

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BIO-RAD LABORATORIES, INC.,
Petitioner,

v.

CALIFORNIA INSTITUTE OF TECHNOLOGY,
Patent Owner

Case No. IPR2024-01451

U.S. Patent No. 11,827,921 B2

**PATENT OWNER'S SUBMISSION OF
DEMONSTRATIVE EXHIBITS FOR ORAL ARGUMENT**

Mail Stop "PATENT BOARD"
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

IPR2024-01451
Patent Owner's Submission of Demonstrative
Exhibits For Oral Argument

In accordance with 37 C.F.R. § 42.70(b) and with the Board's Order Setting Oral Argument dated November 20, 2025 (Paper 34) ("Order"), filed herewith are Patent Owner California Institute of Technology's ("Patent Owner") demonstrative exhibits to be relied upon in the Oral Hearing for IPR2024-01451 scheduled on January 6, 2026. Patent Owner also certifies that on December 24, 2025, Patent Owner served Patent Owner's Demonstratives on Petitioner Bio-Rad Laboratories, Inc. as required by the Order.

Respectfully submitted,

SHEPPARD, MULLIN, RICHTER & HAMPTON LLP

/Jesse A. Salen/

Jesse A. Salen (Reg. No. 72,209)

Counsel for Patent Owner

California Institute of Technology

Date: December 31, 2025

12275 El Camino Real, Suite 100

San Diego, CA 92130

Tel: (858) 720-8900

Fax: (858) 509-3691

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), I hereby certify that on December 31, 2025, a copy of the foregoing **PATENT OWNER'S SUBMISSION OF DEMONSTRATIVE EXHIBITS FOR ORAL ARGUMENT** was served via E-Mail on the Petitioner at the following correspondence address provided by counsel of record for the Petitioner pursuant to 37 C.F.R. § 42.8(b)(4):

Derek C. Walter
dwalter@jonesday.com
Jones Day
555 California Street, 26th floor
San Francisco, CA 94104

Matthew W. Johnson
mwjohnson@jonesday.com
Jones Day
500 Grant Street, Suite 4500
Pittsburgh, PA 15219

/Jesse A. Salen/

Jesse A. Salen (Reg. No. 72,209)
Counsel for Patent Owner
California Institute of Technology

12275 El Camino Real, Suite 100
San Diego, CA 92130
Tel: (858) 720-8900
Fax: (858) 509-3691



Patent Owner Caltech's Demonstratives for '921 Patent Oral Hearing

**IPR2024-01451
Patent 11,827,921 B2**

January 6, 2026

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Caltech

Topics

- | |
|---|
| 1. The '921 Patent and challenged claims |
| 2. Caltech's responses to Grounds |
| 3. Dr. Struhl's testimony should be credited |
| 4. Caltech's motion to strike |
| 5. Construction of "single sample volume" |

Topics

1. The '921 Patent and challenged claims are patentable.

2. Caltech's responses to Grounds

3. Dr. Struhl's testimony should be credited

4. Caltech's motion to strike

5. Construction of "single sample volume"

The '921 Patent and the Challenged Claims



US011827921B2

(12) **United States Patent**
Kartalov et al.

(10) **Patent No.:** **US 11,827,921 B2**
(45) **Date of Patent:** ***Nov. 28, 2023**

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

(71) Applicant: **California Institute of Technology**, Pasadena, CA (US)

(72) Inventors: **Emil P. Kartalov**, Pasadena, CA (US); **Aditya Rajagopal**, Orange, CA (US); **Axel Scherer**, Pasadena, CA (US)

(73) Assignee: **California Institute of Technology**, Pasadena, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 580 days.
This patent is subject to a terminal disclaimer.

(21) Appl. No.: **16/864,744**

(22) Filed: **May 1, 2020**

(65) **Prior Publication Data**
US 2021/0098076 A1 Apr. 1, 2021

Related U.S. Application Data

(63) Continuation of application No. 15/914,356, filed on Mar. 7, 2018, now Pat. No. 10,770,170, which is a (Continued)

(51) **Int. Cl.**
G01N 33/48 (2006.01)
G01N 33/50 (2006.01)
(Continued)

(52) **U.S. CL.**
CPC **C12Q 1/6825** (2013.01); **C12Q 1/68** (2013.01); **C12Q 1/6851** (2013.01);
(Continued)

(58) **Field of Classification Search**
CPC C12Q 1/6825; C12Q 1/68; C12Q 1/6851;
G01N 21/6486; G06F 17/10; G06F 17/11
See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS
5,079,351 A 1/1992 Saindy et al.
5,538,848 A 7/1996 Livak et al.
(Continued)

FOREIGN PATENT DOCUMENTS
CN 1936019 A 3/2007
CN 101570782 A 11/2009
(Continued)

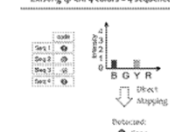
OTHER PUBLICATIONS
Arya, et al., Basic principles of real-time quantitative PCR, 2005, *Expert Rev. Mol. Diagn.*, 5(2), p. 209-219.
(Continued)

Primary Examiner — Russell S Negin
(74) *Attorney, Agent, or Firm* — Sheppard Mullin Richter & Hampton LLP

(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.

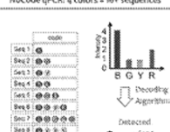
19 Claims, 8 Drawing Sheets
Specification includes a Sequence Listing.

Existing qPCR: 4 colors × 4 sequences



Decoded:
Seq 1
Seq 2
Seq 3
Seq 4

NuCode qPCR: 4 colors × 16+ sequences



Decoded:
Seq 1
Seq 2
Seq 3
Seq 4
Seq 5
Seq 6
Seq 7
Seq 8
Seq 9
Seq 10
Seq 11
Seq 12
Seq 13
Seq 14
Seq 15
Seq 16

B: Blue
G: Green
Y: Yellow
R: Red

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Paper 17 at 14-15; EX1001 at page 1, claim 1

M, F, and coding scheme

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

TABLE 3-continued

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

	Blue	Green	Yellow	Red
F	1	1	1	0
G	1	1	1	1

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Paper 17 at 8-10; EX1001 at table 3, claim 1

All grounds are based on Bio-Rad's theory that Larson teaches $M \geq 3$ and $F \geq 2$

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said **at least M analytes** is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises **F wavelength components**, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Bio-Rad:

Most important for the purposes of the present petition, in paragraph 140, Larson describes detecting **three target polynucleotides** using **two colors**. A single-color probe of the first color is used to detect the first analyte, a second single-color probe of the second color is used to detect the second analyte, and **one or more probes collectively including both the first and second colors** is used to detect **the third analyte**. *Id.* ¶ 140; EX1002 ¶ 113. The particular assay format set forth in paragraph 140 of Larson is relied upon throughout this petition and corresponds to the exact assay format used in Bio-Rad's Wastewater and SARS-CoV-2 kits that Patent Owner accuses of infringing in parallel district court proceedings. See EX1012; EX1002 ¶ 113.

Paper 17 at 12; Paper 1 at 1, 24-29; Paper 20 at 14-15; EX1001, claim 1

6

The claim language regarding plural “components” supports $M \geq 3$ and $F \geq 2$

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,
wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,
wherein said fluorescent signal comprises F wavelength components, and
wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,
wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and
wherein the analytes are amplified in the single sample volume.

“In accordance with common English usage, we presume a plural term refers to two or more items.”

Apple Inc. v. MPH Techs. Oy, 28 F.4th 254, 261
(Fed. Cir. 2022)

Dr. Struhl confirmed $M \geq 3$ and $F \geq 2$

Dr. Struhl:



Q. In view of the claim language, what is your understanding of what a minimum value of F is?

A. I mean, it says it right here, F equals 2 or is greater than 2.

47. It is clear from the language of the claims and the original specification that the '921 Patent can detect three or more analyte molecules ($M \geq 3$) using multiple colors ($F \geq 2$). For example, the '921 Patent states that “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,” and the lowest example number of analytes that the '921 Patent discloses detecting per fluorophore is “1.5” analytes. See EX1001('921 Patent), 12:46-62. For this lower bound of $M=1.5$ analytes per fluorophore disclosed in the '921 Patent, the smallest value of F that yields an integer number of analytes (and thus the smallest value of F that yields a physical result) is $F=2$, for which $M=3$. See EX1001('921 Patent), 12:46-62.

EX1044 at 197:11-14; EX2018 at ¶47

The Board relied on Bio-Rad's $M \geq 3$ and $F \geq 2$ theory in Larson

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Institution Decision:

Here, Petitioner asserts that Larson disclosed conducting a digital PCR assay “using two colors to identify three target analytes, relying on the combination of two colors as the multi-color label for one analyte, with each of the two colors individually as a label for the other two targets.” *Id.* at 51, 54 (referring to Petitioner’s analysis for the anticipation ground), 28–29 (citing Ex. 1017 ¶ 140; Ex. 1002 ¶ 130). Petitioner asserts for this assay “M is three and F is two,” and “Larson . . . detect[s] whether . . . three targets are present in a ‘sample volume,’ specifically, a portion of a sample solution that is subsequently partitioned into droplets.” *Id.* at 29 (citing Ex. 1002 ¶¶ 131–132; EX1017 ¶¶ 11, 13–17, 21–23, 52–68, 154, 164, Figs. 1–3).

In this regard, Petitioner and Dr. Batt refer to Larson’s assay depicted in Figures 9A and 9C, “involving three targets and two colors, wherein one color is used to identify two of the targets and a second color identifies the third target.” *Id.* (citing Ex. 1017 ¶ 121, Fig. 9A; Ex. 1002 ¶ 333). According

Paper 17 at 12-13; Paper 11 at 30-33; EX1001, claim 1

9

Detecting combinations of analytes in single sample volume

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

TABLE 3-continued

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

	Blue	Green	Yellow	Red
F	1	1	1	0
G	1	1	1	1

TABLE 4 (excerpt)

7	3	3	3	pABCDEF
8	4	4	4	pABCDEFGG

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Paper 17 at 8-10; EX1001 at table 3, table 4, claim 1

Detecting combinations of analytes in single sample volume

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,
wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,
wherein said fluorescent signal comprises F wavelength components, and
wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,
wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and
wherein the analytes are amplified in the single sample volume.

Bio-Rad:

The claim language requires merely detecting “M analytes” in a “single sample volume,” with amplification occurring in that same volume. EX1001 67:9–26, 68:19-48. There are no other requirements as to the “single sample volume” in any of the claims. Nothing in any of the claims, for instance, places any restrictions as to the form of the “single sample volume,” including whether it be a tube, a droplet, a partitioned volume, or a non-partitioned volume. See EX1002 ¶¶ 13-14.

Construction of “single sample volume” = plain and ordinary meaning

The Board “need only construe terms that are in controversy, and only to the extent needed to resolve the controversy.”

- Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co., 868 F.3d, 1017 (Fed. Cir. 2017)

Paper 29 at 5-6; Paper 33 at 2-3

12

Detecting combinations of analytes in single sample volume

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Institution Decision:

To begin, we find no dispute between the parties with respect to the meaning of “single sample volume,” recited in claim 1. Both parties agree that the ’921 patent “contemplates both embodiments where the sample volume is separated and those where it is not.” Pet. Reply 1–2; PO Sur-reply 3. Further, the parties agree that the “single sample volume” is not “limited to an individual ‘droplet,’ but rather includes any type or nature of volume, such as a ‘tube or bottle.’” Pet. Reply 1; PO Sur-reply 3.

Caltech:

The Board need not construe the term “single sample volume” because there is no controversy over its construction and the term should be given its plain and ordinary meaning. The claimed “single sample volume” includes a droplet or any other type of volume, such as a tube or bottle. Nonetheless, claim 1’s concluding

Paper 11 at 33 (citing Paper 9 at 1-2; citing Paper 10 at 3); Paper 30 at 1; Paper 33 at 1; EX1001, claim 1

13

Claim 1 also requires that “the analytes are amplified in the single sample volume.”

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,
wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,
wherein said fluorescent signal comprises F wavelength components, and
wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,
wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and
wherein the analytes are amplified in the single sample volume.

Institution Decision:

To begin, we find no dispute between the parties with respect to the meaning of “single sample volume,” recited in claim 1. Both parties agree that the ’921 patent “contemplates both embodiments where the sample volume is separated and those where it is not.” Pet. Reply 1–2; PO Sur-reply 3. Further, the parties agree that the “single sample volume” is not “limited to an individual ‘droplet,’ but rather includes any type or nature of volume, such as a ‘tube or bottle.’” Pet. Reply 1; PO Sur-reply 3.

Caltech:

The Board need not construe the term “single sample volume” because there is no controversy over its construction and the term should be given its plain and ordinary meaning. The claimed “single sample volume” includes a droplet or any other type of volume, such as a tube or bottle. Nonetheless, claim 1’s concluding

Paper 11 at 33 (citing Paper 9 at 1-2; citing Paper 10 at 3); Paper 30 at 1; Paper 33 at 1; EX1001, claim 1

14

Larson discloses that the analytes are amplified in a droplet

In Larson, the “single sample volume” must be a “droplet,” and Larson teaches diluting the sample such that there is only one target per droplet.

US 2011/0250597 A1

4

Oct. 13, 2011

cence intensities; FIG. 11b shows a comparison of gene copy numbers measured by microfluidic dPCR.

[0032] FIG. 12 is a schematic for tuning the intensity of a detectable label to a particular target with a microfluidic device.

[0033] FIG. 13 is a line graph depicting the linear dependence of droplet fluorescence intensity on probe concentration (Line, best linear fit ($y = -0.092x + 0.082$, $R^2 = 0.995$)).

[0034] FIG. 14 depicts a 5-plex dPCR assay for spinal muscular atrophy with only two fluorophores. FIG. 14a is a 2D histogram of droplet fluorescence intensities, shown as a heat map, for the 5-plex assay against the synthetic model chromosome for validation. The six well resolved droplet populations corresponded to the five individual assays plus the empty droplets; FIG. 14b shows the results of the SMA pilot study.

[0035] FIG. 15 depicts a 9-plex dPCR assay for spinal muscular atrophy with only two fluorophores, showing the process of optimizing droplet intensities. FIGS. 15a and 15b show 2-D histograms of droplet fluorescence intensity, shown as heat maps with hotter colors representing higher droplet counts, for the 9-plex assay against the synthetic model chromosome (a) Before optimization; (b) after optimization.

[0036] FIG. 16 depicts an optical schematic for combining optical labels with multiplexing.

[0037] FIG. 17 depicts a dPCR assay combining multiplexing with optical labels using co-flow microfluidics. The contributions from all droplets are shown, that is, from three different triplex assays. (Both panels) 2-D histograms shown as heat maps with hotter colors representing higher droplet counts. (Left panel) histogram of optical labels, i.e. fluorescence intensities of droplets measured at wavelengths for the two fluorophores comprising the optical labels. (Right panel) assay histogram, i.e. fluorescence intensities of droplets measured at wavelengths suitable for FAM detection (x-axis), and VIC detection (y-axis). Both histograms were compensated for spectral overlap by standard techniques.

[0038] FIG. 18 shows single assay selections using optical labels. Selections were taken from all of the droplets from FIG. 17. Each of the three different selections in panels A-C were for optical labels encoding the same assay (TERT, SMN1, and SMN2). Histograms are as described in FIG. 17. (Left histograms, optical labels) Superimposed lines demarcate the bounding box for selecting a single optical label. (Right histograms, assay) Only droplets containing the selected optical label are displayed.

[0039] FIG. 19 shows single assay selections using optical labels. Selections were taken from all of the droplets from FIG. 17. Each of the three different selections in panels A-C were for optical labels encoding the same assay (TERT, c-SC from SMN1, and HCKD1A). Histograms are as described in FIG. 17. (Left histograms, optical labels) Superimposed lines demarcate the bounding box for selecting a single optical label. (Right histograms, assay) Only droplets containing the selected optical label are displayed.

[0040] FIG. 20 shows single assay selections using optical labels. Selections were taken from all of the droplets from FIG. 17. Each of the three different selections in panels A-C were for optical labels encoding the same assay (TERT, c-88G from SMN1, and RNaseP). Histograms are as described in FIG. 17. (Left histograms, optical labels) Superimposed lines demarcate the bounding box for selecting a single optical label. (Right histograms, assay) Only droplets containing the selected optical label are displayed.

[0041] FIG. 21 depicts a dPCR assay combining multiplexing with optical labels using droplet merging.

[0042] FIG. 22 is a schematic showing haplotype detection in droplets.

DETAILED DESCRIPTION

[0043] The invention provides materials and methods for analysis of biomolecules. In one aspect, the invention provides for digital analysis in droplets, such as microfluidic droplets. The invention allows digital PCR to be conducted and provides for signal amplification or signal amplification.

[0044] Ideally, the sensitivity of digital PCR is limited only by the number of independent amplifications that can be analyzed, which has motivated the development of several ultra-high throughput miniaturized methods allowing millions of single molecule PCR reactions to be performed in parallel (discussed in detail elsewhere). In a preferred embodiment, the invention provides for digital PCR in aqueous droplets separated by oil using a microfluidic system. In another preferred embodiment, the oil is a fluorinated oil such as the Fluorinert oils (3M). In a still more preferred embodiment the fluorinated oil contains a surfactant, such as PEG-PPG-PPG triblock copolymer, to stabilize the droplets against coalescence during the amplification step or at any point where they contact each other. Microfluidic approaches allow the rapid generation of large numbers (e.g. 10^6 or greater) of very uniformly sized droplets that function as picoliter volume reaction vessels (see reviews of droplet-based microfluidics). But as will be described, the invention is not limited to dPCR performed in water-in-oil emulsions, but rather is general to all methods of reaction compartmentalization for dPCR. In the description that follows, the invention is described in terms of the use of droplets for compartmentalization, but it is understood that this choice of description is not limiting for the invention, and that all of the methods of the invention are compatible with all other methods of reaction compartmentalization for dPCR.

[0045] Methods of the invention involve novel strategies for performing multiple different amplification reactions on the same sample simultaneously to quantify the abundance of multiple different DNA targets, commonly known to those familiar with the art as “multiplexing”. Methods of the invention for multiplexing dPCR assays promise greater flexibility than existing qPCR or dPCR techniques. It is based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in any one droplet even when multiple primers/probes targeting different alleles are present. This alleviates the complications that otherwise plague simultaneous competing reactions, such as varying arrival time into the exponential phase and unintended interactions between primers.

[0046] In one aspect, the invention provides materials and methods for improving amplification yield while maintaining the sensitivity and specificity in droplet based digital PCR. More specifically, the invention provides droplets containing a single nucleic acid template and multiplexed PCR primers and methods for detecting a plurality of targets in a biological sample by forming such droplets and amplifying the nucleic acid templates using droplet based digital PCR.

[0047] Reactions within microfluidic droplets yield very uniform fluorescence intensity at the end point, and ultimately the intensity depends on the efficiency of probe hydrolysis. Thus, in another aspect of the methods of the

[0044] Ideally, the sensitivity of digital PCR is limited only by the number of independent amplifications that can be analyzed, which has motivated the development of several ultra-high throughput miniaturized methods allowing millions of single molecule PCR reactions to be performed in parallel (discussed in detail elsewhere). In a preferred

existing qPCR or dPCR techniques. It is based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in any one droplet even when multiple primers/probes targeting different alleles are present. This alleviates the complications that otherwise plague simultaneous competing reactions, such as varying arrival time into the exponential stage and unintended interactions between primers.

BIO-RAD EX.1017.045

Paper 17 at 17 (citing EX1017, ¶¶44-45; EX2018, ¶55; EX2017, 100:9-21, 127:12-130:4, 136: 4-10)

Analytes are amplified in the same single sample volume where detected



Dr. Struhl:

with similar limitations. EX1001('921 Patent), 68:31-38. While the single sample volume may not necessarily contain *all* analyte molecules at the same time, the power of the '921 Patent lies in its flexibility to detect *any possible combination* of the analyte molecules within the single sample volume within which that combination of analyte molecules is amplified, in a manner that each analyte may be uniquely distinguished from each other analyte. See ¶¶37-49 of this Declaration.

At the time of the '921 Patent and its priority applications, no such solution was available to solve the problem of how to increase the number of analytes which could be non-degenerately detected in a single sample volume in any combination in a single sample volume (such as a vial or droplet) within which amplification is performed. See ¶¶37-49 of this Declaration; *see also* EX1001('921 Patent), 1:46-47 ("There is a need for multiplexed reactions that can be carried out *in a single solution.*")

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, **in a single sample volume**, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and **wherein the analytes are amplified in the single sample volume.**

Paper 17 at 30; Paper 29 at 7; EX2018 at ¶¶ 30, 37-49, 90-92; EX1001 at 36:58-67, claim 1

16

Analytes are amplified in the same single sample volume where detected

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.



Dr. Batt:

the proximity allows the quencher to suppress the signal. This type of probe is generally referred to as a TaqMan probe and was described in literature as early as 1991. EX1011. The TaqMan probe is used with two PCR primers and is designed to bind to a region between the two primers. In this method, a target-bound probe is cleaved during extension by the DNA polymerase, which has an exonuclease activity. The cleavage frees the quencher and fluorophore which both diffuse away and the signal from the fluorophore is no longer quenched, thereby allowing detection of signal emitted from the fluorophore. This process is depicted in the figure below:

EX1002 at ¶42; Paper 29 at 7; EX1001, claim 1

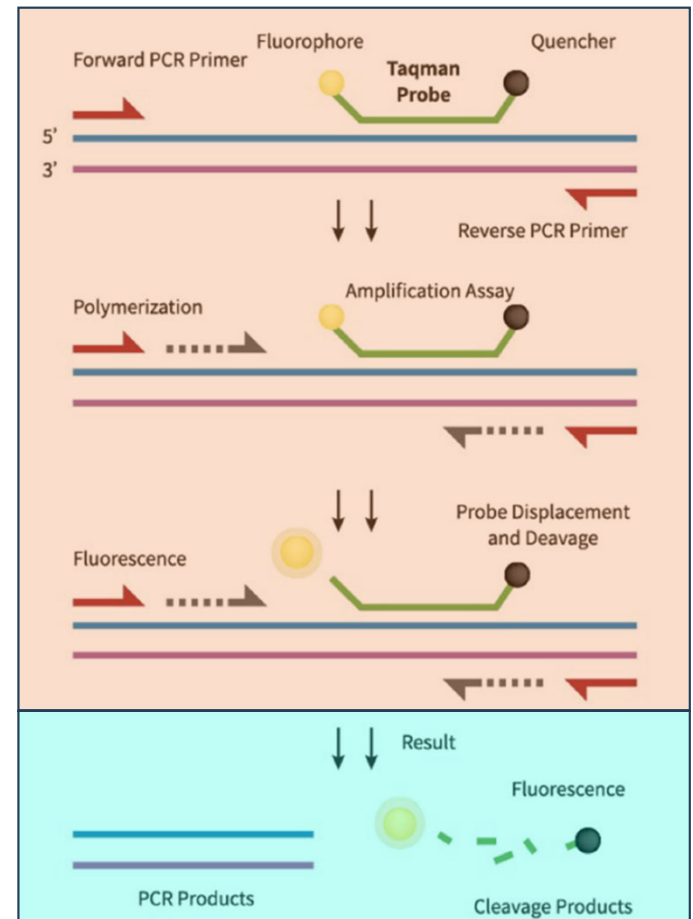
17

Analytes are amplified in the same single sample volume where detected

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Dr. Batt:



EX1002 at ¶42; Paper 29 at 7; EX1001, claim 1

18

Analytes are amplified in the same single sample volume where detected

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Bio-Rad:

and digital PCR (“dPCR”). EX1002 ¶¶ 43-47. One form of qPCR uses TaqMan probes that hybridize to the target sequence and generate a signal when degraded by a polymerase. *Id.* ¶ 45. The signal generated is proportional to the amount of the target polynucleotide in a sample, allowing quantification of analytes. *Id.* ¶ 46.

In dPCR, the PCR solution including sample is partitioned into small droplets or other partitions, where separate PCR reactions occur. *Id.* ¶ 47. TaqMan probes

The claim language requires merely detecting “M analytes” in a “single sample volume,” with amplification occurring in that same volume. EX1001 67:9–26, 68:19-48. There are no other requirements as to the “single sample volume” in any of the claims. Nothing in any of the claims, for instance, places any restrictions as to the form of the “single sample volume,” including whether it be a tube, a droplet, a partitioned volume, or a non-partitioned volume. *See* EX1002 ¶¶ 13-14.

Paper 1 at 4-5; Paper 25 at 5; Paper 29 at 7; EX1001, claim 1

19

Topics

1. The '921 Patent and challenged claims

2. Bio-Rad failed to meet its burden of demonstrating unpatentability under any of its alleged grounds.

3. Dr. Struhl's testimony should be credited

4. Caltech's motion to strike

5. Construction of "single sample volume"

Grounds in Petition

Ground 1	Alleged anticipation by Larson (US 2011/0250597) under pre-AIA 35 U.S.C. § 102	Claims 1-10, 13-19
Ground 2	Alleged obviousness over Larson under pre-AIA 35 U.S.C. § 103	Claims 4, 6, 7, 14-19
Ground 3	Alleged obviousness over Larson in view of Saxonov (US 2013/0040841) under pre-AIA 35 U.S.C. § 103	Claims 1-10, 12, 19
Ground 4	Alleged obviousness over Larson in view of Saxonov and Silverbrook (US 2011/0312704) under pre-AIA 35 U.S.C. § 103	Claim 11

Ground 1: Bio-Rad's Arguments are Factually and Legally Flawed

1. Bio-Rad cannot meet its burden to show Larson anticipates claim 1:

Bio-Rad ignores claim 1's plain language and the understanding of a POSA as established by Bio-Rad's own expert, in favor of non-binding extrinsic evidence (PO's infringement contentions)

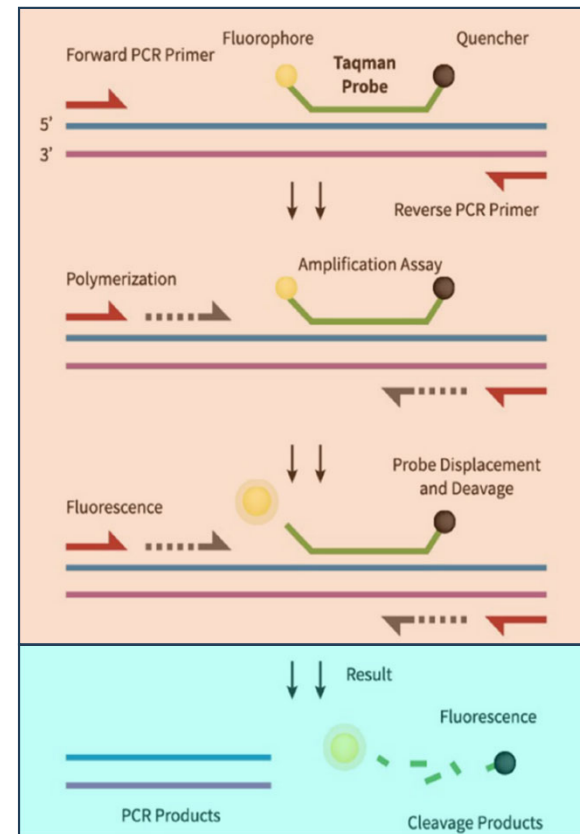
2. Dr. Batt's testimony regarding infringement contentions should not be credited:

Dr. Batt was unaware of, and did not apply, the correct legal standard to interpret the '921 Patent claims in light of such non-binding extrinsic evidence

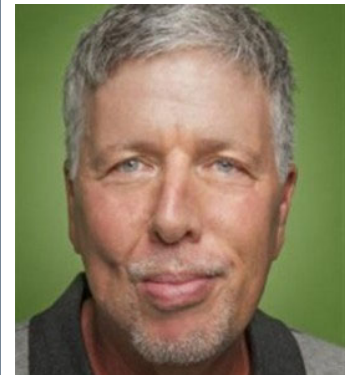
Analytes are amplified in the same single sample volume where detected

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.



Dr. Batt:



EX1002 at ¶42; Paper 29 at 7; EX1001, claim 1

Bio-Rad's theory replaces the claim language with non-binding extrinsic evidence

Bio-Rad's Ground 1 anticipation theory for “the analytes are amplified in the single sample volume” is improperly based solely on PO's infringement contentions:

6. wherein the analytes are amplified in the single sample volume.

Larson discloses PCR assays that amplify target polynucleotide analytes in a single sample volume, specifically a portion of the reaction mixture that is subsequently partitioned into droplets. *See supra* Sections X.A.1, 5; EX1002

¶¶ 144-147.

As detailed above, Patent Owner relies on Petitioner's droplet digital PCR assays as infringing and contends that the claimed “sample volume” is a portion of the sample mixture that is subsequently partitioned into droplets. EX1002 ¶ 145.

As Patent Owner states with regard to Petitioner's products, “the detection is performed in a single *sample volume* that is *subsequently* partitioned into multiple

droplets.” EX1012 at 20. Patent Owner goes on to state that “analytes are amplified in the single sample volume” where “the single sample is partitioned into individual droplets” and that “[f]ollowing droplet generation, the sample is amplified in a thermal cycler” prior to reading “each droplet to determine the fraction of positive droplets in the original sample and us[ing] Poisson statistical formulas to determine the absolute quantity.” *Id.* at 27-29. Thus, Larson again discloses the exact thing that Patent Owner now accuses of infringing in district court. EX1017 ¶¶ 2, 9, 11, 13, 14, 16, 17, 20, 47, 49, 91, 93, 119-121, 150, 195, Figs. 1-4, 7-11, 15; EX1002 ¶ 146.

Paper 1 at 31-32; Paper 17 at 33; EX1002

24

Infringement contentions are of limited value

“We do not regard the infringement contentions in the District Court as a claim construction proposed by Patent Owner in the proceeding before us.”

- Intex Recreation Corp. v. Bestway (USA), Inc., No. IPR2019-00454, Paper 34 at 11 (PTAB June 22, 2020)

Extrinsic evidence “of what a party [allegedly] subjectively thought was a proper construction is of limited value.”

- Great W. Cas. Co. v. Intell. Ventures II LLC, IPR2025-01706, Paper 69 at 13 (PTAB Feb. 14, 2020)

Paper 17 at 25; Paper 29 at 8

25

Dr. Batt did not know the proper legal standard for evaluating extrinsic evidence

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BIO-RAD LABORATORIES, INC.,
Petitioner,

v.

CALIFORNIA INSTITUTE OF TECHNOLOGY,
Patent Owner.

U.S. PATENT NO. 11.827,921 B2

DECLARATION OF CARL A. BATT, PH.D. IN SUPPORT
OF PETITIONER FOR *INTER PARTES* REVIEW

Q. Now, your invalidity opinions in this IPR are based in part on Caltech's infringement contentions, correct, the infringement contentions from the parallel litigation, correct?

A. Right, from the district court, yes, yes, the ChromaCode cases, I sometimes refer to it, yes.

Q. But section 4B on claim construction doesn't say anything about the legal standard for interpreting claims in an IPR based on the patent owner's infringement contentions in a parallel litigation, correct?

A. It does not state that, it doesn't exclude it. There is no statement here do not include.

Dr. Batt:



Q. Do you know what the legal standard is for interpreting claims in an IPR based on the patent owner's infringement contentions in a parallel litigation?

A. I do not have that information off the top of my head.

BIO-RAD EX.1002.001

Paper 17 at 26; Paper 29 at 8; EX2017 at 47:8-49:11

26

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Ground 2: Bio-Rad's Arguments Lack Merit

1. Dr. Batt agrees with Caltech that Larson's goal is one target per droplet and that Larson teaches many problems associated with multiple occupancy.

2. Dr. Batt did not properly analyze motivation to modify Larson in view of Larson's admitted teachings on the difficulties of multiple occupancy.

3. Bio-Rad should not be permitted to shift its theories and rely on new evidence after failing to meet its burden to establish obviousness in the Petition.

4. Bio-Rad's current representations to the Board about Larson's teachings, for purposes of attacking the '921 Patent, entirely contradict those it made in a in a different IPR for purposes of preserving Larson's validity.

Bio-Rad's theory: Larson FIG 9 and ¶121 teach $M \geq 3$, $F \geq 2$, and coding

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,

wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,

wherein said fluorescent signal comprises F wavelength components, and

wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,

wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single sample volume.

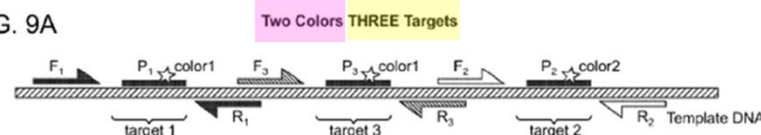
Bio-Rad:

2. wherein each...coding scheme

As documented above, Larson teaches a scheme wherein each of two or three analytes is encoded by an intensity of light in either one or two colors. *See supra* Section XI.A.1-4; EX1002 ¶¶ 324-327.

For example, Larson teaches a coding scheme wherein one detects three targets using only two colors, as depicted in Fig. 9:

FIG. 9A



EX1017 Fig. 9A. As shown, targets 1-3 are detected using corresponding forward and reverse primer pairs F₁₋₃ and R₁₋₃, respectively, and labeled probes P₁₋₃ for each of targets 1, 2, and 3. *Id.* ¶ 121. The probes for targets 1 and 3, probes P₁ and P₃, are labeled with the same color (color1), but provide different intensity signals, and probe P₂ is labeled with a different color (color2). *Id.* ¶ 121; EX1002 ¶ 325. This

Paper 1 at 53-57; Paper 17 at 46-54; EX1001, claim 1

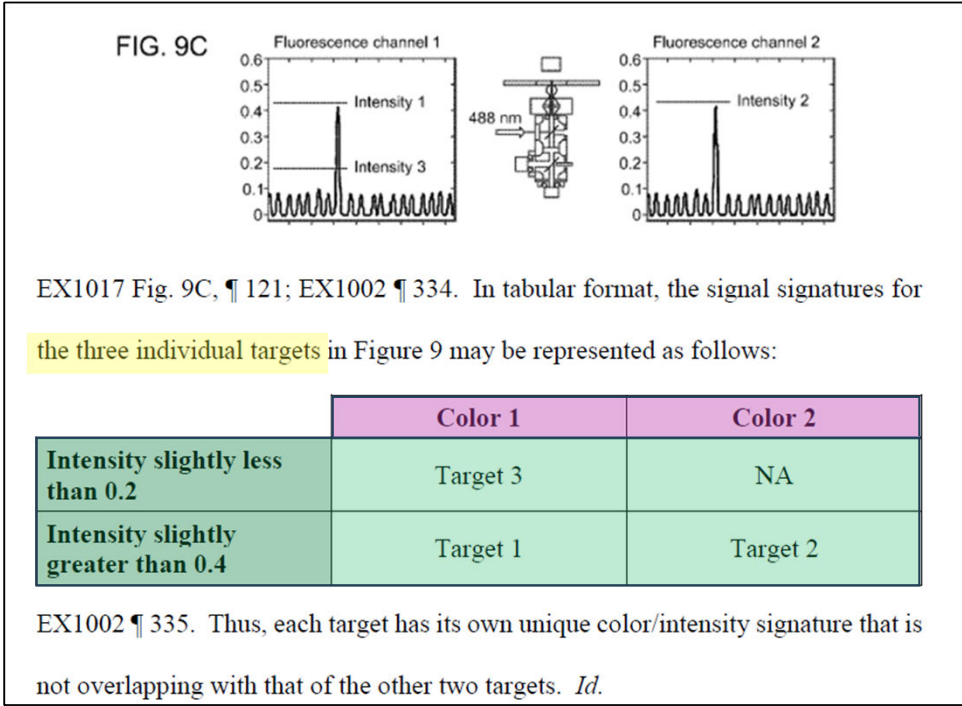
28

Bio-Rad's theory: Larson FIG 9 and ¶121 teach $M \geq 3$, $F \geq 2$, and coding

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Bio-Rad:



Larson's FIG 9 and ¶121 do not teach multiple occupancy

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,

wherein said fluorescent signal comprises F wavelength components, and

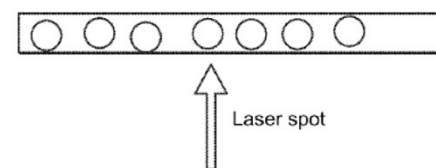
wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,

wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single sample volume.

Caltech:

FIG. 9B



Larson's FIG. 9B is expressly directed to "*single molecule* amplification," which directly contradicts Petitioner and Dr. Batt's "multiple occupancy" theory.

EX1017, FIG. 9B; EX2018, ¶114; *see, e.g.*, Petition, 60-61; EX1002, ¶312 et seq.

So while this figure's omission from the Petition and Dr. Batt's declaration is noteworthy, it is also not surprising.

Larson's FIG 9 and ¶121 do not teach multiple occupancy

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,

wherein said fluorescent signal comprises F wavelength components, and

wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,

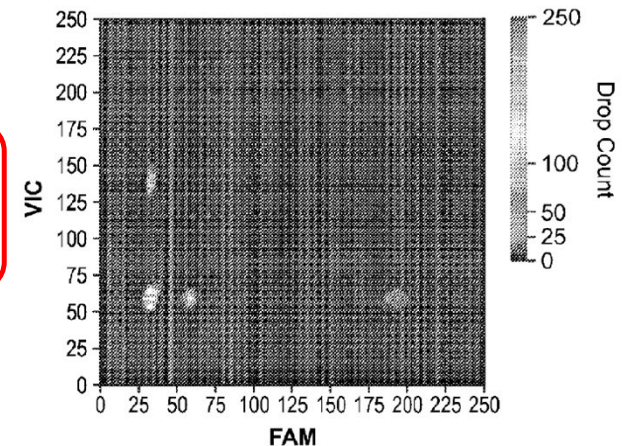
wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single sample volume.

Caltech:

FIG. 9D

Histogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets.



EX1017, FIG. 9D; EX2018, ¶119.

According to Larson, FIG. 9D illustrates a “[h]istogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets.” EX1017, ¶121; EX2018, ¶119. Moreover, Larson’s legend to FIG. 9D exclusively uses the disjunctive “or” to refer to the targets. EX1017,

Paper 17 at 53-54; EX1001, claim 1

31

Larson's FIG 9 and ¶121 do not teach multiple occupancy

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Dr. Batt:



Q. And in paragraph 121, the last sentence, Larson doesn't say anything about microdroplets containing targets 1, 2 and 3, does he?

A. Containing all three of them, is that what you are asking?

Q. He doesn't say that, correct?

A. He doesn't account for those, he doesn't say that per se. It doesn't mean that they don't exist.

Paper 27 at 14; EX2017 at 114:13-25; EX1001, claim 1

32

Larson's FIG 9 and ¶121 do not teach multiple occupancy

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,

wherein said fluorescent signal comprises F wavelength components, and

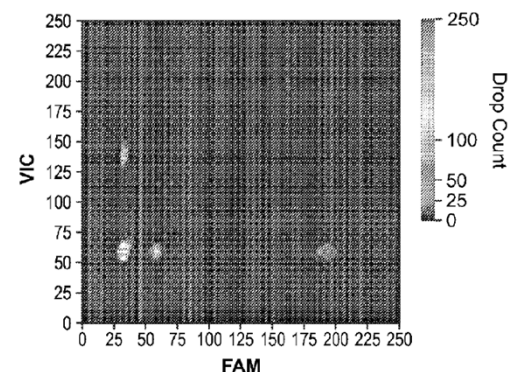
wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,

wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single sample volume.

FIG. 9D

Histogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets.



Dr. Batt:

There may be a droplet floating around at 250, let's say a FAM, let's pick up what appears to be a blank level, 125 on the FAM, 125 on the VIC, right, it looks pretty blank to me, does that mean there is no droplets there? I don't know.

Q. You don't know?

A. No, you don't know, right, because the analysis of the original sample volume is dependent upon these clusters.

Paper 27 at 14; Paper 17 at 53; EX2017 at 113:10-19; EX1001, claim 1

33

Bio-Rad's bigger problem: Larson's "goal" is single-occupancy droplets

Dr. Batt



Q. And those are issues that Larson avoids by having only one template molecule per droplet, that's what he says in paragraph 100, correct?

A. So if you are looking at that phrase that says these issues are avoided in droplet PCR, that is correct. And it says there is only one. In other words, you can't exclude only one a priori, but that would be your aspiration.

Q. That's how Larson sought to avoid the issues of having multiple alleles, right?

A. Yes, digital PCR in general, Larson being an example of that, that is the goal of digital PCR.

Paper 27 at 3-6; EX2017 at 129:13-130:4; *id.* at 106:6-22, 107:18-108:3, 127:6-11, 127:6-11; EX2025 at 39:12-19, 50:19-23 14; *accord* EX2018 at ¶¶76, 108-119

Dr. Batt explains why Larson's goal is single-occupancy droplets

Dr. Batt:



Q. Now we touched on this before, but Larson describes several downsides of detecting multiple analytes in a droplet; is that correct?

A. As a general statement, yes.

Q. And that includes bias from allele-specific PCR; is that correct?

A. That is correct, yes.

Q. One of the downsides Larson describes with detecting multiple analytes in a droplet is bias from competition between different amplicons, correct?

A. It states specifically these result in varying yields between different -- I am sorry, these results in

varying yields between competing amplicon which can result in a non-uniform amplicon yield. So in other words, yes, there is competition. I am not sure if this states it exactly the way you phrased it.

Q. It is a complexity, correct?

A. Yes, it is a consideration.

Q. In paragraph 129 of Larson, Larson describes another challenge as the possibility of cross reactivity, correct?

A. Yes, I don't like the word "cross reactivity," but yes.

Q. And Larson in paragraph 129 describes the downside of an error that results from overlapping probes, correct?

A. Yes, it does speak to overlapping, yes.

Paper 27 at 3-6; EX2017 at 182:4-183:18; accord EX2018 at ¶¶52, 55, 58, 105

35

Dr. Batt explains how Larson achieves single-occupancy droplets

Dr. Batt:



21 Q. Larson alleviates the
22 complications of simultaneous reactions by
23 having only a single target allele per
24 droplet, correct?

25 A. Yes, so the problem in PCR in
2 general is that when you have multiple
3 events going on, they obviously, not they
4 obviously, but they can compete for one
5 another and the products of those reactions.
6 So the more simplistic, the lower the number
7 of targets you have in a sample, the less
8 likely it is that those competing reactions
9 would cause problems.

10 Q. That's what Larson teaches, right?

11 A. Yes. And it goes on, there is a

Paper 27 at 5-6; EX2017 at 103:21-104:11, 168:21-169:23

36

Bio-Rad's representations in a different IPR concerning a patent related to (continuation of) Larson

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DROPWORKS, INC.,
Petitioner,

v.

BIO-RAD LABORATORIES, INC.,
Patent Owner.

Case IPR2021-00212
U.S. Patent No. 9,127,310

PATENT OWNER'S PRELIMINARY RESPONSE TO PETITION FOR
INTER PARTES REVIEW OF U.S. PATENT NO. 9,127,310

Mail Stop "PATENT BOARD"
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Larson (US 2011/0250597)



US009127310B2

(12) **United States Patent**
Larson et al.

(10) **Patent No.:** **US 9,127,310 B2**
(45) **Date of Patent:** **Sep. 8, 2015**

(54) **DIGITAL ANALYTE ANALYSIS**

(71) Applicant: **Raindance Technologies, Inc.**, Billerica, MA (US)

(72) Inventors: **Jonathan William Larson**, Chelsea, MA (US); **Qun Zhong**, Lexington, MA (US); **Darren R. Link**, Lexington, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

Pub. No.: **14/264,686**

(22) Filed: **Apr. 29, 2014**

(65) **Prior Publication Data**
US 2014/0329239 A1 Nov. 6, 2014

Related U.S. Application Data
(63) Continuation of application No. 13/026,120, filed on Feb. 11, 2011.

(60) Provisional application No. 61/388,937, filed on Oct. 1, 2010, provisional application No. 61/347,158, filed on May 21, 2010, provisional application No. 61/331,490, filed on May 5, 2010, provisional application No. 61/304,163, filed on Feb. 12, 2010.

(51) **Int. Cl.**
C12P 19/34 (2006.01)
C12Q 1/68 (2006.01)

(52) **U.S. Cl.**
CPC **C12Q 1/6816** (2013.01); **C12Q 1/686** (2013.01); **C12Q 1/6827** (2013.01)

(58) **Field of Classification Search**
USPC 435/6.12, 91.2
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,143,496 A * 11/2000 Brown et al. 435/6.11
6,440,760 B1 * 8/2002 Cho et al. 438/16
7,041,481 B2 * 5/2006 Anderson et al. 435/91.2
2009/0317798 A1 * 12/2009 Heid et al. 435/6

OTHER PUBLICATIONS

Kiss et al. Anal. Chem., 2008 vol. 80, p. 8975-8981.*
DiMatteo et al. Experimental Cell Research, 2008, vol. 314(4), p. 878-886.*

* cited by examiner

Primary Examiner — Kenneth R. Horlick
Assistant Examiner — Joyce Tung
(74) **Attorney, Agent, or Firm** — Brown Rudnick LLP; Thomas C. Meyers

(57) **ABSTRACT**

The invention generally relates to droplet based digital PCR and methods for analyzing a target nucleic acid using the same. In certain embodiments, methods of the invention involve forming sample droplets containing, on average, a single target nucleic acid, amplifying the target in the droplets, excluding droplets containing amplicon from the target and amplicon from a variant of the target, and analyzing target amplicons.

28 Claims, 40 Drawing Sheets

Paper 33 at 4-5; *Dropworks, Inc. v. Bio-Rad Labs., Inc.*, IPR2021-00212, Paper 6 at 36 (PTAB Mar. 8, 2021)

37

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Bio-Rad argued a key aspect of Larson's disclosure is having one target per droplet

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DROPWORKS, INC.,
Petitioner,

v.

BIO-RAD LABORATORIES, INC.,
Patent Owner.

Case IPR2021-00212
U.S. Patent No. 9,127,310

PATENT OWNER'S PRELIMINARY RESPONSE TO PETITION FOR
INTER PARTES REVIEW OF U.S. PATENT NO. 9,127,310

Mail Stop "PATENT BOARD"
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Bio-Rad:

Additionally, partitioning DNA such that only one allele is present in each droplet, a key aspect of the '310 patent's multiplexing approach, is taught away from in Beer. For instance, Beer, whose main aim is successful PCR in picoliter droplet sizes, acknowledges that its most successful embodiment is one in which seven template strands are included per droplet. Ex. 1007 at 8474 ("Very repeatable amplification was observed at seven starting DNA copies per droplet, whereby all droplets showed successful PCR (Figure 3a)."). This directly contradicts the

Paper 33 at 4-5; *Dropworks, Inc. v. Bio-Rad Labs., Inc.*, IPR2021-00212, Paper 6 at 36 (PTAB Mar. 8, 2021)

38

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Bio-Rad argued that in Larson's disclosure, each droplet "must" contain "only one" target

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DROPWORKS, INC.,
Petitioner,

v.

BIO-RAD LABORATORIES, INC.,
Patent Owner.

Case IPR2021-00212
U.S. Patent No. 9,127,310

PATENT OWNER'S PRELIMINARY RESPONSE TO PETITION FOR
INTER PARTES REVIEW OF U.S. PATENT NO. 9,127,310

IV. CONCLUSION

For these reasons, Dropwork's Petition for *Inter Partes* Review should be denied.

Respectfully submitted,

Dated: March 8, 2021

/s/ Derek C. Walter
Derek C. Walter
Reg. No. 74,656

*Attorney for Patent Owner Bio-Rad
Laboratories, Inc.*

Bio-Rad:

droplets showed successful PCR (Figure 3a)."). This directly contradicts the teachings of the '310 patent, which state that, in order to successfully perform multiplex PCR, not only must each droplet contain only one DNA template but it should preferentially be single stranded. See Ex. 1001 at 19:51-53 ("Having only one haploid genome (i.e., one allele) per droplet gives droplet PCR advantages over standard singleplex or multiplex PCR in tubes or microwells."). While Davies does acknowledge that its droplets should only contain "one copy of the target," it does not acknowledge the difficulties of including double stranded DNA when performing multiplex PCR. Ex. 1005 at ¶ 24.

Paper 33 at 4-5; *Dropworks, Inc. v. Bio-Rad Labs., Inc.*, IPR2021-00212, Paper 6 at 36 (PTAB Mar. 8, 2021)

39

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Batt's reasons for modifying Larson should be rejected

Dr. Batt:

338. Additionally, a POSA would have been motivated to use this strategy because limiting an experiment to only single-target droplets severely impacts the total data collected in that experiment. These assays, especially at the time of the '921 patent, were quite costly to set up and run, for example, as discussed in Larson, many reagents are required. *See, e.g.*, EX1017 ¶ 68. The ability to acquire data not just from a fraction of the droplets, but from all droplets in the experiment would thus be extremely cost effective. Additionally, by allowing more DNA into each droplet, the dilution factor for the sample decreases, meaning less sample exists and less run-time on the machine is necessary. *See* EX1017 ¶ 96.

Dr. Batt's first theory is to maintain dilution but acquire data from rare multi-analyte droplets, which wouldn't affect cost:

Q. In this paragraph are you referring to keeping the number of multiple analyte droplets the same, but just acquiring data from them?
A. I believe so, yes.

Dr. Batt's second, contradictory theory is to decrease dilution to increase multiple occupancy, which Larson teaches away from:

how to deal with that inevitability. So if I was practicing Larson, I would do a less dilute sample so I could look at multiple occupancies, because I had the capabilities of doing it.

Paper 17 at 54-62; EX1002 at ¶338; EX2017 at 176:19-23, 221:4-8

40

Dr. Batt did not consider the drawbacks of modifying Larson

“The Board ***must*** weigh the benefits ***and drawbacks*** of the modification against each other, to determine whether there would be a motivation to combine.”

- *Arctic Cat Inc. v. Polaris Indus., Inc.*, 795 Fed. App’x 827, 833 (Fed. Cir. 2019) (emphasis added)

Dr. Batt:



18 Q. When discussing the reasonable
19 expectation of success, you don't discuss
20 any of the downsides that Larson teaches
21 with respect to detecting multiple analytes
22 in a droplet, correct?
23 A. Is that my responsibility? I am
24 saying, as I said here, a POSA would have a
25 reasonable expectation of success,

2 reasonable, not perfect. And your
3 expectation is that I am going to sit here
4 and discuss why it is not going to work, is
5 that what you are asking me to do?
6 Q. You didn't account for any of the
7 reasons why it might not work, correct?
8 A. Right, because the overwhelming

Paper 27 at 14-15; EX2017 at 183:19-185:15

41

The improperly raised new embodiment of Larson also fails

Dr. Batt:



The Board should not consider FIG. 11a and ¶¶130-31 of Larson because Petitioner did not raise them in the Petition. See Petitioner's Motion to Strike (Paper 26).

Even if considered, Larson's new embodiment does not teach unambiguously detecting at least three targets in a single sample volume using less colors channels:

- Petitioner alleges Larson's disclosure of a "very small peak...at ~0.9 V...corresponded to droplets occupied by both genes" teaches the "unambiguous detection of multiple analytes in a single droplet" but conceded this peak is "not visible on the scale of FIG. 11a." Paper 20 at 15 (emphasis added).
- FIG. 11a uses only one channel to ostensibly detect two targets. EX2025, 42:24-43:6, 72:9-73:5.

Q. Figure 11 is talking about a single wavelength, right? Or single change, correct?

A. Figure 11 is talking about a single channel and is talking about

Paper 27 at 11; EX2025 at 42:24-43:4

42

Ground 3: Bio-Rad's Arguments Lack Merit

1. Ground 3 is deficient for same reasons as Grounds 1 and 2: Larson does not disclose unambiguously detecting three targets in a single droplet using two color channels, and Saxonov does not cure this deficiency.

2. The disclosures of Saxonov relied on in the Petition are not prior art.

3. Petitioner improperly raised new arguments regarding Saxonov FIG. 4 and ¶¶20, 41.

4. There is no motivation to combine the new portions of Saxonov with Larson.

The Board correctly found the Petitioned portions of Saxonov are not prior art

The disclosures of Saxonov relied on in the Petition are not prior art.

Petitioner's Reply does not attempt establish otherwise.

Thus, because Petitioner has not shown sufficiently that the Petition explains how each of the relied-upon disclosures in Saxonov Figure 10, and Saxonov paragraphs 18 and 78–79, which are not present in the '013 provisional, are supported by the '013 provisional, we determine that Petitioner has not shown that those cited portions of Saxonov are entitled to the priority date of the '013 provisional. Accordingly, on this record, we preliminarily determine that those relied-upon portions of Saxonov do not qualify as prior art to the '921 patent.

Even if the improperly raised portions of Saxonov are considered, Petitioner's motivation to combine argument is deficient

While Petitioner asserts Larson and Saxonov disclose the same “exact” scheme, Petitioner does not explain why any similarities between Larson’s FIG. 11a and Saxonov’s FIG. 4 would have motivated a POSA to combine Larson and Saxonov.

- *See Microsoft Corp. v. Enfish, LLC*, 662 Fed. Appx. 981, 990 (Fed. Cir. 2016) (asserting references are similar is insufficient).

30. Most importantly, Saxonov Figure 4 depicts the exact scheme taught by Larson Figure 11a. A POSA would have understood to combine these references and would have done so with a reasonable expectation of success.

37. A POSA would be motivated to combine Saxonov and Larson because, in part, Saxonov discloses the same scenarios taught in Larson. *Id.* A POSA would have a reasonable expectation of success because Saxonov and Larson both teach methods for unambiguous detection of multiple targets in a single droplet.

Dr. Batt:



Paper 27 at 16-17; EX1043 at ¶¶30, 37

45

Petitioner has not established a motivation to combine Saxonov with Larson

Dr. Batt:



Q. Is there any problem with the method in Larson with respect to multiplex PCR that are cured or resolved by Saxonov?

A. I can't answer that.

Q. And I guess what I am asking, is there any shortcoming of the methodology for detecting multiple targets in a single wavelength that are thought by Larson that Saxonov cures?

A. As I stated before, I haven't done that analysis and I haven't compared those two at that level of questioning.

Q. Okay. And you don't have any opinion about any particular shortcoming of the methodology disclosed in Saxonov that you believe Larson cures, correct?

A. I haven't done that analysis.

Paper 27 at 14-15; EX2025 at 70:22-79:5

46

Topics

1. The '921 Patent and challenged claims

2. Caltech's responses to Grounds

3. Dr. Struhl's testimony should be credited.

4. Caltech's motion to strike

5. Construction of "single sample volume"

Bio-Rad's criticism's of Dr. Struhl's testimony should be rejected

- | |
|---|
| 1. Priority date: Dr. Struhl applied the correct concept of priority date, and refers to this as the “relevant timeframe.” |
| 2. “Historical perspective”: Dr. Struhl performed at least the same analysis as Bio-Rad’s expert. |
| 3. Claim construction standard and understanding the claims: Dr. Struhl’s applied the correct standard. |
| 4. Familiarity with PO’s infringement contentions: Dr. Struhl’s purported lack of familiarity PO’s the infringement contentions does not render his opinions unreliable. |
| 5. Familiarity with the Petition: Dr. Struhl reviewed the correct IPR Petition. |

Paper 27 at 24-28

48

Bio-Rad's criticisms of Dr. Struhl's testimony should be rejected

1. Priority date: Dr. Struhl applied the correct concept of priority date, and refers to this as the “relevant timeframe.”

2. “Historical perspective”: Dr. Struhl performed at least the same analysis as Bio-Rad’s expert.

3. Claim construction standard and understanding the claims: Dr. Struhl’s applied the correct standard.

4. Familiarity with PO’s infringement contentions: Dr. Struhl’s purported lack of familiarity PO’s the infringement contentions does not render his opinions unreliable.

5. Familiarity with the Petition: Dr. Struhl reviewed the correct IPR Petition.

Paper 27 at 24-28

49

Priority date: Dr. Struhl’s declaration set forth the patent’s priority date (i.e., the “relevant timeframe”)

STRUHL DECL.
IPR2024-01451

- Exhibit 2011: Plaintiff’s Opening Claim Construction Brief, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.); and
- Exhibit 2012: Reply Claim Construction Brief, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.).

IV. UNDERSTANDING OF THE LAW

12. I am not an attorney. In preparing and expressing my opinions and considering the subject matter of the ’921 Patent, I am relying on certain basic legal principles that have been explained to me. These principles are discussed below.

A. A Person Having Ordinary Skill in the Relevant Field During the Relevant Time

13. I understand that the disclosures of patents and prior art references are to be viewed from the perspective of a mythical person of ordinary skill in the relevant field (sometimes referred to by lawyers as “the relevant art”) at the relevant timeframe (a “POSA”). I understand that, for purposes of this IPR proceeding, I should interpret the term POSA as referring to a mythical person to whom an expert in the relevant field could have assigned a routine task with reasonable confidence that the task would be successfully carried out.

-9-

IPR2024-01451
California Institute of Technology - Ex. 2001 Page 12

STRUHL DECL.
IPR2024-01451

14. I have been asked to provide my opinion regarding the “level of ordinary skill in the art” at the time of the invention, which I understand is on or before February 3, 2012.

15. I understand that the hypothetical person of ordinary skill in the art is considered to have the normal skills and knowledge of a person in a certain technical field. I understand that factors that may be considered in determining the level of ordinary skill in the art include: (1) the education level of the inventor; (2) the types of problems encountered in the art; (3) the prior art solutions to those problems; (4) rapidity with which innovations are made; (5) the sophistication of the technology; and (6) the education level of active workers in the field. I also understand that “the person of ordinary skill” is a hypothetical person who is presumed to be aware of the universe of available prior art.

16. I also understand the level of ordinary skill in the art can be evidenced by the prior art. Accordingly, I have also considered the prior art discussed herein in determining the level of ordinary skill in the art.

17. Petitioner asserts that a POSA relevant to the ’921 Patent as of February 3, 2012 would have a Ph.D. in molecular biology, genetics, biochemistry, or a related discipline, with at least a year of experience in quantitative and/or digital PCR and fluorescence-based analyte detection. Petition, 9-10. Alternatively, the Petitioner asserts that a POSA would have been someone with

-10-

IPR2024-01451
California Institute of Technology - Ex. 2001 Page 13

STRUHL DECL.
IPR2024-01451

claims, specification (including drawings), and prosecution history (the “intrinsic” evidence). I understand that if the claim is unclear in view of the intrinsic evidence, then outside (“extrinsic”) evidence also may be considered.

C. Anticipation

22. I understand that a patent claim is unpatentable as anticipated if a POSA during the relevant timeframe would have understood a single prior art reference to disclose an embodiment that expressly or inherently teaches every limitation contained in the claim. The disclosure in a reference does not have to be in the same words as the claim, but all of the requirements of the claim must be described in enough detail, or necessarily inherent in an embodiment disclosed in the reference, to enable a POSA looking at the reference to practice the claimed invention.

D. Obviousness

23. I understand that a patent claim also, or alternatively, is unpatentable if it would have been obvious to a POSA during the relevant timeframe. I understand that the following factors must be evaluated to determine whether the claimed subject matter was obvious: (1) the scope and content of the prior art; (2) the difference or differences, if any, between the subject matter defined by the claim of the patent under consideration and what is disclosed in the prior art; (3) the level of ordinary skill in the relevant field at the time of the patent’s earliest

-12-

IPR2024-01451
California Institute of Technology - Ex. 2001 Page 15

Paper 27 at 24-28; EX2001 at ¶¶ 14, 17, 22, 23

Dr. Struhl confirmed that he relied on the correct priority date

Dr. Struhl:



And I just want to ask you, do you have an understanding now or what is your understanding of what the relevant time is that you refer to in subheading A?

A. Well, see -- um -- at the time it's the -- oh, okay. It's in my patent. I forgot about this, okay. It says, "at the time of the invention," which I understand is on February 3rd, 2012.

And then recall, we went through several bullet points, the four bullet points --

A. Yes.

Q. -- that Mr. Walter showed you about the understanding of a person of the ordinary skill in the art?

A. Yes.

Q. Did you have those four bullet points, would you have understood those concepts as of

February 3, 2012?

A. Yeah, because that's what I was judging it upon, and I would have judged it on that.

EX1044 at 193:23-195:4; Paper 27 at 24

Bio-Rad's criticism's of Dr. Struhl's testimony should be rejected

1. **Priority date:** Dr. Struhl applied the correct concept of priority date, and refers to this as the “relevant timeframe.”

2. **“Historical perspective”:** Dr. Struhl performed at least the same analysis as Bio-Rad's expert.

3. **Claim construction standard and understanding the claims:** Dr. Struhl's applied the correct standard.

4. **Familiarity with PO's infringement contentions:** Dr. Struhl's purported lack of familiarity PO's the infringement contentions does not render his opinions unreliable.

5. **Familiarity with the Petition:** Dr. Struhl reviewed the correct IPR Petition.

Paper 27 at 24-28

52

Historical perspective: Dr. Struhl applied the correct legal analysis

As required, Dr. Struhl considered the thinking of a POSA guided by the prior art and then-accepted wisdom in the field.

- *In re Kotzab*, 217 F.3d 1365, 1369 (Fed. Cir. 2000).

Dr. Struhl:



13. I understand that the disclosures of patents and prior art references are to be viewed from the perspective of a mythical person of ordinary skill in the relevant field (sometimes referred to by lawyers as “the relevant art”) at the relevant timeframe (a “POSA”). I understand that, for purposes of this IPR proceeding, I should interpret the term POSA as referring to a mythical person to whom an expert in the relevant field could have assigned a routine task with reasonable confidence that the task would be successfully carried out.

16. I also understand the level of ordinary skill in the art can be evidenced by the prior art. Accordingly, I have also considered the prior art discussed herein in determining the level of ordinary skill in the art.

Paper 27 at 24-28; EX2001 at ¶¶ 13, 16

Dr. Batt did not do a historical perspective either

If Dr. Struhl's testimony is discounted based on not doing a "historical perspective," Dr. Batt's should be as well because he also did not do one.

Dr. Batt:



Q. Did you do anything to determine the history of technological improvements for multiplex PCR between 2002 and 2011 in preparing your declaration in this case?

A. No, I did not.

Q. You didn't do a historical analysis of that?

A. Across all of science, no, I did not.

Q. How about just across multiplex PCR related science?

A. No.

Paper 27 at 25-26; EX2025 at 19:6-18

Bio-Rad's criticism's of Dr. Struhl's testimony should be rejected

1. **Priority date:** Dr. Struhl applied the correct concept of priority date, and refers to this as the “relevant timeframe.”

2. **“Historical perspective”:** Dr. Struhl performed at least the same analysis as Bio-Rad’s expert.

3. Claim construction standard and understanding the claims: Dr. Struhl’s applied the correct standard.

4. **Familiarity with PO’s infringement contentions:** Dr. Struhl’s purported lack of familiarity PO’s the infringement contentions does not render his opinions unreliable.

5. **Familiarity with the Petition:** Dr. Struhl reviewed the correct IPR Petition.

Paper 27 at 24-28

55

Claim construction standard: Dr. Struhl used the correct standard

Dr. Struhl:



20. I understand that the first step in determining the validity of a claim is for the claim to be properly construed. I understand that “claim construction” is the process of determining a patent claim’s meaning. I also understand that the proper construction of a claim term is the meaning that a POSA would have given to that term during the relevant timeframe.

21. For purposes of *Inter Partes Review*, I understand that the Board applies the *Phillips* standard, under which claims are to be given their ordinary and customary meaning as understood by a POSA at the time the application was filed. I understand that this analysis is performed by primarily looking to the patent’s claims, specification (including drawings), and prosecution history (the “intrinsic” evidence). I understand that if the claim is unclear in view of the intrinsic evidence, then outside (“extrinsic”) evidence also may be considered.

EX2018 at ¶¶20-21; Paper 27 at 26-27

56

Dr. Struhl understood the claims (e.g., “components”)

techniques described above. *Id.*; see ¶¶28-33 of this Declaration. Instead, “each analyte to be detected is encoded as a value in each of two components of a signal (e.g., intensity and wavelength).” these components are cumulatively measured for the sample, and the “presence or absence of an analyte may then be determined based on the values used to encode the presence of the analyte (i.e., those values in the coding scheme).” *Id.*, 8:66-9:5, 9:41-47.

Dr. Struhl:



Q. Well, it says -- it says, "each of said at least M analyte is encoded as a value of one component of a fluorescent signal," it says. So each of the analyte is encoded as one component of a fluorescent signal. How might you do that?

A. I mean, the analyte is encoded by the components that are the light and the intensity.

EX2018 at ¶38; Paper 27 at 26-27; EX1044 at 97:16-22.

Bio-Rad's criticism's of Dr. Struhl's testimony should be rejected

1. **Priority date:** Dr. Struhl applied the correct concept of priority date, and refers to this as the “relevant timeframe.”

2. **“Historical perspective”:** Dr. Struhl performed at least the same analysis as Bio-Rad’s expert.

3. **Claim construction standard and understanding the claims:** Dr. Struhl’s applied the correct standard.

4. Familiarity with PO’s infringement contentions: Dr. Struhl’s purported lack of familiarity PO’s the infringement contentions does not render his opinions unreliable.

5. **Familiarity with the Petition:** Dr. Struhl reviewed the correct IPR Petition.

Paper 27 at 24-28

58

Dr. Struhl's familiarity with the infringement contentions is unimportant

“We do not regard the infringement contentions in the District Court as a claim construction proposed by Patent Owner in the proceeding before us.”

- Intex Recreation Corp. v. Bestway (USA), Inc., No. IPR2019-00454, Paper 34 at 11 (PTAB June 22, 2020)

Extrinsic evidence “of what a party [allegedly] subjectively thought was a proper construction is of limited value.”

- Great W. Cas. Co. v. Intell. Ventures II LLC, IPR2025-01706, Paper 69 at 13 (PTAB Feb. 14, 2020)

Paper 17 at 25; Paper 29 at 8

59

Bio-Rad's criticism's of Dr. Struhl's testimony should be rejected

- | |
|---|
| 1. Priority date: Dr. Struhl applied the correct concept of priority date, and refers to this as the “relevant timeframe.” |
| 2. “Historical perspective”: Dr. Struhl performed at least the same analysis as Bio-Rad’s expert. |
| 3. Claim construction standard and understanding the claims: Dr. Struhl’s applied the correct standard. |
| 4. Familiarity with PO’s infringement contentions: Dr. Struhl’s purported lack of familiarity PO’s the infringement contentions does not render his opinions unreliable. |
| 5. Familiarity with the Petition: Dr. Struhl reviewed the correct IPR Petition. |

Paper 27 at 24-28

60

Dr. Struhl was familiar with the Petition

Dr. Struhl:



23 Q. Okay. And I just want to clarify something
24 from earlier. I think you were asked some questions
25 about the word "petition" or what a petition, and you
1 didn't remember what that referred to in your
2 declaration, and I just want to ask you if you have
3 any clarifications to your answer about what petition
4 you may be referring to?

5 A. I see it in my own document. My point was
6 before that I didn't see it in my list, so I didn't
7 see it on my list, but now I'm seeing it in my
8 document, so it's obviously there.

9 Q. So which -- which -- so just to clarify,
10 what -- what document were you referring to, do you
11 refer to in your declaration when you cite to a
12 petition?

13 A. It's this document. Whatever. It's, you
14 know, IPR2024-01451.

EX1044 at 190:23-192:5; Paper 27 at 27

Topics

1. The '921 Patent and challenged claims

2. Caltech's responses to Grounds

3. Dr. Struhl's testimony should be credited

4. Caltech's motion to strike should be granted.

5. Construction of "single sample volume"

Caltech's motion to strike should be granted

A “challenger [is] obliged to make an adequate case *in its Petition* and the Reply [is] limited to a true rebuttal role.”

- *Ariosa Diagnostics v. Verinata Health, Inc.*, 805 F.3d 1359, 1367 (Fed. Cir. 2015) (emphasis added).

Bio-Rad's *new* arguments and evidence in its Reply should be stricken:

- | |
|---|
| 1. Larson, FIG. 11a and ¶¶130-31 |
| 2 Saxonov, FIG. 4 and ¶¶20, 41
Corresponding Saxonov Provisional, 5:19-6:3, 9:14-15, 10:18-11:13, 15:12-17:3 |
| 3. Dr. Struhl's testimony concerning the above, which was outside the scope of direct |

Petitioner's discussion of Larson FIG. 11a and ¶¶130-31 should be stricken

Petitioner impermissibly relies on new portions of Larson to fill gaps identified in the PO Response:

Petition: relies on Larson ¶¶121, 141 and FIG. 9C to argue Larson alone or combined with Saxonov teaches unambiguously detecting three analytes in a droplet using two colors.

POR: establishes FIG. 9C is directed to single-occupancy droplets and Petitioner's rational for modifying Larson alone or in view of Saxonov is deficient.

Reply: relies *for the first time* on a *completely different embodiment* of Larson (¶¶130-31 and FIG. 11a) and new theory based on identifying two targets with one color.

Paper 26 at 1-9; Paper 1 at 53; Paper 17 at 27-29, 51-62; Paper 20 at 15-16.

64

Petitioner's discussion of Saxonov FIG. 4 and ¶¶20, 41 should be stricken

Petitioner impermissibly relies on new portions of Saxonov to argue a new “motivation to combine” theory for the first time in Reply:

Petition: cites Saxonov FIG. 4 only one time for proposition that Saxonov discloses using probes labeled with the same color at different intensities to distinguish among different polynucleotide analytes.

PO response: because the Board found Petitioner failed to establish the relied-upon portions of Saxonov qualify as prior art, PO's Response did not discuss Saxonov FIG. 4 and ¶¶20, 41.

Reply: newly asserts that FIG. 4 is “substantively identical to the one disclosed in Figure 11a of Larson” as to provide an alleged motivation to combine Larson and Saxonov.

Paper 26 at 9-10; Paper 1 at 14; Paper 11; Paper 17 at 27, 40, 63; Paper 20 at 18-22

65

Petitioner's discussion of Dr. Struhl's objectional testimony regarding the new embodiments of Larson and Saxonov should be stricken for the same reason

Bio-Rad's *new* arguments and evidence in its Reply should be stricken:

- | |
|---|
| 1. Larson, FIG. 11a and ¶¶130-31 |
| 2 Saxonov, FIG. 4 and ¶¶20, 41
Corresponding Saxonov Provisional, 5:19-6:3, 9:14-15, 10:18-11:13, 15:12-17:3 |
| 3. Dr. Struhl's testimony concerning the above, which was outside the scope of direct |

Topics

1. The '921 Patent and challenged claims

2. Caltech's responses to Grounds

3. Dr. Struhl's testimony should be credited

4. Caltech's motion to strike

5. Construction of “single sample volume” is not necessary; but Larson only discloses a “droplet” as “single sample volume.”

Detecting combinations of analytes in single sample volume

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Institution Decision:

To begin, we find no dispute between the parties with respect to the meaning of “single sample volume,” recited in claim 1. Both parties agree that the ’921 patent “contemplates both embodiments where the sample volume is separated and those where it is not.” Pet. Reply 1–2; PO Sur-reply 3. Further, the parties agree that the “single sample volume” is not “limited to an individual ‘droplet,’ but rather includes any type or nature of volume, such as a ‘tube or bottle.’” Pet. Reply 1; PO Sur-reply 3.

Caltech:

The Board need not construe the term “single sample volume” because there is no controversy over its construction and the term should be given its plain and ordinary meaning. The claimed “single sample volume” includes a droplet or any other type of volume, such as a tube or bottle. Nonetheless, claim 1’s concluding

Paper 11 at 33 (citing Paper 9 at 1-2; citing Paper 10 at 3); Paper 30 at 1; Paper 33 at 1; EX1001, claim 1

68

Analytes are amplified in the same single sample volume where detected



Dr. Struhl:

with similar limitations. EX1001('921 Patent), 68:31-38. While the single sample volume may not necessarily contain *all* analyte molecules at the same time, the power of the '921 Patent lies in its flexibility to detect *any possible combination* of the analyte molecules within the single sample volume within which that combination of analyte molecules is amplified, in a manner that each analyte may be uniquely distinguished from each other analyte. See ¶¶37-49 of this Declaration.

At the time of the '921 Patent and its priority applications, no such solution was available to solve the problem of how to increase the number of analytes which could be non-degenerately detected in a single sample volume in any combination in a single sample volume (such as a vial or droplet) within which amplification is performed. See ¶¶37-49 of this Declaration; *see also* EX1001('921 Patent), 1:46-47 ("There is a need for multiplexed reactions that can be carried out *in a single solution.*")

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, **in a single sample volume**, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and **wherein the analytes are amplified in the single sample volume.**

Paper 17 at 30; Paper 29 at 7; EX2018 at ¶¶ 30, 37-49, 90-92; EX1001 at 36:58-67, claim 1

69

Analytes are amplified in the same single sample volume where detected

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,

wherein said fluorescent signal comprises F wavelength components, and

wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,

wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single sample volume.



Dr. Batt:

the proximity allows the quencher to suppress the signal. This type of probe is generally referred to as a TaqMan probe and was described in literature as early as 1991. EX1011. The TaqMan probe is used with two PCR primers and is designed to bind to a region between the two primers. In this method, a target-bound probe is cleaved during extension by the DNA polymerase, which has an exonuclease activity. The cleavage frees the quencher and fluorophore which both diffuse away and the signal from the fluorophore is no longer quenched, thereby allowing detection of signal emitted from the fluorophore. This process is depicted in the figure below:

EX1002 at ¶42; Paper 29 at 7; EX1001, claim 1

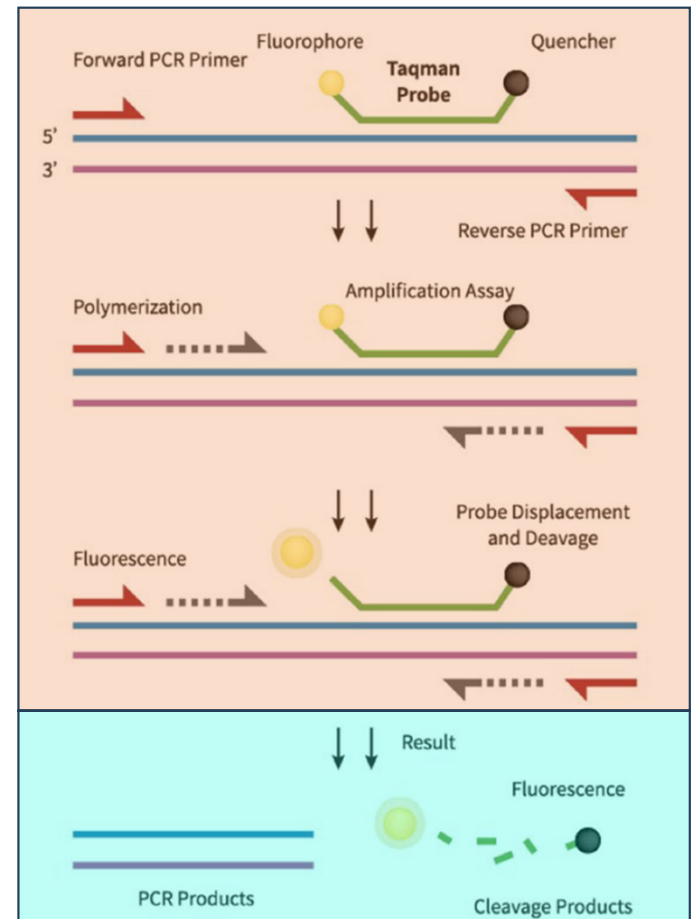
70

Analytes are amplified in the same single sample volume where detected

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Dr. Batt:



EX1002 at ¶42; Paper 29 at 7; EX1001, claim 1

71

Analytes are amplified in the same single sample volume where detected

Claim 1:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and wherein the analytes are amplified in the single sample volume.

Bio-Rad:

and digital PCR (“dPCR”). EX1002 ¶¶ 43-47. One form of qPCR uses TaqMan probes that hybridize to the target sequence and generate a signal when degraded by a polymerase. *Id.* ¶ 45. The signal generated is proportional to the amount of the target polynucleotide in a sample, allowing quantification of analytes. *Id.* ¶ 46. In dPCR, the PCR solution including sample is partitioned into small droplets or other partitions, where separate PCR reactions occur. *Id.* ¶ 47. TaqMan probes

The claim language requires merely detecting “M analytes” in a “single sample volume,” with amplification occurring in that same volume. EX1001 67:9–26, 68:19-48. There are no other requirements as to the “single sample volume” in any of the claims. Nothing in any of the claims, for instance, places any restrictions as to the form of the “single sample volume,” including whether it be a tube, a droplet, a partitioned volume, or a non-partitioned volume. See EX1002 ¶¶ 13-14.

Paper 1 at 4-5; Paper 25 at 5; Paper 29 at 7; EX1001, claim 1

72

Key Term: “the analytes are amplified in the single sample volume”

The Board “need only construe terms that are in controversy, and only to the extent needed to resolve the controversy.”

- Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co., 868 F.3d, 1017 (Fed. Cir. 2017)

Paper 29 at 5-6; Paper 33 at 2-3

73

Topics

- 1. The '921 Patent and challenged claims are patentable.**
- 2. Bio-Rad failed to meet its burden of demonstrating unpatentability under any of its alleged grounds.**
- 3. Dr. Struhl's testimony should be credited.**
- 4. Caltech's motion to strike should be granted.**
- 5. Construction of "single sample volume" is not necessary; but Larson only discloses a "droplet" as "single sample volume."**

Questions?