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(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: Xiaowei Zhuang
Application No.: 17/374,000
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For: SYSTEMS AND METHODS FOR DETERMINING NUCLEIC
ACIDS
Examiner: J. T. Woitach
Art Unit: 1672

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Dated: January 26, 2024

Signature: /Katelyn Taylor/ (Katelyn Taylor)

RESPONSE

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

Dear Commissioner:

INTRODUCTORY COMMENTS

In response to the Office Action dated September 28, 2023, reconsideration is respectfully requested in view of the following:

The Claims are reflected in the listing of claims which begins on page 2 of this response.

Remarks begin on page 8 of this response.

IN THE CLAIMS

Submitted below is a complete listing of the current claims, including marked-up claim(s) with insertions indicated by underlining and deletions indicated by strikeouts and/or double bracketing. This listing of claims replaces all prior versions, and listings, of claims in the application:

1-94. (Cancelled)

95. (Withdrawn) A composition, comprising:

a plurality of nucleic acid probes, each comprising a first portion comprising a target sequence and a plurality of read sequences, wherein the plurality of read sequences are distributed on the plurality of nucleic acid probes so as to define an error-correcting code.

96. (Withdrawn) The composition of claim 95, wherein the plurality of nucleic acid probes defines a code space with a Hamming distance of at least 2 or at least 3.

97. (Cancelled)

98. (Withdrawn) The composition of claim 96, wherein the code is a Hamming(7, 4) code, a Hamming(15, 11) code, a Hamming(31, 26) code, a Hamming(63, 57) code, or a Hamming(127, 120) code.

99. (Withdrawn) The composition of claim 95, wherein the code is a SECDED code.

100-101. (Cancelled)

102. (Withdrawn) The composition of claim 99, wherein the code is a SECDED (8, 4) code, a SECDED (16, 4) code, SECDED (16, 11) code, a SECDED (22, 16) code, a SECDED (39, 32) code, or a SECDED (72, 64) code.

103. (Cancelled)

104. (Withdrawn) The composition of claim 99, wherein the code is MHD4 code or MHD2 code.

105. (Cancelled)

106. (Withdrawn) The composition of claim 95 , wherein the plurality of nucleic acid probes comprises no more than 100, no more than 64, no more than 32, no more than 16, or no more than 8, no more than 6, no more than 4, or no more than 2 possible read sequences.

107-110. (Cancelled)

111. (Withdrawn) The composition of claim 95 , wherein

- (a) the target sequence of the plurality of nucleic acid probes has an average length of between 10 and 200 nucleotides;
- (b) the plurality of read sequences have an average length of between 5 nucleotides and 50 nucleotides;
- (c) the plurality of nucleic acid probes have an average length of between 10 and 300 nucleotides; or
- (d) combinations of one or more of (a)-(c).

112-116. (Cancelled)

117. (Withdrawn) The composition of any one of claims 95-116, wherein at least some of the plurality of nucleic acid probes comprise DNA, RNA, PNA, LNA, or combinations thereof.

118-121. (Cancelled)

122. (Currently amended) A method for imaging spatial organization of a nucleic acid target in a sample, comprising:
- a. contacting the sample comprising associating a plurality of distinct nucleic acid targets in situ with a plurality of primary nucleic acid probe pools each of the nucleic acid probes comprising a target sequence[[s]] and one or more read sequences, wherein each pool of nucleic acid probes hybridize to a distinct nucleic acid target and each pool of probes encode a N-bit ~~a plurality of~~ codeword[[s]] that was assigned to each distinct nucleic acid target and each N-bit codeword is a valid codeword, wherein each of the read sequences correspond to a bit value of 1 for the valid codewords ~~the codewords comprise a number of positions and values for each position~~, and the valid codewords form an error-checking and/or error-correcting code space;
- ~~associating a plurality of distinguishable read sequences with the plurality of codewords such that each distinguishable read sequence represents a value of a position within the codewords; and~~
- ~~forming a plurality of nucleic acid probes, each comprising a target sequence and one or more read sequences~~
- b. contacting the bound primary nucleic acid probes with a plurality of readout probes comprising a fluorescent label, wherein the readout probes hybridize to the read sequences of the primary nucleic probes;
- c. imaging the readout probes bound to the primary nucleic acid probes; and,
- d. repeating steps b) and c) in one or more sequential hybridization and imaging rounds until all N positions in the N-bit codeword have been imaged providing an imaged codeword corresponding to each distinct nucleic acid target in a spatial organization.
123. (Currently amended) The method of claim 122, wherein the code space has a Hamming distance of ~~at least~~ equal to or greater than 2 between valid codewords.
124. (Currently amended) The method of ~~any one of~~ claim[[s]] 122, wherein the ~~plurality of targets~~ nucleic acid target comprises RNA.

125-131. (Cancelled)

132. (Currently amended) The method of claim ~~131~~ 122, ~~comprising wherein~~ imaging ~~the signaling entity~~ comprises using a super-resolution imaging technique.

133-134. (Cancelled)

135. (Currently amended) The method of claim ~~125~~ 122, comprising determining binding of the ~~plurality of secondary nucleic acids~~ readout probes within the sample at a resolution better than 100 nm.

136-176. (Cancelled)

177. (New) The method of claim 122, wherein the valid codewords were assigned using a Single Error Correction Double Error Detection (SECDED) code.

178. (New) The method of claim 122, wherein the valid codewords were assigned using a Hamming code.

179. (New) The method of claim 122, wherein each of the valid codewords contains a parity bit.

180. (New) The method of claim 122, wherein the nucleic acid target comprises DNA.

181. (New) The method of claim 122, comprising determining transcriptome spatial organization from a single cell.

182. (New) The method of claim 122, wherein the N-bit codeword has a Hamming weight of 4.

183. (New) The method of claim 122, wherein the imaged codeword is matched to a valid codeword assigned to a distinct nucleic acid target.
184. (New) The method of claim 122, wherein the imaged codewords are matched to valid codewords or discarded.
185. (New) The method of claim 122, wherein each primary nucleic acid probe pool comprises at least 10 different primary nucleic acid probes.
186. (New) The method of claim 122, wherein each primary nucleic acid probe pool comprises at least four distinct read sequences.
187. (New) The method of claim 122, wherein the primary nucleic acid probes comprise a target sequence, with an average length of between 10 and 200 nucleotides, that hybridize the distinct nucleic acid targets.
188. (New) The method of claim 122, wherein after each hybridization and imaging round the fluorescent readout probe is quenched to inactivate.
189. (New) The method of claim 122, wherein after each hybridization and imaging round the fluorescent readout probe is inactivated by removal of the readout probe hybridized to the read sequences of the primary nucleic probes or by chemically or enzymatically cleaving the fluorescent label from the readout probe.
190. (New) The method of claim 122, wherein the N-bit codeword comprises at least a 16-bit codeword.
191. (New) The method of claim 122, wherein each assigned N-bit codeword is a valid codeword with a Hamming distance equal to or greater than 2 between valid codewords.

192. (New) A method for imaging spatial organization of a nucleic acid target in a sample, comprising:
- a. contacting the sample comprising a plurality of distinct nucleic acid targets in situ with a plurality of primary nucleic acid probe pools each of the nucleic acid probes comprising a target sequence and one or more read sequences, wherein each pool of nucleic acid probes hybridize to a distinct nucleic acid target and each pool of probes encode a N-bit codeword with a Hamming weight of at least 2 that was assigned to each distinct nucleic acid target and each N-bit codeword is a valid codeword with a Hamming distance equal to or greater than 2 between valid codewords and wherein each of the read sequences correspond to a bit value of 1 for the valid codewords;
 - b. contacting the bound primary nucleic acid probes with a plurality of readout probes comprising a fluorescent label, wherein the readout probes hybridize to the read sequences of the primary nucleic acid probes;
 - c. imaging the readout probes bound to the primary nucleic acid probes; and,
 - d. repeating steps b) and c) in one or more sequential hybridization and imaging rounds until all N positions in the N-bit codeword have been imaged providing an imaged codeword corresponding to each distinct nucleic acid target in a spatial organization.

REMARKS

Claims 122-124, 126-132, and 135 are currently pending for examination. Claims 95, 96, 98, 99, 102, 104, 106, 111, 117, 122-124, 126-132, and 135 have been withdrawn.

No new matter has been added.

I. Status of the Claims

Prior to entry of this amendment, claims 95-96, 98-99, 102, 104, 106, 111, 117, 122-124, 126-132 and 135 were pending in the present application. Claims 95, 96, 98-99, 102, 104, 106, 111 and 117 have been withdrawn. Claims 1-94, 97, 100, 101, 103, 105, 107-110, 112-116, 118-121, 125, 133-134 and 136-176 were previously cancelled.

II. The Amendments and Support

Claims 126-131 are herein cancelled without prejudice. Claims 122-124, 132 and 135 are herein amended to clarify embodiments. New claims 177-192 are herein added. Support for those amendments can be found, for example, at paragraph [0005]-[0025] and [0160] of the specification as published. Additional support for the amendments can be found in the claims as filed and throughout the specification. It is believed that no new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

The amendments to the claims are made solely to obtain expeditious allowance of the instant application and not for reasons related to patentability. Amendment of the claims is made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. The right to file one or more continuing applications hereof containing the cancelled or unamended claims is expressly reserved. The amendments to the claims are fully supported by the applications as filed.

III. (Provisional) Obviousness-type Double Patenting Rejection

A. Claims 122-124, 126-132, and 135 were rejected on the grounds of alleged non-statutory obviousness-type double patenting over the claims of U.S. Patent No. 11,098,303.¹

B. Claims 122-124, 126-132, and 135 were rejected on the grounds of alleged non-statutory obviousness-type double patenting over the claims of co pending U.S. Ser. No. 18/046,409.

C. Claims 122-124, 126-132, and 135 were rejected on the grounds of alleged non-statutory obviousness-type double patenting over the claims of co-pending U.S. Ser. No. 18/184,967.

D. Claims 122-124, 126-132, and 135 were rejected on the grounds of alleged non-statutory obviousness-type double patenting over the claims of co-pending U.S. Ser. No. 18/185,048.

E. Claims 122-124, 126-132, and 135 were rejected on the grounds of alleged non-statutory obviousness-type double patenting over the claims of co-pending U.S. Ser. No. 18/297,378.

Each of the above rejections is traversed. It is asserted that the present claims are patentably distinct and non-obvious over the claims of U.S. Pat. No. 11,098,303 and U.S. Ser. No. 18/046,409; 18/184,967; 18/185,048; and 18/297,378.

However, should the Examiner maintain the rejection, it is respectfully requested that the rejections be further held in abeyance until notification of allowable subject matter. The undersigned will then either file a terminal disclaimer that complies with 37 C.F.R. § 1.321(c) or § 1.321(d), explain why the present application is patentably distinct from the asserted claims, or request issuance of the claims without the need of a terminal disclaimer pursuant to § 804 of the M.P.E.P.

¹ The Office Action actually recites U.S. Pat. No. 11,098,683, but this is directed to a “Fluid conduit coupling having securing clamp,” which appears to be assigned to Mahle International GmbH, and does not seem to be reasonably related to the present application or the claims as pending. It is thus assumed that this is an inadvertent typographical error, and that U.S. Pat. No. 11,098,303 was intended. Clarification is respectfully requested if this assumption is incorrect.

IV. Rejection under 35 U.S.C. § 101

Claims 122-124, 126-132, and 135 were rejected under 35 U.S.C. 101 as allegedly being directed to non-statutory subject matter (an abstract idea) without significantly more.

According to the Examiner, claim 122 is directed to “associating target sequences with codewords” wherein “the judicial exception of the claims are the steps of associating a detected signal as a form of a data with a codeword” and “a set of instructions for analysis of sequence data in the form of a probe interaction and appear to be in the category of Mental Processes, that is concepts performed in the human mind.”

However, it is noted that judicial exceptions have been categorized as “laws of nature,” “natural phenomena,” and “abstract ideas” and include further groupings such as “mental processes.” See M.P.E.P. § 2106.04(I). Abstract ideas are enumerated in the following groupings as “mathematical concepts,” “certain methods of organizing human activity,” and “mental processes.” See M.P.E.P. § 2106.04(a). Courts have acknowledged that while “all inventions at some level embody, use, reflect, rest upon, or apply laws of nature, natural phenomenon or abstract idea, not all claims recite an abstract idea” (*Alice Corp. v. CLS Bank Int’l*, 573 U.S. 208 (2014)) but that to be accurately determined subject matter ineligible a claim must be “directed to” a judicial exception. Accordingly, USPTO guidance requires a claim to **recite** (i.e., set forth or describe) an abstract idea to be deemed ineligible in Step 2A Prong One of the subject matter eligibility analyses, thereby distinguishing claims reciting abstract ideas from those that are merely based on or involve an abstract idea. See M.P.E.P. § 2016.04(a)(1). The grouping of “mental processes” is defined **as concepts performed in the human mind**, and examples of mental processes include observations, evaluations, judgements, and opinions. See M.P.E.P. § 2106.04(a)(2)(III). Moreover, a claim with limitations that cannot practically be performed in the human mind does not recite a mental process. *Id.*

It is submitted the claimed subject matter: a) does not “recite,” “set forth,” or “describe” an abstract idea; and b) is directed to a *process* reciting active steps using tangible products that *cannot practically be performed in the human mind*. As is clear from reading the claimed subject matter, and remarks provided below, when viewed as a whole, the **eligibility** of the claims is self-evident.

Accordingly, it is submitted that the pending claims are subject matter **eligible** under 35 U.S.C. § 101.

Initially, claim 122, as amended, recites the following steps:

- a. **contacting the sample comprising a plurality of distinct nucleic acid targets in situ with a plurality of primary nucleic acid probe pools each of the nucleic acid probes comprising a target sequence and one or more read sequences, wherein each pool of nucleic acid probes hybridize to a distinct nucleic acid target and each pool of probes encode a N-bit codeword that was assigned to each distinct nucleic acid target and each N-bit codeword is a valid codeword, wherein each of the read sequences correspond to a bit value of 1 for the valid codewords, and the valid codewords form an error-checking and/or error-correcting code space;**

In this step, the sample is **contacted** with “a plurality of primary nucleic acid pools” wherein those probes are further defined in the recited step. This step cannot practically be performed in the human mind, as it is a physically active step performed with tangible reagents (e.g. primary nucleic acid probes and a biological sample). Accordingly, this step does not “recite,” “set forth,” or “describe” a mental process.

- b. **contacting the bound primary nucleic acid probes with a plurality of readout probes comprising a fluorescent label, wherein the readout probes hybridize to the read sequences of the primary nucleic probes;**

In this step, the primary nucleic acid probes, hybridized to the nucleic acid target of the sample from step a, are **contacted** with fluorescent labeled “readout probes,” which hybridize to the readout sequences of the primary nucleic acid probes. This step cannot practically be performed in the human mind as it is a physically active step performed with tangible reagents (e.g., readout nucleic acid probes). Accordingly, this step does not “recite,” “set forth,” or “describe” a mental process.

- c. **imaging the readout probes bound to the primary nucleic acid probes; and,**

In this step, as well understood in the art, **imaging** the readout probes involves the use of a fluorescent instrument with appropriate excitation and emission filters for a given fluorescent label providing a fluorescent signal and image. It is impossible to perform the step of acquiring an image

without an appropriate imaging instrument, which is a physically active step involving a tangible instrument. Accordingly, this step does not “recite,” “set forth,” or “describe” a mental process.

d. repeating steps b) and c) in one or more sequential hybridization and imaging rounds until all N positions in the N-bit codeword have been imaged providing an imaged codeword corresponding to each distinct nucleic acid target in a spatial organization.

In this step, steps b and c are repeated (which have been established above as not mental processes) “until all N positions in the N-bit codeword have been imaged providing an imaged codeword corresponding to each distinct nucleic acid target in a spatial organization.” In this final step, steps b and c are simply repeated until all N positions (e.g., 16 positions in a 16-bit code) have been imaged providing an image of nucleic acid targets in a spatial organization. While based on use of an algorithm or a “set of instructions” to match an imaged codeword with a valid codeword, not only can that not be performed in a human mind, but that the unrecited underlying process does not meet the threshold for “reciting,” “setting forth,” or “describing” a mental process.

To this point, further instructive are the Examples provided by the USPTO in the USPTO guidance, e.g., Example 37, 38, 39 and 46 (claim 4). Each of these examples is directed to a process and some of which recite a judicial exception (e.g., mental process) but all of which were deemed to be subject matter **eligible**. For example, claim 1 of Example 38 (“Stimulating an Analog Audio Mixer” was deemed to **eligible** because “the claim does not recite a mental process,” which is similar to the present claims that recite active steps even when some of the limitations may be based on an abstract idea. The same analysis applies to Example 39, “Method of Training a Neural Network for Facial Detection” wherein claim 1 was deemed **eligible** because “the claim does not recite a mental process because the steps are not practically performed the human mind.” See also M.P.E.P. § 2106.04(a)(1) “Examples of Claims that Do Not Recite Abstract Ideas.”

Based on the analysis provided above, it is believed that claim 122, as well as any claims dependent thereupon, are **eligible** because the claims do not recite a judicial exception. Withdrawal of the rejection is requested. In summary:

Step	Analysis of claim 122
1: Statutory Category?	Yes. The claim recites a series of steps and, therefore, is a process. “A method for imaging spatial organization of a nucleic acid target in a sample.”
2A: Prong 1: Judicial Exception Recited?	No. The claim does not recite any of the judicial exceptions enumerated in M.P.E.P. § 2106.04(a)(2). For instance, the claim does not recite any mathematical relationships, formulas, or calculations. While some of the limitations may be based on mathematical concepts, the mathematical concepts are not recited in the claims. <i>Further, the claim does not recite a mental process because the steps are not practically performed in the human mind.</i> Finally, the claim does not recite any method of organizing human activity such as a fundamental economic concept or managing interactions between people. Thus, the claim is eligible because it does not recite a judicial exception.
2A – Prong 2: Integrated into a Practical Application	N/A
2B – Claim provides an Inventive Concept?	N/A

Thus, based on the above analysis, and guidance provided by M.P.E.P. § 2106.04(a), it is believed that claims 122-124, 132, 135 and 177-192 qualify as **eligible** subject matter under 35 U.S.C. 101. Withdrawal of this rejection is accordingly respectfully requested.

V. Rejection under 35 U.S.C. § 103

Claims 122-124, 126-132 and 135 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Göransson, *et al.*, “A single molecule array for digital targeted molecular analyses,” *Nucleic Acids Res.*, 37(1):e7, 2009 (“Göransson”),² Su, *et al.*, U.S. Pat. Apl. Pub. No. 2005/0064435 (“Su”), and Winther, *et al.*, U.S. Pat. No. 8,741,566 (“Winther”).

² The Examiner is thanked for the courtesy of a telephone call on October 19, 2023, in which he clarified that the rejection should have been over Göransson (as identified above), rather than “Gransson” as was recited in the Office Action due to an inadvertent typographical error.

Briefly, the Examiner asserted that Göransson teaches all of the elements of the claims other than “specifically stat[ing] that the signals are codewords, but clearly provide the basis of interpreting with a decoding scheme the association of a signal and target gene.” According to the Examiner, Su “is provided for the guidance and evidence that probe design which takes components and creates code components were known” and Winther “is provided to demonstrate variations in the design of recognition units, adapter units and detection units for detection of targets for applications such as in situ hybridizations ([010]) and the application of a multi-layer systems for detection.” Thus, the Examiner asserted it would have been obvious to one of ordinary skill in the art to “associate target probe sets that detect nucleic acids in a sample, and based on the signals detected decode the results.”

However, when determining whether a claim is obvious, an examiner must make “a searching comparison of the claimed invention—including *all its limitations*—with the teaching of the prior art.” *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis added). Thus, “obviousness requires a suggestion of all limitations in a claim.” *CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). Moreover, as the Supreme Court recently stated, “*there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.*” *KSR Int’l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) (emphasis added)).

As described in more detail below, it is submitted that the pending claims are non-obvious and patentably distinct from the cited art because those references fail to disclose, teach, or reasonably suggest all of the claim elements. In particular, the claimed subject matter is a patentably distinct advancement over the methods taught in the cited art for multiplexed *in situ* nucleic acid spatial imaging, as described more fully below.

Claim 122 is directed to a method for imaging spatial organization of a nucleic acid target in a sample, wherein the claims recite, in part, “... a. contacting the sample comprising a plurality of distinct nucleic acid targets in situ with a plurality of primary nucleic acid probe pools each of the nucleic acid probes comprising a target sequence and one or more read sequences, wherein each pool of nucleic acid probes hybridize to a distinct nucleic acid target and each pool of probes encode

a N-bit codeword that was assigned to each distinct nucleic acid target and each N-bit codeword is a valid codeword, wherein each of the read sequences correspond to a bit value of 1 for the valid codewords, and the valid codewords form an error-checking and/or error-correcting code space; b. contacting the bound primary nucleic acid probes with a plurality of readout probes comprising a fluorescent label, wherein the readout probes hybridize to the read sequences of the primary nucleic acid probes; c. imaging the readout probes bound to the primary nucleic acid probes; and, d. repeating steps b) and c) in one or more sequential hybridization and imaging rounds until all N positions in the N-bit codeword have been imaged providing an imaged codeword corresponding to each distinct nucleic acid target in a spatial organization.” Rejected claims 123-124, 132 and 135 depend from and incorporate the patentable subject matter of Independent claim 122. Newly added claims 177-191 also depend from and incorporate the patentable subject matter of claim 122.

Those underlined claim recitations are not taught or suggested by Goransson, Su, and/or Winther, either alone, or in combination.

The present specification discloses and teaches the use of error-robust *in situ* hybridization nucleic acid probes (“encoding probes”) that reduce misidentification of nucleic acid targets in a sample wherein probe pools for distinct targets encode a N-bit codeword. Those nucleic acid probes comprise a target sequence and one or more read sequences wherein the read sequence corresponds to a position within the N-bit codeword, which is imaged in sequential hybridization rounds using fluorescent readout probes that bind to the read sequences of the nucleic acid probes. *See, e.g.,* Example 1; Figure 5E, 11 and 12A-12C for non-limiting illustrative purposes. In other words, the claimed subject matter utilizes two sets of probes which provide for the sequential imaging and generation of a codeword using a combinatorial strategy with readout probes binding to read sequences of the primary nucleic acid probes.

Those figures (Figure 5E, 6D, 11 and 12A-12C) illustrate the “plurality of primary nucleic acid probe pools” wherein “each of the nucleic acid probes comprising a target sequence and one or more read sequences” that “hybridize to a distinct nucleic acid target and each pool of probes encode a N-bit codeword.” Moreover, “each assigned N-bit code is a valid codeword wherein each of the read sequences correspond to a bit value of 1 for the valid codewords, and the valid codewords form an error-checking and/or error-correcting code space.” Further, so that each

distinct nucleic acid target is imaged in a spatial organization *in situ*, the distinct nucleic acid targets are each assigned a valid codeword. None of those elements are disclosed, taught, or reasonably suggested by any of the cited art.

Moreover, the inventors of the present specification published a paper exemplifying certain embodiments within the scope of the claimed subject matter. *See* Chen, *et al.*, “Spatially resolved, highly multiplexed RNA profiling in single cells,” *Science*, 348(6233), 2015 (“Chen”). Chen demonstrates that multiplexing requires an error robust coding scheme to accurately image spatial organization of RNA transcripts *in situ* and that the methods of seqFISH (*See* Lubeck and Cai, *Nat. Methods*, 9(7):743-748, 2012 and, Lubeck and Cai, *Nat. Methods*, 11:360-361, 2014) are inadequate for those purposes (“scaling up the throughput of smFISH to the systems scale faces significant challenge because not only does the addressable RNA species increases exponentially with N [each RNA species by multiple (N) distinct signals], but the detection error rates also grow exponentially with N). *See* Chen at p. 2, “Combinatorial labeling with error-robust encoding schemes.” Moreover, “as N increases, the fraction of RNAs properly detected (the calling rate) would rapidly decrease and, more troubling, the fraction of RNAs that are identified as incorrect species (the misidentification rate) would rapidly increase,” wherein “the majority of RNA molecules would be misidentified after 16 rounds of hybridizations!”

To address that problem of using smFISH probes in multiplex assays, Chen developed error-robust encoding schemes, in which only a subset of $2^N - 1$ words separated by a certain Hamming distance are used to encode RNAs. This was exemplified with a coding scheme “in which the number of 1 bits [Hamming weight] were kept both constant and relatively low—only four per word in this work—so as to reduce error and avoid biased detection.” *See* Chen at p. 1, col. 3, first full paragraph. In other words, the present inventors devised a unique two-step labeling scheme to encode and readout cellular nucleic acid to perform multiplexed error-robust fluorescence *in situ* hybridization (MERFISH). *See* Chen at Figure 1. This error-robust multiplexed method using encoding and readout probes is known in the art as “MERFISH.” The claimed subject matter, which includes MERFISH, is distinct from those methods using smFISH probes and provide an advancement over seqFISH (multiplexed methods using smFISH probes) or methods wherein codewords do not “form an error-checking and/or error-correcting code space.”

None of Goransson, Su, and/or Winther disclose, teach, or reasonably suggest valid codewords assigned to a plurality of nucleic acid targets wherein those valid codewords form an error-checking and/or error-correcting code space. The presently claimed subject matter is directed to use of primary probes that tile across the nucleic acid target and which comprise one or more readout sequences. These readout sequences correspond to a specific position in the N-bit codeword assigned to that nucleic acid target, but which are randomly located across the length of the distinct nucleic acid target via the primary nucleic acid probes. Those readout sequences are then imaged using readout probes which comprise a fluorophore and hybridize to the readout sequence only during the corresponding hybridization round for that position of the N-bit codeword. That combination of primary and secondary probes with readout sequences corresponding to a specific position in the N-bit codeword provide a unique coding scheme for multiplex imaging of single molecules *in situ*.

For those reasons, it is submitted that the teachings of the references are insufficient to make a *prima facie* case of obviousness. Even if combined, the aggregate teachings, legally, do not relate to the subject matter of interest. It is therefore respectfully requested that the rejections of claims 122-124, 126-132 and 135 be withdrawn.

CONCLUSION

In view of the foregoing remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after the foregoing remarks, that the application is not in condition for allowance, the Examiner is requested to call the undersigned at 617-646-8000.

If this response is not considered timely filed and if a suitable request for an extension of time is otherwise absent, any necessary extension of time is hereby requested. If there is a fee occasioned by this response, including an extension fee, the Director is hereby authorized to charge any deficiency or credit any overpayment in the fees filed, asserted to be filed or which should have been filed herewith to Deposit Account No. 23/2825, under Docket No. H0498.70509US03, from which the undersigned is authorized to draw.

Dated: January 26, 2024

Respectfully submitted,

Electronic signature: /Tani Chen/

Tani Chen, Sc.D.

Registration No.: 52,728

WOLF, GREENFIELD & SACKS, P.C.

600 Atlantic Avenue

Boston, Massachusetts 02210-2206

617.646.8000