

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INVENTORS : Emil KARTALOV *et al.*

CONFIRMATION 9929
No. :

SERIAL 16/864,744
NUMBER :

EXAMINER : Russell Scott Negin

FILING DATE : May 1, 2020

ART UNIT : 1672

FOR : SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

RESPONSE TO NON-FINAL OFFICE ACTION

MAIL STOP AMENDMENT

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

Dear Sir:

In response to the Non-Final Office Action mailed **December 28, 2022**, please amend the above-identified application as follows:

Listing of Claims begin on page 2 of this paper.

Remarks begin on page 5 of this paper.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to Deposit Account No. 504561 (**Ref. No. 88DG-330783-US4**).

LISTING OF CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

- 1.– 20. (Canceled)
21. (Currently Amended) An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,
wherein each of said 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 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 without immobilization, separation, mass spectrometry, or melting curve analysis, based on detection of said F wavelength components, when M is greater than F.
22. (Previously Presented) The assay of claim 21, wherein each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.
23. (Previously Presented) The assay of claim 22, wherein said first value is an intensity or range of intensities.
24. (Previously Presented) The assay of claim 21, wherein said detecting is performed with reagents comprising hybridization probes.
25. (Previously Presented) The assay of claim 24, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.
26. (Previously Presented) The assay of claim 21, wherein said signal is generated during a polymerase chain reaction.
27. (Previously Presented) The assay of claim 21, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group

- consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.
28. (Currently Amended) The assay of ~~claim 21~~ claim 24, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.
 29. (Previously Presented) The assay of claim 21, wherein at least one of said analytes comprises a polynucleotide.
 30. (Currently Amended) The assay of claim 21, further comprising transmitting information concerning the presence or absence of at least one of said ~~analyte~~ analytes through a computer network.
 31. (Previously Presented) The assay of claim 21, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.
 32. (Previously Presented) The assay of claim 21, wherein said coding scheme is innately non-degenerate or non-degenerate by construction.
 33. (Currently Amended) The assay of claim 22, wherein said coding scheme is made non-degenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.
 34. (Currently Amended) A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:
 - a. 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 each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and
 - b. each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the ~~first~~ value.
 35. (Currently Amended) The kit of claim 34, wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes ~~the plurality of analytes~~ in a single sample volume, in any combination of presence or absence, without immobilization, separation, or melting curve analysis.

36. (Currently Amended) The kit of claim 34, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F.
37. (Currently Amended) The kit of ~~any of claims 34~~ claim 34, wherein said analyte-specific reagents comprise hybridization probes.
38. (Previously Presented) The kit of claim 37, wherein said hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes.
39. (Currently Amended) The kit of claim 38, wherein said number of hybridization probes in the kit is greater than M ~~the number of the analytes of the plurality of analytes~~.
40. (Currently Amended) The kit of ~~any of claims 34~~ claim 34, wherein the analyte-specific reagents are each present at a different amount in the kit.

REMARKS

Claims 21-40 are pending. Claims 21, 30, 34-36 and 39 are amended for clarity. Claims 28, 33, 37 and 40 are amended to correct claim dependency and/or to correct minor typographical issues. No new matter is added. In view of the amendments and following remarks, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, Applicant respectfully requests that the Examiner reconsider all outstanding rejections and allow the claims.

Objection to Claims 33 and 37

Applicant respectfully submits that the amendments to claims 33 and 37 render moot the objections to these claims.

Rejection of Claims 22-31 under 35 U.S.C. § 112, second paragraph

Claims 30 and 32-40 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite for the reasons described below.

Claim 30 is rejected as indefinite for allegedly lacking antecedent for the recitation “said analyte”. Applicant respectfully submits that this rejection is rendered moot by the amendment of claim 30 to recite “at least one of said analytes”, for which antecedent can be found in claim 21.

Claims 32 and 33 are rejected as indefinite for allegedly lacking antecedent for the recitation “said coding scheme”. Reconsideration and withdrawal of this rejection respectfully is requested in view of the amendment of claim 21 to add the recitation “thereby generating a coding scheme”, thus providing antecedent.

Claim 34 is rejected as indefinite for allegedly lacking antecedent for the recitation “the first value”. Reconsideration and withdrawal of this rejection respectfully is requested in view of the amendment of claim 34 to delete recitation of “first” before “value”.

Claim 36 is rejected as indefinite for allegedly lacking antecedent for the recitation of variable “F”. Reconsideration and withdrawal of this rejection respectfully is requested in view of the amendment of claim 36 to add the recitation “said fluorescent signal comprises F wavelength components”, thus describing, and providing antecedent for, variable “F”.

Claim 39 is rejected as indefinite for allegedly lacking antecedent for the recitations “the number of analytes” and “the plurality of analytes”. As amended, claim 39 does not recite “the number of the analytes of the plurality of analytes”, thus rendering this rejection moot.

Claims Rejections Under 35 U.S.C. §103

Rejection of Claims 21-25, 27, 29-30, and 32-40 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin

Claims 21-25, 27, 29-30, and 32-40 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han et al. (Nature Biotechnology, volume 19, 2001, pages 631-635) (“Han”) in view of International Patent Publication No. 2012/058628 (“Lin”).

This rejection is respectfully traversed. Han teaches a method of multiplexing in which “Optical readout is accomplished by measuring the fluorescence spectra of single beads.” (Han at Figure 1, page 632, emphasis added). Furthermore, Han teaches the immobilization of single-color signal beads on a polylysine-coated glass slide, as expressly illustrated in Figure 2 (Han, at Figure 2, Page 632). Han’s single-bead multiplex approach is again emphasized in Fig. 5, where Han describes the necessary coding scheme to identify the presence and abundance of each individual bead (i.e., single targets). Specifically, Han on page 634 teaches:

To demonstrate the use of QD-tagged beads for biological assays, we designed a model DNA hybridization system using oligonucleotide probes and triple-color encoded beads, as shown in Figure 5. Target DNA molecules are either directly labeled with a fluorescent dye or with a biotin (for binding to fluorescently tagged avidin). Optical spectroscopy at the single-bead level yields both the coding and the target signals. The coding signals identify the DNA sequence, whereas the target signal indicates the presence and the abundance of that sequence. [emphasis added]

Han does **not** teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal, without immobilization, separation, mass spectrometry, or melting curve analysis**. Han also

does **not** teach or suggest a kit, as recited in claim 34, for detecting the presence or absence of at least M analytes by generating **a single cumulative measurement, in a manner that eliminates degeneracy**, wherein each of said M analytes is encoded as a value of one component of a fluorescent signal. The Office Action acknowledges that “Han et al. does not teach detecting cumulative non-degenerate combinations of analytes.” *Office Action*, p. 6.

Lin does not cure the deficiencies in the teachings of Han. Lin teaches DNA nanostructure barcode probes to detect a nucleic acid. Lin teaches that “it is preferable for the barcode probe and/or target to remain stationary during image acquisition”. Page 20, ll. 27-28. Figure 2 of Lin, which is cited in the Office Action as describing “the cumulative non-degenerate combination of each of 27 different combinations of the three fluorophores (R, G, and B) within an image”, illustrates how identification is based, at least in part, on the **positions** of barcodes. The immobilized barcodes taught by Lin produce **multiple separate** fluorescent signals that are analyzed, at least in part, based on their position. Thus, Lin does not teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal, without immobilization, separation, mass spectrometry, or melting curve analysis**. Similarly, Lin does not teach or suggest a kit, as recited in claim 34, for detecting the presence or absence of at least M analytes by generating **a single cumulative measurement, in a manner that eliminates degeneracy**, wherein each of said M analytes is encoded as a value of one component of a fluorescent signal.

Therefore, for at least this reason, there is no teaching or suggestion in Lin that would have led one of ordinary skill in the art to modify Han to result in the claimed assays and kits. Thus, the combination of Han and Lin fails to teach or suggest all elements of claim 21 or claim 34. Claims 22-25, 27, 29-30, 32-33 and 35-40 depend on claim 21 or claim 34 and incorporate the limitations therein. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 21-25, 27, 29-30, and 32-40 under 35 U.S.C. § 103.

Rejection of Claims 26 and 31 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin and Albani

Claims 26 and 31 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and U.S. Patent Publication No. 2006/0039918 (“Albani”).

The Examiner states that the combination of Han and Lin teach or suggest all features of claim 22, but does not teach or suggest a signal generated during PCR, or using a computer connected to a thermal cycler to perform at least one step of said assay, as recited in claims 26 and 31, respectively. The Examiner states that this deficiency is cured by Albani, which allegedly “relates fluorophores, PCR, and thermal cyclers”.

As described above, the combination of Han and Lin does not teach or suggest the method of claim 21, upon which claims 26 and 31 depend. Albani does not cure this deficiency. Albani teaches peptides that modulate, block, or inhibit inflammatory responses by altering T cell functional phenotype. Paragraph 184 of Albani, which is cited by the Examiner, teaches **quantifying** gene expression by measuring relative mRNA for a target gene. Albani does not teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal**. Therefore, for at least this reason, the combination of Han, Lin and Albani does not teach or suggest the method of claim 26 or 31. Therefore, the Examiner has failed to set forth a *prima facie* rejection for obviousness.

Furthermore, any *prima facie* rejection for obviousness is rebutted by the unexpectedly advantageous results in the application, demonstrating unambiguous detection of polynucleotide markers from pathogens, in which the number of analytes is greater than the number of wavelength components. *See, e.g.*, Example 3, and, for example, ¶¶ [0188]-[0190] and Figure 7.

Therefore, for at least the above reasons, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 26 and 31 under 35 U.S.C. § 103.

Rejection of Claim 28 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin and Hardin

Claim 28 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and U.S. Patent Publication No. 2010/0317005 (“Hardin”).

The Examiner states that the combination of Han and Lin teach or suggest all features of claim 22, but do not teach or suggest using the fluorescent signal from to construct a chromatogram, as recited in dependent claim 28. The Examiner states that this deficiency is cured by Hardin, which allegedly teaches use of a chromatogram to monitor the mobility of fluorescently labeled molecules.

As described above, the combination of Han and Lin does not teach or suggest the method of claim 21, upon which claim 28 depend. Hardin does not cure this deficiency. Hardin teaches gamma phosphate labeled nucleotides, and their use in labeling target biomolecules. Hardin does not teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal**. Therefore, for at least this reason, the combination of Han, Lin and Hardin does not teach or suggest the method of claim 28.

Obviousness-type Double Patenting

Rejection of claims 21, 23, 26, 27 and 29-34

Claims 21, 23, 26, 27 and 29-34 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 7-8, 11-12, 15-17, and 20, respectively, of U.S. Patent No. 8,838,394. Obviousness-type double patenting cannot be finally determined until there is allowable subject matter. Accordingly, Applicant requests deferral of this issue until there is an indication of allowable claims.

Rejection of claims 21, 26, and 30-31

Claims 21, 26, and 30-31 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 4-5, and 9, respectively, of U.S. Patent No. 10,068,051. Obviousness-type double patenting cannot be finally determined until there is allowable subject matter. Accordingly, Applicant requests deferral of this issue until there is an indication of allowable claims.

CONCLUSION

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number

provided. Prompt and favorable consideration of this Response is respectfully requested. The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit account No. 50-4561 (Attorney Docket No. 88DG-330783-US4)

Respectfully submitted,

SHEPPARD MULLIN RICHTER & HAMPTON LLP

By / James M. Turner /
James M. Turner
Registration No.: 74,602

Customer No.

30764

12275 El Camino Real, Suite 100
San Diego, CA 92130-4092
Telephone: (858) 720-8900
Facsimile: (858) 509-3691

Dated: March 28, 2023