucleotides with attached chromophore in polymerization techniques and measuring signals to detect presence or absence of analytes in samples (paragraph [0011], [0045], [0082]), but Caltech does not disclose amplification of the first target nucleic acid with the polymerase chain reaction wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and correlating the intensity of the first signal to the first ratio. Further there is no motivation to combine the references of record without the advantage of permissible hindsight.

Claim 44 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest method of identifying a first target molecule comprising, providing a sample comprising the first target molecule, providing a first set of paired capture molecules that bind to the first target molecule, said first set of paired capture molecules comprising a first ratio of (a) first competitive capture molecules to (b) first signal capture molecules comprising a signal tag, wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target molecule.
Wave discloses method of identifying a first target molecule (method of detection of a target nucleic acids (first target molecule); page 2, lines 10-14);comprising, providing a sample comprising the first target molecule (providing a sample with target nucleic acids (first target molecule); page 2, lines 10-14), providing a first set of paired capture molecules that bind to the first target molecule (first and second probe oligonucleotides (capture molecules) that occupy probe hybridization site on the target nucleic acid (first target molecule); page 2, lines 23-31), said first set of paired capture molecules comprising a first ratio (ratio of paired oligonucleotides (capture molecules), first probe oligonucleotides to second probe oligonucleotides; page 3, lines 13-20; page 6, lines 28-30) of (a) first capture molecules (first probe oligonucleotides; page 2, lines 23-31) to (b) first signal capture molecules comprising a signal tag (first probe oligonucleotides (capture molecules) have a first label; page 5, lines 5-8), generating the first signal (generating a first signal, page 16, lines 14-16), measuring intensity of the first signal (measuring the strength of the signal; page 18, lines 22-26), and thereby identifying the first target molecule (identifying the target nucleic acid; page 7, lines 14-19), but Wave does not disclose (a) first competitive capture molecules, wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule, and correlating the intensity of the first signal to the first ratio.
Blair discloses method of identifying a first target molecule (method of identifying a target nucleic acid (first target molecule; paragraph [0018]) comprising, providing a sample comprising the first target molecule (one target nucleic acid (first target molecule) is present in a given sample; paragraphs [0035], [0041]), providing a first set of paired capture molecules that bind to the first target molecule (providing a set (first) of paired nucleic acid probes (capture molecules) that can hybridize (complementarity) to target (first) nucleic acids; paragraphs [0041]-[0042]), said first set of paired capture molecules comprising a first ratio (set of paired probe and competitive labeled probe nucleic acids (first set paired capture molecules), would inherently have a ratio; paragraphs [0041]-[0042]) of (a) first competitive capture molecules (competitive probe nucleic acid; paragraphs [0041]-[0042]) to (b) first signal capture molecules comprising a signal tag (labeled competitor nucleic acids (first signal); paragraphs [0041] [0042]), wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule (probe nucleic acids (capture molecule) competes with labeled competitor nucleic acids (labeled capture molecule); paragraphs [0041]-[0042]), but Blair does not disclose generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target molecule.

-***-Continued Within the Next Supplemental Box-***-

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2544

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-
Caltech discloses method of identifying a first target molecule (method of detecting at least one polynucleotide analyte (first target molecule); paragraph [0003]) comprising, providing a sample comprising the first target molecule (providing a sample comprising a first polynucleotide analyte (first target molecule); paragraph [0003]), providing a first set of paired capture molecules that bind to the first target molecule (providing a set of first primer and a first oligonucleotide specific (complementarity) for binding a first polynucleotide analyte (first target molecule); paragraph [0003]), said first set of paired capture molecules comprising a first ratio of (set of paired probe labeled nucleic acids (first set), would inherently have a ratio; paragraph [0003]), to (b) first signal capture molecules comprising a signal tag (first primer oligonucleotide (first capture molecules) comprises a signal tag; paragraph [0011]), generating the first signal (generating a first signal; paragraph [0011]), measuring intensity of the first signal (measuring a first signal; paragraph [0011]), and thereby identifying the first target molecule (identifying the first polynucleotide analyte (target); paragraph [0011]), but Caltech does not disclose (a) first competitive capture molecules, wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule, and correlating the intensity of the first signal to the first ratio.
Lode discloses method of identifying a first target molecule (method of detecting target nucleic acids (first target molecule); paragraphs [0031]) comprising, providing a sample comprising the first target molecule (providing a sample with target nucleic acids (first target molecule); paragraph [0032]), providing a first set of paired capture molecules that bind to the first target molecule providing a set (first) of paired oligonucleotides (capture molecule) that are complementary to the target nucleic acids (first target molecule); paragraph [0032]), said first set of paired capture molecules comprising a first ratio (set (first) of paired oligonucleotides (capture molecules) would inherently have a ratio; paragraph [0032]) of (a) first capture molecules (quencher probe oligonucleotide; paragraph [0032]-[0033]) to (b) first signal capture molecules comprising a signal tag (label probe oligonucleotide for target nucleic acids (first); paragraphs [0032]-[0033]), but Lode does not disclose (a) first competitive capture molecules, wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target molecule.
Wave, Blair, Caltech, and Lode, fail to disclose either solely or in combination the method of correlating the intensity of the first signal to the first ratio. Wave discloses ratios of a first probe oligonucleotide (capture molecule) with a label and a second probe oligonucleotide (capture molecule) with a label, pair of oligonucleotides, that are complimentary to a target nucleic acids (target molecule), amplifying the target nucleic acids, detecting the cleavage (degradation) of first and second probes (page 5, lines 23-30; page 7, lines 14-19; page 15, lines 25-31; page 16, lines 1-3), but Wave does not disclose wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule and correlating the intensity of the first signal to the first ratio. Caltech discloses method of detecting a plurality of polynucleotide analytes using oligonucleotides with attached chromophore in polymerization techniques and measuring signals to detect presence or absence of analytes in samples (paragraph [0011], [0045], [0082]), but Caltech does not disclose wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule and correlating the intensity of the first signal to the first ratio. Further there is no motivation to combine the references of record without the advantage of permissible hindsight

Claim 45-47 and 48/45-47 meet the criteria set out in PCT Article 33(2)-(3), based on their dependency on positive claim 44.

Claim 83 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a composition comprising: a first set of paired capture molecules with complementarity to a first target molecule, said first set of paired capture molecules comprising a first ratio of(a) competitive capture molecules to (b) signal capture molecules comprising a signal tag, wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule, wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio.
Wave discloses a composition (composition; page 2, lines 15-17) comprising: a first set of paired capture molecules with complementarity to a first target molecule (first and second probe oligonucleotides (capture molecules) that occupy probe hybridization site (complementary) on the target nucleic acid (first target molecule); page 2, lines 23-31), said first set of paired capture molecules comprising a first ratio (ratio of paired oligonucleotides (capture molecules), first probe oligonucleotides to second probe oligonucleotides; page 3, lines 13-20; page 6, lines 28-30) of (a) capture molecules (first probe oligonucleotides (capture molecules); page 2, lines 23-31) to (b) signal capture molecules comprising a signal tag (first oligonucleotides (capture molecules) have a first label; page 5, lines 5-8), but Wave does not disclose (a) competitive capture molecules, wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule, wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio.
Blair discloses a composition (composition; paragraph [0016]) comprising: a first set of paired capture molecules with complementarity to a first target molecule (providing a set of paired probe nucleic acids and labeled probe nucleic acids that competitive to target nucleic acids (target molecule); paragraph [0041]-[0042]), said first set of paired capture molecules comprising a first ratio (set of paired probe and competitive labeled probe nucleic acids (capture molecules), would inherently be in a ratio; paragraphs [0042]-[0043]) of (a) competitive capture molecules (competitive probe nucleic acid (capture molecules); paragraphs [0041]-[0042]) to (b) signal capture molecules comprising a signal tag (labeled competitor nucleic acids (signal capture molecules); paragraphs [0041]-[0042]), wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule (probe nucleic acids (capture molecules) competes with labeled competitor nucleic acids (signal capture molecules) for binding to target nucleic acids (target molecule); paragraphs [0041]-[0042], [0072]), but Blair does not disclose wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio.

-***-Continued Within the Next Supplemental Box-***-

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2545

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-
Caltech discloses a composition (compositions; paragraph [0003]) comprising: a first set of paired capture molecules with complementarity to a first target molecule (providing a set of first primer and a first oligonucleotide (capture molecules) specific (complementarity) for binding a first polynucleotide analyte (first target molecule); paragraph [0003]), said first set of paired capture molecules comprising a first ratio (set of paired probe labeled nucleic acids (first set of paired capture molecules) would inherently have a ratio; paragraph [0003]) of (b) signal capture molecules comprising a signal tag (first primer oligonucleotide comprises a first signal tag (signal capture molecules; paragraph [0011]), but Caltech does not disclose (a) competitive capture molecule, wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule, wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio.
Lode discloses a composition (composition; paragraph [0054]) comprising: a first set of paired capture molecules with complementarity to a first target molecule (providing a set (first) of paired oligonucleotides (capture molecules) that are complementary to the target nucleic acids (first target molecule); paragraph [0032]), said first set of paired capture molecules comprising a first ratio (set (first) of paired oligonucleotides (capture molecules) would inherently have a ratio; paragraph [0032]) of (a) competitive capture molecules (quencher probe oligonucleotide; paragraph [0032]-[0033]) to (b) signal capture molecules comprising a signal tag (label probe oligonucleotide (signal capture molecule); paragraphs [0032]-[0033]), but Lode does not disclose (a) competitive capture molecules, wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule, wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio.
Wave, Blair, Caltech, and Lode, fail to disclose either solely or in combination wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio. Wave discloses ratios of a first probe oligonucleotide (capture molecules) with a label and a second probe oligonucleotide with a label, pair of oligonucleotides, that are complimentary to a target nucleic acids (target molecules), amplifying the target nucleic acids, detecting the cleavage (degradation) of first and second probes (page 5, lines 23-30; page 7, lines 14-19; page 15, lines 25-31; page 16, lines 1-3), but Wave does not disclose wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule, wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio. Caltech discloses method of detecting a plurality of polynucleotide analytes using oligonucleotides with attached chromophore in polymerization techniques and measuring signals to detect presence or absence of analytes in samples (paragraph [0011], [0045], [0062]), but Caltech does not disclose wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule, wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio. Further there is no motivation to combine the references of record without the advantage of permissible hindsight.

Claim 84-87, 88/83-87, 89/83-87, and 90/83-87 meet the criteria set out in PCT Article 33(2)-(3), based on their dependency on positive claim 83.

Claim 91 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly method of altering amplification level of a target nucleic acid comprising, providing a sample comprising the target nucleic acid, providing a set of paired oligonucleotides with complementarity to the target nucleic acid, said set of paired oligonucleotides comprising a ratio of (a) competitive oligonucleotides to (b) functional oligonucleotides, wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR
Wave discloses method of altering amplification level of a target nucleic acid (method of altering amplification level of target nucleic acid; page 6, lines 24-30) comprising, providing a sample comprising the target nucleic acid (providing a sample with target nucleic acids (first); page 2, lines 10-14),, providing a set of paired oligonucleotides with complementarity to the target nucleic acid (first and second probe oligonucleotides that occupy probe hybridization site on the target nucleic acid; page 2, lines 23-31), said set of paired oligonucleotides comprising a ratio (ratio of paired oligonucleotides, first probe oligonucleotides to second probe oligonucleotides; page 3, lines 13-20; page 6, lines 28-30) of (a) competitive oligonucleotides to (b) functional oligonucleotides (oligonucleotides having a label (functional); page 5, lines 5-8), but Wave does not disclose wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR.
Blair discloses method of altering amplification level of a target nucleic acid (method of altering amplification of target nucleic acids; paragraphs [0072], [0074]) comprising, providing a sample comprising the target nucleic acid (one target nucleic acid is present in a given sample; paragraphs [0035], [0041]), providing a set of paired oligonucleotides with complementarity to the target nucleic acid (providing a set (first) of paired nucleic acid probes that can hybridize (complementarity) to target (first) nucleic acids; paragraphs [0041]-[0042]), said set of paired oligonucleotides comprising a ratio (set of paired probe and competitive labeled probe nucleic acids (first set) would inherently have a ratio; paragraphs [0041]-[0042]) of (a) competitive oligonucleotides (competitive probe nucleic acid, paragraphs [0041]-[0042]) to (b) functional oligonucleotides (labeled competitor nucleic acids (functional); paragraphs [0041]-[0042]), but Blair does not disclose wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR.

-***-Continued Within the Next Supplemental Box-***-

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2546

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-
Caltech discloses method of altering amplification level of a target nucleic acid (method of amplification of polynucleotide analyte (target nucleic acid); paragraph [0003]) comprising, providing a sample comprising the target nucleic acid (providing a sample comprising a first polynucleotide analyte (first target molecule); paragraph [0003]), providing a set of paired oligonucleotides with complementarity to the target nucleic acid (providing a set of first primer and a first oligonucleotide specific (complementarity) for binding a first polynucleotide analyte (first target molecule); paragraph [0003]), said set of paired oligonucleotides comprising a ratio (set of paired probe labeled nucleic acids (first set), would inherently have a ratio; paragraph [0003]) of (a) competitive oligonucleotides to (b) functional oligonucleotides (nucleotide with signal (functional oligonucleotide; paragraph [0011]), but Caltech does not disclose (a) competitive oligonucleotides, wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR.
Lode discloses method of altering amplification level of a target nucleic acid (method for amplification of detected nucleic acids; paragraph [0030]) comprising, providing a sample comprising the target nucleic acid (providing a sample with target nucleic acids; paragraph [0032]), providing a set of paired oligonucleotides with complementarity to the target nucleic acid (providing a set (first) of paired oligonucleotides that are complementary to the target nucleic acids (first); paragraph [0032]), said set of paired oligonucleotides comprising a ratio (set (first) of paired oligonucleotides would inherently have a ratio: paragraph [0032]) of (a) competitive oligonucleotides (quencher probe oligonucleotide; paragraph [0032]-[0033]) to (b) functional oligonucleotides (label probe oligonucleotide (functional); paragraphs [0032]-[0033]), but Lode does not discloses wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR.
Wave, Blair, Caltech, and Lode, fail to disclose either solely or in combination the method wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR. Wave discloses ratios of a first probe oligonucleotide with a label and a second probe oligonucleotice with a label (functional), pair of oligonucleotides, that are complimentary to a target nucleic acids, amplifying the target nucleic acids, detecting the cleavage (degradation) of first and second probes (page 5, lines 23-30; page 7, lines 14-19; page 15, lines 25-31; page 16, lines 1-3), but Wave does not disclose wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR. Caltech discloses method of detecting a plurality of polynucleotide analytes using oligonucleotides with attached chromophore in polymerization techniques and measuring signals to detect presence or absence of analytes in samples (paragraph [0011], [0045], [0062]), but Caltech does not disclose wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR. Further there is no motivation to combine the references of record without the advantage of permissible hindsight.

Claim 92-97 and 98/91-97 meet the criteria set out in PCT Article 33(2)-(3), based on their dependency on positive claim 91.

Claim 99 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly a composition comprising: a set of paired oligonucleotides with complementarity to a target nucleic acid, said set of paired oligonucleotides comprising a ratio of (a) competitive oligonucleotides to (b) functional oligonucleotides, wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.
Wave discloses a composition (composition: page 2, lines 15-17) comprising: a set of paired oligonucleotides with complementarity to a target nucleic acid (first and second probe oligonucleotides that occupy probe hybridization site on the target nucleic acid (first); page 2, lines 23-31), said set of paired oligonucleotides comprising a ratio (ratio of paired oligonucleotides, first probe oligonucleotides to second probe oligonucleotides; page 3, lines 13-20; page 6, lines 28-30) of (b) functional oligonucleotides (first oligonucleotides have a first label; page 5, lines 5-8), but Wave does not disclose (a) competitive oligonucleotides, wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.
Blair discloses a composition (composition; paragraph [0016]) comprising: a set of paired oligonucleotides with complementarity to a target nucleic acid molecule (providing a set of paired probe nucleic acids and labeled probe nucleic acids that are competitive to target nucleic acids (target molecule); paragraph [0041]-[0042]), said set of paired oligonucleotides comprising a ratio of (set of paired probe and competitive labeled probe nucleic acids (capture molecules), would inherently be in a ratio; paragraphs [0042]-[0043]) (a) competitive oligonucleotides (competitive probe nucleic acid (capture molecules); paragraphs [0041]-[0042]) to (b) functional oligonucleotides (labeled competitor nucleic acids (functional oligonucleotides); paragraphs [0041]-[0042]), wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides (probe nucleic acids (capture molecules) competes with labeled competitor nucleic acids (signal capture molecules) for binding to target nucleic acids (target molecule); paragraphs [0041]-[0042], [0072]), but Blair does not disclose for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.

-***-Continued Within the Next Supplemental Box-***-

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2547

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/24933

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-

Caltech discloses a composition (compositions; paragraph [0003]) comprising: a set of paired oligonucleotides with complementarity to a target nucleic acid (providing a set of first primer and a first oligonucleotide (capture molecules) specific (complementarity) for binding a first polynucleotide analyte (first target molecule); paragraph [0003]), said set of paired oligonucleotides comprising a ratio (set of paired probe labeled nucleic acids (first set of paired capture molecules) would inherently have a ratio; paragraph [0003]) of (b) functional oligonucleotides (primer oligonucleotide (functional); paragraph [0011]), but Caltech does not disclose (a) competitive oligonucleotide, wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.
Lode discloses a composition (composition; paragraph [0054]) comprising: a set of paired oligonucleotides with complementarity to a target nucleic acid (providing a set of paired oligonucleotides that are complementary to the target nucleic acids); paragraph [0032]), said set of paired oligonucleotides comprising a ratio (set (first) of paired oligonucleotides (capture molecules) would inherently have a ratio; paragraph [0032]) of (a) competitive oligonucleotides (quencher probe oligonucleotide; paragraph [0032]-[0033]) to (b) functional oligonucleotides (label probe oligonucleotide (functional oligonucleotides); paragraphs [0032]-[0033]), but Lode does not disclose wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.
Wave, Blair, Caltech, and Lode, fail to disclose either solely or in combination the composition wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase. Wave discloses ratios of a first probe oligonucleotide with a label and a second probe oligonucleotide with a label (functional), pair of oligonucleotides, that are complimentary to a target nucleic acids, amplifying the target nucleic acids, detecting the cleavage (degradation) of first and second probes (page 5, lines 23-30; page 7, lines 14-19; page 15, lines 25-31; page 16, lines 1-3), but Wave does not disclose wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.
Caltech discloses method of detecting a plurality of polynucleotide analytes using oligonucleotides with attached chromophore in polymerization techniques and measuring signals to detect presence or absence of analytes in samples (paragraph [0011], [0045], [0082]), but Caltech does not disclose wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.

Claims 1-4, 43-47, 46/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, and 99 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2548

PCT Recordation of Search History

Case/PCT Application Number: PCT/US17/24933

CLIN Number/Technical Field of PCT Application: 008 Chemistry

Dates During Which the Search was Conducted: 25 May – 03 June 2017

Date of Completion of Recordation of Search History Form: 03 June 2017

Research Analyst Initials: TFW

Search Approval Official (SAO) Initials: DGF

Field of Search/Classification Information:

IPC(8) Classifications: A61K 31/7088, 31/711, 31/713; C07H 21/00, 21/04; C12Q 1/68; G01N 21/64, 33/532, 33/533, 33/542 (2017.01)

CPC Classifications: A61K 31/7088, 31/711, 31/713; C07H 21/00, 21/04; C12Q 1/68, 1/686, 1/6806, 1/6851, 2561/101, 2561/113; G01N 21/64, 33/532, 33/533, 33/542

Databases Searched (Patent and Non-Patent Literature (NPL), Including Sub-Databases and Files Searched) and Search Terms Used:

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH), EBSCO, Google Scholar, PubMed;

target nucleic acid, PCR, qPCR, competitive oligonucleotides, competitive probes, dimming probes, non-fluorescing probes, target probes, signal oligonucleotides, fluorescent probe, fluorophore, functional oligonucleotides,

Database Search String Recordation, Including Dates of Searches:

Patent Database Search Strategy/Results:

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH);

ID	Search String	Records	Date
L36	TACD:((DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs") AND ((ampli*) W10 (DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs")) AND (competit*_probe* OR competit*_oligonucleotid* OR competit*_captur*_molecul*) AND ((function*_oligonucleotid* OR signal*_oligonucleotid* OR fluoresc*_probe* OR fluoresc*_oligonucleotid*) w10 (extend*)))	97	3-Jun-17
L35	TACD:((DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs") AND ((ampli*) W10 (DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs")) AND (competit*_probe* OR competit*_oligonucleotid* OR competit*_captur*_molecul*) AND (function*_oligonucleotid* OR signal*_oligonucleotid* OR fluoresc*_probe* OR fluoresc*_oligonucleotid*))	824	3-Jun-17
L34	TAC:((DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs") AND ((ampli*) W10 (DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs")) AND (competit*_probe* OR competit*_oligonucleotid* OR competit*_captur*_molecul*) AND (function*_oligonucleotid* OR signal*_oligonucleotid* OR fluoresc*_probe* OR fluoresc*_oligonucleotid*))	2	3-Jun-17
L33	TA:((DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs") AND ((ampli*) W10 (DNA OR nucleic*_acid* OR RNA OR deoxyribonucl*_acid* OR DNAs OR "DNA" OR ribonucl*_acid* OR "DNAs" OR "RNA" OR RNAs OR "RNAs")) AND (competit*_probe* OR competit*_oligonucleotid* OR competit*_captur*_molecul*) AND (function*_oligonucleotid* OR signal*_oligonucleotid* OR fluoresc*_probe* OR fluoresc*_oligonucleotid*))	0	3-Jun-17
L32	TACD: ((amplific*) w10 ("nucleic acid" or oligonucleotides)) and ((paired or set) w5 (oligonucleotides or "nucleic acids" or probes)) and (competitive w5 (oligonucleotides or "nucleic acids" or probes)) and (functional w5 (oligonucleotides or "nucleic acids" or probes)) and ("polymerase chain reaction" or PCR or qPCR) and (extend* w10 polymerase)	197	1-Jun-17
L31	TACD: ((amplific*) w10 ("nucleic acid" or oligonucleotides)) and ((paired or set) w5 (oligonucleotides or "nucleic acids" or probes)) and (competitive w5 (oligonucleotides or "nucleic acids" or probes)) and (functional w5 (oligonucleotides or "nucleic acids" or probes)) and ("polymerase chain reaction" or PCR or qPCR)	352	1-Jun-17
L30	TACD: ((amplific*) w10 ("nucleic acid" or oligonucleotides)) and ((paired or set) w5 (oligonucleotides or "nucleic acids" or probes)) and (competitive w5 (oligonucleotides or "nucleic acids" or probes)) and (functional w5 (oligonucleotides or "nucleic acids" or probes))	356	1-Jun-17

L29	TACD: ((amplific*_level) w10 ("nucleic acid" or oligonucleotides)) and ((paired or set) w5 (oligonucleotides or "nucleic acids" or probes)) and (competitive w5 (oligonucleotides or "nucleic acids" or probes)) and (functional w5 (oligonucleotides or "nucleic acids" or probes))	0	1-Jun-17
L28	TAC: ((amplific*_level) w10 ("nucleic acid" or oligonucleotides)) and ((paired or set) w5 (oligonucleotides or "nucleic acids" or probes)) and (competitive w5 (oligonucleotides or "nucleic acids" or probes)) and (functional w5 (oligonucleotides or "nucleic acids" or probes))	0	1-Jun-17
L27	TA: ((amplific*_level) w10 ("nucleic acid" or oligonucleotides)) and ((paired or set) w5 (oligonucleotides or "nucleic acids" or probes)) and (competitive w5 (oligonucleotides or "nucleic acids" or probes)) and (functional w5 (oligonucleotides or "nucleic acids" or probes))	0	1-Jun-17
L26	TA:(target*_nucleic*_acid* or target_molecule or target_DNA or target_gene or target_polynucleotide) AND TACD: ((paired w3 (primers or oligonucleotides or "capture molecules")) and ((competit*_oligonucleotid* or competit*_polynucleotide OR competit*_probe* or Competit*_primer or capture_molecul*) AND (signal*_oligonucleotid* OR fluoresc*_oligonucleotid* OR fluoresc*_probe* or functional_oligonucleotid* or functional_primer or signal_primer or fluresc*_primer or (signal w5 capture_molecule)) and (((signal or first) w5 intensity) or amplif*)))	113	30-May-17
L25	TAC:(target*_nucleic*_acid* or target_molecule or target_DNA or target_gene) AND TAC: ((competit*_oligonucleotid* OR competit*_probe* or Competit*_primer or capture_molecul*) AND (signal*_oligonucleotid* OR fluoresc*_oligonucleotid* OR fluoresc*_probe* or functional_oligonucleotid* or functional_primer or signal_primer or fluresc*_primer or (signal w5 capture_molecule)) and (((signal or first) w5 intensity) or amplif*))	26	30-May-17
L24	TA:(target w5 (oligonucleotide or "Nucleic acid" or DNA)) AND TACD: ((competitive w5 (oligonucleotide or probe)) and (signal w5 (oligonucleotide or probe)) and (signal w5 intensity))	195	27-May-17
L23	TAC:(oligonucleotide or "Nucleic acid" or DNA) AND TACD: ((competitive w5 (oligonucleotide or probe)) and (signal w5 (oligonucleotide or probe)) and (signal w5 intensity))	917	27-May-17
L22	TAC:(oligonucleotide or "Nucleic acid" or DNA) AND TACD: (competitive w5 (oligonucleotide or probe))	4336	27-May-17
L21	TAC:(oligonucleotide or "Nucleic acid" or DNA) AND TACD: ("multi_analyte" w5 coding)	0	27-May-17
L20	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND TACD: ("multi_analyte" w5 coding)	0	27-May-17
L19	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND TACD:((competitive w5 (oligonucleotide or probes)) and ((signal or fluorscen*) w5 (probe or oligonucleotide))) AND CPC:("C12Q2561/101" or "C12Q2561/113")	76	27-May-17
L18	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND CPC:("C12Q2561/101" or "C12Q2561/113")	1925	27-May-17
L17	CPC:("C12Q2561/101" or "C12Q2561/113")	3090	27-May-17
L16	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND TACD:(("real_time" or "multi_plex" or multiplex) w5 (pcr or qpcr or "polymerase chain reaction") and (competitive w5 (oligonucleotide or probes)) and ((signal or fluorscen*) w5 (probe or oligonucleotide))) AND CPC:(("A61K31/7088" or "A61K31/711" or "A61K31/713" or "C07H21/00" or "C07H21/04") and ("C12Q1/68" or "C12Q1/686" or "C12Q1/6806" or "C12Q1/6851" or "G01N21/64" or "G01N33/532" or "G01N33/533" or "G01N33/542"))	10	27-May-17
L15	CPC:(("A61K31/7088" or "A61K31/711" or "A61K31/713" or "C07H21/00" or "C07H21/04") and ("C12Q1/68" or "C12Q1/686" or "C12Q1/6806" or	4579	27-May-17

	"C12Q1/6851" or "G01N21/64" or "G01N33/532" or "G01N33/533" or "G01N33/542"))		
L14	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND TACD:(("real_time" or "multi_plex" or multiplex) w5 (pcr or qpcr or "polymerase chain reaction") and (competitive w5 (oligonucleotide or probes)) and ((signal or fluorscen*) w5 (probe or oligonucleotide))) AND IC:(("A61K31/7088" or "A61K31/711" or "A61K31/713" or "C07H21/00" or "C07H21/04") and ("C12Q1/68" or "G01N21/64" or "G01N33/532" or "G01N33/533" or "G01N33/542"))	109	27-May-17
L13	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND TACD:(("real_time" or "multi_plex" or multiplex) w5 (pcr or qpcr or "polymerase chain reaction") and ((competitive or signal) w5 (oligonucleotide or probes))) AND IC:(("A61K31/7088" or "A61K31/711" or "A61K31/713" or "C07H21/00" or "C07H21/04") and ("C12Q1/68" or "G01N21/64" or "G01N33/532" or "G01N33/533" or "G01N33/542"))	2071	27-May-17
L12	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND TACD:(("real_time" or "multi_plex" or multiplex) w5 (pcr or qpcr or "polymerase chain reaction")) AND IC:(("A61K31/7088" or "A61K31/711" or "A61K31/713" or "C07H21/00" or "C07H21/04") and ("C12Q1/68" or "G01N21/64" or "G01N33/532" or "G01N33/533" or "G01N33/542"))	4904	27-May-17
L11	TAC:((target or detect*) w5 ("Nucleic acid" or DNA)) AND IC:(("A61K31/7088" or "A61K31/711" or "A61K31/713" or "C07H21/00" or "C07H21/04") and ("C12Q1/68" or "G01N21/64" or "G01N33/532" or "G01N33/533" or "G01N33/542"))	27321	27-May-17
L10	IC:(("A61K31/7088" or "A61K31/711" or "A61K31/713" or "C07H21/00" or "C07H21/04") and ("C12Q1/68" or "G01N21/64" or "G01N33/532" or "G01N33/533" or "G01N33/542"))	104578	27-May-17
L9	TA: (target w5 ("nucleic acids" or DNA)) and TACD: ((competitive w5 (probe or oligonucleotide or hybridization)) and ((detectable or signal or reporter) w5 (oligonucleotide or probe)) and (PCR or qPCR or "polymerase chain reaction") and ((signal or flourescence) w5 intensity))	74	27-May-17
L8	TA: (target w5 ("nucleic acids" or DNA)) and TAC: ((competitive w5 (probe or oligonucleotide or hybridization)) and ((detectable or signal or reporter) w5 (oligonucleotide or probe)) and (PCR or qPCR or "polymerase chain reaction") and ((signal or flourescence) w5 intensity))	0	27-May-17
L7	TACD:((target*_nucleic*_acid* or target_molecule) AND ((competit*_oligonucleotid* OR competit*_probe*) AND (signal*_oligonucleotid* OR fluoresc*_oligonucleotid* OR fluoresc*_probe*)) AND (pcr OR qpcr OR polymerase_chain_reaction) and (signal w5 intensi*))	369	27-May-17
L6	TACD:((target*_nucleic*_acid* or target_molecule) AND ((competit*_oligonucleotid* OR competit*_probe*) AND (signal*_oligonucleotid* OR fluoresc*_oligonucleotid* OR fluoresc*_probe*)) AND (pcr OR qpcr OR polymerase_chain_reaction))	762	27-May-17
L5	TAC:((target*_nucleic*_acid* or target_molecule) AND ((competit*_oligonucleotid* OR competit*_probe*) AND (signal*_oligonucleotid* OR fluoresc*_oligonucleotid* OR fluoresc*_probe*)) AND (pcr OR qpcr OR polymerase_chain_reaction))	2	27-May-17
L4	TA:((target*_nucleic*_acid* or target_molecule) AND ((competit*_oligonucleotid* OR competit*_probe*) AND (signal*_oligonucleotid* OR fluoresc*_oligonucleotid* OR fluoresc*_probe*)) AND (pcr OR qpcr OR polymerase_chain_reaction))	0	27-May-17
L3	ASN:(chromacode)	0	27-May-17
L2	TACD:("multi-analyte" or ((competitive or signal) w5 (probes or oligonucleotides))) AND INV:((Jacky w3 Lucien) or (Rajagopal w3 Aditya) or (Menge w3 Karen) or (Gosch w3 Gregory))	29	27-May-17

L1	INV:((Jacky w3 Lucien) or (Rajagopal w3 Aditya) or (Menge w3 Karen) or (Gosch w3 Gregory))	152	27-May-17

Non-Patent Literature (NPL) Search Strategy/Results:

Google Scholar; 25 May 2017 (25.05.2017)

Search Terms	Results
"target nucleic acids") AND (("competitive oligonucleotides" OR "competitive probes") AND ("signal oligonucleotides" OR "fluorescent oligonucleotides" OR "fluorescent probes")) AND (PCR OR qPCR OR "polymerase chain reaction")	3
Identification target nucleic acids competitive oligonucleotides signal oligonucleotides PCR	48,900
Identification multiple target nucleic acids "fluorescence intensity" oligonucleotides PCR	27,700
multi-plex "real-time PCR" "competitive hybridization" probes ratio-metric coding of signal intensity	2,560
qPCR multi-analyte coding techniques	827
qPCR multi-analyte coding techniques ratiometric "signal intensity"	4

PubMed; 25 May 2017 (25.05.2017)

Search Terms	Results
((((PCR) AND real-time) AND multiplex)) AND signal oligonucleotides	15
((PCR) AND Real-time) AND competitive hybridzation	57
(identification target nucleic acids) AND competitive oligonucleotides	21
(((Jacky[Author]) OR Rajagopal A[Author]) OR Menge K[Author]) OR Gosch G[Author]	47

EBSCO; 01 June 2017 (01.06.2017)

Search ID#	Search Terms	Results
S8	AB (("nucleic acids" or oligonucleotide) N5 (detection or identification or target)) AND ((paired or set) N5 (oligonucleotides or "nucleic acids" or "capture molecules)) AND signal n5 intensity	119
S7	AB (("nucleic acids" or oligonucleotide) N5 (detection or identification or target)) AND ((paired or set) N5 (oligonucleotides or "nucleic acids" or "capture molecules))	779
S6	(("nucleic acids" or oligonucleotide) N5 (detection or identification or target)) AND ((paired or set) N5 (oligonucleotides or "nucleic acids" or "capture molecules))	117,761
S5	amplification N10 (target nucleic acid) AND (paired N5 (oligonucleotides	568

	or "nucleic acids")) AND (functional n5 (oligonucleotides or "nucleic acids"))	
S4	(Target N5 ("nucleic acids" or DNA or molecules)) AND (competitive N5 (oligonucleotides or "capture molecules" or antibodies)) AND polymerase chain reaction AND (ratio N10 (label or oligonucleotides)) AND signal N5 intensity	413
S3	(Target N5 ("nucleic acids" or DNA or molecules)) AND (competitive N5 (oligonucleotides or "capture molecules" or antibodies)) AND polymerase chain reaction AND (ratio N10 (label or oligonucleotides))	2,214
S2	(Target N5 ("nucleic acids" or DNA or molecules)) AND (competitive N5 (oligonucleotides or "capture molecules" or antibodies)) AND polymerase chain reaction	6,356
S1	(Target N5 ("nucleic acids" or DNA or molecules)) AND (competitive N5 (oligonucleotides or "capture molecules" or antibodies))	20,756

Electronic Acknowledgement Receipt	
EFS ID:	30977195
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	Lee Alan Johnson/Daniel Buehler
Filer Authorized By:	Lee Alan Johnson
Attorney Docket Number:	38075-716.301
Receipt Date:	16-NOV-2017
Filing Date:	05-AUG-2014
Time Stamp:	19:07:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		IDS38075-716-301-11-16-2017.pdf	285345 1e4c34c8a6e19798cc9304550784e2c021d7f389	yes	8

	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	4	
	Information Disclosure Statement (IDS) Form (SB08)		5	8	
Warnings:					
Information:					
2	Foreign Reference	WO2013116780A1_EFS.PDF	5144926 f6b7d5713e95f9e824a2030ca30950117f4f607e	no	84
Warnings:					
Information:					
3	Non Patent Literature	HOLLAND.PDF	5119339 86b050f2685b50fc8a29282ae9427280101c7a48	no	5
Warnings:					
Information:					
4	Non Patent Literature	Z-ISR-WO-6-22-2017-PCTUS2017-24933.PDF	2753488 cdaf294d08eeb4cf2b66e4487c0750a7e84635c6	no	20
Warnings:					
Information:					
		Total Files Size (in bytes):	13303098		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	KARTALOV; Emil et al.	Group Art Unit:	1631
Serial Number:	14/451,876	Examiner:	RUSSELL SCOTT NEGIN
Filing or 371 (c) Date:	2014-08-05	**CONFIRMATION NO:**	**1065**
Title:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS		

FILED ELECTRONICALLY ON: November 16, 2017

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2557

A. ☒ *37 CFR § 1.97 (b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☐ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☒ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☐ *37 CFR § 1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☐ a fee of $180.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR § 1.97 (d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of $180.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97 (e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2558

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☐ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. ☐ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☐ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2559

I. ☒ *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of $0.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No.38075-716.301).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: November 16, 2017

By: /Lee A. Johnson/
Lee A. Johnson
Reg. No. 75,671

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2560

Wilson Sonsini Goodrich & Rosati
PROFESSIONAL CORPORATION

12235 El Camino Real, Suite 200
San Diego, California 92130
FAX (858) 350-2399

www.wsgr.com

MEMORANDUM

To: Examiner Russell Scott Negin

From: Lee Alan Johnson

Date: October 31, 2017

Re: US Patent Application Serial No. 14/451,876

WSGR reference no. 38075-716.301

Proposed Agenda for Interview scheduled for November 1, 2017

(Lee Johnson to dial 571-272-1083)

Proposed Agenda:

1. Discuss potential claim amendments and the rejections raised in the Final Office Action mailed June 6th, 2017 and the Advisory Action mailed August 23rd, 2017.

   a. L. Johnson to provide arguments as to the patent eligibility of the claimed methods. L. Johnson to solicit input from Examiner Negin as to potential arguments and/or claim amendments. In particular, L. Johnson to discuss potential patentability of the proposed independent claim 49 presented on Schedule A to this Memorandum.

Sincerely,

WILSON SONSINI GOODRICH & ROSATI
Professional Corporation

/Lee A. Johnson/

Lee A. Johnson, PhD, Esq.
Reg # 75,671
(858) 350-2344

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2561

SCHEDULE A

Proposed amendments to independent claim 49 of US Pat. App. Ser. No. 14/451,876

49. (Currently Amended) A method of detecting the presence or absence of at least seven polynucleotide~~each~~ analytes, in any combination of presence or absence, in a single sample solution volume ~~of a plurality of analytes~~, the method comprising:

~~a)[[a.]] encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by one of said first values, wherein said encoding is performed in a manner that reduces or eliminates degeneracy;~~

a)[[b.]] providing a sample solution volume comprising, or potentially comprising, at least one of said polynucleotide analytes, and comprising at least seven hybridization probes, each hybridization probe corresponding to one of the at least seven polynucleotide analytes, wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores, and wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal, further wherein each generated cumulative intensity signal comprises at least one signal generated by excitement of at least one of the four fluorophores;

b)[[c.]] contacting said sample solution volume with said plurality of hybridization probes and exciting said hybridization probes to generate said cumulative intensity signal(s) if one or more of said seven polynucleotide analytes is present in the sample volume~~analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present~~;

c)[[d.]] cumulatively measuring said cumulative intensity signal(s), thereby generating a cumulative signal measurement, wherein the cumulative signal measurement non-degenerately indicates the presence or absence of said at least seven polynucleotide analytes, in any combination of presence or absence,~~cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement~~; and

~~e)[[e.]] determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme~~;

wherein said method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in [[a]]said single sample solution volume, in any combination of presence or absence, without requiring any step of immobilization of said polynucleotide analytes, physical separation of said polynucleotide analytes, or mass ~~spectometry~~spectrometry.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2562

Applicant-Initiated Interview Summary	Application No. 14/451,876	Applicant(s) Kartalov et al.		
	Examiner RUSSELL S NEGIN	Art Unit 1631	AIA (First Inventor to File) Status No	Page 1 of 2

All participants (applicant, applicants representative, PTO personnel):

1. RUSSELL S NEGIN(Examiner); Telephonic
2. Marjorie A. Moran(TC 1600 SME); Telephonic
3. Lee A. Johnson(Attorney); Telephonic
4. Adrianna Zhang(Attorney); Telephonic

Date of Interview: 01 November 2017

Exhibit shown or demonstration conducted (If so, please describe): An attachment with a proposed amended claim was discussed.

Claims Discussed: Claim 49.

Amendment proposed: The proposed amendments in the attachment were discussed.

Brief Description of the main topic(s) of discussion: Discussion of overcoming the rejections of record occurred.

Issues Discussed:

Item(s) under 35 U.S.C. 101:
The only possible judicial exception in the claim is the cumulatively measuring step. If this step is part of the experiment, it is not a judicial exception. If this step is mathematical analysis after the experiment, it is a judicial exception. Whether or not the other elements of the claim are routine and conventional depend on the interpretation of steps a and b of proposed claim 49.

Item(s) under 35 U.S.C. 112:
Enablement and written description issues were discussed because the claims encompass a single probe with a single fluorophore distinguishing between seven analytes wherein the probes are not immobilized, separated, or put through mass spectrometry.

Item(s) under Election/Restriction:
The proposed amendments may recite a shift in terms of the species election in the restriction requirement if the claims read on a plurality of different fluorophores.

Attachment(s): Proposed Amendments,

/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1631	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

U.S. Patent and Trademark Office
PTOL-413/413b (Rev. 01/01/2015) **Interview Summary** Paper No. 20171101

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/451,876	08/05/2014	Emil Kartalov	38075-716.301	1065

21971 7590 11/24/2017

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER	
NEGIN, RUSSELL SCOTT	

ART UNIT	PAPER NUMBER
1631	

NOTIFICATION DATE	DELIVERY MODE
11/24/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-20070072211	03-29-2007	NEWTON; Nick et al.	
	002	US-20100233686	09-16-2010	HIGUCHI; Russell et al.	
	003	US-20130040841	02-14-2013	SAXONOV; Serge et al.	
	004	US-20140171341	06-19-2014	JOUVENOT; Yann et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	WO-2017173035-A1	10-05-2017	CHROMACODE INC [US]		☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	001	Co-pending US patent application No. US15/914,356, filed on 03-07-2018.	☐
	002	GANDELMAN, et al., Novel Bioluminescent Quantitative Detection of Nucleic Acid Amplification in Real-Time, PLoS One, 2010, 5(11):e14155, 14 pages.	☐
	003	International Search Report and Written Opinion dated 11/16/2017 for International PCT Patent Application No. PCT/US2017/37682.	☐
	004	U.S. Serial No. 15/623,974 Office Action dated February 22, 2018.	☐
	005	U.S. Serial No. 15/677,772 Office Action dated February 08, 2018.	☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 3 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Kyle Bruner/	Date (YYYY-MM-DD)	2018-03-23
Name/Print	Kyle Bruner	Registration Number	75,716

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 October 2017 (05.10.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/173035 A1

(51) International Patent Classification:
C07H 21/00 (2006.01) *G01N 21/64* (2006.01)
C07H 21/04 (2006.01) *G01N 33/533* (2006.01)
C12Q 1/68 (2006.01) *G01N 33/542* (2006.01)

(21) International Application Number:
PCT/US2017/024933

(22) International Filing Date:
30 March 2017 (30.03.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/317,151 1 April 2016 (01.04.2016) US

(71) Applicant: **CHROMACODE INC.** [US/US]; 2075 Corte Del Nogal, Carlsbad, California 92011 (US).

(72) Inventors: **JACKY, Lucien A.E.**; 166 N Waverly Street, Orange, California 92866 (US). **RAJAGOPAL, Aditya**; 221 W. Sparkleberry Avenue, Orange, California 92865 (US). **MENGE, Karen L.**; 987 Gardenia Court, San Marcos, California 92078 (US). **GOSCH, Gregory**; 14445 Caminito Lazanja, San Diego, California 92127 (US).

(74) Agent: **JOHNSON, Lee A.**; WILSON SONSINI GOODRICH & ROSATI, 650 Page Mill Road, Palo Alto, California 94304 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

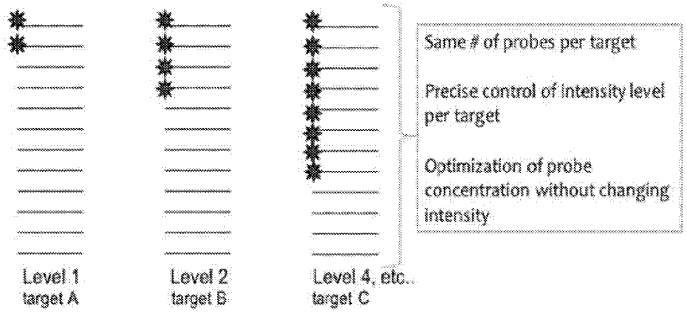
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: COMPETITIVE PROBES FOR ENGINEERING SIGNAL GENERATION

FIG. 1



(57) **Abstract**: A method of identifying a first target nucleic acid comprising, providing a sample comprising the first target nucleic acid, providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid, the first set of paired oligonucleotides comprising a first ratio of (a) first competitive oligonucleotides to (b) first signal oligonucleotides comprising a signal tag, wherein the competitive oligonucleotides compete with the signal oligonucleotides for binding to the first target nucleic acid, amplifying the first target nucleic acid with the polymerase chain reaction, thereby degrading the first signal oligonucleotide and permitting generation of a first signal, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target nucleic acid.

WO 2017/173035 A1

COMPETITIVE PROBES FOR ENGINEERING SIGNAL GENERATION

CROSS-REFERENCE

[001] This application claims the benefit of U.S. Provisional Patent Application No. 62/317,151, filed April 1, 2016, which is hereby incorporated by reference in its entirety.

BACKGROUND

[002] Real-Time PCR is a process of monitoring a PCR reaction by recording the fluorescence generated either by an intercalating dye such as SYBR Green or a target-specific reporter probe at each cycle. This is generally performed on a Real-Time PCR instrument that executes thermal cycling of the sample to complete the PCR cycles and at a specified point in each cycle measures the fluorescence of the sample in each channel through a series of excitation/emission filter sets.

[003] In the age of whole genome sequencing and high-throughput analysis, there is a need for methods of detecting multiple qPCR targets at once. A limitation of current qPCR machines is the number of channels available for target detection. Modern instruments have between two and six channels, meaning that only two to six reporters can be detected in each experiment. Contemporary multiplexing technology provides for two to four different fluorescent reporters, and therefore two to four targets, to be detected in a single reaction. Unfortunately, accurately distinguishing each of the two to four targets from one another is thwarted by overlapping fluorescence signals of the different reporters. Therefore, a need still remains for a reliable and precise method for distinguishing multiple reporter signals during multiplex qPCR reactions.

SUMMARY

[004] The real-time polymerase chain reaction or quantitative PCR (qPCR) is a common molecular biology technique that uses fluorescent reporters to monitor the progress of the exponential amplification of target nucleic acids in a reaction. Traditionally the process is limited to differentiating the presence of one target per fluorescent emission filter (color channel) on a qPCR instrument.

[005] Recent advances made at the California Institute of Technology nanofabrication lab allow for detection of multiple analytes per emission filter using mathematical algorithms on real-time PCR amplification curve characteristics such as amplitude and curve shape (US Patent 8,838,394 issued September 16, 2014 and US Patent Application Publication Number US2015-0057178 published February 26, 2015, both of which are incorporated herein in their entirety). Targets in the same color channel are "coded" to exhibit these amplification curve characteristics. Coding includes setting the intensity level of each target in a color channel.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2571

[006] Disclosed herein are methods by which the effective relative fluorescent intensity generated by a qPCR probe is adjusted independent of the total probe concentration for each target. This is a key improvement to the multi-analyte coding technique.

[007] Disclosed herein are methods and compositions for uniquely and unambiguously detecting multiple targets in a single color detection channel. One aspect of the disclosed methods is the ability to differentiate multiple targets by ratio-metric coding of signal intensity (brightness) of any given color for each target and mathematical strategies to circumvent degeneracy. When used within each of the available color channels on a qPCR instrument, the disclosed methods significantly increase the number of target measurements that can be interrogated.

[008] Provided herein are methods of identifying a first target nucleic acid comprising, providing a sample comprising the first target nucleic acid, providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid, said first set of paired oligonucleotides comprising a first ratio of (a) first competitive oligonucleotides to (b) first signal oligonucleotides comprising a first signal tag, wherein the first competitive oligonucleotides compete with the first signal oligonucleotides for binding to the first target nucleic acid, amplifying the first target nucleic acid with the polymerase chain reaction, thereby degrading the first signal oligonucleotide and permitting generation of a first signal, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target nucleic acid. In some aspects, the sample comprises a second target nucleic acid. In some aspects, the method further comprises identifying the second target nucleic acid by providing a second set of paired oligonucleotides with complementarity to the second target nucleic acid, said second set of paired oligonucleotides comprising a second ratio of (a) second competitive oligonucleotides to (b) second signal oligonucleotides comprising a second signal tag, wherein the second competitive oligonucleotides compete with the second signal oligonucleotides for binding to the second target nucleic acid, amplifying the second target sequence with the polymerase chain reaction, thereby degrading the second signal oligonucleotide and permitting generation of a second signal, generating the second signal, measuring intensity of the second signal, and correlating the intensity of the second signal to the second ratio, thereby identifying the second target nucleic acid. In some aspects, the first signal oligonucleotides and the second signal oligonucleotides comprise the same signal tag. In some aspects, the first target nucleic acid and the second target nucleic acid are identified from differing signal intensities generated during the same polymerase chain reaction. In some aspects, the first signal intensity is different from the second signal intensity. In some aspects, the paired oligonucleotides comprise qPCR probes. In

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2572

some aspects, the signal oligonucleotides comprise a signal tag and a signal quenching tag. In some aspects, the signal tag comprises a fluorophore. In some aspects, the signal quenching tag blocks the generation of the signal corresponding with the signal tag until the signal quenching tag is separated from the signal tag by oligonucleotide degradation during the polymerase chain reaction, thereby permitting the signal to be generated. In some aspects, generating the signal comprises exciting the signal tag. In some aspects, exciting comprises directing at the signal tag at least one of illumination, electromagnetic radiation, and energy emitted from an energy source. In some aspects, the competitive oligonucleotides do not comprise a signal tag. In some aspects, the competitive oligonucleotides comprise at least one modified end. In some aspects, the modified end prevents enzymatic extension during amplification. In some aspects, the modified end comprises a 3' end lacking a hydroxyl group. In some aspects, the modified end comprises a 5' end lacking a phosphate group. In some aspects, the modified end comprises a 5' capped end. In some aspects, the modified end comprises a 3' capped end. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:1. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:2. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:3. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:4. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:5. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:6. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:7. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:8. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:9. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:10. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:16. In some aspects, the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:20. In some aspects, the paired oligonucleotides comprise only

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2573

signal oligonucleotides or only competitive oligonucleotides. In some aspects, the total concentration of the first set of paired oligonucleotides is the same as the total concentration of the second set of paired oligonucleotides. In some aspects, the methods further comprise identifying up to thirty target nucleic acid sequences using up to thirty sets of paired oligonucleotides, wherein each set of paired oligonucleotides possesses complementarity to one of the up to thirty target nucleic acid sequences, wherein each of the up to thirty sets of paired oligonucleotides comprises a unique ratio of (a) competitive oligonucleotides to (b) signal oligonucleotides comprising a signal tag, wherein the competitive oligonucleotides within each set compete with the signal oligonucleotides within the same set for binding to a corresponding target nucleic acid sequence, amplifying the up to thirty target nucleic acid sequences with the polymerase chain reaction, thereby degrading the signal oligonucleotides and permitting generation of up to thirty signals, generating up to thirty signals, measuring the intensities of the up to thirty signals, and correlating the intensities of the up to thirty signals to the up to thirty ratios, thereby identifying the up to thirty target nucleic acid sequences. In some aspects, the signal tag of the signal oligonucleotides within each of the up to thirty sets of paired oligonucleotides are the same In some aspects, the up to thirty target nucleic acid sequences are identified from signal intensities generated during the same polymerase chain reaction. In some aspects, each of the up to thirty ratios differs from every other of the up to thirty ratios. In some aspects, each of the up to thirty signal intensities differs from every other of the up to thirty signal intensities In some aspects, the polymerase chain reaction is carried out by use of a nucleic acid polymerase having 5' to 3' exonuclease activity, an exo-polymerase, or a molecular beacon. In some aspects, the up to five signals are measured in a single fluorescence channel. In some aspects, the total concentration of each of the up to five sets of paired oligonucleotides is the same. In some aspects, a total number of molecules in the reaction remains constant

[009] Provided herein are compositions comprising a first set of paired oligonucleotides with complementarity to a first target nucleic acid, said first set of paired oligonucleotides comprising a first ratio of (a) competitive oligonucleotides to (b) signal oligonucleotides comprising a signal tag, wherein the competitive oligonucleotides are configured to compete with the signal oligonucleotides for binding to the first target nucleic acid during amplification of the first target nucleic acid with the polymerase chain reaction, wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and excited by an energy source, and wherein the first intensity correlates with the first ratio.

[010] Provided herein are methods of identifying a first target molecule comprising, providing a sample comprising the first target molecule, providing a first set of paired capture molecules

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2574

that bind to the first target molecule, said first set of paired capture molecules comprising a first ratio of (a) first competitive capture molecules to (b) first signal capture molecules comprising a signal tag, wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule, generating the first signal, measuring intensity of the first signal, and correlating the intensity of the first signal to the first ratio, thereby identifying the first target molecule. In some aspects, the sample comprises a second target molecule. In some aspects, the methods further comprise identifying the second target molecule by providing a second set of paired capture molecules that binds to the second target molecule, said second set of paired capture molecules comprising a second ratio of (a) second competitive capture molecules to (b) second signal capture molecules comprising the signal tag, wherein the second competitive capture molecules compete with the second signal capture molecules for binding to the second target molecule, generating the second signal, measuring intensity of the second signal, and correlating the intensity of the second signal to the second ratio, thereby identifying the second target molecule. In some aspects, the first signal capture molecules and the second signal capture molecules comprise the same signal tag. In some aspects, the first target capture molecules and the second target capture molecules are identified from differing signal intensities generated. In some aspects, the first signal intensity is different from the second signal intensity. In some aspects, the signal tag comprises a fluorophore. In some aspects, generating the signal comprises exciting the signal tag. In some aspects, exciting comprises directing at the signal tag at least one of illumination, electromagnetic radiation, and energy emitted from an energy source. In some aspects, the competitive capture molecules do not comprise a signaling tag. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:1 In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:2. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:3. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:4. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:5. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:6. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:7. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2575

ratio of competitive capture molecules to signal capture molecules is about 1:8. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:9. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:10. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:16. In some aspects, the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:20. In some aspects, the first paired capture molecules comprise only signal capture molecules or only competitive capture molecules. In some aspects, the methods further comprise identifying up to one hundred target molecules using up to one hundred sets of paired capture molecules, wherein each set of paired capture molecules that binds to one of the up to one hundred target molecules, wherein each of the up to one hundred sets of paired capture molecules comprises a unique ratio of (a) competitive capture molecules to (b) signal capture molecules comprising a signal tag, wherein the competitive capture molecules within each set compete with the signal capture molecules within the same set for binding to a corresponding target molecule, generating up to one hundred signals, measuring the intensities of the up to one hundred signals, and correlating the intensities of the up to one hundred signals to the up to one hundred ratios, thereby identifying the up to one hundred target molecules. In some aspects, the signal tag of the signal capture molecules within each of the up to one hundred sets of capture molecules are the same. In some aspects, the up to one hundred target molecules are identified from signal intensities generated at the same time. In some aspects, each of the up to one hundred ratios differs from every other of the up to one hundred ratios. In some aspects, each of the up to one hundred signal intensities differs from every other of the up to one hundred signal intensities. In some aspects, the up to one hundred signals are measured in a single fluorescence channel. In some aspects, the target molecule is fixed to a surface. In some aspects, the capture molecule is fixed to a surface. In some aspects, the target molecule comprises a protein or a functional fragment thereof. In some aspects, the target molecule comprises an antigen. In some aspects, the target molecule comprises an aptamer. In some aspects, the target molecule comprises a nucleic acid. In some aspects, the capture molecule comprises a protein a protein or a functional fragment thereof. In some aspects, the capture molecule comprises an antibody a protein or a functional fragment thereof. In some aspects, the capture molecule comprises an aptamer. In some aspects, a total number of molecules in the reaction remains constant.

[011] Provided herein are compositions comprising a first set of paired capture molecules with complementarity to a first target molecule, said first set of paired capture molecules comprising

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2576

a first ratio of (a) competitive capture molecules to (b) signal capture molecules comprising a signal tag, wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule, wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source, and wherein the first intensity correlates with the first ratio. In some aspects, the target molecule comprises a protein or a functional fragment thereof. In some aspects, the target molecule comprises a antigen. In some aspects, the target molecule comprises a aptamer. In some aspects, the target molecule comprises a nucleic acid. In some aspects, the capture molecule comprises a protein or a functional fragment thereof. In some aspects, the capture molecule comprises an antibody or a functional fragment thereof. In some aspects, the capture molecule comprises an aptamer.

[012] Provided herein are methods of altering amplification level of a target nucleic acid comprising, providing a sample comprising the target nucleic acid, providing a set of paired oligonucleotides with complementarity to the target nucleic acid, said set of paired oligonucleotides comprising a ratio of (a) competitive oligonucleotides to (b) functional oligonucleotides, wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR), and amplifying the target nucleic acid with PCR. In some aspects, the competitive oligonucleotides comprise at least one modified end. In some aspects, the modified end prevents enzymatic extension during amplification. In some aspects, the modified end comprises a 3' end lacking a hydroxyl group. In some aspects, the modified end comprises a 5' end lacking a phosphate group. In some aspects, the modified end comprises a 5' capped end. In some aspects, the modified end comprises a 3' capped end. In some aspects, a total number of molecules in the reaction remains constant.

[013] Provided herein are compositions comprising a set of paired oligonucleotides with complementarity to a target nucleic acid, said set of paired oligonucleotides comprising a ratio of (a) competitive oligonucleotides to (b) functional oligonucleotides, wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2577

BRIEF DESCRIPTION OF THE DRAWINGS

[014] **Figure 1** illustrates three example probe sets with different ratios of competitive to fluorescent probes

[015] **Figure 2** illustrates an example PIV3 benchmark assay titration performed at four distinct coding levels.

[016] **Figures 3A-3B** illustrate an example RT-qPCR experiment with five distinct ratio levels of labeled and unlabeled competitive probes.

[017] **Figures 4A-4B** illustrate an example RT-qPCR experiment with three distinct ration levels of labeled and unlabeled competitive probes.

DETAILED DESCRIPTION

[018] Without being bound by theory, Polymerase Chain Reaction is a method of exponential amplification of specific target DNA in a reaction mix with a DNA polymerase and primers. Primers are short single stranded DNA oligonucleotides which are complementary to the 3' sequences of the positive and negative strand of the target sequence. The reaction mix is cycled in repeated heating and cooling steps. The heating cycle denatures or split the double stranded DNA target into single stranded templates. In the cooling cycle, the primers bind to complementary sequence on the template. After the template is primed the DNA polymerase creates a copy of the original template. Repeated cycling exponentially amplifies the target 2 fold with each cycle leading to approximately a billion-fold increase of the target sequence in 30 cycles (Saiki et al 1988).

[019] Real-Time PCR is a process of monitoring a PCR reaction by recording the fluorescence generated either by an intercalating dye such as SYBR Green or a target-specific reporter probe at each cycle. This is generally performed on a Real-Time PCR instrument that executes thermal cycling of the sample to complete the PCR cycles and at a specified point in each cycle measures the fluorescence of the sample in each channel through a series of excitation/emission filter sets

[020] Frequently, the target specific reporter probe is a short oligonucleotide complementary to one strand of the amplified target. The probe lacks a 3' hydroxyl and therefore is not extendable by the DNA polymerase. TaqMan (ThermoFisher Scientific) chemistry is a common reporter probe method used for multiplex Real-Time PCR (Holland et al. 1991). The TaqMan oligonucleotide probe is covalently modified with a fluorophore and a quenching tag. In this configuration the fluorescence generated by the fluorophore is quenched and is not detected by the real time PCR instrument. When the target of interest is present, the probe oligonucleotide base pairs with the amplified target. While bound, it is digested by the 5'->3' exonuclease

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2578

activity of the Taq polymerase thereby physically separating the fluorophore from the quencher and liberating signal for detection by the real time PCR instrument. Other methods, such as molecular beacon probes, are also used in real-time PCR and amenable to this competitive probe technique.

[021] For certain qPCR, multiplexed qPCR, and coded qPCR methods, there is a need to adjust signal level in a predictable fashion. This is typically done by increasing or decreasing the number of fluorometric reporters added to a reaction. While this does adjust signal level to the first order, it also risks altering the chemical behavior of the reaction mixture as the total number of participating reactants has also changed. Often, this adversely affects the performance of other components within the reaction mixture. In particular, this is a critical failure mode for multiplexed qPCR. Disclosed herein are methods and compositions for detecting multiple target sequences with the same fluorophore within a single coded or multiplexed qPCR reaction.

[022] Disclosed herein are methods and compositions for adjusting fluorescent intensity without adjusting the reactant concentrations in a mixture. In some embodiments, combinations of both light-emitting and non-light-emitting chemical reporters are used to independently adjust the fluorescent intensity of a qPCR reaction without altering the chemical interactivity of the qPCR reaction mixture. In some embodiments, this is achieved by creating a mixture of non-fluorescent oligo probes and the TaqMan reporter probes for every target interrogated in a qPCR reaction.

[023] The herein disclosed competitive probes (also referred to as dimming probes, or non-fluorescing probes) are oligonucleotides similar to target probes with the exception of the covalently attached fluorophore and quencher. In some embodiments, the competitive probes do not have the fluorophore or the quencher. In some embodiments, the competitive probes have capped 5' and 3' ends to prevent enzymatic extension. In some embodiments, the competitive probes have a 5' end lacking a phosphate group. In some embodiments, the competitive probes have a 3' end lacking a hydroxyl group. In general, competitive probes behave identically to the specific target probes in binding to the amplified target sequence, and in the case of TaqMan style assay, the bound probes are digested by the 5'->3' exonuclease activity of Taq polymerase. However, because the competitive probes do not contain a fluorophore, no signal is generated.

[024] Disclosed herein are methods and compositions for using the disclosed non-fluorescing, competitive binding probes to adjust the signal intensity for a single gene by ratiometic titration of the competitive binding probe. In some examples, these methods are performed using a TaqMan probe. Figure 1 diagrams three different probes mixtures with differing proportions of competitive probes and signal probes. In some embodiments, the titration of the intensity allows

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2579

for control of intensity level ratio-metric coding and the ability to adjust the total probe concentration of a given target without affecting the intensity level coding.

[025] In some embodiments, during the probe annealing portion of the PCR reaction the competitive probes will bind to the amplified target at a rate proportional to the ratio of competitive probes to the total probes both labeled and unlabeled in the reaction yielding a fluorescent signal proportional to the ratio of labeled probes to the total probes.

[026] In some embodiments, the competitive probes are un-extendable probes. For example, the competitive probes are unable to initiate DNA synthesis by the Taq polymerase by employing a variety of 3' modifications including but not limited to the phosphorylation or the addition of a quenching molecule.

[027] In some embodiments, different purification methods and probe sequences are used to yield dramatically different fluorescence intensities. In some examples, differences in the quality of purification leads to more or less unlabeled product (similar to a competitive probe) reducing the fluorescence of a preparation. In some embodiments, the oligo sequence makeup of a probe affects the fluorescence, particularly guanine bases proximal to the fluorophore which can act as a quencher reducing the intensity of fluorescence. In some embodiments, the competitive probes are used to adjust the signal intensity to be consistent between preparations for more consistent lot to lot performance. In some embodiments, competitive probes are used if a dye's intensity is overloading the detector on a particular real-time PCR instrument.

[028] In some embodiments, competitive probes are used to engineer the optical characteristic of a PCR curve without altering its chemical characteristic. In some examples, this is achieved because competitive probes competitively interact with other sequences, along with their TaqMan probe equivalents. In these examples, this competitive interaction allows for the unique capability to fine tune the signal to noise of a PCR reaction without changing the chemical performance of the PCR itself.

[029] Disclosed herein are methods and compositions for using competitive primers for multiplex quantitation by normalizing the efficiency of multiplexed reactions. In some embodiments, the herein disclosed methods are used to drive the PCR reaction into producing more of one strand than the other which can lead to an earlier plateau phase of the PCR reaction.

[030] In some embodiments, competitive probes are used to replace a portion of priming oligonucleotides in a PCR reaction. In many of these examples, the competitive probes are rendered un-extendable by 3' phosphorylation or other methodology. In these cases, the competitive primer will compete with the extendable primer leading to a quantifiable fraction of template strands to fail to replicate during the extension phase of the PCR. One advantage of

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2580

these embodiments is that it allows the adjustment of amplification efficiency of either one or both of the replication strands.

[031] In some embodiments, the herein disclosed competitive probes are used in TaqMan style PCR. In some embodiments, the herein disclosed competitive probes are used in traditional PCR.

[032] Disclosed herein are methods for identifying a target by detecting a corresponding signal, such as a fluorescent signal. In some examples, multiple targets can be detected in a single experiment, wherein the corresponding signal from each is the same. For example, multiple targets can be identified, where each target has a corresponding fluorescent signal, wherein the signal generating molecule, such as a fluorophore, is the same among each of the different targets to be detected.

[033] Using methods disclosed herein, multiple targets can be detected using the same fluorophore by providing sets of target-binding molecules, such as probes, wherein each set of molecules comprises a unique ratio of competitive probes to signal probes. Signal probes can comprise a signal generating molecules, such as a fluorophore. Competitive probes often do not contain the signal generating molecule. In these cases, the total number of probes within each set of probes is the same, which the ratio of competitive probes to signal probes is unique for each target to be detected. This allows for the same level or concentration of total probes to be used for each target while still being able to distinguish each individual target.

[034] Disclosed herein are methods and compositions for detecting multiple target sequences with the same fluorophore. Also disclosed herein are methods and compositions for detecting multiple target specific reporter signals in a single reaction using fluorophore baring oligonucleotide probes in combination with non-fluorophore baring oligonucleotide probes. In some examples, each target sequence is detected using a set of oligonucleotide probes. Each oligonucleotide set can comprise two populations of oligonucleotide probes, each comprising the same nucleic acid sequence. In these examples, one population of oligonucleotides comprises a reporter fluorophore while the second population of oligonucleotides does not comprise a fluorophore. In some embodiments, up to five sets of oligonucleotide probes are used to detect up to five target sequences in a single reaction. In many of these examples, the fluorophore of the up to five sets of probes is the same. In some aspects, within each set of oligonucleotide probes the ratio of fluorophore baring and non-fluorophore baring oligonucleotides is set to a predetermined ratio. In some embodiments, the ratio within each set of oligonucleotides is different for each of the up to five sets of oligonucleotides. In these examples, the different ratios allow the reporter signal for each of the up to five targets to be distinguished from one another within a single reaction. In these examples, the total

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2581

concentration of probes in each set of probes is the same, while the ratio of the two populations of probes within the probe set are different between each of the sets of probes. A fluorophore baring probe can be a signal probe. A non-fluorophore baring probe can be a competitive probe.

[035] In some embodiments, up to 100 sets of oligonucleotide probes are used to detect up to 100 target sequences in a single reaction. For example, there can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 sets of oligonucleotide probes used to detect 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 target sequences in a single reaction. In many of these examples, the fluorophore of the up to 100 sets of probes is the same. In some aspects, within each set of oligonucleotide probes the ratio of fluorophore baring and non-fluorophore baring oligonucleotides is set to a predetermined ratio. In some embodiments, the ratio within each set of oligonucleotides is different for each of the up to 100 sets of oligonucleotides. In these examples, the different ratios allow the reporter signal for each of the up to 100 targets to be distinguished from one another within a single reaction. In these examples, the total concentration of probes in each set of probes is the same, while the ratio of the two populations of probes within the probe set are different between each of the sets of probes. A fluorophore baring probe can be a signal probe. A non-fluorophore baring probe can be a competitive probe.

[036] In some embodiments greater than 100 oligonucleotide probes are used to detect greater than 100 target sequences in a single reaction. In some embodiments, greater than 100 oligonucleotide probes are used to detect up to 100 target sequences in a single reaction. In some embodiments, up to 100 oligonucleotide probes are used to detect greater than 100 target sequences in a single reaction.

[037] Disclosed herein are methods and compositions for using an unlabeled analog to the corresponding labeled entity that generates signal for detection in the presence of a particular analyte in any system. In some examples, the system is an ELISA (enzyme-linked immunosorbent assay), in which case the labeled entity is a labeled antibody. In these cases, the unlabeled analog is an unlabeled antibody. In some embodiments, the ratio of labeled and unlabeled antibodies within a target set are predetermined as discussed previously. In some embodiments, multiple targets can be detected in a single ELISA reaction using multiple target

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2582

sets of antibodies. In many of these examples, each set of target antibodies has a different ratio of labeled and unlabeled antibodies which allow the independent target reporting signal to be distinguished from the others. In these examples, the total concentration of antibodies in each set of antibodies is the same, while the ratio of the two populations of antibodies within the antibodies set are different between each of the sets of antibodies. A fluorophore baring antibody can be a signal antibody. A non-fluorophore baring antibody can be a competitive antibody.

[038] In some embodiments, the total number of molecules in a reaction remains constant despite that the ratio of functional to competitive entities changes between different sets. In some aspects, the total number of molecules refers to the proteins, nucleic acids, solution volume, other entities required for the reaction to take place. In some aspects the reaction is a qPCR, ELISA, PCR, or sequencing reaction. In some aspects, the entities are oligonucleotides, proteins, antibodies, qPCR probes, aptamers, nucleic acid or other appropriate molecule. In some aspects function al comprises performing its intended purpose, such as PCR extension or emitting of fluorescence signal. In some aspects, competitive comprises binding to the target molecule, whether that be a protein, nucleic acid, or another molecule, but not being able to perform the function of the function molecule, such as extend during PCR or emit a fluorescence signal when excited.

Antibody labeling

[039] ELISA is a standard analytical technique for identifying proteins using fluorescence-labelled antibody markers. Without being bound by theory, in a sandwich ELISA, two antibodies, a primary antibody and a fluorescently labeled secondary antibody, are used to bind and identify a protein of interest (antigen). In general, the two antibodies bind to distinct regions of the protein. The primary antibody is adhered to a surface or a reaction vessel, with its binding domain exposed to allow for protein capture. When a sample is introduced, the protein of interest will diffuse through the chamber and favorably bind to the primary antibody. After a wash procedure, the fluorescently labeled secondary antibody is introduced. This fluorescently labeled secondary antibody binds to a distinct epitope of the fixed antigen. After washing the excess, unbound secondary antibody, the sample is interrogated. The presence and intensity of the fluorescence signal correlates with the concentration of antigen that has been captured. However, the binding dynamics of the antibodies are not readily engineerable. Unlike nucleic acids, antibodies cannot be inorganically assembled and even simple changes to the antibody structure result in dramatic changes in binding efficiencies. This critically limits the efficiency of antibody multiplexing for ELISA.

[040] Disclosed herein are methods and compositions for using competitive, non-reporting probes for fluorescence reporting using antibodies. In some embodiments, the fluorescence

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2583

reporting is in the context of an ELISA assay. In some embodiments, a primary antibody is adhered to a surface or a reaction vessel and the protein of interest favorably binds to the primary antibody. In these cases, after a wash procedure, a fluorescently labeled secondary antibody is introduced and binds to a distinct epitope of the fixed antigen. In these cases, after a wash procedure, the sample is interrogated and the presence and intensity of the fluorescence signal correlates with the concentration of antigen that has been captured.

[041] In some embodiments, a set of secondary antibodies is introduced and binds to the fixed antigen. In some aspects, the antigen is fixed by being bound to a primary antibody, which is in turn adhered to a surface or a reaction vessel. In some aspects, the set of secondary antibodies comprises a labeled population and an unlabeled population. In some aspects, the secondary antibodies in the labeled and unlabeled populations are identical except for the presence or absence of the label. In some aspects, secondary antibodies being identical means they comprise the same protein sequence. In some aspects, identical means they bind to the same epitope. In some aspects, identical means they bind to the same antigen. In some embodiments, the label is a fluorescent label. In some embodiments, the ratio of labeled and unlabeled secondary antibodies within a set of secondary antibodies is set to a predetermined ratio. In some aspects, the ratio of labeled to unlabeled secondary antibodies correlated to a signal intensity. In some aspects, by measuring the signal intensity, the signal intensity can be correlated back to the ratio of labeled to unlabeled secondary antibodies.

[042] In some embodiments, the ratio of labeled to unlabeled secondary antibodies within a set of secondary antibodies is used to set the signal level for an ELISA immunoassay. In some aspects, the ratio is set without changing the sensitivity or the number of antigens captured. In some embodiments, the primary antibody determines capture sensitivity and sensitivity. In some embodiments, multiple distinct antigens can be detected in a single ELISA reaction using the methods disclosed herein. In some aspects, a set of secondary antibodies able to bind to each of the multiple antigens is used in the ELISA. In many cases, the same label is used to label the labeled secondary antibody within each of the multiple sets of secondary antibodies. In some aspects the ratio of labeled to unlabeled secondary antibodies is unique within each of the multiple sets of secondary antibodies. In many examples, the unique ratios correspond to unique signal intensities. In these and other examples, the unique signal intensities are measured and correlated back to the predetermined ratios, thereby identifying the set of secondary antibodies that generated the signal intensity. In this way, a specific antigen can be identified in an ELISA assay based on the unique signal generated by the unique ratio of labeled to unlabeled secondary antibodies within the unique set of secondary antibodies used to bind to the specific antigen. In many examples, these methods disclosed herein allow identification of multiple antigens despite

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2584

using the same label on the labeled secondary antibodies for each of the multiple antigens. This is achieved by using unique ratios of labeled to unlabeled secondary antibodies for each of the multiple antigens. The unique ratios correlate to unique signal intensities, thereby allowing identification of each of the multiple antigens.

Sequencing bias

[043] While genomic sequencing is poised to be the predominant clinical tool in the coming future, it has particularly susceptible to sample-preparation bias. Without being bound by theory, before nucleic acids are sequenced, they are enriched using highly multiplexed broad-binding PCR primers. However, this enrichment generates a large bias in the amplified targets. This bias results in ineffective sequencing. For applications such as cancer tissue genotyping and tumor heterogeneity assessment, this makes the technique inapplicable for many clinical use cases.

[044] Disclosed herein are methods and compositions for using competitive oligonucleotides to reduce sequencing bias. In some embodiments, sequencing bias is reduced by using a set of oligonucleotide primers during amplification. In some aspects, amplification comprises a polymerase chain reaction. In some embodiments, a set of primers comprises a population of functional primers and a population of competitive primers. In some aspects, functional primers comprise primers capable of annealing to a complementary sequence and being extended during a polymerase chain reaction. In some aspects, competitive primers comprise are identical in sequence to their corresponding functional primer, are able to bind to the same complementary sequence, but are not able to be extended during a polymerase chain reaction. In some embodiments, the ratio of functional and competitive primers is adjusted to alter the level of sequencing bias without changing the chemical interactivity of the primer sets themselves. In some embodiments, reducing or increasing the ratio of functional primers to competitive primers, increase or decrease the enrichment of DNA to be sequenced. In some aspects, this ratio adjustment is performed without affecting the enrichment of other portions of the genome. In some aspects, this ratio adjustment allows a fine control of PCR bias. In some aspects, this ratio adjustment allows for the compensation of the deficiencies of current PCR methods for sequencing prep.

[045] In some embodiments, the ratio of functional primers to competitive primers is increased. In some aspects, the ratio of functional primers to competitive primers is increased in order to increase the sequencing bias of a target sequence. In some aspects, altering sequencing bias comprises increasing the amplification level of the target sequence. In some aspects, increasing the amplification level of the target sequence leads to an increased level of the target sequence in the final sample to be sequenced.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2585

[046] In some embodiments, the ratio of functional primers to competitive primers is decreased. In some aspects, the ratio of functional primers to competitive primers is decreased in order to decrease the sequencing bias of a target sequence. In some aspects, altering sequencing bias comprises decreasing the amplification level of the target sequence. In some aspects, decreasing the amplification level of the target sequence leads to an decreased level of the target sequence in the final sample to be sequenced.

[047] In some embodiments, competitive primers are not able to be extended. In some aspects, they are not able to be extended due to a competitive, capped, or otherwise modified end, such as those described and disclosed herein.

EXAMPLES

Example 1

[048] A Real-Time PCR reaction with an equimolar ratio of competitive probes and fluorogenic probes would yield approximately half the fluorescent signal at each reading as compared to a reaction with a similar amount of fluorogenic probes. Figure 2 shows the same TaqMan Assay with 4 differing proportions of fluorogenic probes to total probes. The 100% fluorescent probe reaction in yellow (solid top line) has an amplitude approximately 8 fold that of the assay in grey (long dashed line) which has 1 part fluorescent probes with 7 parts competitive probes. See Table 1 for the reaction make up and Table 2 for the PCR cycling parameters.

Table 1: Reaction components for Figure 2 reactions

Reaction Mix	Part #	12.5% fluorescent probe	25% fluorescent probe	50% fluorescent probe	100% fluorescent probe	No Template
ABI TaqMan Universal Master Mix	4304437	10 µL	11 µL	12 µL	13 µL	14 µL
Ambion Nuclease Free Water	AM9937	4 µL	4 µL	4 µL	4 µL	4 µL
Promega TE 1x (diluant)	V6231	6 µL	6 µL	6 µL	6 µL	6 µL
Oligo (concentation in final reaction)						
Forward Primer 1	FBF2	200 nM	200 nM	200 nM	200 nM	200 nM
Reverse Primer 1	FBR2	200 nM	200 nM	200 nM	200 nM	200 nM
FAM Labeled Probe 1	FBP2-1	25 nM	25 nM	25 nM	25 nM	25 nM

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2586

Blunt Probe 1	FBP2-0	175 nM	175 nM	175 nM	175 nM	175 nM
Forward Primer 2	FAF1	200 nM	200 nM	200 nM	200 nM	200 nM
Reverse Primer 2	FAR1	200 nM	200 nM	200 nM	200 nM	200 nM
FAM Labeled Probe 2	FAP1-1	50 nM	50 nM	50 nM	50 nM	50 nM
Blunt Probe 2	FAP1-0	150 nM	150 nM	150 nM	150 nM	150 nM
Forward Primer 3	RBF1	200 nM	200 nM	200 nM	200 nM	200 nM
Reverse Primer 3	RBR1	200 nM	200 nM	200 nM	200 nM	200 nM
FAM Labeled Probe 3	RBP1-1	100 nM	100 nM	100 nM	100 nM	100 nM
Forward Primer 4	RBP1-0	100 nM	100 nM	100 nM	100 nM	100 nM
Reverse Primer 4	RAF2	200 nM	200 nM	200 nM	200 nM	200 nM
FAM Labeled Probe 4	RAR2	200 nM	200 nM	200 nM	200 nM	200 nM
Blunt Probe 4	RAP2-1	200 nM	200 nM	200 nM	200 nM	200 nM
template (copies per reaction)						
Template 1	FBT2	1000 copies	-	-	-	-
Template 2	FAT1	-	1000 copies	-	-	-
Template 3	RBT1	-	-	1000 copies	-	-
Template 4	RAT2	-	-	-	1000 copies	-
total volume [µL]		20 µL	20 µL	20 µL	20 µL	20 µL

Table 2: Temperature Schedule on ABI Viia7 for figure 2 reactions

Temperature Schedule:	Temperature [°C]	Time [s]	Data Collection
UNGase	50	120	N
Initial Denature	95	600	N
Number of Cycles	75		
Denature	95	15	N
Anneal	60	30	Y

Example 2

[049] A Real-Time PCR reaction is performed to detect three nucleic acid sequences of interest within a single reaction. In addition to the other components required for the RT-PCR reaction,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2587

three sets of probes are added. One of each of the sets of probed corresponds to and is able to uniquely bind to one of the three transcripts of interest.

[050] Set 1 comprises 25% unlabeled competitive probes and 75% labeled probes, which are labeled with a green fluorophore and a quencher molecule. The probes within Set 1 are identical other than the presence of the fluorescent label and quencher molecule.

[051] Set 2 comprises 50% unlabeled competitive probes and 50% labeled probes, which are labeled with a green fluorophore and a quencher molecule. The probes within Set 2 are identical other than the presence of the fluorescent label and quencher molecule. The green fluorophore used in Set 2 is the same green fluorophore used in Set 1.

[052] Set 3 comprises 75% unlabeled competitive probes and 25% labeled probes, which are labeled with a green fluorophore and a quencher molecule. The probes within Set 3 are identical other than the presence of the fluorescent label and quencher molecule. The green fluorophore used in Set 3 is the same green fluorophore used in Set 1 and Set 2.

[053] The probes within each of Sets 1-3 are distinct from each other since they are selected for their ability to specifically bind to their corresponding nucleic acid target. However, the fluorophore used to label the labeled probes within each set is the same. Also, the ratio of unlabeled to labeled probes within each set are different and unique compared to the other sets.

[054] When the reaction first starts, no fluorescent signal is detected since the signals from each fluorophore are blocked by the quencher molecule. As the reaction proceeds, unlabeled and labeled probes within each set compete for binding to their complementary nucleic acid target sequence. Because the labeled and unlabeled probes within each set are basically identical, their binding and annealing kinetics are basically identical. When a labeled probe anneals, it is subsequently degraded by the 5' to 3' exonuclease activity of the polymerase. This degradation step separates the fluorophore and the quenching molecule, allowing for a fluorescence signal to be emitted upon excitation by the appropriate excitation source.

[055] An excitation source excites the green fluorophores and a signal is emitted from each of the released green fluorophores. Three distinct signal intensities are detected and measured. Each signal intensity correlated to a specific ratio. Because each set of probes comprised a unique ratio of labeled to unlabeled probes, a unique signal intensity can be correlated to that specific set of probes. Therefore, each unique signal intensity is used to identify one of each of the three nucleic acid targets.

Example 3

[056] Figures 3A and 3B demonstrate an example experiment exemplifying consistent leveling with competitive probes versus diluted probes.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2588

[057] Ten RT-qPCR Master Mixes were made for a system containing three TaqMan™ assays in a single color channel for human rhinovirus, respiratory syncytial virus A (RSV A), and respiratory syncytial virus B (RSV B). All three assays utilized probes with the same fluorophore quencher combination. The total probe concentration of the mixtures was conserved for five of these reactions. Table 3 summarizes how the fluorescent and competitive probes were diluted to the same concentration. Table 4 summarizes how the primer sets were diluted to the same concentration. Table 5 displays the master mix composition for reactions containing both fluorescent and competitive probes. Table 5 displays the master mix composition for reactions containing fluorescent probes only with no competitive probes. Table 7 displays the concentration of the three templates included in each reaction.

[058] The fluorescent probe concentrations for two of the targets (RSV A and RSV B) were fixed but different. For the final target (human rhinovirus, RH), the fluorescent probe concentration was varied from 25nM. All reactions contained the same enzyme formulation and primer concentrations, as shown in Tables 5 and 6.

[059] The qPCR protocol for each of the 10 master mixes were tested with known single-stranded DNA templates at DNA templates with a fixed concentration of 10,000 copies per reaction (Table 7). The qPCR reactions were performed on a standard qPCR instrument with the cycling parameters shown in Table 8. The resulting data is depicted in Figure 3A and Figure 3B.

Table 3. Oligo mix

Oligo mix	Oligo	µM	Oligo volume to make 10x stock	TE Buffer	Total Volume
RH 100% Fluorescent	RHP1-1	101.1	2.47	997.53	1000.0
RH 100% Competitive	RHP1-0	95.2	2.63	997.37	1000.0
RSV B 100% Fluorescent	RBP1-1	101.1	2.24	997.76	1000.0
RSV A 25% Fluorescent	RAP2-1	95.9	0.50	999.50	1000.0
RSV A 75% Competitive	RAP2-0	93.8	2.00	998.00	1000.0

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2589

Table 4. 6plex primer mix

Primer set	Material	µM	Desired µM in 20X stock	Oligo volume to make 10x stock (µL)
Set 1	cdcFAUniF1	93.1	0.4	17.19
	cdcFAUniR1	99.2	0.4	16.13
Set 2	ccFAH1_2009F2	117	0.4	13.68
	ccFAH1_2009R2	114	0.4	14.04
Set 3	cdcFAH3F3	114.6	0.4	13.96
	cdcFAH3R3	97.23	0.4	16.46
Set 4	RHF1	97.7	0.4	16.38
	RHR1	96.7	0.4	16.55
Set 5	RAF2	102.2	0.4	15.66
	RAR2	94.7	0.4	16.90
Set 6	RBF1	94.2	0.4	16.99
	RBR1	94.7	0.4	16.90
Set 7	RBF1	94.2	0.4	42.46
	RBR1	94.7	0.4	42.24
	Promega V6231, TE	n/a	n/a	9.2

Table 5. Reaction Master Mix – with competitive probes

Material	100 FP	80 FP + 20 CP	60 FP + 40 CP	40 FP + 60 CP	20 FP + 80 CP
TaqPath ABI A28626	60 µL	60	60	60	60
20X 6plex primer mix	12	12	12	12	12
10X RH 100% Fluorescent	24	19.2	14.4	9.6	4.8
10X RH Competitive	0	4.8	9.6	14.4	19.2
10X RSV B 100% Fluorescent	24	24	24	24	24
10X RSV A 25% Fluorescent	24	24	24	24	24
10X RSV A Competitive	24	24	24	24	24
water RxBiosciences	12	12	12	12	12

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2590

Table 6. Reaction Master Mix – without competitive probes

Material	100 FP - BP	80 FP	60 FP	40 FP	20 FP
TaqPath ABI A28626	60 µL	60	60	60	60
20X 6plex primer mix	12	12	12	12	12
10X RH 100% Fluorescent	24	19.2	14.4	9.6	4.8
10X RSV B 100% Fluorescent	24	24	24	24	24
10X RSV A 25% Fluorescent	24	24	24	24	24
water RxBiosciences	12	12	12	12	12
TE Promega V6231	24	28.8	33.6	38.4	43.2

Table 7. Templates

Name	Concentration
RHT1	2E3 copies/µL
RAT2	2E3 copies/µL
RBT1	2E3 copies/µL
NTC TE	NA (buffer only)

Table 8

Cycling Parameters

Step	time(seconds)	temperature(°C)	
1	120	25	
2	600	53	
3	120	95	
4	3	95	
5	30	60	detection
	repeat step 4 & 5 90 cycles		

Example 4

[060] The following example experiments and resulting data displayed in Figure 4A and Figure 4B demonstrate the ability to deterministically generate a 3plex assay with the same

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2591

reporting fluorophore and quencher combination with the binary separation based upon initial 100% fluorescent probe data.

[061] To generate the initial fluorescence data a RT-qPCR Master Mix was made containing three TaqMan™ assays for human rhinovirus (Target A), respiratory syncytial virus A (Target B) and respiratory syncytial virus B (Target C). No non-fluorescent, competitive probes were used in these assays. All three assays were labeled with the same fluorophore quencher combination. The primer concentration was selected to be in excess of the total probe concentration. The same concentration of fluorogenic probe, and therefore total probe, was used for each target.

[062] Table 9 displays the composition of the oligo master mix, which contained the indicated fluorescent probes and no competitive probes. Table 10 displays the composition of the primer master mix. Table 11 displays the master mix composition for reactions containing only 100% fluorescent probes and no competitive probes. Table 12 displays the concentration of the three templates included in each reaction. Table 13 displays the cycling parameters used for the RT-qPCR reaction.

Table 9. 25X fluorescence probe Oligo Mix

Oligo Mix	Oligo	Stock Conc, µM	Conc. of 1X, µM	Vol, µL
Target A RH 100% Fluorescent	RHP1-1	79.9	0.025	3.13
Target B RSV A 100% Fluorescent	RAP2-1	95.9	0.025	2.61
Target C RSV B 100% Fluorescent	RBP1-1	101.1	0.025	2.47
	Promega V6231, TE	n/a	n/a	391.79
	Total			400.00

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2592

Table 10. 25X Primer Mix

Primer set	Name	Stock Conc, µM	Conc of 1X, µM	Vol, µL
Target A set	RHF1	97.7	0.1	10.24
	RHR1	96.7	0.1	10.34
Target B set	RAF2	102.2	0.1	9.78
	RAR2	94.7	0.1	10.56
Target C set	RBF1	94.2	0.1	10.62
	RBR1	94.7	0.1	10.56
	Promega V6231, TE	n/a	n/a	337.90
	Total			400.00

Table 11. Reaction Master Mix

Name	1 rxn	24 rxns
4x TaqPath with MP	5 µL	120
25X Primer mix	0.8	19.2
25X Fluorescent probe oligo mix	0.8	19.2
water	8.4	201.6

Table 12. Templates

Target	Name	Conc
Target A	RHT1	2E3 copies/µL
Target B	RAT2	2E3 copies/µL
Target C	RBT1	2E3 copies/µL
No template control	NTC TE	NA

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2593

Table 13. Cycling parameters

Step	time(seconds)	temperature(°C)	
1	120	25	
2	600	53	
3	120	95	
4	3	95	
5	30	60	detection
	repeat step 4 & 5 90 cycles		

[063] The qPCR protocol for each of the 10 master mixes were tested with known single-stranded DNA templates at DNA templates with a fixed concentration of 10,000 copies per reaction (Table 12). The qPCR reactions were performed on a standard qPCR instrument with the parameters displayed in Table 13. The results from this experiment are displayed in Table 14 and in Figure 4A. Six replicates were performed along with a no template control (NTC).

Table 14. Data from RT-qPCR with no competitive probes

Target	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	NTC
Target A	0.4016	0.4028	0.3967	0.4018	0.4057	0.4056	0.0631
Target B	0.5291	0.5235	0.4415	0.5147	0.5324	0.5331	NA
Target C	0.4535	0.4415	0.4449	0.4445	0.4438	0.4381	0.0600

Table 15. Fluorescence score for experiment without competitive probes

Target	Name	Score
Target A	RH T1	0.4023
Target B	RSV A T2	0.5263
Target C	RSV B T1	0.4441

[064] To calculate the desired ratio of fluorogenic probe to completive probe for a leveled mix, the median fluorescence level from the 6 replicates was determined from the data from the fluorescent probe only experiment (Table 15). Target C was set to the highest intensity level and left at 100% of the initial concentration of fluorogenic probe. The levels for Target A and Target B were engineered to be distinct by replacing a proportion of the fluorescent probe with a non-fluorescent, competitive probe. The total amount of probe (fluorescent plus non-fluorscent, competitive) was the same for every target and identical to the total probe concentrations for the experiments shown in Figure 4A.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2594

[065] Table 16 displays the composition of the oligo master mix, which contained the indicated fluorescent probes and competitive probes. The primer master mix and reaction master mix used was the same as those displayed in Table 10 and Table 11 respectively. The templates and cycling parameters used were the same as those displayed in Tables 12 and Table 13 respectively.

[066] Six replicates along with a no template control (NTC) were performed. The resulting data is displayed in Table 17 and Figure 4B. The fluorescence score calculated for each target is displayed in Table 18.

Table 16. Fluorescent and Competitive probe oligo mix

Probe	Name	Stock Conc, µM	Conc of 1X, µM	Vol, µL
Target A - Fluorescent	RHP1-1	53.7	0.0125	4.67
Target A - Competitive	RHP1-0	90.2	0.0125	2.76
Target B - Fluorescent	RAP2-1	9.59	0.0044	9.11
Target B - Competitive	RAP2-0	93.8	0.0206	4.40
Target C - Fluorescent	RBP1-1	101.1	0.0250	4.95
	Promega V6231, TE	n/a	n/a	774.11
	Total			800.00

Table 17. Data from RT-qPCR with competitive probes

Target	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	NTC
Target A	0.2154	0.2121	0.2072	0.2129	0.2142	0.2161	0.0369
Target B	0.1192	0.1174	0.1145	0.1169	0.1129	0.1214	0.0359
Target C	0.4323	0.4247	0.4195	0.4190	0.4217	0.4243	0.0374

Table 18. Fluorescence score for experiment with competitive probes

Target	Name	Score
Target A	RH T1	0.2135
Target B	RSV A T2	0.1171
Target C	RSV B T1	0.4230

Example 5

[067] An ELISA assay is performed to detect three proteins of interest within a single reaction vessel. The sets of primary antibodies are adhered to a surface of a reaction vessel. One of each

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2595

sets is able to uniquely bind to one of each of the three proteins of interest. A sample comprising the three proteins of interest is added to the reaction vessel and each favorably binds to the corresponding primary antibody. A wash procedure is performed to removed unbound sample. Next, three sets of secondary antibodies are added to the reaction vessel, one of the sets corresponding to each of the three proteins of interest.

[068] Set 1 comprises 25% unlabeled competitive secondary antibodies and 75% labeled secondary antibodies, which are labeled with a green fluorophore. The secondary antibodies in Set 1 are identical other than the presence of the fluorescent label.

[069] Set 2 comprises 50% unlabeled competitive secondary antibodies and 50% labeled secondary antibodies, which are labeled with a green fluorophore. The secondary antibodies in Set 2 are identical other than the presence of the fluorescent label. The green fluorophore used in Set 2 is the same green fluorophore used in Set 1.

[070] Set 3 comprises 75% unlabeled competitive secondary antibodies and 25% labeled secondary antibodies, which are labeled with a green fluorophore. The secondary antibodies in Set 3 are identical other than the presence of the fluorescent label. The green fluorophore used in Set 3 is the same green fluorophore used in Set 1 and Set 2.

[071] The secondary antibodies within each of Sets 1-3 are distinct from each other since they are selected for their ability to specifically bind to their corresponding protein of interest. However, the fluorophore used to label the labeled secondary antibodies within each set is the same. Also, the ratio of unlabeled to labeled secondary antibodies within each set are different and unique compared to the other sets.

[072] The three sets of secondary antibodies are added to the reaction vessel. Labeled and unlabeled secondary antibodies from each set compete for binding to their corresponding protein epitope. Because the labeled and unlabeled secondary antibodies within each set are identical other than the presence of the fluorophore, their binding kinetics are basically the same. After the binding step, a wash procedure is performed to remove unbound secondary antibody.

[073] An excitation source excites the green fluorophores and a signal is emitted from each of the bound labeled secondary antibodies. Three distinct signal intensities are detected and measured. Each signal intensity correlated to a specific ratio. Because each set of secondary antibodies comprised a unique ratio of labeled to unlabeled secondary antibodies, a unique signal intensity can be correlated to that specific set of secondary antibodies. Therefore, each unique signal intensity is used to identify one of each of the three proteins of interest.

Example 6

[074] mRNA is isolated from a sample following an experiment and processed for sequencing. The mRNA sample reverse transcribed to cDNA and then PCR amplified in order to prepare the

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2596

sequencing library. Sequencing is performed to detect a target of interest and to determine how the target level has changed due to the conditions of the experiment compared to a control gene. Unfortunately, the target is from a very highly expressed gene and is over-represented in the sequencing library compared to the control gene. This makes it difficult to accurately calculate the impact of the experimental conditions on the expression level of the gene.

[075] The frustrated scientists go back to the original cDNA sample and re-amplify it by PCR. However, this time, they include a different set of target specific primers in the PCR reaction mix. The set of target primers comprises 25% functional primers capable of being extended during PCR, and 75% of competitive primers which cannot be extended due to a capped end.

[076] The resulting sequencing results has a lower level of the first transcript, which allows for a more accurate calculation of the transcription level caused by the conditions used in the original experiment.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2597

CLAIMS

What is claimed is:

1. Method of identifying a first target nucleic acid comprising,

providing a sample comprising the first target nucleic acid,

providing a first set of paired oligonucleotides with complementarity to the first target nucleic acid, said first set of paired oligonucleotides comprising a first ratio of (a) first competitive oligonucleotides to (b) first signal oligonucleotides comprising a first signal tag,

wherein the first competitive oligonucleotides compete with the first signal oligonucleotides for binding to the first target nucleic acid,

amplifying the first target nucleic acid with the polymerase chain reaction, thereby degrading the first signal oligonucleotide and permitting generation of a first signal,

generating the first signal,

measuring intensity of the first signal, and

correlating the intensity of the first signal to the first ratio, thereby identifying the first target nucleic acid.

2. The method of claim 1, wherein the sample comprises a second target nucleic acid.

3. The method of claim 2, further comprising identifying the second target nucleic acid by:

providing a second set of paired oligonucleotides with complementarity to the second target nucleic acid, said second set of paired oligonucleotides comprising a second ratio of (a) second competitive oligonucleotides to (b) second signal oligonucleotides comprising a second signal tag,

wherein the second competitive oligonucleotides compete with the second signal oligonucleotides for binding to the second target nucleic acid,

amplifying the second target sequence with the polymerase chain reaction, thereby degrading the second signal oligonucleotide and permitting generation of a second signal,

generating the second signal,

measuring intensity of the second signal, and

correlating the intensity of the second signal to the second ratio, thereby identifying the second target nucleic acid.

4. The method of claim 3, wherein the first signal oligonucleotides and the second signal oligonucleotides comprise the same signal tag.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2598

5. The method of any of claims 3-4, wherein the first target nucleic acid and the second target nucleic acid are identified from differing signal intensities generated during the same polymerase chain reaction.

6. The method of any of claims 3-5, wherein the first signal intensity is different from the second signal intensity.

7. The method of claim 1, wherein the paired oligonucleotides comprise qPCR probes.

8. The method of any of claims 1-7, wherein the signal oligonucleotides comprise a signal tag and a signal quenching tag.

9. The method of claim 8, wherein the signal tag comprises a fluorophore.

10. The method of and of claims 8-9, wherein the signal quenching tag blocks the generation of the signal corresponding with the signal tag until the signal quenching tag is separated from the signal tag by oligonucleotide degradation during the polymerase chain reaction, thereby permitting the signal to be generated.

11. The method of any of claims 1-10, wherein generating the signal comprises exciting the signal tag.

12. The method of claim 11, wherein exciting comprises directing at the signal tag at least one of illumination, electromagnetic radiation, and energy emitted from an energy source.

13. The method of any of claims 1-12 wherein the competitive oligonucleotides do not comprise a signal tag.

14. The method of any of claims 1-13, wherein the competitive oligonucleotides comprise at least one modified end.

15. The method of claim 14, wherein the modified end prevents enzymatic extension during amplification.

16. The method of claim 15, wherein the modified end comprises a 3' end lacking a hydroxyl group.

17. The method of claim 15, wherein the modified end comprises a 5' end lacking a phosphate group.

18. The method of claim 15, wherein the modified end comprises a 5' capped end.

19. The method of claim 15, wherein the modified end comprises a 3' capped end.

20. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:1.

21. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:2.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2599

22. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:3.

23. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:4.

24. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:5.

25. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:6.

26. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:7.

27. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:8.

28. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:9.

29. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:10.

30. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:16.

31. The method of any of claims 1-19, wherein the ratio of signal oligonucleotides to competitive oligonucleotides or the ratio of competitive oligonucleotides to signal oligonucleotides is about 1:20.

32. The method of any of claims 1-19, wherein the paired oligonucleotides comprise only signal oligonucleotides or only competitive oligonucleotides.

33. The method of any of the claims 1-32, wherein the total concentration of the first set of paired oligonucleotides is the same as the total concentration of the second set of paired oligonucleotides.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2600

34. The method of any of claims 1-33, further comprising identifying up to thirty target nucleic acid sequences using up to thirty sets of paired oligonucleotides,

wherein each set of paired oligonucleotides possesses complementarity to one of the up to thirty target nucleic acid sequences,

wherein each of the up to thirty sets of paired oligonucleotides comprises a unique ratio of (a) competitive oligonucleotides to (b) signal oligonucleotides comprising a signal tag,

wherein the competitive oligonucleotides within each set compete with the signal oligonucleotides within the same set for binding to a corresponding target nucleic acid sequence,

amplifying the up to thirty target nucleic acid sequences with the polymerase chain reaction, thereby degrading the signal oligonucleotides and permitting generation of up to thirty signals,

generating up to thirty signals,

measuring the intensities of the up to thirty signals, and

correlating the intensities of the up to thirty signals to the up to thirty ratios, thereby identifying the up to thirty target nucleic acid sequences.

35. The method of claim 34, wherein the signal tag of the signal oligonucleotides within each of the up to thirty sets of paired oligonucleotides are the same.

36. The method of any of claims 34-35, wherein the up to thirty target nucleic acid sequences are identified from signal intensities generated during the same polymerase chain reaction.

37. The method of any of claims 34-36, wherein each of the up to thirty ratios differs from every other of the up to thirty ratios.

38. The method of any of claims 34-37, wherein each of the up to thirty signal intensities differs from every other of the up to thirty signal intensities.

39. The method of any of claims 1-38, wherein the polymerase chain reaction is carried out by use of a nucleic acid polymerase having 5’ to 3’ exonuclease activity, an exo-polymerase, or a molecular beacon.

40. The method of any of claims 34-39, wherein the up to five signals are measured in a single fluorescence channel.

41. The method of any of the claims 34-40, wherein the total concentration of each of the up to five sets of paired oligonucleotides is the same.

42. The method of any of claims 1-41, wherein a total number of molecules in the reaction remains constant

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2601

43. A composition comprising:

a first set of paired oligonucleotides with complementarity to a first target nucleic acid,

said first set of paired oligonucleotides comprising a first ratio of (a) competitive oligonucleotides to (b) signal oligonucleotides comprising a signal tag,

wherein the competitive oligonucleotides are configured to compete with the signal oligonucleotides for binding to the first target nucleic acid during amplification of the first target nucleic acid with the polymerase chain reaction,

wherein the signal oligonucleotides are adapted to generate a first signal having a first intensity when degraded by a nucleic acid polymerase during the polymerase chain reaction and excited by an energy source,

and wherein the first intensity correlates with the first ratio.

44. Method of identifying a first target molecule comprising,

providing a sample comprising the first target molecule,

providing a first set of paired capture molecules that bind to the first target molecule, said first set of paired capture molecules comprising a first ratio of (a) first competitive capture molecules to (b) first signal capture molecules comprising a signal tag,

wherein the competitive capture molecules compete with the signal capture molecules for binding to the first target molecule,

generating the first signal,

measuring intensity of the first signal, and

correlating the intensity of the first signal to the first ratio, thereby identifying the first target molecule.

45. The method of claim 44, wherein the sample comprises a second target molecule.

46. The method of claim 45, further comprising identifying the second target molecule by:

providing a second set of paired capture molecules that binds to the second target molecule, said second set of paired capture molecules comprising a second ratio of (a) second competitive capture molecules to (b) second signal capture molecules comprising the signal tag,

wherein the second competitive capture molecules compete with the second signal capture molecules for binding to the second target molecule,

generating the second signal,

measuring intensity of the second signal, and

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2602

correlating the intensity of the second signal to the second ratio, thereby identifying the second target molecule.

47. The method of claim 46, wherein the first signal capture molecules and the second signal capture molecules comprise the same signal tag.

48. The method of any of claims 45-47, wherein the first target capture molecules and the second target capture molecules are identified from differing signal intensities generated.

49. The method of any of claims 45-48, wherein the first signal intensity is different from the second signal intensity.

50. The method of any of claims 44-49, wherein the signal tag comprises a fluorophore.

51. The method of any of claims 44-50, wherein generating the signal comprises exciting the signal tag.

52. The method of claim 51, wherein exciting comprises directing at the signal tag at least one of illumination, electromagnetic radiation, and energy emitted from an energy source.

53. The method of any of claims 44-52 wherein the competitive capture molecules do not comprise a signaling tag.

54. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:1.

55. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:2.

56. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:3.

57. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:4.

58. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:5.

59. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:6.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2603

60. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:7.

61. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:8.

62. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:9.

63. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:10.

64. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:16.

65. The method of any of claims 44-53, wherein the ratio of signal capture molecules to competitive capture molecules or the ratio of competitive capture molecules to signal capture molecules is about 1:20.

66. The method of any of claims 44-53, wherein the first paired capture molecules comprise only signal capture molecules or only competitive capture molecules.

67. The method of any of claims 44-66, further comprising identifying up to one hundred target molecules using up to one hundred sets of paired capture molecules,

wherein each set of paired capture molecules that binds to one of the up to one hundred target molecules,

wherein each of the up to one hundred sets of paired capture molecules comprises a unique ratio of (a) competitive capture molecules to (b) signal capture molecules comprising a signal tag,

wherein the competitive capture molecules within each set compete with the signal capture molecules within the same set for binding to a corresponding target molecule,

generating up to one hundred signals,

measuring the intensities of the up to one hundred signals, and

correlating the intensities of the up to one hundred signals to the up to one hundred ratios, thereby identifying the up to one hundred target molecules.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2604

68. The method of claim 67, wherein the signal tag of the signal capture molecules within each of the up to one hundred sets of capture molecules are the same.

69. The method of any of claims 67-68, wherein the up to one hundred target molecules are identified from signal intensities generated at the same time.

70. The method of any of claims 67-69, wherein each of the up to one hundred ratios differs from every other of the up to one hundred ratios.

71. The method of any of claims 67-70, wherein each of the up to one hundred signal intensities differs from every other of the up to one hundred signal intensities.

72. The method of any of claims 67-71, wherein the up to one hundred signals are measured in a single fluorescence channel.

73. The method of any of claims 44-72, wherein the target molecule is fixed to a surface.

74. The method of any of claims 44-73, wherein the capture molecule is fixed to a surface.

75. The method of any of claims 44-74, wherein the target molecule comprises a protein or a functional fragment thereof.

76. The method of any of claims 44-74, wherein the target molecule comprises an antigen.

77. The method of any of claims 44-74, wherein the target molecule comprises an aptamer.

78. The method of any of claims 44-74, wherein the target molecule comprises a nucleic acid.

79. The method of any of claims 44-78, wherein the capture molecule comprises a protein a protein or a functional fragment thereof.

80. The method of any of claims 44-78, wherein the capture molecule comprises an antibody a protein or a functional fragment thereof.

81. The method of any of claims 44-78, wherein the capture molecule comprises an aptamer.

82. The method of any of claims 44-81, wherein a total number of molecules in the reaction remains constant.

83. A composition comprising:

a first set of paired capture molecules with complementarity to a first target molecule,

said first set of paired capture molecules comprising a first ratio of (a) competitive capture molecules to (b) signal capture molecules comprising a signal tag,

wherein the competitive capture molecules are configured to compete with the signal capture molecules for binding to the target molecule,

wherein the signal capture molecules are adapted to generate a first signal having a first intensity when excited by an energy source,

and wherein the first intensity correlates with the first ratio.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2605

84. The composition of claim 83, wherein the target molecule comprises a protein or a functional fragment thereof.

85. The composition of claim 83, wherein the target molecule comprises a antigen.

86. The composition of claim 83, wherein the target molecule comprises a aptamer.

87. The composition of claim 83, wherein the target molecule comprises a nucleic acid.

88. The composition of any of claims 83-87, wherein the capture molecule comprises a protein or a functional fragment thereof.

89. The composition of any of claims 83-87, wherein the capture molecule comprises an antibody or a functional fragment thereof.

90. The composition of any of claims 83-87, wherein the capture molecule comprises an aptamer.

91. Method of altering amplification level of a target nucleic acid comprising,

providing a sample comprising the target nucleic acid,

providing a set of paired oligonucleotides with complementarity to the target nucleic acid, said set of paired oligonucleotides comprising a ratio of (a) competitive oligonucleotides to (b) functional oligonucleotides,

wherein the competitive oligonucleotides compete with the functional oligonucleotides for binding to the target nucleic acid and are not extendible by a nucleic acid polymerase, wherein the functional oligonucleotides are extendible by a nucleic acid polymerase as primers for amplifying the target nucleic acid by the polymerase chain reaction (PCR),

and

amplifying the target nucleic acid with PCR.

92. The method of claim 91, wherein the competitive oligonucleotides comprise at least one modified end.

93. The method of claim 92, wherein the modified end prevents enzymatic extension during amplification.

94. The method of claim 93, wherein the modified end comprises a 3' end lacking a hydroxyl group.

95. The method of claim 93, wherein the modified end comprises a 5' end lacking a phosphate group.

96. The method of claim 93, wherein the modified end comprises a 5' capped end.

97. The method of claim 93, wherein the modified end comprises a 3' capped end.

98. The method of any of claims 91-97, wherein a total number of molecules in the reaction remains constant.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2606

99. A composition comprising:

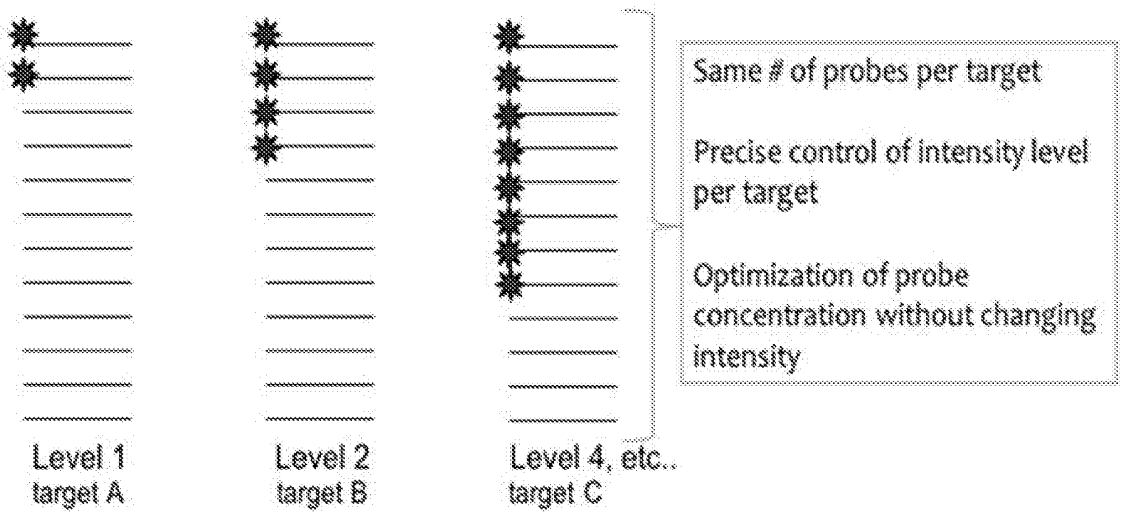
a set of paired oligonucleotides with complementarity to a target nucleic acid,

said set of paired oligonucleotides comprising a ratio of (a) competitive oligonucleotides to (b) functional oligonucleotides,

wherein the competitive oligonucleotides are configured to compete with the functional oligonucleotides for binding to the nucleic acid target during amplification of the first target nucleic acid with the polymerase chain reaction and are not extendible by a nucleic acid polymerase.

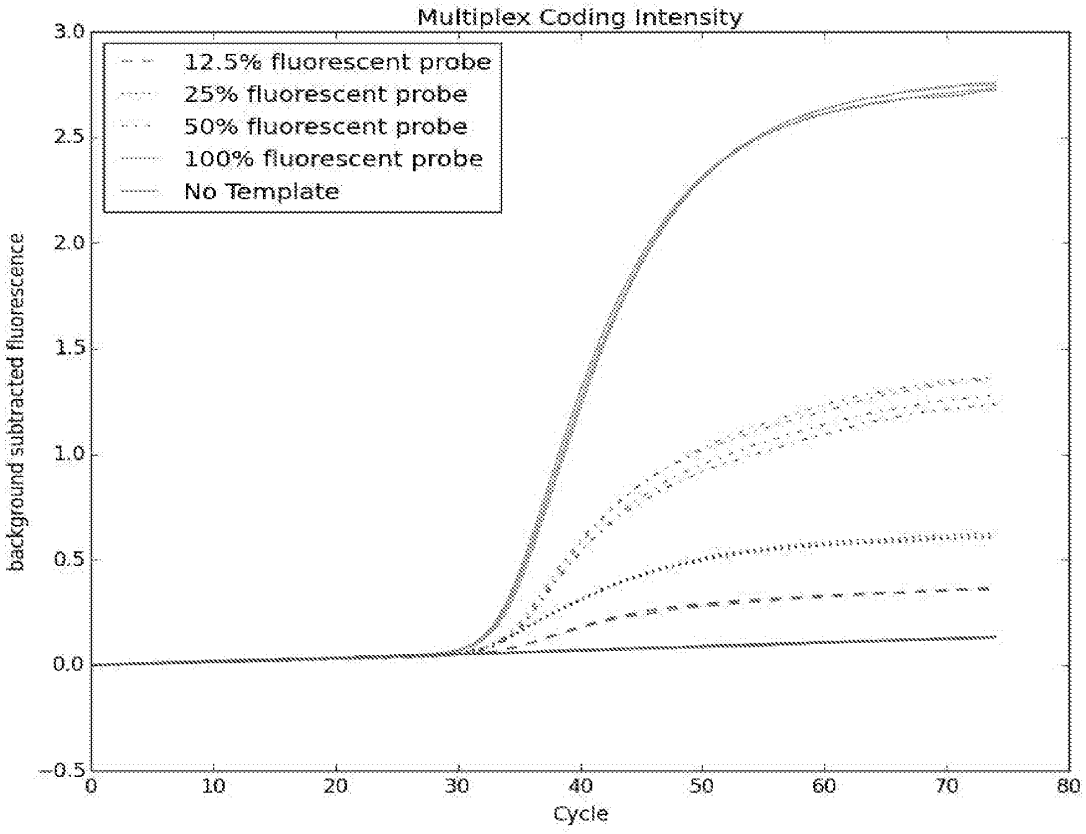
IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2607

FIG. 1



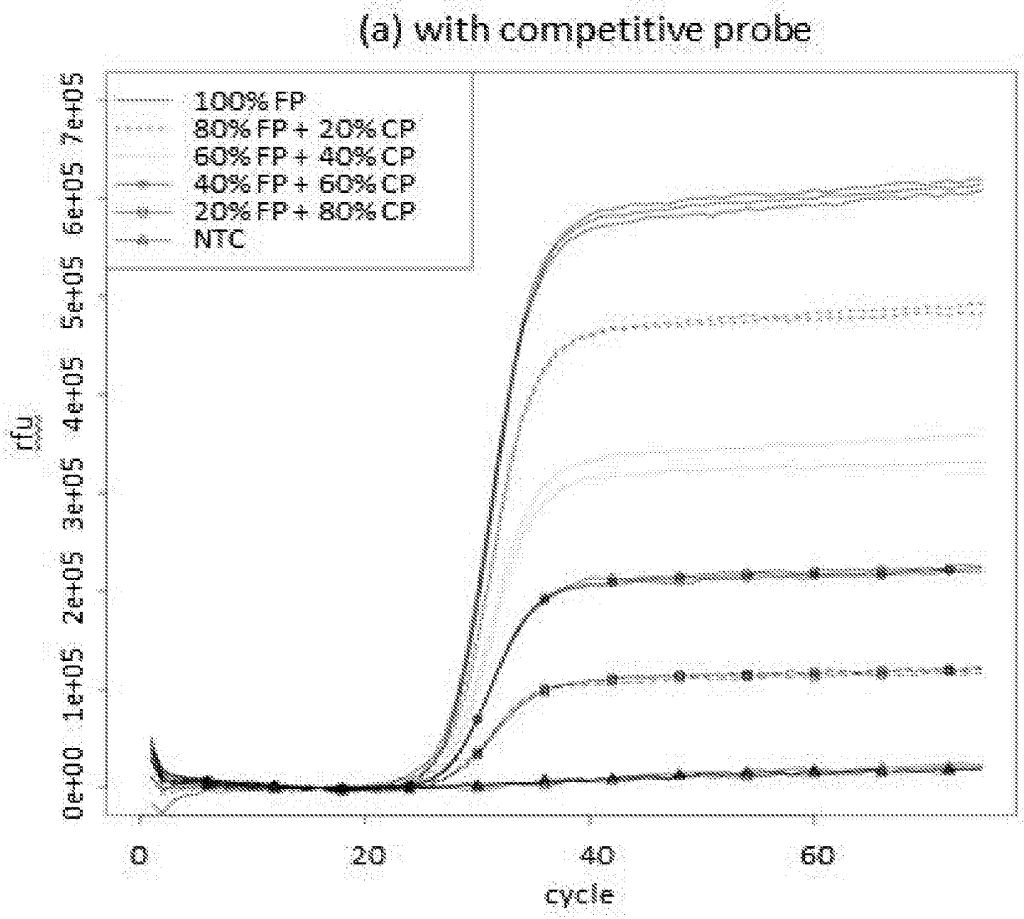
IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2608

FIG. 2



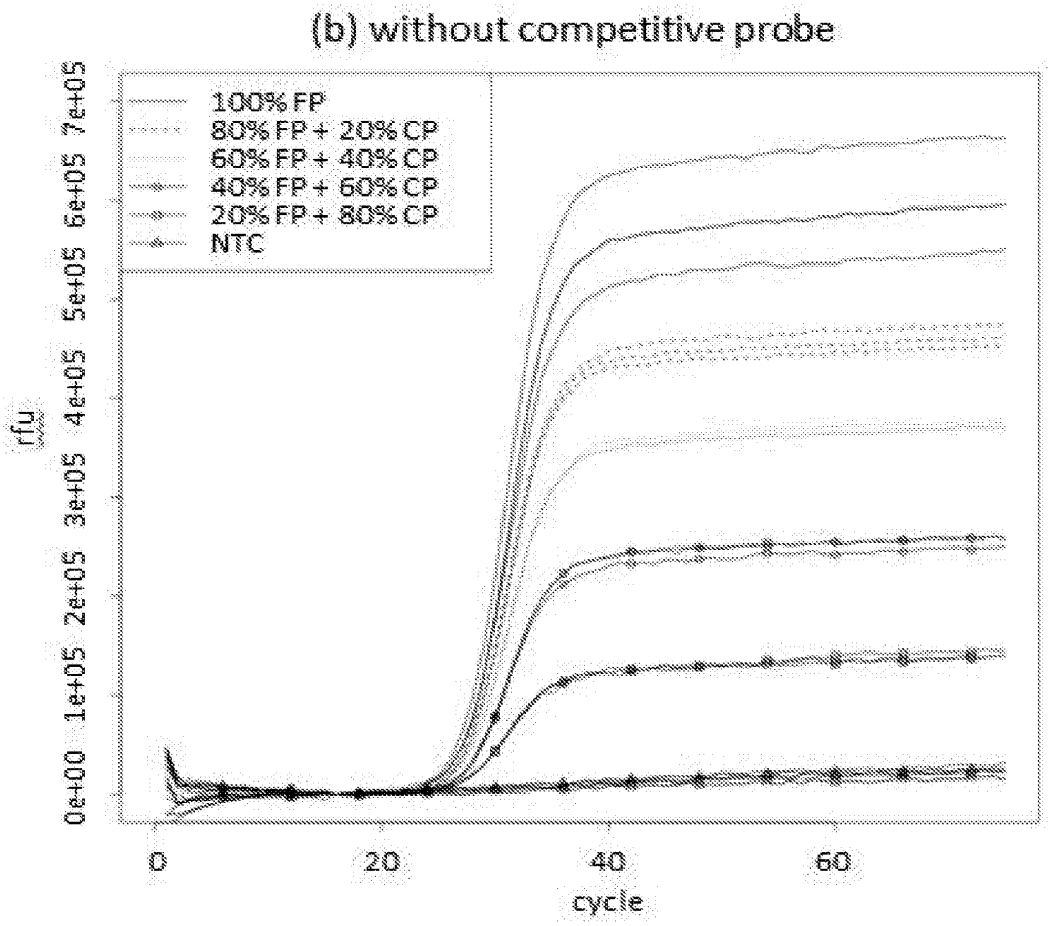
IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2609

FIG. 3A



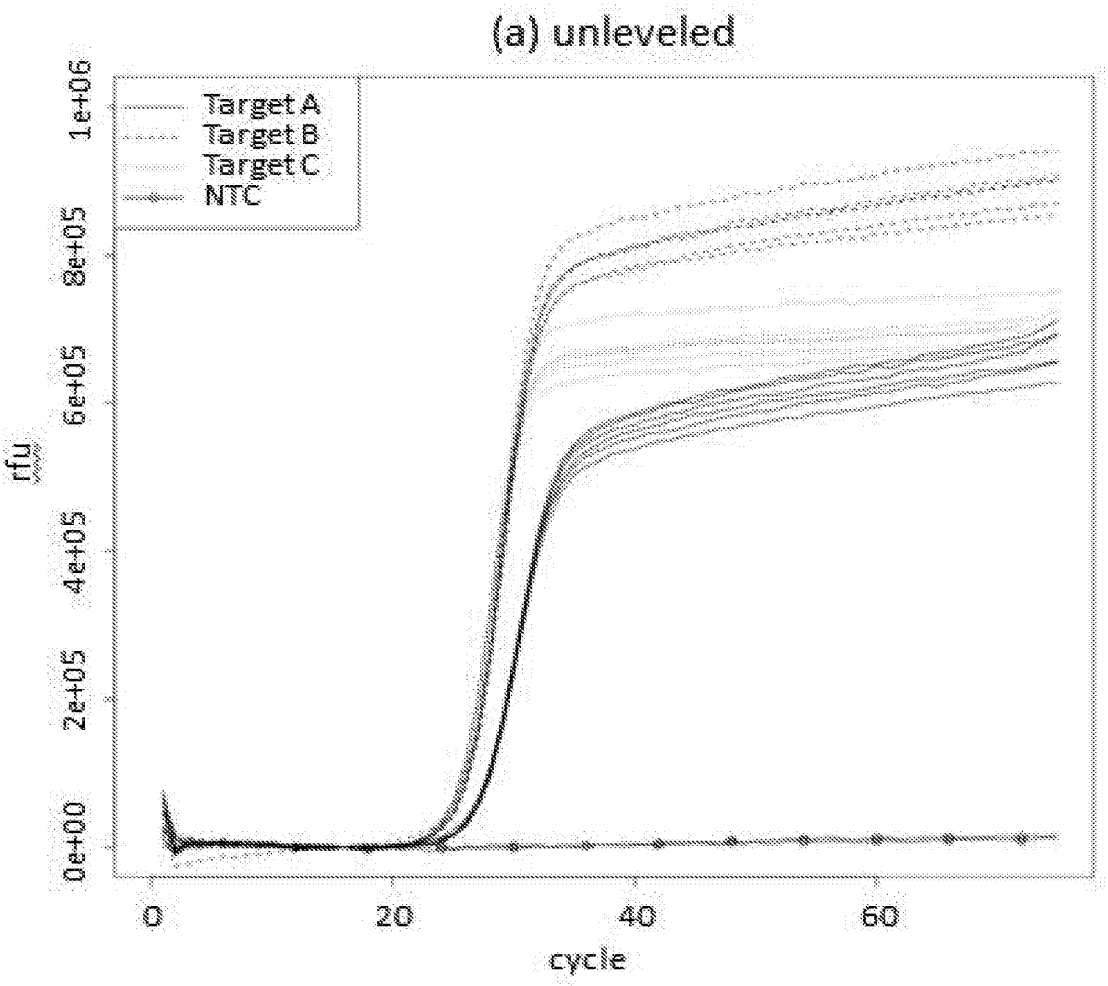
IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2610

FIG. 3B



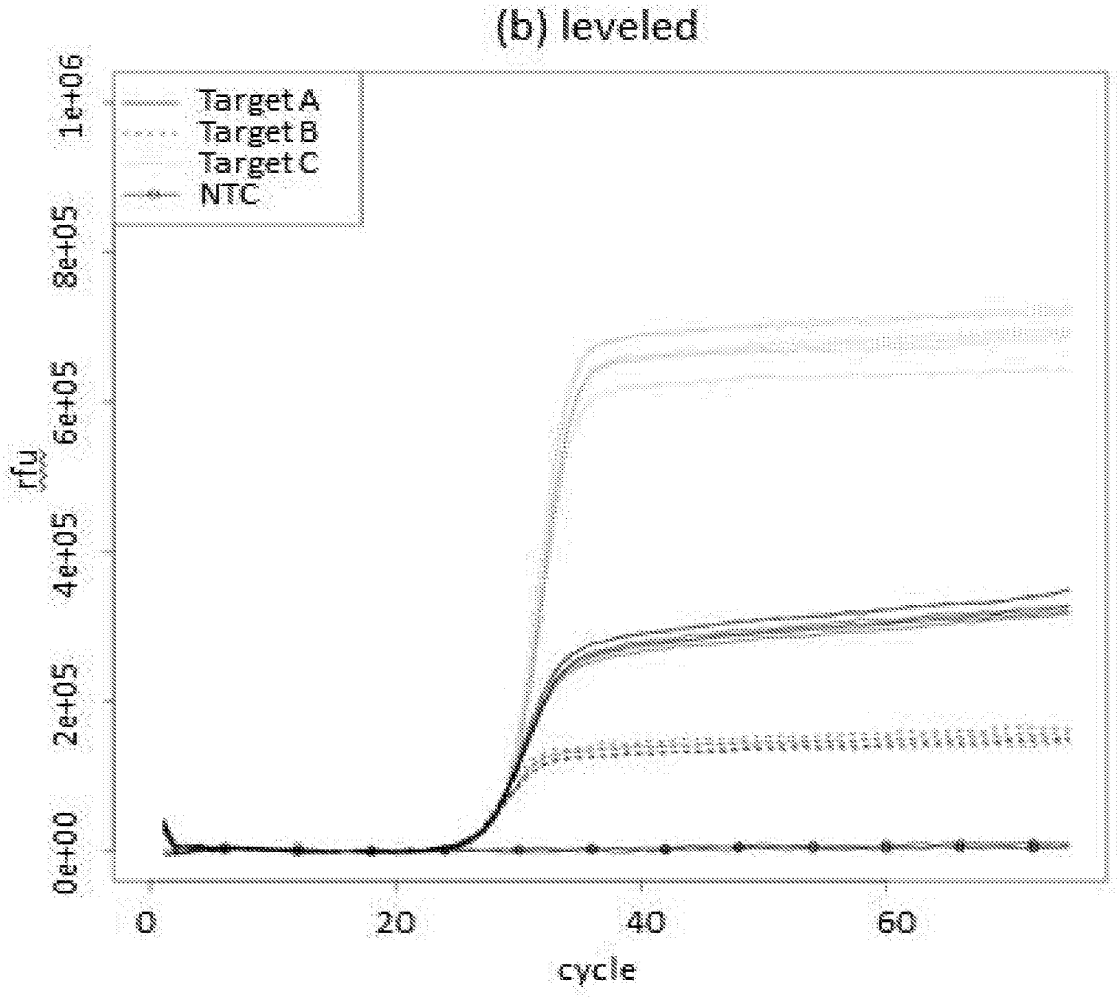
IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2611

FIG. 4A



IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2612

FIG. 4B



IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2613

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/24933

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-42 and 49-82
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/24933

A. CLASSIFICATION OF SUBJECT MATTER
IPC - C07H 21/00, 21/04; C12Q 1/68; G01N 21/64, 33/533, 33/542 (2017.01)
CPC - C07H 21/00, 21/04; C12Q 1/68, 1/686, 1/6806, 1/6851, 2561/101, 2561/113; G01N 21/64, 33/533, 33/542

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/079049 A2 (THIRD WAVE TECHNOLOGIES, INC.) 27 July 2006; page 2, lines 10-17 and 23-31; page 3, lines 13-20, page 5, lines 5-8 and 23-30; page 6, lines 18-30; page 7, lines 14-19; page 15, lines 24-31; page 16, lines 1-3 and 18-28	1-4, 5/3-4, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99
A	US 2009/0042735 A1 (BLAIR SM, et al.) 12 February 2009; paragraphs [0018], [0035], [0041]-[0043], [0072], [0074]	1-4, 5/1-3, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99
A	US 2014/0213471 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 31 July 2014; paragraphs [0003], [0010]-[0011], [0045], [0082])	1-4, 5/1-3, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99
A	US 2012/0040349 A1 (LODE PV, et al.) 16 February 2012; paragraphs [0030]-[0034], [0054]	1-4, 5/1-3, 43-47, 48/45-47, 83-87, 88/83-87, 89/83-87, 90/83-87, 91-97, 98/91-97, 99

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 2017 (03.06.2017)	22 JUN 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: LEE JOHNSON
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing *(day/month/year)*	16 NOV 2017
Applicant's or agent's file reference: 38075-729601	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/US 17/37682	International filing date *(day/month/year)*: 15 June 2017 (15.06.2017)
Applicant: CALIFORNIA INSTITUTE OF TECHNOLOGY	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **22 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 38075-729601	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 17/37682	International filing date *(day/month/year)* 15 June 2017 (15.06.2017)	(Earliest) Priority Date *(day/month/year)* 17 June 2016 (17.06.2016)
Applicant CALIFORNIA INSTITUTE OF TECHNOLOGY		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 6

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2617

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/37682

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

---------Please see continuation in first extra sheet----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2618

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/37682

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, C12P 19/34 (2017.01)
CPC - C12Q 1/6844, C12N 15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0072211 A1 (NEWTON et al.,) 29 March 2007 (29.03.2007) Claim 1; Claim 3; para [0007]	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 August 2017	16 NOV 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/37682

Continuation of Box No III Observations where unity of invention is lacking

Group I: Claims 1-6, drawn to a method of detecting an analyte comprising generating a kinetic signature from a signal generated during an amplification reaction .

Group II: Claims 7-17, 27-31, drawn to methods of detecting target analytes comprising performing an amplification reaction by using a primer set that comprises at least one limiting primer.

Group III: Claims 18-26, drawn to a method of detecting an analyte comprising performing a amplification on a sample by using a primer set that comprises primer pairs with different annealing temperatures.

Group IV: Claims 32-33, drawn to a method of determining an amount of nucleic acid analyte, comprising generating transformed signal by transforming a signal generated during an amplification reaction into a frequency domain.

The inventions listed as Groups I, II, III, and IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires generating a kinetic signature from a signal generated during an amplification reaction, not required by Groups II, III, and IV.

Group II requires the detection of target analytes by using a set of primers comprising at least one limiting primers, not required by Groups I, III, and IV.

Group III requires the detection of target analytes by using a set of primers by using a primer set that comprises primer pairs with different annealing temperatures, not required by Groups I, II, and IV.

Group IV requires generating transformed signal by transforming a signal generated during an amplification reaction into a frequency domain, not required by Groups I, II, and III.

Common Technical Features

The feature shared by Groups I, II, III, and IV is a method of detecting a nucleic acid analyte in a sample, the method comprising:
performing an amplification reaction on the sample;
measuring a signal generated during the amplification reaction; and
detecting the presence or absence of the target nucleic acid analyte in the sample.

Another feature shared by Groups I and IV is the method step of: comparing a test value to a reference value and determining whether the test value corresponds with the reference value.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2007/0072211 A1 to Newton et al., (hereinafter 'Newton').

Newton teaches a method of detecting the presence or absence of a target nucleic acid analyte in a sample (para [0011] "the invention comprises methods for identifying one or more nucleic acid targets in a sample"), the method comprising:
performing an amplification reaction on the sample (para [0011] "Such methods comprise: performing asymmetric kinetic PCR in a reaction mixture containing one or more labeled 5'-nuclease probes and one or more labeled hybridization probes");
measuring a signal generated during the amplification reaction (para [0011] "monitoring one or more growth curves from the kinetic PCR"); and
detecting the presence or absence of the target nucleic acid analyte in the sample (para [0011] "thus, identifying the nucleic acid targets in the sample").

Newton further teaches the method step of: comparing a test value to a reference value and determining whether the test value corresponds with the reference value (para [0011] "correlating the melting curve or annealing curve, thus produced, to standard melting or annealing curves of completely or partially complementary probes of known nucleic acid targets or the same probe tested against a known sample, thus, identifying the nucleic acid targets in the sample").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

-----Please see continuation in next extra sheet----------

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2620

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/37682

Continuation of Box No III Observations where unity of invention is lacking

Another feature shared by Group II and III is the detection of target analytes by using a set of primers.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2010/0233686 A1 to Higuchi et al., (hereinafter 'Higuchi').

Higuchi teaches detection of target analytes by using a set of primers (para [0012] "The present invention is a method for amplification, detection and quantification of one or more target nucleic acids in a single sample container comprising the steps of contacting a sample, suspected of containing one or more target nucleic acids, with at least one set of oligonucleotides, each oligonucleotide within the set labeled with the same one or more reporter moieties"; para [0042] "amplification of several target nucleic acid sequences in the same reaction vessel. Each tube contains one or more sets of probes, each set labeled with the same fluorescent reporter. However, within the set, each probe is characterized by a unique melting temperature with its target nucleic acid. For the maximum multiplex ability, several sets of probes are present in the same reaction vessel"; para [0057] "In some embodiments, the present invention involves asymmetric PCR. In an asymmetric PCR mixture, one of the amplification primers is present in greater amount than the other primer. The primers are referred to as "excess primer" and "limiting primer" respectively. The nucleic acid strands resulting from the extension of these primers are referred to as "excess strand" and "limiting strand" respectively").

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I, II, III, and IV therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2621

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: LEE JOHNSON
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 16 NOV 2017

Applicant's or agent's file reference
38075-729601

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 17/37682	15 June 2017 (15.06.2017)	17 June 2016 (17.06.2016)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68, C12P 19/34 (2017.01)
CPC - C12Q 1/6844, C12N 15/10

Applicant CALIFORNIA INSTITUTE OF TECHNOLOGY

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [x] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	22 August 2017	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 17/37682

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2623

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 17/37682

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

☐ paid additional fees.

☐ paid additional fees under protest and, where applicable, the protest fee.

☐ paid additional fees under protest but the applicable protest fee was not paid.

☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-6, drawn to a method of detecting an analyte comprising generating a kinetic signature from a signal generated during an amplification reaction .

Group II: Claims 7-17, 27-31, drawn to methods of detecting target analytes comprising performing an amplification reaction by using a primer set that comprises at least one limiting primer.

Group III: Claims 18-26, drawn to a method of detecting an analyte comprising performing a amplification on a sample by using a primer set that comprises primer pairs with different annealing temperatures.

Group IV: Claims 32-33, drawn to a method of determining an amount of nucleic acid analyte, comprising generating transformed signal by transforming a signal generated during an amplification reaction into a frequency domain.

The inventions listed as Groups I, II, III, and IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires generating a kinetic signature from a signal generated during an amplification reaction, not required by Groups II, III, and IV.

Group II requires the detection of target analytes by using a set of primers comprising at least one limiting primers, not required by Groups I, III, and IV.

Group III requires the detection of target analytes by using a set of primers by using a primer set that comprises primer pairs with different annealing temperatures, not required by Groups I, II, and IV.

Group IV requires generating transformed signal by transforming a signal generated during an amplification reaction into a frequency domain, not required by Groups I, II, and III.

----------Please see continuation in first supplemental box----------

4. Consequently, this opinion has been established in respect of the following parts of the international application:

☐ all parts.

☒ the parts relating to claims Nos. 1-6

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2624

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 17/37682

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-6	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-6	NO
Industrial applicability (IA)	Claims	1-6	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-6 lack an inventive step under PCT Article 33(3) as being obvious over US 2007/0072211 A1 to Newton et al., (hereinafter 'Newton').

Regarding Claim 1, Newton teaches a method of detecting the presence or absence of a target nucleic acid analyte in a sample (Claim 1 - 'A method for identifying one or more nucleic acid targets in a sample,'), the method comprising:
a) performing an amplification reaction on the sample (Claim 1 - 'the method comprising: a) performing an asymmetric kinetic PCR in a reaction mixture');
b) measuring a signal generated during the amplification reaction and generating a kinetic signature from the measured signal (Claim 1 - 'b) monitoring one or more growth curves from the kinetic PCR'); and
c) comparing the generated kinetic signature to a reference signature and determining whether the generated kinetic signature corresponds with the reference signature, thereby detecting the presence or absence of the target nucleic acid analyte in the sample (Claim 1 - 'e) correlating the melting curve or annealing curve of step d) to a melting or annealing curve of a completely complementary probe of one or more known nucleic acid targets, thus, identifying the one or more nucleic acid targets in the sample.'). Newton does not state expressly that the growth curve from kinetic PCR is a kinetic signature. However, since Newton teaches the kinetic PCR is aimed at differentiating between closely related nucleic acid molecules (para [0007] - 'For example, many diseases and infections are caused by a number of, often related, pathogens. While the disease symptoms may present as similar, it can be of utmost importance to determine the actual causative pathogen in order to present an effective treatment. Not only is this true in terms of differentiation between different infectious species, but is also true, and can be even more difficult to resolve, when trying to discriminate between closely related agents, e.g., different strains of a pathogen such as the subtypes of hepatitis C virus (HCV).'), it would have been obvious to one of ordinary skill in the art that the growth curve from the kinetic PCR of Newton represents a kinetic signature enabling specific identification of a nucleic acid target.

Regarding Claim 2, Newton teaches the method of claim 1, wherein the kinetic signature comprises a signal selected from an electromagnetic signal, a wavelength, and a fluorescence emission signal (Claim 1 - 'c) monitoring one or more fluorescent signals generated from the one or more labeled hybridization probes thereby producing a melting curve or annealing curve').

Regarding Claim 3, Newton teaches the method of claim 1, wherein the kinetic signature is plotted as a curve (Claim 1 - 'd) monitoring one or more fluorescent signals generated from the one or more labeled hybridization probes thereby producing a melting curve or annealing curve').

Regarding Claim 4, Newton teaches the method of claim 1 wherein the reference signature corresponds with a kinetic signature generated by amplification of the target nucleic acid analyte under one or more members of a set of constrained amplification parameters, the set of constrained amplification parameters comprising any combination of: (i) a range of forward and/or reverse primer concentrations; (ii) a range of polymerase concentrations; (iii) polymerase type; (iv) a range of nucleotide concentrations; (v) chromophore concentration; and (vi) chromophore type; (vii) a number of thermocycles; (viii) rate of thermocycling; (ix) one or more thermocycle stage temperatures; and (x) one or more thermocycle stage time lengths; (xi) a range of target-specific molecular probes; (xii) primers associated with one or more targetspecific probes; and (xiii) a concentration of one or more enzyme cofactors (Claim 1 - 'e) correlating the melting curve or annealing curve of step d) to a melting or annealing curve of a completely complementary probe of one or more known nucleic acid targets'; Claim 3 - 'The method of claim 1, wherein the asymmetric kinetic PCR comprises a first primer and at least a second primer, wherein the amount of the first primer is greater than the amount of the second primer and wherein the hybridization probe is present in an equal or greater amount than the second primer.').

----------Please see continuation in second supplemental box----------

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2625

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. IV.3. Lack of unity of invention

Common Technical Features

The feature shared by Groups I, II, III, and IV is a method of detecting a nucleic acid analyte in a sample, the method comprising:
performing an amplification reaction on the sample;
measuring a signal generated during the amplification reaction; and
detecting the presence or absence of the target nucleic acid analyte in the sample.

Another feature shared by Groups I and IV is the method step of: comparing a test value to a reference value and determining whether the test value corresponds with the reference value.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2007/0072211 A1 to Newton et al., (hereinafter 'Newton').

Newton teaches a method of detecting the presence or absence of a target nucleic acid analyte in a sample (para [0011] "the invention comprises methods for identifying one or more nucleic acid targets in a sample"), the method comprising:
performing an amplification reaction on the sample (para [0011] "Such methods comprise: performing asymmetric kinetic PCR in a reaction mixture containing one or more labeled 5'-nuclease probes and one or more labeled hybridization probes");
measuring a signal generated during the amplification reaction (para [0011] "monitoring one or more growth curves from the kinetic PCR"); and
detecting the presence or absence of the target nucleic acid analyte in the sample (para [0011] "thus, identifying the nucleic acid targets in the sample").

Newton further teaches the method step of: comparing a test value to a reference value and determining whether the test value corresponds with the reference value (para [0011] "correlating the melting curve or annealing curve, thus produced, to standard melting or annealing curves of completely or partially complementary probes of known nucleic acid targets or the same probe tested against a known sample, thus, identifying the nucleic acid targets in the sample").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Another feature shared by Group II and III is the detection of target analytes by using a set of primers.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2010/0233686 A1 to Higuchi et al., (hereinafter 'Higuchi').

Higuchi teaches detection of target analytes by using a set of primers (para [0012] "The present invention is a method for amplification, detection and quantification of one or more target nucleic acids in a single sample container comprising the steps of contacting a sample, suspected of containing one or more target nucleic acids, with at least one set of oligonucleotides, each oligonucleotide within the set labeled with the same one or more reporter moieties"; para [0042] "amplification of several target nucleic acid sequences in the same reaction vessel. Each tube contains one or more sets of probes, each set labeled with the same fluorescent reporter. However, within the set, each probe is characterized by a unique melting temperature with its target nucleic acid. For the maximum multiplex ability, several sets of probes are present in the same reaction vessel"; para [0057] "In some embodiments, the present invention involves asymmetric PCR. In an asymmetric PCR mixture, one of the amplification primers is present in greater amount than the other primer. The primers are referred to as "excess primer" and "limiting primer" respectively. The nucleic acid strands resulting from the extension of these primers are referred to as "excess strand" and "limiting strand" respectively").

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I, II, III, and IV therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2626

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No V2 Citations and Explanations

Regarding Claim 5, Newton teaches the method of claim 4, wherein the amplification reaction is carried out within one or more of the constrained amplification parameters (Claim 3 - 'The method of claim 1, wherein the asymmetric kinetic PCR comprises a first primer and at least a second primer, wherein the amount of the first primer is greater than the amount of the second primer and wherein the hybridization probe is present in an equal or greater amount than the second primer.').

Regarding Claim 6, Newton teaches the method of claim 3, wherein the at least one of an exponential region, a linear region, and a plateau region of the curve occurs over at least 4 thermocycles or over at least 8 thermocycles (para [0068] - 'FIG. 3 provides growth curves generated during an asymmetric kPCR'; and data shown in Figure 3). Newton does not state expressly at least 4 thermocycles or over at least 8 thermocycles. However, data shown in Figure 3 illustrates at least 4 thermocycles or over at least 8 thermocycles.

Claims 1-6 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2627

SEARCH HISTORY

Application Number	PCT/US 17/37682
Search Conducted By	AEM
Search Approved By	PP

CPC/IPC Classifications Searched	IPC(8) - C12 Q1/68, C12P 19/34 (2017.01) CPC - C12Q 1/6844, C12N 15/10
Date Conducted	22 August 2017 (22.08.2017)

Documentation Searched	Keyword limited; search terms below
Search Terms Used	Amplification, kinetic PCR or polymerase chain reaction, plot or curve, primer, electromagnetic or wavelength or fluorescence, reference or control or standard, primer set, 2 forward primers, 2 reverse primers, nested primer, limiting primer, annealing temperature, thermocycling or temperature cycle
Date Conducted	22 August 2017 (22.08.2017)

Electronic Database Searched	Patbase
Files Searched	Full-text: AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO Bibliographic: (Europe) AT BA BE BG CH CS CY CZ DD DK EE ES FI GE GR HR HU IE IS IT LT LU LV MC MD MT NL NO PL PT RO RS SE SI SK SM TR UA YU (Asia) EA GC HK ID IL IN KZ MN MY PH RU SG SU TH TJ TW UZ VN (North America) CA CR CU DO GT HN MX NI PA SV TT (South America) AR BR CL CO EC PE UY (Australasia) AU NZ (Africa) AP DZ EG KE MA MW OA ZA ZM ZW
Date Conducted	22 August 2017 (22.08.2017)
Search Logic: Classification based search	

Search 1: IC=(C12Q1/68 OR C12P19/34) (Results 128654)
Search 2: CPC=(C12Q1/6844 OR C12N15/10 OR C12Q1/68 OR C12Q1/686 OR C12Q1/6853 OR G01N21/6486 OR G01N2021/6439) (Results 14465)
Search 3: 1 OR 2 (Results 131488)
Search 4: 3 AND FT=(KINETIC W1 PCR) (Results 509)
Search 5: 4 AND FT=(LIMIT* W1 PRIMER%) (Results 9)
Search 6: 4 AND FT=(CONSTRAINED NEAR (PCR OR AMPLIFICATION OR PARAMETER%)) (Results 0)
Search 7: 3 AND FT=(CONSTRAINED NEAR (PCR OR AMPLIFICATION OR PARAMETER%)) (Results 79)
Search 8: 7 AND FT=(KINETIC NEAR PCR) (Results 0)
Search 9: 7 AND FT=(ASYMMETRIC NEAR (PCR OR AMPLIFICATION)) (Results 6)
Search 10: 4 AND FT=(NESTED NEAR PCR) (Results 159)
Search 11: 10 AND FT=(TWO NEAR (REVERSE OR FORWARD)) (Results 6)
Search 12: 11 NOT (5 OR 9) (Results 6)
Search 13: 4 AND FT=(PCR NEAR SIGNATURE) (Results 3)

Keyword only search

Search 14: FT=(KINETIC W1 PCR) (Results 639)
Search 15: 14 AND FT=(LIMIT* W1 PRIMER%) (Results 9)
Search 16: 15 NOT (5 OR 9 OR 13) (Results 0)
Search 17: 14 AND FT=(CONSTRAINED NEAR (PCR OR AMPLIFICATION OR PARAMETER%)) (Results 0)
Search 18: FT=(CONSTRAINED NEAR (PCR OR AMPLIFICATION OR PARAMETER%)) (Results 2434)
Search 19: 18 AND FT=(KINETIC NEAR PCR) (Results 0)
Search 20: 18 AND FT=(ASYMMETRIC NEAR (PCR OR AMPLIFICATION)) (Results 6)
Search 21: 20 NOT (5 OR 9 OR 13) (Results 0)
Search 22: FT=(NESTED NEAR PCR) (Results 9272)
Search 23: 22 AND FT=(TWO NEAR (REVERSE OR FORWARD)) (Results 579)
Search 24: 23 AND FT=(KINETIC W1 PCR) (Results 7)
Search 25: FT=(PCR NEAR SIGNATURE) (Results 604)
Search 26: 25 AND FT=(ASYMMETRIC NEAR (PCR OR AMPLIFICATION)) (Results 10)
Search 27: 26 NOT (5 OR 9 OR 13) (Results 9)

Forward and backward search

Search 28: PN=(US2007072211 OR US2010233686 OR US8367325) (Results 3)
Search 29: CTF 28 OR CTB 28 (Results 194)
Search 30: 29 AND FT=(KINETIC W1 PCR) (Results 6)
Search 31: 30 NOT (5 OR 9 OR 13) (Results 2)

Electronic Database Searched	Google
Files Searched	Patents
Date Conducted	22 August 2017 (22.08.2017)

Search Logic:

kinetic PCR

About 67,500 results (0.34 seconds)

kinetic PCR signature limiting primer

About 12,700 results (0.34 seconds)

constrained PCR amplification parameters

About 36,900 results (0.38 seconds)

constrained PCR amplification parameters kinetic PCR

About 16,100 results (0.61 seconds)

constrained PCR amplification kinetic PCR limiting primer

About 16,400 results (0.52 seconds)

constrained nested PCR amplification kinetic PCR limiting primer

About 2,650 results (0.52 seconds)

constrained nested PCR amplification kinetic PCR signature limiting primer

About 554 results (0.47 seconds)

kinetic PCR with 2 reverse primers

About 65,000 results (0.93 seconds)

kinetic nested PCR with 2 reverse primers

About 9,020 results (0.48 seconds)

asymmetric kinetic nested PCR with 2 reverse primers

About 1,820 results (0.52 seconds)

asymmetric kinetic nested PCR with 2 forward primers

About 1,290 results (0.58 seconds)

Electronic Database Searched	Google
Files Searched	Scholar
Date Conducted	22 August 2017 (22.08.2017)

Search Logic:

kinetic PCR
About 466,000 results (0.11 sec)

kinetic PCR signature limiting primer
About 21,700 results (0.07 sec)

constrained PCR amplification parameters
About 45,900 results (0.09 sec)

constrained PCR amplification parameters kinetic PCR
About 21,400 results (0.09 sec)

constrained PCR amplification kinetic PCR limiting primer
About 18,800 results (0.11 sec)

constrained nested PCR amplification kinetic PCR limiting primer
About 4,580 results (0.15 sec)

constrained nested PCR amplification kinetic PCR signature limiting primer
About 1,450 results (0.07 sec)

kinetic pcr with 2 reverse primers
About 235,000 results (0.09 sec)

kinetic nested pcr with 2 reverse primers
About 21,800 results (0.15 sec)

asymmetric kinetic nested pcr with 2 reverse primers
About 3,340 results (0.06 sec)

asymmetric kinetic nested pcr with 2 forward primers
About 2,050 results (0.08 sec)

==========

Supplemental Search

Electronic Database Searched	PatBase
Files Searched	Full-text: AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO Bibliographic: (Europe) AT BA BE BG CH CS CY CZ DD DK EE ES FI GE GR HR HU IE IS IT LT LU LV MC MD MT NL NO PL PT RO RS SE SI SK SM TR UA YU (Asia) EA GC HK ID IL IN KZ MN MY PH RU SG SU TH TJ TW UZ VN (North America) CA CR CU DO GT HN MX NI PA SV TT (South America) AR BR CL CO EC PE UY (Australasia) AU NZ (Africa) AP DZ EG KE MA MW OA ZA ZM ZW
Date Conducted	30 October 2017
Search Logic:	
1) IC=(C12Q1/68 or C12P19/34) (130470) 2) CPC=(C12Q1/6844 or C12N15/10 or C12Q1/68 or C12Q1/686 or C12Q1/6853 or G01N21/6486 or G01N2021/6439) (15934) 3) 1 or 2 (133809) 4) (PCR or polymerase chain reaction) and amplif* (168197) 5) 4 and kinetic (16544) 6) 5 and (plot or curve) (9932) 7) TAC=(detect* w5 (nucleic acid or DNA or RNA or nucleotide or oligonucleotide or polynucleotide)) (31528) 8) 7 and 6 (1344) 9) 3 and 8 (1120) 10) kinetic signature (29) 11) 10 and 8 (6) 12) 3 and 11 (6)	

Electronic Acknowledgement Receipt	
EFS ID:	32143378
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	J Kyle Bruner/Judy Wan
Filer Authorized By:	J Kyle Bruner
Attorney Docket Number:	38075-716.301
Receipt Date:	23-MAR-2018
Filing Date:	05-AUG-2014
Time Stamp:	15:26:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		IDS-38075-716-301-03-23-18.pdf	286295 7e8fbbda31655fe82156215e191d1195b1f7a688	yes	8

	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	4	
	Information Disclosure Statement (IDS) Form (SB08)		5	8	
Warnings:					
Information:					
2	Foreign Reference	WO2017173035A1.PDF	2525583 5d8df6d2adf9a5ea0672cf50f162771e50c622d9	no	46
Warnings:					
Information:					
3	Non Patent Literature	GANDELMAN-NOVEL-BIOLUMINESCENT-2010.pdf	840908 c62121b6479dc5b53b7bcf548c1736075c7533fb	no	14
Warnings:					
Information:					
4	Non Patent Literature	OA-02-08-18-US15677772.PDF	641624 c6c59fbe29d0eeb51fa44a70df0659470fc322ef	no	16
Warnings:					
Information:					
5	Non Patent Literature	OA-02-22-18-US15623974.PDF	145707 31d6b2384e567f395ecd33c312e03374a4549fc0	no	6
Warnings:					
Information:					
6	Non Patent Literature	USApp-15914356.pdf	4503303 cec59fb478ca17899e62fe004d8b0a56aeb0b8dd	no	81
Warnings:					
Information:					
7	Non Patent Literature	ISR-WO-11-16-17-US1737682.pdf	1047446 4593a50ed9bcd7a6df9c082fbd2b9b42f3bb3dbc	no	17
Warnings:					

Information:	
Total Files Size (in bytes):	9990866

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	KARTALOV; Emil et al.	Group Art Unit:	1631
Serial Number:	14/451,876	Examiner:	RUSSELL SCOTT NEGIN
Filing or 371 (c) Date:	2014-08-05	**CONFIRMATION NO:**	**1065**
Title:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS		

FILED ELECTRONICALLY ON: March 23, 2018

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2636

A. ☒ *37 CFR § 1.97 (b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☐ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☒ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☐ *37 CFR § 1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☐ a fee of $240.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR § 1.97 (d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of $240.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97 (e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2637

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☒ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. ☐ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☐ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2638

I. ☒ *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of $0.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No.38075-716.301).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: March 23, 2018

By: /Kyle Bruner/
Kyle Bruner, Patent Agent
Register No.: 75,716

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2639

Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-9366632	06-14-2016	LINK; Darren Roy et al.	
	002	US-9441266	09-13-2016	LARSON; Jonathan William et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		None				

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
		None	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 3 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Kyle Bruner/	Date (YYYY-MM-DD)	2018-04-26
Name/Print	Kyle Bruner	Registration Number	75,716

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	32460025
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	Lee Alan Johnson/Judy Wan
Filer Authorized By:	Lee Alan Johnson
Attorney Docket Number:	38075-716.301
Receipt Date:	26-APR-2018
Filing Date:	05-AUG-2014
Time Stamp:	19:21:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		IDS-38075-716-301-04-26-18.pdf	283639 29da99bbe97e09157aefb8795d1dde59cf8c43db	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	4
	Information Disclosure Statement (IDS) Form (SB08)	5	8
Warnings:			
Information:			
	Total Files Size (in bytes):	283639	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	KARTALOV; Emil et al.	Group Art Unit:	1631
Serial Number:	14/451,876	Examiner:	RUSSELL SCOTT NEGIN
Filing or 371 (c) Date:	2014-08-05	**CONFIRMATION NO:**	**1065**
Title:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS		

FILED ELECTRONICALLY ON: April 26, 2018

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2646

A. ☒ *37 CFR § 1.97 (b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☐ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☒ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☐ *37 CFR § 1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☐ a fee of $240.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR § 1.97 (d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of $240.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97 (e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2647

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

☐ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. ☐ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☐ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2648

I. ☒ *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of $0.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No.38075-716.301).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: April 26, 2018

By: /Kyle Bruner/
Kyle Bruner, Patent Agent
Register No.: 75,716

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2649

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/451,876	Filing Date 08/05/2014	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	06/12/2018	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 9	Minus	** 74	= 0	X $50 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***14	= 0	X $230 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
MOLIKI MAY

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

21971 7590 06/14/2018

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CALIFORNIA 94304-1050
UNITED STATES OF AMERICA

EXAMINER
NEGIN, RUSSELL SCOTT

ART UNIT	PAPER NUMBER
1631	

DATE MAILED: 06/14/2018

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/451,876	08/05/2014	Emil Kartalov	38075-716.301	1065

TITLE OF INVENTION: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$500	$0.00	$0.00	$500	09/14/2018

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2651

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

21971 7590 06/14/2018

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CALIFORNIA 94304-1050
UNITED STATES OF AMERICA

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/451,876	08/05/2014	Emil Kartalov	38075-716.301	1065

TITLE OF INVENTION: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$500	$0.00	$0.00	$500	09/14/2018

EXAMINER	ART UNIT	CLASS-SUBCLASS
NEGIN, RUSSELL SCOTT	1631	702-019000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____
2_____
3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2652

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/451,876	08/05/2014	Emil Kartalov	38075-716.301	1065

21971 7590 06/14/2018

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CALIFORNIA 94304-1050
UNITED STATES OF AMERICA

EXAMINER	
NEGIN, RUSSELL SCOTT	
ART UNIT	**PAPER NUMBER**
1631	

DATE MAILED: 06/14/2018

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2653

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	**Application No.** 14/451,876	**Applicant(s)** Kartalov et al.	
	Examiner RUSSELL S NEGIN	**Art Unit** 1631	**AIA Status** No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the communication of 6 November 2017.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file areply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 11/16/17; 3/23/18; 4/26/18.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1631

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2655

Continuation of 3. The allowed claim(s) is/are: 49,81-83,87-88 and 93-95

CONTINUED EXAMINATION UNDER 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 6 November 2017 has been entered.

INFORMATION DISCLOSURE STATEMENTS

The IDSs filed on 11/16/2017, 3/23/2018, and 4/26/2018 have been considered.

ELECTION/RESTRICTIONS

Claim 49 is allowable. The species election requirement , as set forth in the Office action mailed on 12 May 2016 , has been reconsidered in view of the allowability of claims to the elected invention pursuant to MPEP § 821.04(a). **The species election requirement is hereby withdrawn as to any claim that requires all the limitations of an allowable claim.** Specifically, the species election requirement is withdrawn. Claim 82, directed to a non-elected species no longer withdrawn from consideration because the claim(s) requires all the limitations of an allowable claim. However, claims 1-2, 4-5, 9, 13, 17-19, 22, 27-29, 34-35, 37-39, 42-43, 45, and 47, directed to a non-elected Group remain withdrawn from consideration because these claims do not require all the limitations of an allowable claim. The elections were made on 3 November 2016 were made **without** traverse.

In view of the above noted withdrawal of the restriction requirement, applicant is advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

Once a restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See In re Ziegler, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

The application has been amended as follows:

In the claims:

Cancel claims 1-2, 4-5, 9, 13, 17-19, 22, 27-29, 34-35, 37-39, 42-43, 45, and 47.

REASONS FOR ALLOWANCE

The claims do not recite any judicial exceptions. The claims recite a new and non-obvious assay to detect the presence/absence pattern of seven different analytes using non-degenerate hybridization techniques.

CONCLUSION

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Russell Negin, whose telephone number is (571) 272-1083. This Examiner can normally be reached from Monday through Thursday from 10:30 am to 5 pm and variable hours on Fridays.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's Supervisor, Peter Paras, Supervisory Patent Examiner, can be reached at (571) 272-4517.

Information regarding the status of the application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1631
12 June 2018

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/451,876	Kartalov et al.
	Examiner	Art Unit
	RUSSELL S NEGIN	1631

CPC - Searched*		
Symbol	Date	Examiner
G06F 19/12 with modified terms	06/10/2018	RSN
G06F 19/18 with modified terms	06/10/2018	RSN
G06F 19/34 with modified terms	06/10/2018	RSN
G06F 19/3437 with modified terms	06/10/2018	RSN

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
see attached search history printouts	06/10/2018	RSN
EAST prior art search	06/10/2018	RSN
EAST inventor search on all inventors	06/10/2018	RSN
EAST assignee search	06/10/2018	RSN
PALM inventor search on all inventors	06/10/2018	RSN

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	see attached interference search history printouts	06/10/2018	RSN

/RUSSELL S NEGIN/ Primary Examiner.Art Unit 1631	

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2660

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/451,876	Kartalov et al.
	Examiner	Art Unit
	RUSSELL S NEGIN	1631

/RUSSELL S NEGIN/ Primary Examiner.Art Unit 1631	

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2661

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/451,876	Kartalov et al.
	Examiner	**Art Unit**
	RUSSELL S NEGIN	1631

CPC							
Symbol						**Type**	**Version**
G06F	/	19	/	20		F	2013-01-01
G06F	/	19	/	12		I	2013-01-01
C12Q	/	1	/	68		I	2013-01-01
G01N	/	21	/	6486		I	2013-01-01
C12Q	/	1	/	6825		I	2013-01-01
C12Q	/	1	/	6851		I	2013-01-01
G06F	/	19	/	24		A	2013-01-01
G06F	/	17	/	10		A	2013-01-01
G06F	/	17	/	11		A	2013-01-01
Y02A	/	50	/	53		A	2018-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
C12Q	/	1	/	6851	I	1	1	2013-01-01
C12Q	/	2537	/	101	A	1	2	2013-01-01
C12Q	/	2537	/	143	A	1	3	2013-01-01
C12Q	/	2537	/	165	A	1	4	2013-01-01
C12Q	/	2563	/	107	A	1	5	2013-01-01

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1631	10 June 2018	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	49	1

U.S. Patent and Trademark Office — Part of Paper No.: 20180610

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2662

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/451,876	Kartalov et al.
	Examiner	**Art Unit**
	RUSSELL S NEGIN	1631

INTERNATIONAL CLASSIFICATION					
CLAIMED					
G01N	/	33	/	48	
G01N	/	33	/	50	

NON-CLAIMED					
	/		/		

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**
702	19

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1631	10 June 2018	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	49	1

U.S. Patent and Trademark Office — Part of Paper No.: 20180610

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2663

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/451,876	Kartalov et al.
	Examiner	**Art Unit**
	RUSSELL S NEGIN	1631

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	49														
2	81														
3	82														
4	83														
5	87														
6	88														
7	93														
8	94														
9	95														

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1631	10 June 2018	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	49	1

U.S. Patent and Trademark Office — Part of Paper No.: 20180610

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2664

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

BIB DATA SHEET

CONFIRMATION NO. 1065

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/451,876	08/05/2014 RULE	702	1631	38075-716.301

APPLICANTS

California Institute of Technology, Pasadena, CA;

INVENTORS

Emil Kartalov, Pasadena, CA;
Aditya Rajagopal, Irvine, CA;
Axel Scherer, Barnard, VT;

**** CONTINUING DATA ***************************

This application is a CON of 13/756,760 02/01/2013 PAT 8838394
which claims benefit of 61/703,093 09/19/2012
and claims benefit of 61/594,480 02/03/2012

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
08/14/2014

Foreign Priority claimed	❑ Yes ☑ No		STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	❑ Yes ☑ No	❑ Met after Allowance				
Verified and Acknowledged	/RUSSELL SCOTT NEGIN/ Examiner's Signature	/RSN/ Initials	CA	8	27	3

ADDRESS

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050
UNITED STATES

TITLE

SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

FILING FEE RECEIVED		
1800	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	❑ All Fees ❑ 1.16 Fees (Filing) ❑ 1.17 Fees (Processing Ext. of time) ❑ 1.18 Fees (Issue) ❑ Other _____ ❑ Credit

BIB (Rev. 05/07).

/RSN/ 6/10/2018

EAST Search History

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L29	182814	polynucleotide	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:15
L30	247382	hybridization	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:15
L31	128340	l29 and l30	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:15
L32	50789	fluorophores	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:16
L33	18300	l31 and l32	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:16
L34	267598	cumulative	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:16
L35	2423	l33 and l34	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:16
L36	37	"non-degenerately"	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:17
L37	101390	immobilization	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:17
L38	817	l35 and l37	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:17
L39	184005	mass adj spectrometry	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:17
L40	968	l35 and l39	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:17
L41	72273	analytes	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:18
L42	0	l40m and l41	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:18
L43	235	l40 and l41	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:18
L44	235	l30 and l43	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:19
L45	3707608	computer	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:19
L46	227	l44 and l45	US-PGPUB; USPAT	OR	OFF	2018/06/10 09:20

6/ 10/ 2018 9:20:26 AM
C:\ Users\ rnegin\ Documents\ EAST\ Workspaces\ 14451876.wsp

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2666

/RSN/ 6/10/2018

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6	(("9366632") or ("9441266") or ("20070072211") or ("20100233686") or ("20130040841") or ("20140171341")).PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2018/06/10 08:56
L2	83	kartalov.in. near emil.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L3	127	rajagopal.in. near aditya.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L4	750	scherer.in. near axel.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L5	4883	"california institute of technology".as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L6	5672	g06f19/12.cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L7	14025	g06f19/18.cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L8	2581	g06f19/34.cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L9	4535	g06f19/3437.cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L10	809	l2 l3 l4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:07
L11	24862	l6 l7 l8 l9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:08
L12	9	l10 and l11	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:08

L13	54821	fluorophores	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:08
L14	24	hybrization near probes	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:08
L15	49239	hybridization near probes	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:08
L16	7597	l13 and l15	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:09
L17	197816	polynucleotides	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:09
L18	4674	l16 and l17	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:09
L19	94	l11 and l18	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:09
L20	176504	analyte	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:09
L21	1426	l18 and l20	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:09
L22	326258	cumulative	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:10
L23	220	l21 and l22	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:10
L24	47	"non-degenerately"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:10
L25	6277784	signals	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:10
L26	1232	l21 and l25	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:10
L27	229075	mass adj spectrometry	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	OFF	2018/06/10 09:10

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2668

			DERWENT			
L28	449	l26 and l27	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	OFF	2018/06/10 09:11

6/10/2018 9:11:44 AM
C:\Users\rnegin\Documents\EAST\Workspaces\14451876.wsp

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2669

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		None			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
/RSN/	001	WO-2013116780-A1	08-08-2013	CALIFORNIA INST OF TECHN [US], et al.		☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	06/10/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
/RSN/	001	Holland, et al., Detection of specific polymerase chain reaction product by utilizing the 5´ to 3´ exonuclease activity of Thermus aquaticus DNA polymerase. PNAS (USA) 88:7276-7280, 1991.	☐
/RSN/	002	International search report and written report dated 06/22/2017 for PCT Application No. PCT/US2017/24933.	☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	06/10/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

14451876
Google Scholar Search
/RSN/ 6/10/2018

polynucleotides non-degenerate
analytes non-degenerate
(DNA or analytes) non-degenerate combination
(DNA or analytes) non-degenerate hybridization
(DNA or analytes) non-degenerate "mass spectrometry"
(DNA or analytes) non-degenerate cumulative
(DNA or analytes) non-degenerate immobilization

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2672

Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
/RSN/	001	US-9366632	06-14-2016	LINK; Darren Roy et al.	
/RSN/	002	US-9441266	09-13-2016	LARSON; Jonathan William et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		None				

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	06/10/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
		None	

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	06/10/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
/RSN/	001	US-20070072211	03-29-2007	NEWTON; Nick et al.	
/RSN/	002	US-20100233686	09-16-2010	HIGUCHI; Russell et al.	
/RSN/	003	US-20130040841	02-14-2013	SAXONOV; Serge et al.	
/RSN/	004	US-20140171341	06-19-2014	JOUVENOT; Yann et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
/RSN/	001	WO-2017173035-A1	10-05-2017	CHROMACODE INC [US]		☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	06/10/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 4

Complete if Known	
Application Number	14451876
Filing Date	08-05-2014
First Named Inventor	KARTALOV; Emil
Art Unit	1631
Examiner Name	RUSSELL SCOTT NEGIN
Attorney Docket Number	38075-716.301

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
/RSN/	001	Co-pending US patent application No. US15/914,356, filed on 03-07-2018.	☐
/RSN/	002	GANDELMAN, et al., Novel Bioluminescent Quantitative Detection of Nucleic Acid Amplification in Real-Time, PLoS One, 2010, 5(11):e14155, 14 pages.	☐
/RSN/	003	International Search Report and Written Opinion dated 11/16/2017 for International PCT Patent Application No. PCT/US2017/37682.	☐
/RSN/	004	U.S. Serial No. 15/623,974 Office Action dated February 22, 2018.	☐
/RSN/	005	U.S. Serial No. 15/677,772 Office Action dated February 08, 2018.	☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	06/10/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/451,876	08/05/2014	Emil Kartalov	38075-716.301	1065

21971 7590 06/20/2018

WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER	
NEGIN, RUSSELL SCOTT	

ART UNIT	PAPER NUMBER
1631	

NOTIFICATION DATE	DELIVERY MODE
06/20/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

PTOL-90A (Rev. 04/07)

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Application No. : 14451876
Applicant : Kartalov
Filing Date : 08/05/2014
Date Mailed : 06/20/2018

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given two (2) months from the mail date of this Notice within which to respond. This time period for reply is extendable under 37 CFR 1.136(a) for only TWO additional MONTHS.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED.

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Chris Harmon/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 14451876

NON-COMPLIANT ILLUSTRATIONS IN SPECIFICATION OR CLAIMS

☐ **The illustration(s) in the location(s) referenced below do not come within the exceptions of 37 CFR 1.58(a). Please delete the illustration(s) from the specification or claims, provided the illustration(s) as part of the formal drawing(s) in accordance with 37 CFR 1.84, and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):**

- ☐ Specification document coded dated , page(s) .
- ☐ Claims document coded dated , claim(s) .
- ☐ Amendment document coded dated .
- ☐ Other: .

☒ **The chemical formula(s), mathematical formula(s), or table(s) in the location(s) referenced below contain shading (color/grayscale) and therefore do not comply with 37 CFR 1.52 and do not come within the exceptions of 37 CFR 1.58(a). Please delete chemical formula(s), mathematical formula(s), or table(s) with shading from the specification or claims, provide them as formal color drawing(s) in accordance with 37 CFR 1.84, including petition, fee, and amendment in accordance with 37 CFR 1.84(a)(2), and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):**

- ☒ Specification document coded SPEC dated 08/05/2014, page(s) 26-29,62-67.
- ☐ Claims document coded dated , claim(s) .
- ☒ Amendment document coded SPEC dated 11/14/2014.
- ☒ Other: The amendment to paragraph 00174 contains shading.

PTO/AIA/26 (04-13)
Approved for use through 04/30/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) 38075-716.301

In re Application of: EMIL P. KARTALOV, *et al.*

Application No.: 14/451,876

Filed: AUGUST 5, 2014

For: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

The applicant, CALIFORNIA INSTITUTE OF TECHNOLOGY, owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. 8,838,394 as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for any during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. ☒ The undersigned is an attorney or agent of record. Reg. No. 75,716

/Kyle Bruner/ — Signature
June 21, 2018 — Date

J. Kyle Bruner — Typed or printed name

Agent of Record — Title
512.338.5444 — Telephone Number

☒ Terminal disclaimer fee under 37 CFR 1.20(d) included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Patent Application Fee Transmittal					
Application Number:	14451876				
Filing Date:	05-Aug-2014				
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS				
First Named Inventor/Applicant Name:	Emil Kartalov				
Filer:	Vernon A. Norviel/katherine lopez				
Attorney Docket Number:	38075-716.301				
Filed as Small Entity					
Filing Fees for Utility under 35 USC 111(a)					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
Pages:					
Claims:					
Miscellaneous-Filing:					
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					
Extension-of-Time:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	2814	1	160	160
		Total in USD ($)		**160**

Electronic Acknowledgement Receipt	
EFS ID:	32968127
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	Vernon A. Norviel/katherine lopez
Filer Authorized By:	Vernon A. Norviel
Attorney Docket Number:	38075-716.301
Receipt Date:	21-JUN-2018
Filing Date:	05-AUG-2014
Time Stamp:	16:30:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$160
RAM confirmation Number	062218INTEFSW00003308232415
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Terminal Disclaimer Filed	CIT_38075_716_301_Signed_TD.pdf	126307 c9e5893456559214d7347b117aa892e55e18f58f	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30782 98f5e472ec0a282060930456c76520728308c70a	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	157089		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	14/451,876	KARTALOV ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☐ APPROVED	☒ DISAPPROVED
Date Filed : 21 June, 2018	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

CRYSTAL QUEEN

Technology Center: PLRC

Telephone: _____

The person who signed the terminal disclaimer is not the applicant, patentee or an attorney or agent of record. 37 CFR 1.321(a) and (b). (See FP 14.26.08)

(Note: PoA can be given to a customer number, wherein all practitioners listed under the customer number have PoA. given to a list of practitioners by registration number, the list may not comprise more than 10 practitioners or a separate paper signed by a 37 CFR l.33(b) party must be in the record identifying which of the practitioners, up to 10, are recognized as having PoA. If the applicant is a juristic entity (e.g., corporation), a representative of the applicant cannot sign the TD unless it is established that the representative is a party authorized to act on behalf of the applicant.)

NOTE: signature and printed name must match and person signing name must match the name under that registration number.

Also resubmit Td with this paper, NO FEE is required.

PTO/AIA/26 (04-13)
Approved for use through 04/30/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) 38075-716.301

In re Application of: EMIL P. KARTALOV, *et al.*

Application No.: 14/451,876

Filed: AUGUST 5, 2014

For: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

The applicant, CALIFORNIA INSTITUTE OF TECHNOLOGY, owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. 8,838,394 as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for any during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. ☒ The undersigned is an attorney or agent of record. Reg. No. 75,716

/J. Kyle Bruner/ — Signature
June 26, 2018 — Date

J. Kyle Bruner — Typed or printed name

Agent of Record — Title
512.338.5444 — Telephone Number

☒ Terminal disclaimer fee under 37 CFR 1.20(d) included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

10048184_1.doc

Electronic Acknowledgement Receipt	
EFS ID:	33006859
Application Number:	14451876
International Application Number:	
Confirmation Number:	1065
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil Kartalov
Customer Number:	21971
Filer:	J Kyle Bruner/Katherine Lopez
Filer Authorized By:	J Kyle Bruner
Attorney Docket Number:	38075-716.301
Receipt Date:	26-JUN-2018
Filing Date:	05-AUG-2014
Time Stamp:	14:29:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Terminal Disclaimer Filed	CIT_38075_716_301_Signed_TD.pdf	126298 fdaca023fee38fc442d05bceb5f30f3a64749a6b	no	1
Warnings:					

Information:					
2	Terminal Disclaimer Filed	CIT_38075_716_401_TD_Disapproved.pdf	35188 a924124b3da61ee5526161475032e2153634afec	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	161486		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	14/451,876	KARTALOV ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 26 June, 2018	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

/CRYSTAL QUEEN/

Technology Center: PLRC

Telephone: _____

U.S. Patent and Trademark Office

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Inventors: Emil KARTALOV et al. Serial No.: 14/451,876 Filed: August 5, 2014 Title: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS	Group Art Unit: 1631 Confirmation No.: 1065 Examiner: RUSSELL SCOTT NEGIN Customer No.: 21971 Certificate of Electronic Filing I hereby certify that the attached Response to Notice to File Corrected Application Papers is being deposited by Electronic Filing on July 3, 2018, by using the EFS – Web patent filing system and addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450. By: /Katherine A. Lopez/

Mail Stop Issue Fee
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

Dear Commissioner:

Applicant hereby submits a response to the Notice to File Corrected Application Papers mailed June 20, 2018. This Response is being filed on or before August 20, 2018, which is within the two-month statutory period for reply. Applicant believes this response is timely filed. Applicant respectfully requests entry of the proposed amendments prior to examination.

Amendments to the Specification begin on page **2** of this paper
Remarks and Conclusion begin on page **3** of this paper.

AMENDMENTS TO THE SPECIFICATION

Applicant submits herewith Substitute Specification (clean copy only) in compliance with 37 CFR 1.121. The specification is amended to remove the grey background from certain cells of Tables 6-7 on pages 26-29 and Tables 9-14 on pages 53-55, and to modify the background color and text formatting of the header row of tables 18-24 on pages 62-67 pursuant to 37 CFR 1.52. Since this amendment is not able to be marked, a marked version of the Substitute Specification is not provided. No new matter has been introduced by this amendment.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2691

REMARKS

Applicant submits herewith Substitute Specification (clean copy only) in compliance with 37 CFR 1.121. The specification is amended to correct certain cells of Tables 6-7 and 9-14 on pages 26-29 and 53-55 respectively, and to correct the header row of Tables 18-24 on pages 62-67. Specifically, the grey background of certain cells has been removed in Tables 6-7 and 9-14, and the black background has been changed to white while the white text has been changed to bolded black in the header row of Tables 18-24, to ensure that the text is readable and sufficiently clear as is required by 37 CFR 1.52. No new matter has been introduced by these amendments.

CONCLUSION

Applicants respectfully solicit the Examiner to enter the amendments and expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to contact the undersigned at (512) 338-5444.

The Commissioner is hereby authorized to charge any fees that may be required, or credit any overpayment to Deposit Account No. 23-2415, referencing Attorney Docket No. 38075-716.301.

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI
A Professional Corporation

Date: July 3, 2018 By: /Kyle Bruner/
J. Kyle Bruner
Reg. No. 75,716

650 Page Mill Road
Palo Alto, CA 94304
(512) 338-5444
USPTO Customer No. 021971

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2692

SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

CROSS-REFERENCE

[0001] This application is a continuation of U.S. Patent Application No. 13/7561760, filed February 1, 2013, which claims the benefit of U.S. provisional applications 61/594,480, filed February 3, 2012 and 61/703,093, filed September 19, 2012. The content of the three applications are incorporated herein by reference in entirety.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 20, 2014, is named 38075-716-301-Seqlist.txt and is 9,260 bytes in size.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0001.5] This invention was made with the support of the United States government under Grant No. 1144469 awarded by NSF. The government has certain rights in the invention.

BACKGROUND

[0002] Multiplexed reactions offer significant advantages over traditional uniplex reactions, including performance of parallel reactions on the same sample, use of the same chamber to perform multiple reactions, and the ability to extract rich information from a sample in a fast and efficient manner. However, to achieve these benefits, multiplexed assays generally require complex reporting mechanisms, namely spectrally resolved fluorescence or chemiluminescence (e.g., PCR, ELISA), spatially resolved signals (e.g., microarrays, gel electrophoresis), temporally resolved signals (e.g., capillary electrophoresis), or combinations thereof (e.g., Sanger sequencing). There is a need for multiplexed reactions that can be carried out in a single solution.

SUMMARY OF THE INVENTION

[0003] This disclosure provides methods, compositions, systems, and kits for the multiplexed detection of analytes. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2693

single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis. In some examples, each of the analytes is encoded as a value of one (or at least one) component of a signal.

[0004] In some examples, an assay provided in this disclosure is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of one component of a signal (e.g., an intensity) when all the analytes are present.

[0005] In some cases, each analyte is encoded as at least one first value in a first component of a signal (e.g., at least one intensity or range of intensities) and at least one second value in a second component of a signal (e.g., at least one wavelength or range of wavelengths). In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths).

[0006] In some examples, an assay provided herein is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = C * \log_2 (F + 1)$, where C is the number of the second values used to encode the analytes and F is the maximum cumulative value of the first component of the signal, for any second value, when all of the analytes are present.

[0007] In some cases, an assay provided in this disclosure is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = (P * T) + 1$, where P is the number of codes per tier in a coding scheme and T is the number of tiers. In some cases, the number of tiers $T = \log_4 (F + 1)$, and F is the maximum cumulative value of a first component of a signal, for any second value, when all of the analytes are present.

[0008] In some examples, the first value is an intensity or range of intensities. In some cases, a coding scheme comprises at least three intensities or ranges of intensities.

[0009] In some cases, the second value is a wavelength or range of wavelengths. In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths.

[0010] In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths.

[0011] In some cases, an assay provided herein is performed with reagents that are lyophilized prior to use.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2694

[0012] In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores. In some examples, a sample is contacted with at least 18 of said hybridization probes. In some examples, a detecting step is performed with reagents comprising at least one pair of primers. In some cases, at least one pair of primers are capable of amplifying a region complementary to at least three of said hybridization probes.

[0013] In some examples, the signal that is measured is generated during a polymerase chain reaction. In some cases, the polymerase chain reaction is selected from the group consisting of an end-point polymerase chain reaction, a real-time polymerase chain reaction, a digital polymerase chain reaction, and combinations thereof.

[0014] In some examples, at least one cumulative measurement is performed on a solution.

[0015] In some examples, at least one analyte is encoded by at least one additional value (i.e., at least two values together), wherein the at least one additional value is selected from the group consisting of a value from at least one additional component of a signal, a value from at least one component of a different signal, and combinations thereof. For example, the at least one additional value may be selected from the group consisting of a fluorescence emission intensity, a fluorescence emission wavelength, a Förster resonance energy transfer (FRET) emission intensity, a FRET emission wavelength, an electrochemical signal, a chemiluminescence wavelength, a chemiluminescence intensity, a fluorescence bleaching rate, a chemiluminescence bleaching rate, and combinations thereof.

[0016] In some cases, a chromatogram is constructed. The chromatogram may be constructed by plotting all possible combinations of first values and second values for positive control samples for each analyte.

[0017] In some examples, at least one of the analytes comprises a polynucleotide. In some cases, the polynucleotide is from a source selected from the group consisting of an animal, a plant, a bacteria, a fungus, a parasite, and a virus. In some examples, the polynucleotide is from a source selected from the group consisting of human immunodeficiency virus, herpes simplex virus, human papilloma virus, Plasmodium, Mycobacterium, dengue virus, hepatitis virus, and influenza virus. In some cases, the polynucleotide is selected from the group consisting of human immunodeficiency virus polyprotease, human immunodeficiency virus p17, human papilloma virus E6, and human papilloma virus E7.

[0018] In some cases, a sample is selected from the group consisting of a clinical sample, a food sample, an environmental sample, a pharmaceutical sample, and a sample from a consumer product.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2695

[0019] In some examples, information concerning the presence or absence of an analyte is transmitted through a computer network.

[0020] In some cases, information concerning the presence or absence of an analyte is provided to a physician. In some examples, a clinical decision is made based on such information.

[0021] In some examples, at least one step of a method provided herein is performed using instructions on a computer readable medium. In some cases, the instructions are located on a remote server. In some examples, the instructions are located on a thermal cycler. In some cases, the instructions are located on a computer in communication with a thermal cycler.

[0022] In some cases, at least one of the analytes is a positive control analyte.

[0023] In some examples, the coding scheme is non-degenerate. In some cases, the coding scheme is designed to be non-degenerate. In some examples, the coding scheme is made non-degenerate by enumerating every legitimate result that can be obtained from the coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from the coding scheme to eliminate degeneracy.

[0024] In some cases, an assay provided herein is an end-point assay. In some examples an assay provided in this disclosure is ended at a threshold number of cycles set by the limit of detection of an instrument.

[0025] In certain examples, an assay provided herein is a liquid phase assay.

[0026] In some cases, an assay provided herein is quantitative.

[0027] In some cases, this disclosure provides a method of detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme.

[0028] In some examples, the method described in the preceding paragraph is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of the first values when all of the analytes are present. In some cases, the first value is an intensity or range of intensities. In some examples, the first

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2696

value has a minimum value in the coding scheme that is selected from the group consisting of at least 1, at least 2, at least 4, at least 8, at least 16, at least 32, and at least 64. In some cases, the first value is incremented in the coding scheme by an amount equal to the cumulative maximum of said preceding first values plus one. In other cases, the first value is incremented in the coding scheme by an amount greater than the cumulative maximum of the preceding first values plus one.

[0029] In some cases, this disclosure provides a method of detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said at least one first value and at least one said second value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme, wherein, when degeneracy is eliminated, said method is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

[0030] In some cases, each of the analytes of the method described in the preceding paragraph is encoded as a first value in a first component of the signal at each of a plurality of second values in a second component of the signal. In some cases, when degeneracy is eliminated, this method is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single volume using four second values. In some examples, when degeneracy is eliminated, the coding scheme comprises T non-degenerate tiers, wherein $T = \log_4 (F + 1)$ and F is the maximum cumulative value of the first component of the signal, for any second value, when all of the analytes are present. In some cases, when degeneracy is eliminated, the method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = (P * T) + 1$ and P is the number of codes per tier. In some examples, when degeneracy is eliminated, the method is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, where $M = C * \log_2 (F + 1)$, C is the number of second values in the coding scheme, and F is

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2697

the maximum cumulative value of the first component of the signal, for any second value, when all of the analytes are present.

[0031] This disclosure also provides non-degenerate coding schemes capable of unambiguously encoding the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis.

[0032] In some examples, a non-degenerate coding scheme of this disclosure is generated by a method comprising: (a) generating a code for each potential analyte, wherein each potential analyte is encoded by at least one value of at least one component of a signal; (b) enumerating every legitimate cumulative result for all possible combinations of presence or absence of each analyte; (c) identifying each legitimate result that is degenerate; and (d) eliminating at least one code to eliminate degeneracy. In some cases, the coding scheme may be expanded by adding additional codes that are at least one unit greater than the sum of all previous codes in the at least one component of said signal. In some cases a coding scheme is generated by a method of mathematical iteration that guarantees non-degeneracy by construction.

[0033] In some cases, this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values of said signal within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme.

[0034] In some cases, this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes; (c) contacting said sample with analyte-specific reagents that generate said at least one first

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2698

value and said at least one second value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme, wherein, when degeneracy is eliminated, said system is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

[0035] In some cases, this disclosure provides methods of detecting the presence or absence of each analyte of a plurality of analytes for a third party, comprising: (a) obtaining the identity of each of said analytes from a party; (b) encoding each of said analytes as a first value of a signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said first value, wherein said encoding is performed in a manner that eliminates degeneracy; (c) providing said party with analyte-specific reagents that generate said first value, as represented in said coding scheme, when each of said analytes is present, said party: (i) contacting a sample comprising, or potentially comprising, at least one of said analytes with said reagents; and (ii) cumulatively measuring said first values within said sample, thereby providing a cumulative measurement; and (d) obtaining said cumulative measurement from said party; (e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme; and (f) providing said party with information about the presence or absence of each of said analytes.

[0036] In some cases, this disclosure provides methods of detecting the presence or absence of each analyte of a plurality of analytes for a third party, comprising: (a) obtaining the identity of each of said analytes from a party; (b) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (c) providing said party with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present, said party: (i) contacting a sample comprising, or potentially comprising, at least one of said analytes with said reagents; and (ii) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; and (d) obtaining said cumulative measurement from said party; (e) determining whether each of said analytes is present or absent based on said

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2699

cumulative measurement and said coding scheme; and (f) providing said party with information about the presence or absence of each of said analytes.

[0037] In some cases, this disclosure provides compositions for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, each reagent generating a signal comprising a first value, wherein said reagents are capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, mass spectrometry, or melting curve analysis.

[0038] In some cases, this disclosure provides compositions for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, each reagent generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said reagents are capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

[0039] In some cases, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, packaging, and instructions, each reagent generating a signal comprising a first value, wherein said kit is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, mass spectrometry, or melting curve analysis.

[0040] In some examples, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, comprising analyte-specific reagents, packaging, and instructions, each reagent generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said kit is capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value.

[0041] In some cases, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, comprising a kit body (601), clamping slots arranged in the kit body for placing bottles, a bottle comprising probes for the detection of an analyte (602), a bottle comprising primers for amplification (603), and a bottle comprising reagents for amplification (604), wherein said kit is capable of unambiguously detecting the presence or absence of each of at least seven analytes in a single sample volume, in any combination of presence or absence, without immobilization, separation, mass spectrometry, or melting curve analysis.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2700

[0042] In some examples, this disclosure provides kits for detecting the presence or absence of each analyte of a plurality of analytes, a kit body (601), clamping slots arranged in the kit body for placing bottles, a bottle comprising probes for the detection of an analyte (602), a bottle comprising primers for amplification (603), and a bottle comprising reagents for amplification (604), each probe generating a signal comprising at least one first value that is a value from a first component of said signal and at least one second value that is a value from a second component of said signal, wherein said kit is capable of detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said probes generates only one second value.

INCORPORATION BY REFERENCE

[0043] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0045] Fig. 1 shows a comparison between a traditional encoding method of detecting four analytes with four colors and an encoding method of the invention able to detect 16 or more sequences with four colors. Colors are indicated by B, G, Y, and R, which indicate blue, green, yellow, and red, respectively.

[0046] Fig. 2 shows a schematic representation of detection of six analytes (including control) with four colors. The analytes are detected using hybridization probes attached to a fluorophore (B, G, Y, R; blue, green, yellow, and red, respectively) and a quencher (oval with "X"). The control sequence and the five other sequences (Dengue Fever = dengue virus; Tuberculosis = *Mycobacterium tuberculosis*; P17 = HIV p17; Malaria = *Plasmodium falciparum;* and Herpes = herpes simplex virus 2) are all detected using a probe labeled with a blue fluorophore. The non-control analytes are each detected using 1-3 additional probes. For example, the dengue virus analyte is detected using three additional probes with green,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2701

yellow, and red fluorophores; the herpes simplex virus 2 analyte is detected using one additional probe with a red fluorophore; and so on.

[0047] **Fig. 3** shows chromatograms of experimental results obtained as described in Example 2.

[0048] **Fig. 4** shows an exemplary embodiment of the invention in which a computer is used to perform one or more steps of the methods provided herein.

[0049] **Fig. 5** shows a schematic of two different methods of detecting wavelength and intensity of a light signal, such as a fluorescence emission signal.

[0050] **Fig. 6** shows a schematic of exemplary kits.

[0051] **Fig. 7** shows chromatograms of experimental results obtained as described in Example 3.

[0052] **Fig. 8** shows a schematic of a legitimate result (top) and an illegitimate result (bottom).

DETAILED DESCRIPTION OF THE INVENTION

[0053] Fluorescence detection has been a preferred technique for multiplexed assays because of several desirable features, including compatibility with biochemical assays, the relatively small size of fluorescent labels, simple means of conjugation to molecules of interest, affordability, low toxicity, stability, robustness, detectability with inexpensive optics, and an ability to be combined with spatial arrays. However, standard fluorophores have wide emission spectra. Therefore, in order to avoid spectral overlap (i.e., to preserve spectral resolution) only a relatively small number of colors (e.g., 4 to 6) are typically used simultaneously in multiplexed fluorescent assays.

[0054] The traditional encoding method for multiplexed fluorescent assays has been to encode each analyte with a single color; i.e., M=N, where M is the number of analytes that can be detected and N is the number of spectrally resolved fluorescent probes. Whenever higher factors of multiplexing are required (i.e., M>N), fluorescence is generally combined with other techniques, such as aliquoting, spatial arraying, or sequential processing. These additional processing steps are labor-intensive and frequently require relatively expensive and complex optical and mechanical systems (e.g., spectrometers, mechanized microscopy stages, microfluidics, droplet generators, scanners, and the like). Such systems are often impractical to deploy in certain settings, particularly point-of care and low-resource settings. Thus, there is a significant need for multiplexed encoding and decoding methods that can provide an inexpensive means of multiplexing while avoiding the use of expensive additional processing steps.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2702

[0055] This disclosure provides methods, systems, compositions, and kits for the detection of multiple analytes in a sample. Analytes are detected based on the encoding, analysis, and decoding methods presented herein. In some examples, each analyte to be detected is encoded as a value of a signal (e.g., intensity), where the values are assigned so that the results of the assay unambiguously indicate the presence or absence of the analytes being assayed. In other examples, each analyte to be detected is encoded as a value in each of at least two components of a signal (e.g., intensity and wavelength). The at least two components of a signal may be orthogonal. Similarly, as described more fully elsewhere in this disclosure, multiple orthogonal signals may be used, such as a combination of a fluorescent signal and an electrochemical signal. The analyte may be any suitable analyte, such as a polynucleotide, a protein, a small molecule, a lipid, a carbohydrate, or mixtures thereof. The signal may be any suitable signal such as an electromagnetic signal, a light signal, a fluorescence emission signal, an electrochemical signal, a chemiluminescent signal, and combinations thereof. The at least two components of the signal may be any suitable two components, such as an amplitude and a frequency or an intensity and a wavelength.

[0056] After encoding of the analytes, a sample is provided wherein the sample comprises or may comprise at least one of the encoded analytes. The sample is contacted with an analyte-specific reagent or reagents that generate a particular signal, as specified for each analyte in the coding scheme, in the presence of an analyte. A reagent may be any suitable reagent that is capable of generating such a signal in the presence of its corresponding analyte, for example, an oligonucleotide probe attached to a fluorophore and a quencher (e.g., a TAQMAN probe). If the reagent is an oligonucleotide probe attached to a fluorophore and a quencher, a nucleic acid amplification may be performed to generate the signal.

[0057] After addition of the reagent(s), the signal is quantified. In some cases, this quantification is performed by measuring one component of the signal (e.g., fluorescence intensity) and determining the presence and absence of certain analytes based on the values used to encode the presence of each analyte and the cumulative value of the signal.

[0058] In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths. The presence or absence of an analyte may then be determined based on the values of each of the at least two components of the signal and the values used to encode the presence of the analyte (i.e., those values in the coding scheme).

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2703

[0059] The encoding may be performed in a manner that reduces or eliminates the number of possible degenerate (e.g., ambiguous) results that can be obtained by the method. As described elsewhere in this specification, the full coding capability of a particular coding scheme may be enumerated, and certain potential analyte codes may be eliminated from the coding scheme to reduce or eliminate degeneracy. Similarly, a coding scheme may be designed to be non-degenerate, so that a reduction or elimination of degeneracy is not necessary. A decoding matrix may be constructed to translate cumulative signal measurements (e.g., intensities or intensities at particular wavelengths) into the presence or absence of certain analytes, corresponding to the constituent signals of the cumulative signal measurement.

I. Definitions

[0060] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0061] As used herein, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including," "includes," "having," "has," "with," "such as," or variants thereof, are used in either the specification and/or the claims, such terms are not limiting and are intended to be inclusive in a manner similar to the term "comprising".

[0062] The term "about," as used herein, generally refers to a range that is 15% greater than or less than a stated numerical value within the context of the particular usage. For example, "about 10" would include a range from 8.5 to 11.5.

[0063] The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component).

[0064] The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes.

[0065] The term "encoding," as used herein, generally refers to the process of representing an analyte using a code comprising values of a signal, such as intensity, or values in each of at least two components of a signal or signals, such as wavelength and intensity.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2704

[0066] The terms "oligonucleotide probe attached to a fluorophore and a quencher" and "TAQMAN probe" generally refer to hydrolysis probes used to detect the presence of an analyte in a polynucleotide amplification assay. These probes comprise an oligonucleotide probe attached to a fluorophore and a quencher. So long as the quencher and the fluorophore are in proximity, the quencher quenches the fluorescence emitted by the fluorophore upon excitation by a light source. The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte. Hybridization of the oligonucleotide probe is performed in a nucleic acid amplification reaction comprising primers (e.g., a polymerase chain reaction). Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present. If no analyte is present, the probe will not hybridize to an analyte, and the fluorophore and quencher will remain in close proximity. Little or no signal will be produced.

[0067] The term "orthogonal," as used herein, generally refers to at least two components of a signal (e.g., wavelength and intensity), or at least two different signals (e.g., fluorescence emission and electrochemical signal), that can be varied independently or approximately independently. For example, wavelength and intensity are considered orthogonal or approximately orthogonal when fluorescent molecules are used. Among other factors, the wavelength of fluorescence emission will depend on the composition of the fluorescent molecule and the intensity of the fluorescence will depend on the amount of molecule present. Although wavelength and intensity are examples of two components of a signal that can be varied approximately independently, the methods described herein are not limited to components that can be varied independently or approximately independently. Components of a signal, or signals, that vary non-independently may also be used, so long as the components or signals are characterized well enough to enable encoding, measurement, and decoding. For example, if the variance in one component or signal affects the variance in another component or signal, the two components or signals may still be used so long as the relationship between the variances is understood.

[0068] The terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides. Exemplary polynucleotides include deoxyribonucleic acids, ribonucleic acids, and synthetic analogues thereof, including peptide nucleic acids.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2705

[0069] The term "probe," as used herein, generally refers to a reagent capable of generating a signal in the presence of a particular analyte. A probe generally has at least two portions: a portion capable of specifically recognizing an analyte, or a portion thereof, and a portion capable of generating a signal in the presence of an analyte, or a portion thereof. A probe may be an oligonucleotide probe attached to a fluorophore and a quencher, as described above and elsewhere in this disclosure. A probe may also be any reagent that generates a signal in the presence of an analyte, such as an antibody that detects an analyte, with a fluorescent label that emits or is quenched upon binding of the antibody to an analyte. Any suitable probe may be used with the methods presented in this disclosure, so long as the probe generates a quantifiable signal in the presence of an analyte. For example, the analyte-specific portion of a probe may be coupled to an enzyme that, in the presence of an analyte, converts an uncharged substrate into a charged product, thereby increasing the electrical conductivity in the medium over time. In this case, different analytes may be encoded by coupling the analyte-specific portion of the probe (e.g., hybridization probe or antibody) to an enzyme at different ratios. The resulting rate of increased conductivity in the medium will be cumulative for all analytes present in the medium. Encoding analytes according to the methods provided herein enables conversion of the conductivity measurements into unambiguous (i.e., non-degenerate) results providing information about the presence or absence of particular analytes. Similarly, a probe may comprise an enzyme producing a chemiluminescent product from a substrate. The amount of chemiluminescence may then be used to encode the presence of particular analytes.

II. Encoding and Decoding Methods

A. Traditional Fluorescent Encoding and Decoding Method

[0070] A commonly used method of determining the presence of an analyte uses four spectrally resolved fluorescent molecules to indicate the presence or absence of four analytes. An example of this method is presented on the left-hand side of **Fig. 1**. The left-hand side of **Fig. 1** shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively). The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher. In the system shown on the left-hand side of **Fig. 1**, there are four different oligonucleotide probes, each comprising a single fluorophore (blue, green, yellow, or red) and a quencher. The presence or absence of an analyte is determined based on the presence or absence of a signal in a particular color.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2706

[0071] The chart on the left-hand side of **Fig. 1** shows intensity versus color for a hypothetical sample containing two analytes: Seq 1 and Seq 3. The presence of these analytes is determined based on the measurement of a blue signal (corresponding to Seq 1) and a yellow signal (corresponding to Seq 3). The absence of Seq 2 and Seq 4 is indicated by the absence of a blue and red signal.

[0072] Table 1 shows a translation of this coding scheme into a binary format. Each analyte is encoded as a value in each of two components of a fluorescent signal: (1) color (also known as wavelength; or range of wavelengths) and (2) intensity (indicated by the numbers within the table). For example, A (e.g., Seq 1) has a color of blue and an intensity of 1; B (e.g., Seq 2) has a color of green and an intensity of 1; and so on. The intensity of the signal within each color range may be quantified as described herein, for example by measuring the intensity of the signal within a particular wavelength range determined by a band pass filter. A result of 1000 indicates that only analyte A is present; a result of 1100 indicates that analytes A and B are present; and so on.

Table 1. Traditional encoding of four analytes with four probes, each probe having a single color, and one probe per analyte.

	Blue	**Green**	**Yellow**	**Red**
A	1	0	0	0
B	0	1	0	0
C	0	0	1	0
D	0	0	0	1

B. Encoding Methods Using More Than One Color Per Analyte

[0073] The traditional method described above suffers from the fact that it is limited by the number of spectrally resolvable fluorophores. More specifically, the number of detectable analytes is equal to the number of spectrally resolvable fluorophores. Therefore, the number of analytes may only be increased by increasing the number of spectrally resolvable fluorophores.

[0074] This disclosure provides methods that overcome this limitation. More specifically, in some cases, by utilizing at least two components of a signal during encoding, the methods described herein may be used to detect more than one analyte per fluorophore. For example, using the method provided herein 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes may be detected per fluorophore. In some cases, the methods provided herein may be used to

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2707

detect at least 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes may be detected per fluorophore. In some cases, the methods provided herein may be used to detect 1.5-2, 2-4, 1.5-4, 4-6, 2-6, 6-10 analytes per fluorophore.

[0075] In some cases, the methods provided in this disclosure may include the use of a control color. The control color may be attached to one or more probes binding a positive control analyte, and each analyte to be detected, in a sample. If the same sequence occurs in the positive control analyte and each analyte to be detected, a single control probe may be used. If the same sequence does not occur in the positive control analyte and each analyte to be detected, different probes may be used, but each probe may still be attached to the control color.

[0076] For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method.

Table 2. Encoding of four analytes, including control, with four colors and up to two colors per analyte.

	Blue	**Green**	**Yellow**	**Red**
Ctrl. (p)	1	0	0	0
A	1	0	0	1
B	1	0	1	0
C	1	1	0	0

[0077] In Table 2, the presence of the control (p) is indicated by a result of 1000. The presence of the control and analyte A is indicated by a result of 2001. The presence of the control and all three other analytes is indicated by a result of 4111. The control color (blue) provides an indication that the assay is functioning properly. The intensity of the blue color reports the number of analytes that are present in a test sample. Of course, any color may be used as the control color. One of skill in the art will recognize that certain practical considerations might make it preferable to use one color over another as the control color. For example, if one color is better detected in a particular optical system (or system of fluorophores), it might be practically preferable to use that color as a control color.

[0078] The coding scheme shown in Table 2 encodes each analyte using one control color and one additional color (up to 2 probes per analyte). However, as shown below, the number of analytes that can

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2708

be encoded increases when up to 4 colors are used per analyte. Table 3 shows an exemplary coding scheme where each analyte is encoded by up to 4 colors. In the scheme shown in Table 3, the control color (blue) is generated in the presence of the control sequence and each of the other seven analytes (A-G). The other seven analytes are each encoded by the presence of one to three additional colors. The colors may be contained on different probes (e.g., oligonucleotide probes attached to a fluorophore and a quencher) or on the same probe (which can have multiple fluorophores and multiple quenchers).

Table 3. Encoding of eight analytes (including control) with four colors and up to four colors per analyte.

	Blue	Green	Yellow	Red
Ctrl. (p)	1	0	0	0
A	1	0	0	1
B	1	0	1	0
C	1	1	0	0
D	1	0	1	1
E	1	1	0	1
F	1	1	1	0
G	1	1	1	1

[0079] In Table 3, the presence of the control and all seven other analytes is indicated by a result of 8444. Three analytes (A, B, and C) are encoded by two colors. Three analytes (D, E, and F) are encoded by three colors, and one analyte (G) is encoded by four colors. The presence of the control (p) is indicated by a result of 1000. The presence of the control and analyte A is indicated by a result of 2001. The presence of the control and analyte G is indicated by a result of 2111. These results express the cumulative intensity of the signal in each color. For example, the result 2111 has a 2X signal intensity in the blue channel, while the result 1000 has only a 1X signal intensity in the blue channel.

[0080] Using Table 3, each of the possible cumulative assay results can be enumerated, in terms of color (blue, green, yellow, red) and intensity (0-8). Table 4 shows a "decoding matrix" generated by enumerating each of the possible cumulative assay results based on the encoding method presented in Table 3 and providing the corresponding decoded result of each assay in terms of the analyte(s) present in the sample. A similar decoding matrix may be generated for any coding scheme described herein, by enumerating each of the possible cumulative assay results based on the coding scheme and providing the corresponding decoded result of each assay in terms of the analyte(s) present in (or absent from) the sample. In some cases, as described below, one or more analytes may be removed from the coding scheme in order to reduce or eliminate degeneracy.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2709

Table 4. Decoding matrix for encoding method presented in Table 3.

Cumulative Assay Result				
Blue	**Green**	**Yellow**	**Red**	**Analyte(s) Present**
1	0	0	0	p
2	0	0	1	pA
2	0	1	0	pB
2	1	0	0	pC
2	0	1	1	pD
2	1	0	1	pE
2	1	1	0	pF
2	1	1	1	pG
3	0	1	1	pAB
3	1	0	1	pAC
3	0	1	2	pAD
3	1	0	2	pAE
3	1	1	1	pAF, pCD, pBE
3	1	1	2	pAG, pDE
3	1	1	0	pBC
3	0	2	1	pBD
3	1	2	0	pBF
3	1	2	1	pBG, pDF
3	2	0	1	pCE
3	2	1	0	pCF
3	2	1	1	pCG, pEF
3	1	2	2	pDG
3	2	1	2	pEG
3	2	2	1	pFG
4	1	1	1	pACB
4	0	2	2	pABD
4	2	2	0	pBCF
4	2	0	2	pACE
4	1	1	2	pABE, pACD
4	1	2	1	pABF, pBCD
4	2	1	1	pACF, pBCE
4	1	2	2	pADF, pABG, pBDE
4	2	1	2	pACG, pCDE, pAEF
4	2	2	1	pBCG, pBEF, pCDF
4	2	2	2	pCDG, pAFG, pBEG, pDEF
4	1	1	3	pADE
4	1	3	1	pBDF
4	3	1	1	pCEF
4	1	2	3	pADG
4	3	2	1	pCFG
4	2	1	3	pAEG

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2710

4	2	3	1	pBFG
4	1	3	2	pBDG
4	3	1	2	pCEG
4	2	2	3	pDEG
4	3	2	2	pEFG
5	1	2	2	pABCD
5	2	1	2	pABCE
5	2	2	1	pABCF
5	2	2	2	pABCG, pABEF, pBCDE, pACDF
5	1	2	3	pABDE
5	2	1	3	pACDE
5	1	3	2	pABDF
5	2	1	3	pBCDF
5	3	1	2	pACEF
5	3	2	1	pBCEF
5	2	2	3	pABEG, pACDG, pADEF
5	2	3	2	pABFG, pBCDG, pBDEF
5	3	2	2	pACFG, pBCEG, pCDEF
5	1	3	3	pABDG
5	3	1	3	pACEG
5	3	3	1	pBCFG
5	2	3	3	pADFG, pBDEG
5	3	3	2	pBEFG, pDEFG
5	3	2	3	pCDEG, pAEFG
5	2	2	4	pADEG
5	2	4	2	pBDFG
5	4	2	2	pCEFG
5	3	3	3	pDEFG
6	2	2	3	pABCDE
6	2	3	2	pABCDF
6	3	2	2	pABCEF
6	2	3	3	pABCDG, pABDEF
6	3	2	3	pABCEG, pACDEF
6	3	3	2	pABCFG, pBCDEF
6	4	3	2	pBCEFG
6	4	2	3	pACEFG
6	3	3	3	pABEFG, pACDFG, pBCDEG
6	3	4	2	pBCDFG
6	2	4	3	pABDFG
6	3	2	4	pACDEG
6	2	3	4	pABDEG
6	4	3	3	pCDEFG
6	3	4	3	pBDEFG
6	3	3	4	pADEFG
7	4	4	3	pBCDEFG
7	4	3	4	pACDEFG

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2711

7	3	4	4	pABDEFG
7	4	3	3	pABCEFG
7	3	4	3	pABCDFG
7	3	3	4	pABCDEG
7	3	3	3	pABCDEF
8	4	4	4	pABCDEFG

[0081] The decoding matrix provided in Table 4 is constructed using two assumptions. First, the decoding matrix assumes that the positive control (p) always produces a positive outcome. Second, the decoding matrix assumes that, within each color, the intensity is additive and scales in the same way with changing probe concentration, regardless of which probe the signal may come from. This essentially means that the signals are additive and digital. The conditions underlying these assumptions may be met by properly preparing the assay. If a fluorescent signal is used, the intensity need only be approximately additive and digital, as demonstrated in the Examples provided herein.

[0082] Table 4 allows the conversion of a cumulative measurement of intensity in four ranges of fluorescent wavelengths (i.e., signal intensity within each color range), into the corresponding analytes present in the sample. For example, a result of 4321 indicates that p, C, F, and G are present and the other analytes are not present. This result is referred to as a "legitimate" result, because it is present in the decoding matrix. By contrast, a result of 4000, while possible to measure, does not occur in the decoding matrix. More specifically, the result of 4000 cannot be achieved by adding any combination of control and analyte codes from Table 3. This result is referred to as an "illegitimate" result. An illegitimate result may indicate that the assay malfunctioned. Thus, the control (p) and the decoding matrix provide a means of verifying that the assay is functioning properly.

[0083] Table 4 is exhaustive for any combination of fluorophores generating four resolvable emission spectra (e.g., colors). The term "rank" is used to describe the number of detected analytes, including the control. In the example provided above, if the assay functions properly, the rank is equal to the value of the blue signal. For example, a rank of 8 indicates that the control and all seven other analytes (A-G) are present. A rank of 2 indicates that the control and only one analyte are present. The lowest rank is a rank of 1, which indicates that only the control is present. The number of possibilities at each rank can be enumerated. For example, continuing to refer to the encoding and decoding method described in Tables 3-4, there are 7 possibilities for rank 2. The number of possibilities at rank 3 can be calculated as a combination of 7 take 2, or $7!/(5! * 2!) = 21$ possibilities. More generally, the number of possibilities for a combination of N take K is $N!/((N-K)! * K!)$. Analogously, at ranks 4, 5, 6, 7, and 8, the number

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2712

of possibilities is 35, 35, 21, 7, and 1, respectively. Referring to Table 4 shows that the table agrees with the theoretical prediction. Thus, Table 4 is an exhaustive decoding matrix for the encoding method provided in Table 3.

C. Reducing or Eliminating Degeneracy

[0084] The terms "degenerate" and "degeneracy," as used herein, generally describe a situation where a legitimate result is not definitive, because it can indicate more than one possibility in terms of the presence or absence of an analyte. For example, with reference to Table 4, result 5233 is degenerate because it can be decoded as either pADFG or pBDEG. Similarly, result 4222 is degenerate because it can be decoded as any of the following: pCDG, pAFG, pBEG, pDEF. By contrast, result 3110 can only indicate pBC and thus is not degenerate.

[0085] This disclosure provides methods of reducing or eliminating degeneracy, thereby increasing the confidence with which an analyte is detected. In one embodiment, degeneracy is eliminated by a method comprising (i) encoding each potential analyte to be detected as a value of a signal and, optionally, as a value in each of at least two components of a signal; (ii) enumerating every legitimate result that can be obtained from the coding scheme; (iii) identifying each legitimate result that is degenerate; and (iv) eliminating at least one potential analyte (or potential analyte code) from the coding scheme, wherein eliminating the at least one potential analyte reduces or eliminates degeneracy. For example, with reference to the coding scheme described in Table 3 and the decoding matrix described in Table 4 (enumerating every legitimate result), eliminating any two of analytes D, E, and F eliminates the degeneracy. Eliminating any one of analytes D, E, and F would not eliminate the degeneracy, but would reduce it.

[0086] With continued reference to the coding scheme described in Table 3, eliminating any two of analytes D, E, and F from the coding scheme results in a scheme where six analytes (including control) can be analyzed, with no degeneracy, using only 4 colors. By contrast, conventional methods of multiplexing would allow for only the reporting of 3 analytes and 1 control using 4 colors. Therefore, the number of analytes that can be analyzed is nearly doubled by using the methods provided herein.

[0087] **Fig. 2** shows an exemplary embodiment of the invention in which 4 colors are used to detect five analytes and a control. The analytes are nucleic acids from dengue virus ("Dengue Fever"), *Mycobacterium tuberculosis* ("Tuberculosis"), human immunodeficiency virus (HIV) p17 (P17), *Plasmodium* ("Malaria"), and herpes simplex ("Herpes"). The "colors" in this example are fluorophores attached to oligonucleotide probes that also comprise a quencher. The oligonucleotide probes will

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2713

generally be different for different analytes, whereas the color is the same. For example, all probes designated as Probe 1 have a blue color but generally will have a different oligonucleotide sequence. Of course, probes designated as Probe 1 could also have the same oligonucleotides sequence, if the complementary sequence was present in each of the analytes. The mechanism of detection with these probes is described elsewhere in this disclosure. Probe 1 (blue) hybridizes to all six analytes, including control. Probe 2 (green) hybridizes to analytes from dengue virus, *Mycobacterium*, and HIV p17. Probe 3 (yellow) hybridizes to analytes from dengue fever and *Plasmodium*. Probe 4 hybridizes to analytes from dengue virus, *Mycobacterium*, and herpes simplex. The coding scheme defined by these probes is illustrated in Table 5. With reference to Table 3, potential analytes D (1011) and F (1110) have been eliminated from the coding scheme. Therefore, the coding scheme presented in Table 5 is non-degenerate and each legitimate result from the assay corresponds to the presence or absence of a unique combination of analytes in a sample.

Table 5. Coding scheme for detection of dengue virus, *Mycobacterium*, HIV, *Plasmodium*, and herpes simplex, as exemplified in Fig. 2.

	Blue	**Green**	**Yellow**	**Red**
Ctrl. (p)	1	0	0	0
Herpes Simplex	1	0	0	1
Plasmodium	1	0	1	0
HIV	1	1	0	0
Mycobacterium	1	1	0	1
Dengue Virus	1	1	1	1

[0088] The methods for encoding and decoding presented above, including the methods for eliminating degeneracy, are all equally applicable to encoding methods using additional colors. For example, Table 3 could be extended by including additional colors and additional intensities (described further below). The decoding matrix is then generated as described above, enumerating every legitimate result that can be obtained from the coding scheme. A decoding matrix analogous to the decoding matrix provided in Table 4 may be constructed for any coding scheme described in this disclosure. The legitimate results that are degenerate are then identified and at least one potential analyte code is eliminated from the coding scheme to reduce or eliminate degeneracy. The method of eliminating degeneracy may be

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2714

carried out using software on a computer readable medium, or hardware configured to carry out the method (e.g., a microchip).

[0089] Although degeneracy can be reduced or eliminated by the methods described above, and elsewhere in this disclosure, this disclosure also provides coding schemes that are non-degenerate by design. For example, after elimination of degeneracy in the coding scheme described in Table 3, the coding scheme may be extended indefinitely in a non-degenerate manner where the non-degeneracy is by design (see, e.g., the coding scheme exemplified in Table 6, described more fully below). Similarly, this disclosure provides coding schemes that are completely non-degenerate by design and therefore do not require any reduction or elimination of degeneracy (see, e.g., the coding scheme exemplified in Table 8, described more fully below). Thus, the coding schemes provided in this disclosure may have reduced or eliminated degeneracy, or be non-degenerate by design.

D. Encoding Methods Using More Than One Color and More Than One Intensity

[0090] The encoding method described above (e.g., Tables 3-5) may be further extended by allowing analytes to be encoded by an intensity greater than 1. For example, each of the analytes encoded in the coding scheme provided in Table 5 is encoded by a fluorescence intensity of either 1 or 0. Allowing higher values for the signal intensity in at least one color further increases the number of analytes that can be encoded by any of the methods provided in this disclosure. In some examples, these higher intensity values may be assigned any color except for the control color, in order to maintain the analyte counting capability of the control color.

[0091] Table 6 shows the first three tiers of an exemplary coding scheme that utilizes four colors and multiple intensities. Tier 1 of Table 6 is a reproduction of Table 3, showing the encoding of seven analytes and a control with four colors. As described above, any two of potential analytes D, E, and F may be eliminated from the coding scheme in order to produce a non-degenerate coding scheme. Tier 1 of Table 6 indicates that potential analytes D (1011) and F (1110) have been eliminated from the coding scheme to eliminate degeneracy. Therefore, the coding scheme presented in Tier 1 of Table 6 is capable of determining the presence of five analytes and one control using four colors, as described above.

[0092] The coding scheme of Tier 1 of Table 6 may be expanded to a second tier (Tier 2) by allowing the intensity in any of the colors to increase. As described above, the intensity of the control color may be maintained at 1, in order to preserve the sequence counting capability of the control. Increasing the intensity of any of the remaining three colors will yield codes 100Y, 10Y0, 1Y00, 10YY, 1Y0Y, 1YY0, and 1YYY, where $Y > 1$. The minimal value of Y for a new tier of encoding is equal to the cumulative

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2715

maximum value from the prior tier(s) plus 1. As described below, a value greater than 1 could also be used, to maximize the differences between the intensities.

[0093] Thus, in this context, the term "tier" is generally used to describe a set of codes that fully utilize the coding capability provided by a particular number of first values (e.g., intensities) and second values (e.g., colors), without degeneracy. For example, Tier 1 of Table 6 fully utilizes the coding capability provided by four colors with up to one intensity in each color, without degeneracy. As shown in Table 6, Tier 1, this results in six encoded analytes, including the control. To introduce a second tier, the minimum value of Y (described above) may be incremented to equal the cumulative maximum result from the prior tier(s) plus one (or more than one). In the example provided in Table 6, the intensity of the blue (control) color is maintained as one, to preserve the sequence counting capability in this color. Thus, Tier 2 consists of five non-degenerate encoding possibilities obtained by incrementing the intensities in the green, yellow, and red channels to equal the cumulative maximum results in each of these channels from Tier 1, plus one. All possibilities of these codes may then be enumerated for Tier 2, and codes resulting in degeneracy may be eliminated, or Tier 2 may be made non-degenerate by design, using the information used to eliminate the corresponding codes from Tier 1. Further coding capacity may then be achieved by adding a third tier, or further tiers, which are constructed according to analogous methods. A coding scheme may have an infinite number of tiers, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100 or more tiers.

[0094] More specifically, with reference to Table 6, Tier 2, analyte H is encoded by 1004. The value of the control color is maintained as 1. The value of the red color is equal to the cumulative maximum result from the prior tier (3) plus 1, or 4. Similarly, analytes I and J are encoded by 1030 and 1400, respectively. Combinations of these codes are used to encode the remaining four analytes (K-N), as was done for analytes D-G in Tier 1. This completes Tier 2. In Tier 2, the inclusion of potential analytes K (1034) and M (1430) results in degeneracy. Therefore, these analytes have been eliminated from the coding scheme, to eliminate degeneracy.

[0095] Continuing to refer to Table 6, a third tier (Tier 3) is constructed using the same principles described above. Analyte O is encoded by 1-0-0-16. The value of the control color is still maintained as 1. The value of the red color is equal to the cumulative maximum result from the prior tiers (15) plus 1, or 16. Similarly, analytes P and Q are encoded by 1-0-9-0 and 1-16-0-0, respectively. Combinations of these codes are used to encode the remaining four analytes (R-U), as was done for analytes D-G in Tier 1 and analytes K-N in Tier 2. In Tier 3, the inclusion of potential analytes R (1-0-9-16) and T (1-16-9-0)

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2716

results in degeneracy. Therefore, these analytes have been eliminated from the coding scheme, to eliminate degeneracy.

[0096] The three-tier coding scheme shown in Table 6 shows the encoding of 15 analytes and one control using four colors. This coding scheme may be indefinitely extended, by adding more intensities to generate additional tiers and/or adding more colors, to generate additional coding capability within the tiers. The methods of reducing or eliminating degeneracy, as described in this disclosure, may be used with the addition of each intensity and/or color, to reduce or eliminate degenerate results.

[0097] More generally, the coding scheme depicted in Table 6 is a non-degenerate, infinite extension of the coding scheme depicted in Tables 3-5. The maximum intensity of the cumulative measurement at the first tier is 6. The maximum intensity of the cumulative measurement at the second tier is 15. The maximum intensity of the cumulative measurement at the third tier is 63, and so on. Given a maximum cumulative intensity value (F), the maximum number of tiers (T) available in this coding scheme is $T = \log_4 (F + 1)$. The coding scheme depicted in Table 6 provides five non-degenerate codes per tier (P). Thus, the maximum number of codes $M = 5 * \log_4 (F + 1)$, or $M = P * T$, where P is the number of non-degenerate codes per tier and T is the number of tiers. For example, given $F = 63$, the maximum number of codes (i.e., analytes) is 15 for 5 non-degenerate codes per tier. This formula does not include the 1000 code, which is reserved for a positive control in Table 6. To include the control in the total number of analytes, one would simply add one, to provide the formula $M = (P * T) + 1$.

[0098] The methods provided in this disclosure may be used to expand this coding scheme infinitely. For example, by utilizing combinations of different intensities (i.e., first values) and colors (i.e., second values) one can encode any number of analytes (M) by varying the number of non-degenerate codes per tier (P) and the number of tiers (T). For example, the number of non-degenerate codes per tier is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more. Similarly, the number of tiers may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more.

Table 6. Encoding of 15 analytes and one control using four colors and multiple intensities.

Tier	Analyte	B	G	Y	R	Comments
1	**Ctrl. (p)**	1	0	0	0	
	A	1	0	0	1	
	B	1	0	1	0	

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2717

	C	1	1	0	0	
	D	1	0	1	1	Eliminated from coding scheme to eliminate degeneracy.
	E	1	1	0	1	
	F	1	1	1	0	Eliminated from coding scheme to eliminate degeneracy.
	G	1	1	1	1	
Cumulative Maximum Result		*6*	*3*	*2*	*3*	
2	**H**	1	0	0	4	
	I	1	0	3	0	
	J	1	4	0	0	
	K	1	0	3	4	Eliminated from coding scheme to eliminate degeneracy.
	L	1	4	0	4	
	M	1	4	3	0	Eliminated from coding scheme to eliminate degeneracy.
	N	1	4	3	4	
Cumulative Maximum Result		*11*	*15*	*8*	*15*	
3	**O**	1	0	0	16	
	P	1	0	9	0	
	Q	1	16	0	0	
	R	1	0	9	16	Eliminated from coding scheme to eliminate degeneracy.
	S	1	16	0	16	
	T	1	16	9	0	Eliminated from coding scheme to eliminate degeneracy.
	U	1	16	9	16	
Cumulative Maximum Result						
Cumulative Maximum Result for Three Non-Degenerate Tiers		***16***	***63***	***26***	***63***	

[0099] The right-hand side of **Fig. 1** shows one exemplary embodiment of the method described above. More specifically, the right-hand side of **Fig. 1** shows an encoding method where nine or more analytes

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2718

(Seq 1 - Seq 9, etc.) are each encoded by at least two colors, with varying intensities within each color. The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher. The system can be designed so that each probe is labeled with single fluorophore, or each probe is labeled with more than one fluorophore. For example, the code for analyte H, in Table 6, is 1004. The intensity of 4 in the red channel may be achieved by either using an H-specific probe comprising 4 red fluorophores, or by using 4 H-specific probes each comprising a single red fluorophore. Of course, any combination of probes and fluorophores producing a result of 4 in the red channel would be equally appropriate, such as 2 probes with 2 red fluorophores each, and 1 probe with 1 red fluorophore and 1 probe with 3 red fluorophores, or simply one probe with one red fluorophore but present at 4x amount in the reaction mixture.

[00100] The coding scheme depicted on the right-hand side of **Fig. 1** may be represented as in Table 7. The result of the analysis shown on the right-hand side of **Fig. 1** is 4112, or an intensity of 4 in the blue channel, 1 in the green channel, 1 in the yellow channel, and 2 in the red channel. Using a decoding matrix constructed as described herein, this result is decoded to indicate the presence of Seq 1, Seq 3, Seq 4, and Seq 5.

Table 7. Coding scheme illustrated on the right-hand side of Fig. 1.

Tier	Analyte	B	G	Y	R	Comments
1	**Seq 1**	1	0	0	0	
	Seq 2	1	1	0	0	
	Seq 3	1	0	1	0	
	Seq 4	1	0	0	1	
	Seq 5	1	1	0	1	
	Seq 6	1	1	1	1	
	~~Eliminated Potential Seq~~	~~1~~	~~0~~	~~1~~	~~1~~	Eliminated from coding scheme to eliminate degeneracy.
	~~Eliminated Potential Seq~~	~~1~~	~~1~~	~~1~~	~~0~~	Eliminated from coding scheme to eliminate degeneracy.
Cumulative Maximum Result		*6*	*3*	*2*	*3*	
2	**Seq 7**	1	4	0	0	
	Seq 8	1	0	3	0	
	Seq 9	1	0	0	4	
Etc. - i.e. Continue as provided in Table 6.						

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2719

E. Encoding Methods Using One Color and One Intensity Per Analyte but Different Intensities Among Analytes

[00101] In some methods provided herein, each analyte is encoded by a single color and intensity combination. For example, in a four color system, the first four analytes may be encoded by 1000, 2000, 4000, and 8000. The next four analytes may be encoded by 0100, 0200, 0400, and 0800. Analytes 9-12 and 13-16 would be assigned analogously, as shown in Table 8.

[00102] Like the encoding method described in Table 6, this coding scheme is theoretically infinite, non-degenerate by construction, and only limited by the bandwidth of the instrument used to measure the signal. However, this coding scheme enables more analytes to be quantified per unit of bandwidth than the encoding method described in Table 6. The reason is that the available multiplicity of signal is used with maximal efficiency, as each level of intensity is utilized in the coding (i.e. there are no gaps in the chromatogram; see below for description of chromatograms). Table 8 shows one embodiment of this method, illustrating four tiers of encoding based on four colors and intensities 1, 2, 4, and 8. The coding scheme is non-degenerate, and the result if all 16 analytes are present is 15-15-15-15. This encoding method is more efficient, in terms of bandwidth utilization, than the encoding methods presented above. However, this method does not have the proofreading capability of the first scheme, as all the results decode to legitimate outcomes in the absence of gaps in the chromatogram.

Table 8. Example of encoding method using one color and one intensity per analyte, but different intensities among analytes.

Tier	Analyte	B	G	Y	R
1	A	1	0	0	0
	B	2	0	0	0
	C	4	0	0	0
	D	8	0	0	0
2	E	0	1	0	0
	F	0	2	0	0
	G	0	4	0	0
	H	0	8	0	0
3	I	0	0	1	0
	J	0	0	2	0
	K	0	0	4	0

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2720

	L	0	0	8	0
4	**M**	0	0	0	1
	N	0	0	0	2
	O	0	0	0	4
	P	0	0	0	8
		15	***15***	***15***	***15***

[00103] The method presented above, and exemplified in Table 8, may be extended by introducing additional colors and/or intensities. For example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more colors may be used. Additional intensity levels, such as 16, 32, 64, 128, 255, 512, 1024, 2048, 4096, 8192, 16384, 32768, 65536, and so on may also be used. The method is generalizable and may be used to encode an infinite number of analytes. Each analyte is represented by a code in a single color, wherein the value of the code in that single color equal to the sum of all previous values plus one. For example, if the first code contains a 1 in a particular color, the next codes are 2, 4, 8, 16, 32, 64, 128 and so on. Other progressions are possible, but will not be as efficient in terms of usage of the bandwidth. However, one of ordinary skill in the art will recognize that less efficient use of bandwidth may be desirable when one wishes to maximize separation between values. Similarly, the value 1 could be excluded from the coding scheme, for example to maximize the difference in the intensity between the first encoded value and instrumental noise. For example, in some cases a coding scheme may begin with a value of 2, which would provide a progression of 2, 4, 8, 16, 32, 64, 128, and so on for the analyte codes, while the progression of cumulative results would be 2, 4, 6, 8, 10, 12, 14, 16, and so on. This approach generates uniform gaps in the possible cumulative results, allowing for higher tolerance to noise in comparison to an analyte code progression of 1, 2, 4, 8, 16, 32, and so on, and its corresponding cumulative result progression of 1, 2, 3, 4, 5, 6, 7, 8, and so on. In this example, the increased robustness in the measurement comes at a cost of a decrease in the number of available codes within a fixed bandwidth of multiplicity. For example, if only 63 multiplicity states can be measured reliably, the coding scheme starting with 1 offers 7 codes per color whereas the coding scheme starting with 2 offers 6 codes per color. If 4 colors are available, the former will offer 28 codes, while the latter will offer 24 codes. In summary, both the starting signal intensity and the progression (i.e., difference between intensity values) may be scaled in order to maximize the ability to distinguish

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2721

over instrumental noise and maximize the differences between the intensity values, thereby enhancing the ability to distinguish between distinct experimental outcomes.

[00104] The coding scheme illustrated in Table 8 is non-degenerate by design. Although the coding scheme illustrated in Table 8 uses both intensity and color to encode each of the 16 analytes, each of the analytes could also be encoded by simply utilizing intensity. For example, given the 16 analytes provided in Table 8 (A-P), a single color coding scheme encoding all 16 analytes may assign the values 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192, and 16384 to analytes A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, and P, respectively. Such a coding scheme is non-degenerate by design and the cumulative intensity result can be unambiguously decoded to indicate the presence or absence of analytes A-P. This coding scheme is capable of detecting the presence or absence of M analytes, where $M = \log_2 (F + 1)$ and F is the maximum cumulative value of a signal (e.g., signal intensity). By adding a second component to this signal (e.g., color, as depicted in Table 8), the capacity of this coding scheme can be increased to $M = C * \log_2 (F + 1)$, where C is the number of colors used in said coding scheme.

[00105] As described throughout this specification, any suitable value may be used for C or F. For example, C may be at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, or more. F may be at least about 2, 4, 8, 16, 32, 64, 128, 255, 512, 1024, 2048, 4096, 8192, 16384, 32768, 65536, 131072, 262144, 524288, 1048576, and so on. As described elsewhere in this disclosure, both the initial starting value of F and the steps in its progression may also be varied.

III. Analytes

[00106] An analyte may be any suitable analyte that may be analyzed using the methods and compositions of the invention, where the analyte is capable of interacting with a reagent in order to generate a signal with at least two components that can be measured. An analyte may be naturally-occurring or synthetic. An analyte may be present in a sample obtained using any methods known in the art. In some cases, a sample may be processed before analyzing it for an analyte. The methods and compositions presented in this disclosure may be used in solution phase assays, without the need for particles (such as beads) or a solid support.

[00107] In some cases, an analyte may be a polynucleotide, such as DNA, RNA, peptide nucleic acids, and any hybrid thereof, where the polynucleotide contains any combination of deoxyribo- and/or

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2722

ribo-nucleotides. Polynucleotides may be single stranded or double stranded, or contain portions of both double stranded or single stranded sequence. Polynucleotides may contain any combination of nucleotides or bases, including, for example, uracil, adenine, thymine, cytosine, guanine, inosine, xanthine, hypoxanthine, isocytosine, isoguanine and any nucleotide derivative thereof. As used herein, the term "nucleotide" may include nucleotides and nucleosides, as well as nucleoside and nucleotide analogs, and modified nucleotides, including both synthetic and naturally occurring species. Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to cDNA, mitochondrial DNA (mtDNA), messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), nuclear RNA (nRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), small Cajal body-specific RNA (scaRNA), microRNA (miRNA), double stranded (dsRNA), ribozyme, riboswitch or viral RNA. Polynucleotides may be contained within any suitable vector, such as a plasmid, cosmid, fragment, chromosome, or genome.

[00108] Genomic DNA may be obtained from naturally occurring or genetically modified organisms or from artificially or synthetically created genomes. Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art. For example, genomic DNA may be isolated with or without amplification. Amplification may include PCR amplification, multiple displacement amplification (MDA), rolling circle amplification and other amplification methods. Genomic DNA may also be obtained by cloning or recombinant methods, such as those involving plasmids and artificial chromosomes or other conventional methods (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*., cited *supra*.) Polynucleotides may be isolated using other methods known in the art, for example as disclosed in *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV) or *Molecular Cloning: A Laboratory Manual*. If the isolated polynucleotide is an mRNA, it may be reverse transcribed into cDNA using conventional techniques, as described in Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*., cited *supra*.

[00109] An analyte may be a protein, polypeptide, lipid, carbohydrate, sugar, small molecule, or any other suitable molecule that can be detected with the methods and compositions provided herein. An analyte may be an enzyme or other protein. An analyte may be a drug or metabolite (e.g., anti-cancer drug, chemotherapeutic drug, anti-viral drug, antibiotic drug, or biologic). An analyte may be any molecule, such as a co-factor, receptor, receptor ligand, hormone, cytokine, blood factor, antigen, steroid, or antibody.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2723

[00110] An analyte may be any molecule from any pathogen, such as a virus, bacteria, parasite, fungus, or prion (e.g., PrP^{Sc}). Examples of viruses include those from the families Adenoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Orthomyxoviridae, Papovaviridae, Paramyxoviridae, Picornaviridae, Polyomavirus, Retroviridae, Rhabdoviridae, and Togaviridae. Specific examples of viruses include adenovirus, astrovirus, bocavirus, BK virus, coxsackievirus, cytomegalovirus, dengue virus, Ebola virus, enterovirus, Epstein-Barr virus, feline leukemia virus, hepatitis virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, herpes simplex virus (HSV), HSV type 1, HSV type 2, human immunodeficiency virus (HIV), HIV type 1, HIV type 2, human papilloma virus (HPV), HPV type 1, HPV type 2, HPV type 3, HPV type 4, HPV type 6, HPV type 10, HPV type 11, HPV type 16, HPV type 18, HPV type 26, HPV type 27, HPV type 28, HPV type 29, HPV type 30, HPV type 31, HPV type 33, HPV type 34, HPV type 35, HPV type 39, HPV type 40, HPV type 41, HPV type 42, HPV type 43, HPV type 44, HPV type 45, HPV type 49, HPV type 51, HPV type 52, HPV type 54, HPV type 55, HPV type 56, HPV type 57, HPV type 58, HPV type 59, HPV type 68, HPV type 69, influenza virus, JC virus, Marburg virus, measles virus, mumps virus, Norwalk virus, parovirus, polio virus, rabies virus, respiratory syncytial virus, retrovirus, rhinovirus, rotavirus, Rubella virus, smallpox virus, vaccinia virus, West Nile virus, and yellow fever virus.

[00111] Examples of bacteria include those from the genuses *Bordetella, Borrelia, Brucella, Campylobacter*, *Chlamydia, Clostridium, Corynebacterium, Enterococcus, Escherichia, Francisella, Haemophilus, Helicobacter, Legionella, Leptospira, Listeria, Mycobacterium*, *Mycoplasma, Neisseria, Pseudomonas*, *Rickettsia, Salmonella*, *Shigella*, *Staphylococcus, Streptococcus*, *Treponema, Vibrio, and Yersinia.* Specific examples of bacteria include *Bordetella par apertussis, Bordetella pertussis, Borrelia burgdorferi, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia psittaci, Chlamydia trachomatix, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium diphtheriae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli*, *Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Leptospira interrogans, Listeria monocytogenes, Mycobacterium leprae, Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella choleraesuis, Salmonella dublin, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Vibrio cholerae, Yersinia pestis, and Yersinia enterocolitica.*

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2724

[00112] Examples of parasites include those from the genuses *Acanthamoeba, Babesia, Balamuthia, Balantidium, Blasocystis, Cryptosporidium, Dientamoeba, Entamoeba, Giardia, Isospora, Leishmania, Naegleria, Pediculus, Plasmodium, Rhinosporidium, Sarcocystis, Schistosoma, Toxoplasma, Trichomonas,* and *Trypanosoma.* Specific examples of parasites include *Babesia divergens, Babesia bigemina, Babesia equi, Babesia microfti, Babesia duncani, Balamuthia mandrillaris, Balantidium coli, Dientamoeba fragilis, Entamoeba histolytica, Giardia lamblia, Isospora belli, Naegleria fowleri, Pediculus humanus, Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax, Rhinosporidium seeberi, Sarcocystis bovihominis, Sarcocystis suihominis, Schistosoma mansoni, Toxoplasma gondii, Trichomonas vaginalis, Trypanosoma brucei,* and *Trypansoma cruzi.*

[00113] Examples of fungi include those from the genuses *Apophysomyces, Aspergillus, Blastomyces, Candida, Cladosporium, Coddidioides, Cryptococcos, Exserohilum, Fusarium, Histoplasma, Pichia, Pneumocystis, Saccharomyces, Sporothrix, Stachybotrys,* and *Trichophyton.* Specific examples of fungi include *Aspergillus fumigatus, Aspergillus flavus, Aspergillus clavatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Crytptococcus neoformans, Exserohilum rostratum, Fusarium verticillioides, Histoplasma capsulatum, Pneumocystis jirovecii, Sporothrix schenckii, Stachybotrys chartarum,* and *Trichophyton mentagrophytes.*

[00114] In some cases, the methods provided in this disclosure may be used to detect any one of the analytes described above, or elsewhere in the specification. In some cases the methods provided in this disclosure may be used to detect panels of the analytes described above, or elsewhere in the specification. For example, a panel may comprise an analyte selected from the group consisting of any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100 or more analytes described above or elsewhere in the specification.

[00115] An analyte may be obtained from any suitable location, including from organisms, whole cells, cell preparations and cell-free compositions from any organism, tissue, cell, or environment. Analytes may be obtained from environmental samples, biopsies, aspirates, formalin fixed embedded tissues, air, agricultural samples, soil samples, petroleum samples, water samples, or dust samples. In some instances, an analyte may be obtained from bodily fluids which may include blood, urine, feces, serum, lymph, saliva, mucosal secretions, perspiration, central nervous system fluid, vaginal fluid, or semen. Analytes may also be obtained from manufactured products, such as cosmetics, foods, personal care products, and the like. Analytes may be the products of experimental manipulation including,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2725

recombinant cloning, polynucleotide amplification, polymerase chain reaction (PCR) amplification, purification methods (such as purification of genomic DNA or RNA), and synthesis reactions.

[00116] More than one type of analyte may be detected in each multiplexed assay. For example, a polynucleotide, a protein, a polypeptide, a lipid, a carbohydrate, a sugar, a small molecule, or any other suitable molecule may be detected simultaneously in the same multiplexed assay with the use of suitable reagents. Any combination of analytes may be detected at the same time.

[00117] Detection of an analyte may be useful for any suitable application, including research, clinical, diagnostic, prognostic, forensic, and monitoring applications. Exemplary applications include detection of hereditary diseases, identification of genetic fingerprints, diagnosis of infectious diseases, cloning of genes, paternity testing, criminal identification, phylogeny, anti-bioterrorism, environmental surveillance, and DNA computing. For example, an analyte may be indicative of a disease or condition. An analyte may be used to make a treatment decision, or to assess the state of a disease. The presence of an analyte may indicate an infection with a particular pathogen, or any other disease, such as cancer, autoimmune disease, cardiorespiratory disease, liver disease, digestive disease, and so on. The methods provided herein may thus be used to make a diagnosis and to make a clinical decision based on that diagnosis. For example, a result that indicates the presence of a bacterial polynucleotide in a sample taken from a subject may lead to the treatment of the subject with an antibiotic.

[00118] In some cases the methods and compositions of the invention may be used to detect at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes. In some cases the methods and compositions of the invention may be used to detect 7-50, 8-40, 9-30, 10-20, 10-15, 8-12, or 7-12 analytes.

[00119] In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45,

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2726

46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1000 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis.

IV. Signals

[00120] The methods presented in this disclosure may be used with any quantifiable signal. In some cases, this disclosure provides coding schemes and methods to encode an infinite number of targets without degeneracy, using a single component of a signal (e.g., intensity). For example, as described above and in Example 3, a coding scheme may rely on a multiplicity of signal intensity without consideration of color. Although fluorescent probes have been used to illustrate this principle, the coding scheme is equally applicable to any other method providing a quantifiable signal, including an electrochemical signal and a chemiluminescent signal, as described elsewhere in this disclosure.

[00121] The methods presented in this disclosure may also utilize the measurement of a signal in at least two dimensions, also referred to as the measurement of at least two components of a signal. In comparison to the coding scheme described in the paragraph above, which relies on, for example, signal intensity to differentiate between analytes, utilization of at least two components of a signal (e.g., color and intensity) allows the generation of more unique codes per unit of signal intensity bandwidth. When at least two components of a signal are utilized, a cumulative measurement of the at least two components may be obtained for a single sample volume. For example in the coding scheme described in Table 5, the presence of each analyte results in a particular intensity (one component of the signal) in each of the four colors (a second component of a signal). The combination of these constituent signals leads to a cumulative signal that may be measured by measuring an intensity at each wavelength or range of wavelengths. The corresponding coding scheme or decoding matrix may then be used to convert the cumulative measurement into a determination of the presence or absence of an analyte.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2727

[00122] In some cases, a quantifiable signal comprises a waveform that has both a frequency (wavelength) and an amplitude (intensity). A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal. In some cases, an electromagnetic signal may be a fluorescent signal, for example a fluorescence emission spectrum that may be characterized in terms of wavelength and intensity.

[00123] In certain portions of this disclosure, the signal is described and exemplified in terms of a fluorescent signal. This is not meant to be limiting, and one of ordinary skill in the art will readily recognize that the principles applicable to the measurement of a fluorescent signal are also applicable to other signals. For example, like fluorescent signals, any of the electromagnetic signals described above may also be characterized in terms of a wavelength and an intensity. The wavelength of a fluorescent signal may also be described in terms of color. The color may be determined based on measuring intensity at a particular wavelength or range of wavelengths, for example by determining a distribution of fluorescent intensity at different wavelengths and/or by utilizing a band pass filter to determine the fluorescence intensity within a particular range of wavelengths. Such band pass filters are commonly employed in a variety of laboratory instrumentation, including quantitative PCR machines. Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel."

[00124] In some cases, the methods provided in this disclosure may be used with any signal where the cumulative signal scales with the constituent signals of the same color, frequency, absorption band, and so on. However, the cumulative signal need not be digital or scale linearly with the number and intensity of the constituent signals. For example, if the physical principle of measurement is absorption, the cumulative attenuation is a product of constituent attenuations while the constituent concentrations are additive, due to the exponential nature of the Beer-Lambert law. The logarithm of the cumulative attenuation will then scale linearly with constituent concentrations in each absorption band (the equivalent of color, if fluorescent detection is used). The methods of the invention are therefore applicable. The methods of the invention may also be used with chemiluminescent signals and electrochemical signals.

[00125] The number of signals, and the number of dimensions or components measured, may also be expanded beyond the numbers shown in the exemplary embodiments of the invention, leading to an expansion in multiplexing capability. The exemplary embodiments provided in this disclosure utilize coding schemes constructed utilizing a fluorescent signal with one or two components measured: wavelength and/or intensity. The number of analytes that can be encoded can be increased by increasing

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2728

the number of wavelengths and/or intensities. The number of analytes that can be encoded can also be increased by increasing the number of signals, for example by combining a fluorescent signal with an electrochemical signal or a FRET signal (fluorescence resonance energy transfer).

[00126] In some cases, more than two components of a signal may be measured. For example, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, 20 or more components of a signal may be measured. At least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, or 20 components of a signal may be measured. At least 2, but fewer than 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, or 20 components of a signal may be measured. In some cases, 2-3, 2-4, 2-5, 2-6, 3-5, 3-6, 3-8, or 5-10 components of a signal may be measured. These additional components may include kinetic components, such as a rate of signal decay and rate of photobleaching.

[00127] If a fluorescent signal is employed, the number of analytes that can be encoded may be further expanded by utilizing additional fluorophores. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more fluorophores may be used. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores may be used. In some cases, fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores may be used. In some cases, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 fluorophores may be used.

[00128] Generally, the number of analytes that can be encoded may be further expanded by utilizing additional first values (e.g., intensities) that are values or ranges of values from a first component of a signal. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more first values that are values or ranges of values from a first component of a signal may be used. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more first values that are values or ranges of values from a first component of a signal may be used. In some cases, fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 first values that are values or ranges of values from a first component of a signal may be used. In some

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2729

cases, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 first values that are values or ranges of values from a first component of a signal may be used.

[00129] In instances in which intensity is a component of a signal that is quantified, such as when a fluorescent signal is utilized, the presence of an analyte may be encoded using a variety of intensities or ranges of intensities. For example, a coding scheme may utilize 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more intensities or ranges of intensities. A coding scheme may utilize at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more intensities or ranges of intensities. A coding scheme may utilize fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 intensities or ranges of intensities. In some cases, a coding scheme may utilize 2-20, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, or 5-10 intensities or ranges of intensities.

[00130] The number of analytes that can be encoded may be further expanded by utilizing additional second values (e.g., wavelengths) that are values or ranges of values from a second component of a signal. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more second values that are values or ranges of values from a second component of a signal may be used. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more second values that are values or ranges of values from a second component of a signal may be used. In some cases, fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 second values that are values or ranges of values from a second component of a signal may be used. In some cases, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 second values that are values or ranges of values from a second component of a signal may be used.

[00131] In instances in which wavelength is a component of a signal that is quantified, such as when a fluorescent signal is utilized, the presence of an analyte may be encoded using a variety of wavelengths or ranges of wavelengths. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more wavelengths or ranges of wavelengths may be used. In some cases, at

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2730

least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more wavelengths or ranges of wavelengths may be used. In some cases, fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 wavelengths or ranges of wavelengths may be used. In some cases, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 wavelengths or ranges of wavelengths may be used.

[00132] In some cases, when degeneracy is eliminated, the methods of the invention are capable of detecting the presence or absence of at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more analytes in a single volume when each reagent used to generate a signal in the volume generates only one second value (e.g., each reagent, emits light at only one wavelength).

[00133] In other cases, when degeneracy is eliminated, the methods of the invention are capable of detecting the presence or absence of at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more analytes in a single volume using a total of four second values in that volume (e.g., a total of four wavelengths or ranges or wavelengths, which might be implemented by using four spectrally resolvable fluorophores).

[00134] As described throughout the specification, the assay provided herein utilizes cumulative measurements on a sample. A cumulative measurement may be, for example, a single measurement of intensity values, or a measurement of intensity values at one or more wavelengths or ranges of wavelengths. A plurality of cumulative measurements may be obtained. For example, an intensity may be measured at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more wavelengths or ranges of wavelengths. An intensity may be measured at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more wavelengths or ranges of wavelengths. An intensity may be measured at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 wavelengths or ranges of wavelengths.

[00135] More generally a cumulative measurement may be obtained for any quantifiable component of a signal, and for any quantifiable component of a signal at another quantifiable component

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2731

of a signal. For example, at least a first component of a signal may be measured at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more second components of a signal. At lest a first component of a signal may be measured at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more second components of a signal. At least a first component of a signal may be measured at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 second components of a signal.

[00136] As is apparent from this disclosure, each analyte to be detected can be encoded as a code utilizing any number of suitable components of a signal or any number of signals. For example, each analyte to be detected can be encoded in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more components of a signal or signals. In some cases, each analyte to be detected can be encoded in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more components of a signal or signals. In some cases, each analyte to be detected can be encoded in fewer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 components of a signal or signals. In some cases, 1-4, 4-20, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 10-15, or 10-20 components of signal or signals may be used to encode each analyte. Each analyte in a coding scheme may be encoded by the same number of components of a signal, or signals, or different numbers of components of a signal, or signals.

A. Probes, Primers, Fluorophores, and Quenchers

[00137] Some of the methods provided in this disclosure utilize a reagent that generates a signal in the presence of an analyte. Any suitable reagent may be used with the invention. Generally, a reagent will have an analyte-specific component and a component that generates a signal in the presence of the analyte. In some cases, these reagents are referred to as probes. The probes may be hybridization probes. The hybridization probes may be an oligonucleotide probe attached to a fluorophore and a quencher (e.g., a TAQMAN probe).

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2732

[00138] The methods of the invention may use one or more reagents or probes to detect the presence or absence of each analyte. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more or probes may be used to detect the presence or absence of each analyte. In some cases, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 probes may be used to detect the presence or absence of each analyte. In some cases, fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, , 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 probes may be used to detect the presence or absence of each analyte. In some cases, the number of probes used to detect the presence or absence of each analyte is 1-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 1-3, or 1-4.

[00139] In some cases, a sample is contacted with 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more probes to detect the presence or absence of all analytes. In some cases, a sample is contacted with at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more probes to detect the presence or absence of all analytes. In some cases, a sample is contacted with fewer than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 probes to detect the presence or absence of all analytes. In some cases, the number of probes that a sample is contacted with to detect the presence or absence of all analytes is 4-50, 4-40, 4-30, 4-20, 5-15, 5-10, or 3-10.

[00140] As described above, oligonucleotide probes attached to a fluorophore and a quencher may be used to detect the presence of an analyte in a polynucleotide amplification assay. So long as the quencher and the fluorophore are in proximity, the quencher quenches the fluorescence emitted by the fluorophore upon excitation by a light source. The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte. Hybridization of the oligonucleotide probe may be performed in a nucleic acid amplification reaction comprising primers (e.g., a polymerase chain reaction). Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2733

present. If no analyte is present, the probe will not hybridize to an analyte, and the fluorophore and quencher will remain in close proximity. Little or no signal will be produced.

[00141] Oligonucleotide probes may have one or a plurality of fluorophores and quenchers per probe. For example, in some embodiments an oligonucleotide probe may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more fluorophores. An oligonucleotide probe may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores. An oligonucleotide probe may comprise fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 fluorophores.

[00142] An oligonucleotide probe may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more quenchers. An oligonucleotide probe may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 quenchers. An oligonucleotide probe may comprise fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 quenchers.

[00143] Attachment of probes and quenchers to a probe may be performed in the same reaction or in serial reactions. A series of reactions may be performed to label probes with at least one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores.

[00144] If an oligonucleotide probe comprises two or more fluorophores, these fluorophores may be arranged to allow Förster (Fluorescence) resonance energy transfer (FRET) to occur between the fluorophores. Briefly, FRET is a mechanism of energy transfer between fluorophores. Using FRET-based probes allows one excitation source to generate two different fluorescence emission signals by excitation of a single fluorophore. For example, the methods described herein may used paired probes, where one probe in the pair is attached to a fluorophore and a quencher and a second probe is attached to two fluorophores (in close enough proximity for FRET to occur) and a quencher. Any combination of fluorophores and quenchers that provide for FRET may be used. Such an approach doubles the number of fluorescent probes that may be used with an excitation source, because a single excitation source can be used to produce two signals: one from the probe with one fluorophore and one from the probe with two fluorophores in close enough proximity to provide for FRET. For example, using the encoding

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2734

method described in Table 8, the number of unambiguously detectable analytes could be increased from 16 to 32 by pairing each of the probes in Table 8 with a corresponding FRET probe.

[00145] The primers may be specific for a particular analyte and capable of amplifying a region complementary to a probe. In some cases, the number of pairs of primers used is equivalent to the number of probes. In other cases, the number of probes used may exceed the number of primer pairs used. In still other cases, the number of primer pairs used may exceed the number of probes used. In some cases a sample to be analyzed is contacted with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more pairs of primers. In some cases a sample to be analyzed is contacted with at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more pairs of primers. In some cases a sample to be analyzed is contacted with fewer than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 pairs of primers. In some cases, the number of pairs of primers is 2-10, 3-15, 4-20, 3-10, 4-10, 5-10, 6-8, or 6-10.

[00146] The primers may amplify regions of a polynucleotide in which different numbers of hybridization probes hybridize. For example, at least one pair of primers may amplify a region complementary to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 hybridization probes. In some cases, all of said pairs of primers may amplify a region complementary to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 hybridization probes.

[00147] In one example, a first probe may be labeled with the fluorophore 5'-fluorescein amidite (5'-FAM) and the quencher black hole quencher 1 (BHQ-1). A second probe may be labeled with 5'-FAM in close proximity (e.g., attached to) to cyanine 5.5 (Cy5.5) and the quencher black hole quencher 3 (BHQ-3). Upon digestion of the probe, via the nuclease activity of the polymerase, two fluorescent signals are generated from a single excitation wavelength (e.g., 470 nm). The fluorophore from the first probe will fluoresce at about 520 nm. The fluorophores on the second probe undergo FRET. The donor fluorophore, FAM is excited by the excitation light source (e.g., 470 nm) and transfers its energy to the acceptor fluorophore, Cy5.5. The acceptor fluorophore emits at about 705 nm. These fluorophores and quenchers are merely exemplary. Any fluorophores that can undergo FRET and any quenchers that can

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2735

quench fluorescence are suitable for use with the invention. Methods for producing FRET-based probes are described in Jothikumar et al., BioTechniques, 2009, 46(7):519-524.

[00148] In some examples, a single hybridization probe may be used for each analyte. In order to utilize multi-color encoding (e.g., as shown in Table 3), each probe may be labeled with a plurality of fluorophores at pre-determined ratios. The ratio may be determined so that a positive control signal provides the same intensity as other positive control signals of the same intensity, in the same color. This approach reduces the number of probes that must be synthesized and may be less expensive to deploy than methods utilizing multiple probes. Moreover, in the case of hybridization probes, it is easier to fit one hybridization probe to an analyte sequence than multiple probes.

[00149] Although many aspects of the invention are exemplified using nucleic acid-based probes, one of ordinary skill in the art will readily recognize that other forms of probes would work equally well with the invention described in this disclosure. For example, a binding molecule specific to an analyte could be used as a probe. Non-limiting exemplary binding molecules include an antibody recognizing an analyte, and generating a signal in the presence of an analyte.

[00150] In embodiments of the invention that utilize fluorescent labels, any suitable fluorescent label may be used. Exemplary fluorescent labels suitable for use with the invention include rhodamine, rhodol, fluorescein, thiofluorescein, aminofluorescein, carboxyfluorescein, chlorofluorescein, methylfluorescein, sulfofluorescein, aminorhodol, carboxyrhodol, chlororhodol, methylrhodol, sulforhodol; aminorhodamine, carboxyrhodamine, chlororhodamine, methylrhodamine, sulforhodamine, and thiorhodamine, cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, merocyanine, cyanine 2, cyanine 3, cyanine 3.5, cyanine 5, cyanine 5.5, cyanine 7, oxadiazole derivatives, pyridyloxazole, nitrobenzoxadiazole, benzoxadiazole, pyrene derivatives, cascade blue, oxazine derivatives, Nile red, Nile blue, cresyl violet, oxazine 170, acridine derivatives, proflavin, acridine orange, acridine yellow, arylmethine derivatives, auramine, crystal violet, malachite green, tetrapyrrole derivatives, porphin, phtalocyanine, and bilirubin. Exemplary quenchers include black hole quenchers, such as BHQ-0, BHQ-1, BHQ-2, BHQ-3; ATTO quenchers, such as ATTO 540Q, ATTO580Q, and ATTO612Q; and the like.

[00151] The fluorophores that may be used with the invention are not limited to any of the fluorophores described herein. For example, fluorophores with improved properties are continually developed, and these fluorophores could readily be used with the methods provided in this disclosure. Such improved fluorophores include quantum dots, which may emit energy at different wavelengths after being excited at a single wavelength. The advantage of using such fluorophores is that only a

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2736

single excitation source is needed, but many different signals may be quantified, for example in terms of color and intensity. Moreover, fluorophores with narrow emission spectra would be particularly useful with the method described herein, as such fluorophores could be included in multiplex assays with minimal or no overlap between their emission spectra, thereby offering many more "colors" and boosting significantly the overall number of coded analytes.

[00152] In some cases, one or more reagents are lyophilized. Any suitable reagent may be lyophilized. For example, probes, primers, enzymes, antibodies, or any other reagent used for detection may be lyophilized. In some cases, a sample comprising an analyte may also be lyophilized. Lyophilization may be useful, for example, when distributing reagents and/or samples in developing regions where access to cold storage is expensive, not readily available, or not reliably available. In one example, lyophilized reagents comprise any probe or analyte-specific reagent described in this disclosure or otherwise suitable for use with the invention. In another example, lyophilized reagents comprise PCR primers. In yet another example, lyophilized reagents comprise reagents suitable for performing a PCR reaction.

V. Analytical Techniques and Instrumentation

[00153] The methods provided herein are suitable for use with a variety of detection methods. For example, the methods may be applied using an analytical technique that measures the wavelength and intensity of a fluorescent signal. This may be accomplished by measuring the intensity of a signal across a spectrum of wavelengths, or by using band pass filters that restrict the passage of certain wavelengths of light, thereby allowing only light of certain wavelengths to reach a photodetector. Many real-time PCR and quantitative PCR instruments comprise an excitation light source and band pass filters that enable the detection of fluorescent signals in four colors (e.g., blue, green, yellow, and red). Therefore, the methods of the invention can be readily applied using instruments widely used in the art. Importantly, the methods and compositions provided herein may be used to detect multiple analytes by obtaining a cumulative measurement on a single solution. No separation is necessary. The invention does not require the use of beads or a solid phase. Of course, one of ordinary skill in the art would understand that the invention could be used with separation, beads, or a solid phase, if desired.

[00154] **Fig. 5** shows two examples of instrumental configurations that may be used to obtain cumulative measurements useful for the methods of the invention. With reference to **Fig. 5**, an instrument with a detector configured to detect both wavelength and intensity (e.g., a fluorometer) is shown in **501.** With reference to **501**, at least one excitation light source **502** is directed into a chamber

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2737

503 containing analytes and reagents that generate a signal in the presence of the analytes **504.** As described elsewhere, the analyte may be a nucleic acid and the reagents generating the signal may be hybridization probes comprising a fluorophore and a quencher. If the analyte is present, an emission signal **505** is generated. In the configuration shown in **501**, the wavelength and intensity of this emission signal are measured across a spectrum by a detector capable of generating a fluorescence emission spectrum **506.**

[00155] The wavelength and intensity may also be determined using a combination of a photodetector and band pass filters. This configuration is used in several thermal cyclers known in the art. With reference to **Fig. 5**, an instrument with band pass filters and a photodetector is depicted in **507.** With reference to **507**, at least one excitation light source **508** is directed into a chamber **509** containing analytes and reagents that generate a signal in the presence of the analytes **510.** As described elsewhere, the analyte may be a nucleic acid and the reagents generating the signal may be hybridization probes comprising a fluorophore and a quencher. If the analyte is present, an emission signal **511** is generated. In the configuration shown in **507**, band pass filters **512, 513,** and **514** are used to restrict the passage of light to light within certain ranges of wavelengths. For example band pass filter **512** may restrict the passage of light to light within wavelength range 1. Band pass filter **513** may restrict the passage of light to light within wavelength range 2. Band pass filter **514** may restrict the passage of light to light within wavelength range 3. Any number of band pass filters (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more) may be used with the invention. In addition, the positions of the band pass filters in **507** are not meant to be limiting. In some cases, emitted light may be passed through more than one band pass filter at any time. In other cases, the band pass filters may be configured so that all emitted light passes through only one band pass filter at a time. After passage through a band pass filter, filtered light **515, 516,** and **517** is detected by a photodetector. For example, filtered light **515** may be light within wavelength range 1, filtered light **516** may be light within wavelength range 2, and filtered light **517** may be light within wavelength range 3. The intensity of each of the filtered lights **515, 516,** and **517** may then be quantified by a photodetector **518.**

[00156] The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, end point PCR methods, reverse transcriptase PCR, and digital PCR methods. Digital PCR methods (e.g., DROPLET DIGITAL (BIORAD) and DYNAMIC ARRAY (FLUIDIGM)) produce highly sensitive quantification of polynucleotide copy numbers. The methods provided herein can be easily integrated into these systems to significantly expand their throughput by allowing multiplexing in a droplet or a dynamic array.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2738

[00157] Similarly, the methods described in this disclosure may be applied in a real-time PCR assay. For example, real-time data may be fully recorded as the PCR runs to completion. The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color. As a particular analyte is amplified, fluorophores are released from its corresponding probes in the same ratios as the color multiplicities in the coding of that sequence. Hence, e.g. a code of 1100 will have the same Ct in blue and green, while a code of 1400 will have its green Ct precede its blue Ct by 2 cycles. This additional data enables determination of the identity and starting quantity of each analyte.

[00158] The methods described herein may also be used directly on a tissue sample. For example, a tissue sample may be obtained and photolithographic methods may be used to build wells directly onto the tissue sample. The tissue sample may be fixed prior to building the wells. The tissue sample may then be analyzed by dispensing appropriate reagents into the wells in the tissue sample. The encoding and decoding methods described in this disclosure may be used for the multiplexed detection of analytes within the wells etched into the tissue. Each well may correspond to a single cell or a few cells, providing excellent spatial resolution when analyzing different portions of a tissue for an analyte. This method may be used to detect analytes in different areas of the same tissue.

[00159] In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum. Including additional excitation sources would allow for the excitation of a larger variety of fluorophores. Including additional band pass filters, or modifying or constructing an instrument capable of determining an entire spectrum allows detection of a wider variety of emissions from fluorophores. These techniques can be used to increase the number of fluorophores that can be used with the methods described in this disclosure and, accordingly, to increase the number of analytes that be simultaneously detected.

[00160] In some cases, the methods described in this disclosure utilize end-point PCR methods. However, in some cases a result may be obtained before the end-point. This may be advantageous when, for example, results are needed as soon as possible. For example, in one case calibration experiments are performed in which different starting concentrations of an analyte are analyzed to determine the cycle number at which the signal becomes saturated, as a function of the starting amount of the analyte. Using this data, a computer monitoring a PCR reaction in real-time can be programmed to search for saturation up to the maximal cycle number according to the limit of detection (LOD) of the

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2739

particular system. If there is no amplification of an analyte (e.g., other than a positive control, if present), by the maximal cycle number, the result for that analyte is negative. If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme. In both cases, there would be no need to run the PCR reaction beyond the number of cycles set by the LOD for that instrument. Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time.

VI. Compositions and Kits

[00161] This disclosure also provides compositions and kits for use with the methods described herein. The compositions may comprise any component, reaction mixture and/or intermediate described herein, as well as any combination. For example, the disclosure provides detection reagents for use with the methods provided herein. Any suitable detection reagents may be provided, including hybridization probes labeled with a fluorophore and a quencher and primers, as described elsewhere in the specification.

[00162] In some cases, compositions comprise reagents for the detection of at least seven analytes using four fluorophores. In some cases, compositions comprise reagents for the detection of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using four fluorophores. In some cases, compositions comprise reagents for the detection of at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using five fluorophores. In some cases, compositions comprise reagents for the detection of at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using six fluorophores. In some cases, compositions comprise reagents for the detection of at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 analytes using seven fluorophores.

[00163] In some cases the compositions comprise primers. The compositions may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 pairs of primers.

IPR2024-01177
California Institute of Technology - Ex. 2003 Page 2740