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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application	)	
Inventors: KARTALOV, Emil P., et al.	)	Confirmation No.: 2457
Application No.: 15/914,356	)	Art Unit: 1631
Filed: March 07, 2018	)	Examiner: NEGIN, Russell Scott
Title: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS	)	Customer No. 021971

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

AMENDMENT AND RESPONSE TO FINAL OFFICE ACTION

AND

REQUEST FOR CONTINUED EXAMINATION

Dear Commissioner:

This paper is filed in response to the Final Office Action mailed July 22, 2019, and is accompanied by a Request for Continued Examination and the requisite fees. Applicant respectfully requests reconsideration of the above-referenced application in view of the following amendments and remarks.

Amendments to the Claims begins on page **2** of this paper

Remarks begin on page **5** of this paper.

Conclusion begins on page **10** of this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings of claims in this application. Applicant reserves the right to pursue any subject matter of any canceled claims in this or any other appropriate patent application. Support for these claims is provided in the remarks following the listing of claims.

Listing of the Claims

1. (Currently Amended) A method of unambiguously detecting any unique combination of presence or absence of at least five polynucleotide analytes in a plurality of droplets, the method comprising:
 - (a) providing a sample comprising, or potentially comprising, at least one of said at least five polynucleotide analytes;
 - (b) forming a mixture of said sample and at least five hybridization probes, wherein each of said at least five hybridization probes further comprises ~~comprising at least one or more~~ fluorophore[[s]] and selected from at most four fluorophores;
 - (c) partitioning said mixture into said plurality of droplets;
 - (d) exciting said at least one fluorophore[[s]] to generate one or more signals if one or more of said at least five polynucleotide analytes is present in said plurality of droplets, wherein said one or more signals comprise at least one signal generated by excitement of said one or more fluorophores; [[and]]
 - (e) measuring said one or more signals to generate a cumulative intensity measurement, wherein said cumulative intensity measurement corresponds to the presence of a unique combination of presence or absence of said at least five polynucleotide analytes in said sample, ~~thereby detecting the presence or absence of said at least five polynucleotide analytes, in any unique combination of presence or absence; and~~
 - (f) determining whether each of said least five polynucleotide analytes is present, in any unique combination of presence or absence, based on said cumulative intensity measurement,wherein the method does not require any step of immobilization of said at least five polynucleotide analytes or mass spectrometry.

2. (Previously Presented) The method of claim 1, wherein each of said at least five hybridization probes is complementary to one of said at least five polynucleotide analytes.
3. (Previously Presented) The method of claim 1, wherein said at least five hybridization probes comprise one or more fluorophores selected from one, two, three, or four fluorophores.
4. (Previously Presented) The method of claim 1, wherein, in (b), a droplet of said plurality of droplets comprises said at least five hybridization probes.
5. (Previously Presented) The method of claim 1, wherein, in (b), a droplet of said plurality of droplets comprises one or more of said at least five polynucleotide analytes.
6. (Previously Presented) The method of claim 1, wherein, in (b), a droplet of said plurality of droplets comprises at most one of said at least five polynucleotide analytes.
7. (Previously Presented) The method of claim 1, wherein said one or more signals is generated during a polymerase chain reaction.
8. (Previously Presented) The method of claim 1, wherein said at least five polynucleotide analytes are at least seven polynucleotide analytes.
9. (Previously Presented) The method of claim 1, wherein said at least five hybridization probes are at least seven hybridization probes.
10. (Previously Presented) The method of claim 1, wherein said one or more polynucleotide analytes comprise cell-free nucleic acid, tumor nucleic acid, fetal nucleic acid, viral nucleic acid, or any combination thereof.
11. (Previously Presented) The method of claim 1, wherein said sample is a biological sample derived from a subject.
12. (Previously Presented) The method of claim 11, wherein detecting the presence or absence of said at least five polynucleotide analytes indicates the presence of cancer or an infection with a pathogen in said subject.
13. (Previously Presented) The method of claim 1, further comprising transmitting information concerning the presence or absence of said at least five polynucleotide analytes through a computer network.
14. (Previously Presented) The method of claim 1, further comprising providing information concerning the presence or absence of said at least five polynucleotide analytes to a health care

professional, wherein the health care professional makes a clinical decision based on said information.

15. (Previously Presented) The method of claim 14, wherein said clinical decision is a treatment decision.
16. (Previously Presented) The method of claim 15, wherein said treatment comprises treatment with an antibiotic or an anticancer therapeutic.
17. (Previously Presented) The method of claim 1, wherein at least part of said method is performed using a computer.
18. (Previously Presented) The method of claim 17, wherein said computer is located on a remote server.
19. (Previously Presented) The method of claim 17, wherein said computer is located on a droplet reader.
20. (Previously Presented) The method of claim 1, wherein each of said at least five hybridization probes are provided at a given concentration such that said cumulative measurement corresponds to said presence of said unique combination.

REMARKS

Status of the Claims

The following remarks are in response to the Office Action mailed on July 22, 2019. Claims 1-20 are pending. Claim 1 is amended. No new matter is added. Reconsideration and allowance is respectfully requested in light of the following remarks.

Claim amendments

Claim 1 has been amended to clarify the claimed subject matter. These amendments are fully supported by the application as originally filed, U.S. Patent Application Serial No. 15/914,356, (“the Application”) at, for example, paragraph [0076], [0103] – [0107], and [0143] of the Application.

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

i. Claims 1-12 and 20 are not obvious over Han in view of Lin in view of Vogelstein

Claims 1-12 and 20 stand rejected under 35 U.S.C. §103 over Han et al., “Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules” Nature Biotechnology volume 19, pages 631–635 (2001) (hereinafter “Han”) in view of Lin et al., “NUCLEIC ACID NANOSTRUCTURE BARCODE PROBES” WO 2012/058638 A2 (hereinafter “Lin”) in view of Vogelstein et al., “Digital PCR” PNAS August 3, 1999 96 (16) 9236-9241 (hereinafter “Vogelstein”). *See* Office Action at pages 4-7.

MPEP 2143 states that obviousness may be established only when three basic criteria have been met. “First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.”

Without conceding in the basis of the rejection, Applicant has amended claim 1 to further clarify the claimed subject matter. Applicant respectfully submits that claims 1-12 and 20 are not obvious over Han, Lin, and Vogelstein for at least the reason that the asserted combination of references does not teach or suggest all the claim limitations of claim 1.

Claim 1 recites, *inter alia*, “[a] method of unambiguously detecting any unique combination of presence or absence of at least five polynucleotide analytes in a plurality of droplets, the method comprising: (a) providing a sample comprising, or potentially comprising, at least one of said at least five polynucleotide analytes; (b) forming a mixture of said sample and at least five hybridization probes, ... (d) **exciting said at least one fluorophore to generate one or more signals** if one or more of said at least five polynucleotide analytes is present... (e) **measuring said one or more signals to generate a cumulative intensity measurement**, wherein said cumulative intensity measurement **corresponds to the presence of a unique combination of presence or absence of said at least five polynucleotide analytes in said sample**; and (f) **determining whether each of said at least five polynucleotide analytes is present, in any unique combination of presence or absence, based on said cumulative intensity measurement**, wherein the method does not require any step of immobilization of said at least five polynucleotide analytes or mass spectrometry.”

Han does not disclose a method of generating or measuring a cumulative measurement across analytes as recited by Applicant in claim 1. Rather, Han teaches a method of multiplexing whereby the fluorescence intensity level of each target (*i.e.*, single bead) is measured individually. Han’s distinctly dissimilar method for optical coding is illustrated in the schematic illustrations in Figures 1 and 5.

Figure 1 illustrates Han’s underlining method, whereby the “**large spheres represent polymer microbeads**, in which small colored spheres (multicolor quantum dots) are embedded according to predetermined intensity ratios. Molecular probes (A–E) are attached to the bead surface for biological binding and recognition, such as DNA–DNA hybridization.... The numbers of colored spheres (red, green, and blue) do not represent individual QDs, but are used to illustrate the fluorescence intensity levels. **Optical readout is accomplished by measuring the fluorescence spectra of single beads [Large spheres]. Both absolute intensities and relative intensity ratios at different wavelengths are used for coding purposes**; for example (1:1:1) (2:2:2), and (2:1:1) are distinguishable codes” (Han at Fig. 1, page 632, teaching (emphasis added)).

Figure 5 on page 634 further illustrates that Han’s coding scheme relies on the identification **of each individual bead** (*i.e.*, single targets). Specifically, Fig. 5 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.... **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).”

Lin does not cure the deficiencies of Han. Lin is cited for disclosing nanostructure barcode probes. In regards to observing the barcodes, Lin discloses “[t]wo distinct features of the barcode were clearly visible from the TIRF images (FIG. 2A, top panel): first, each fluorescently labeled zone on a barcode was resolved as a single-color spot and each complete barcode consisted of three of such spots; Second, two of the neighboring spots were separated by a small gap while the other 7 two neighbors sat closely together. Therefore, one can **visually recognize and decode those geometrically encoded barcodes based on the color identity of the spots and their relative spatial positions**, even without the aid of any specialized decoding software” (Lin, p. 25, lns. 7-14; emphasis added). Lin uses **color-coded patterns** deposited on a nanorod barcode probe to **detect a target of interest**, and does not “determin[e] whether each of [the] analytes is present, in any unique combination of presence or absence, **based on said cumulative intensity measurement**,” as recited in claim 1 of the instant application.

Vogelstein does not cure the deficiencies of Lin or Han. Vogelstein discloses “[s]ingle molecules are isolated by dilution and individually amplified by PCR; each product is then analyzed separately for the presence of mutations by using fluorescent probes” (Vogelstein at Abstract). However, nowhere does Vogelstein disclose, “determining whether each of said least five polynucleotide analytes is present, in any unique combination of presence or absence, based on said cumulative intensity measurement,” as recited in claim 1.

Overall, because Han, Lin, and Vogelstein, alone, or in combination, fail to disclose, “[a] method of unambiguously detecting any unique combination of presence or absence of at least five polynucleotide analytes in a plurality of droplets [by] **measuring said one or more signals to generate a cumulative intensity measurement**, wherein said cumulative intensity measurement **corresponds to the presence of a unique combination of presence or absence of said at least five polynucleotide analytes in said sample**; and (f) **determining whether each of said least five polynucleotide analytes is present, in any unique combination of presence or absence, based on said cumulative intensity measurement**,” as recited by claim 1, the asserted combination of

references fails to meet the requirements under MPEP 2143. Accordingly, Applicant respectfully requests that the rejection of claim 1 under 35 U.S.C. §103 be withdrawn.

As claims 2-12 and 20 depend from and include all of the elements of claim 1 and recite additional elements of particular advantage and utility. The combination of Han, Lin, and Vogelstein similarly fail to meet the combination of claims 2-12 and 20. Accordingly, Applicant respectfully requests that the rejections of dependent claims 2-12 and 20 under 35 U.S.C. §103 be withdrawn.

ii. Claims 13 and 17-19 are not obvious over Han, in view of Lin and Vogelstein in further view of Empedocles

Claims 13 and 17-19 stand rejected under 35 U.S.C. §103 over Han, in view of Lin and Vogelstein in further view of Empedocles et al., “DIFFERENTIABLE SPECTRAL BAR CODE MEHTODS AND SYSTEMS” 2002/0022273A1; on IDS (hereinafter “Empedocles”). See Office Action at pages 7-8.

Applicant submits that claims 13 and 17-19 are not obvious over Han, Lin, and Vogelstein, for at least the reason that they depend from and include all element of claim 1. Because the asserted combination of Han, Lin, and Vogelstein fail to meet all of the elements of claim 1, much less the combination of elements of claims 13 and 17-19, Empedocles does not cure the abovementioned deficiencies of Han, Lin, and Vogelstein.

Accordingly, Applicant respectfully requests that the rejections of dependent claims 13 and 17-19 be withdrawn under 35 U.S.C. §103.

iii. Claims 14-16 are not obvious over Han in view of Lin, Vogelstein, and Empedocles, in further view of Newton

Claims 14-16 stand rejected under 35 U.S.C. §103 over Han in view of Lin, Vogelstein and Empedocles in further view of Newton et al., “ASYMMETRIC PCR COUPLED WITH POST-PCR CHARACTERIZATION FOR THE IDENTIFICATION OF NUCLEIC ACIDS” 2007/0072211A1; on IDS (hereinafter “Newton”). See Office Action at pages. 9-10.

Applicant submits that claims 14-16 are not obvious over Han, Lin, Vogelstein, and Empedocles for at least the reason that they depend from and include all element of claim 1. Because the asserted combination of Han, Lin, Vogelstein, and Empedocles fails to meet all of the elements

of claim 1, much less the combination of elements of claims 14-16, Newton does not cure the abovementioned deficiencies of Han, Lin, Vogelstein, and Empedocles.

Accordingly, Applicant respectfully requests that the rejections of dependent claims 14-16 be withdrawn under 35 U.S.C. §103.

It shall be understood herein that any instance in which Applicant has addressed certain comments set forth by the Office shall not be construed as a concession to other comments or arguments advanced by the Office. Any circumstance in which Applicant has amended or cancelled a claim also does not mean that Applicant concedes to the arguments or positions advanced by the Office with respect to that claim or other claims pending herein.

CONCLUSION

In light of the remarks set forth above, Applicants believe that the pending claims are under condition for allowance. Applicants respectfully solicit the Examiner to expedite the prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned at (650) 849-3327.

The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit account No. 23-2415 (Attorney Docket No. 38075-716.302).

Respectfully submitted,

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By: /Nicholas Tiee/
Nicholas Tiee, Reg. No. 77,907

WILSON SONSINI GOODRICH & ROSATI
650 Page Mill Road
Palo Alto, CA 94304-1050
Phone: (512) 338-5444
Customer No. 021971