

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10X GENOMICS, INC.,
Petitioner,

v.

PRESIDENT AND FELLOWS OF HARVARD COLLEGE,
Patent Owner

Case IPR2023-01299
U.S. Patent No. 11,098,303

**PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 11,098,303**

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EXHIBIT LIST

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1002	Declaration of Paul Spellman, Ph.D.
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1005	U.S. Patent No. 10,829,814 to Fan <i>et al.</i> , issued November 10, 2020 (“Fan”)
1006	U.S. Provisional Application No. 62/014,538 to Fan <i>et al.</i> , filed on June 19, 2014 (“Fan-Prov”)
1007	Gunderson <i>et al.</i> , “Decoding Randomly Ordered DNA Arrays,” <i>Genome Research</i> , 14(5):870-877 (May 2004) (“Gunderson”)
1008	Larsson <i>et al.</i> , “ <i>In situ</i> detection and genotyping of individual mRNA molecules,” <i>Nature Methods</i> , 7(5):395-397 (April 2010) (“Larsson”)
1009	U.S. Patent No. 8,765,642 to Curry, issued July 1, 2014 (“Curry”)
1010	Levsky <i>et al.</i> , “Fluorescence in situ hybridization: past, present and future,” <i>Journal of Cell Science</i> , 116(4):2833-2838 (July 2003) (“Levsky 2003”)
1011	Gozzetti <i>et al.</i> , “Fluorescence In Situ Hybridization: Uses and Limitations,” <i>Seminars in Hematology</i> , 37(4):320-333 (October 2000) (“Gozzetti”)
1012	Femino <i>et al.</i> , “Visualization of Single RNA Transcripts in Situ,” <i>Science</i> , 280:585-590 (April 1998) (“Femino”)
1013	Raj <i>et al.</i> , “Imaging individual mRNA molecules using multiple singly labeled probes,” <i>Nature Methods</i> , 5(10):877-879 (October 2008) (“Raj”)
1014	Levesque <i>et al.</i> , “Single chromosome transcriptional profiling reveals chromosome-level regulation of gene expression,” <i>Nature Methods</i> , 10(3):246-248 (March 2013) (“Levesque”)
1015	Levsky <i>et al.</i> , “Single-Cell Gene Expression Profiling,” <i>Science</i> , 297:836-840 (August 2002), with Supplemental Materials (“Levsky 2002”)
1016	Lubeck <i>et al.</i> , “Single cell systems biology by super-resolution imaging and combinatorial labeling,” <i>Nature Methods</i> , 9(7):743-748 (July 2012) (“Lubeck 2012”)

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1017	Jakt <i>et al.</i> , “A continuum of transcriptional identities visualized by combinatorial fluorescent <i>in situ</i> hybridization,” <i>Development</i> , 140(1):216-225 (January 2013) (“Jakt”)
1018	Shaffer <i>et al.</i> , “Turbo FISH: A Method for Rapid Single Molecule RNA FISH,” <i>PLoS ONE</i> , 8(9):e75120 (September 2013) (“Shaffer”)
1019	Lubeck <i>et al.</i> , “Single-cell <i>in situ</i> RNA profiling by sequential hybridization,” <i>Nature Methods</i> , 11(4):360-361 (April 2014) with Supplemental Materials (“Lubeck 2014”)
1020	U.S. Patent No. 10,457,980 to Cai <i>et al.</i> , issued October 29, 2019 (“Cai”)
1021	International Patent Application Publication No. WO 2013/096851 A1 to Levner <i>et al.</i> , published June 27, 2013 (“Levner”)
1022	U.S. Patent Application Publication No. 2010/0291557 A1 to Livak <i>et al.</i> , published November 18, 2010 (“Livak”)
1023	Jensen, E., “Technical Review: <i>In Situ</i> Hybridization,” <i>The Anatomical Record</i> , 297(8):1349-1353 (May 2014) (“Jensen 2014a”)
1024	Silverman <i>et al.</i> , “Oligonucleotide Probes for RNA-Targeted Fluorescence <i>In Situ</i> Hybridization,” <i>Advances in Clinical Chemistry</i> , 43:79-115 (2007) (“Silverman”)
1025	Kwon, S., “Single-molecule fluorescence <i>in situ</i> hybridization: Quantitative imaging of single RNA molecules,” <i>BMB Reports</i> , 46(2):65-72 (February 2013) (“Kwon”)
1026	U.S. Patent No. 9,896,720 to Raj <i>et al.</i> , issued February 20, 2018 (“Raj 720”)
1027	Deng <i>et al.</i> , “Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming,” <i>Nature Biotechnology</i> , 27(4):353-360 (April 2009) with Supplemental Materials (“Deng”)
1028	Leung <i>et al.</i> , “Review of Super-Resolution Fluorescence Microscopy for Biology,” <i>Applied Spectroscopy</i> , 65(9):967-980 (September 2011) (“Leung”)
1029	Henriques <i>et al.</i> , “PALM and STORM: Unlocking Live-Cell Super-Resolution,” <i>Biopolymers</i> , 95(5):322-331 (May 2011) (“Henriques”)
1030	Chi, K., “Super-resolution microscopy: breaking the limits,” <i>Nature Methods</i> , 6(1):15-18 (January 2009) (“Chi”)

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1031	Cai, L., “Turning single cells into microarrays by super-resolution barcoding,” <i>Briefings in Functional Genomics</i> , 12(2):75-80 (March 2013) (“Cai 2013”)
1032	Kamiyama <i>et al.</i> , “Development in the STORM,” <i>Developmental Cell</i> , 23(6):1103-1110 (December 2012) (“Kamiyama”)
1033	Jensen, E., “Technical Review: Types of Imaging – Direct STORM,” <i>The Anatomical Record</i> , 297(12):2227-2231 (December 2014) (“Jensen 2014b”)
1034	U.S. Patent Application Publication No. 2007/0231824 A1 to Chee <i>et al.</i> , published October 4, 2007 (“Chee”)
1035	Shannon, C., “A Mathematical Theory of Communication,” <i>The Bell System Technical Journal</i> , 27(3):379-423 (July 1948) (“Shannon 1948a”)
1036	Shannon, C., “A Mathematical Theory of Communication,” <i>The Bell System Technical Journal</i> , 27(4):623-656 (October 1948) (“Shannon 1948b”)
1037	Hamming, R., <i>Coding and Information Theory</i> , 2 nd Ed., Chapters 1 through 3, Prentice-Hall (1986) (“Hamming”)
1038	Krishnan <i>et al.</i> , “Barcodes for DNA sequencing with guaranteed error correction capability,” <i>Electronics Letters</i> , 47(4):236-237 (March 2011) (“Krishnan”)
1039	Bystrykh, L., “Generalized DNA Barcode Design Based on Hamming Codes,” <i>PLoS ONE</i> , 7(5):e36852 (May 2012) (“Bystrykh”)
1040	U.S. Patent No. 6,654,696 to Davies, issued November 25, 2003 (“Davies”)
1041	U.S. Patent Application Publication No. 2013/0053256 to Hubbell, published February 28, 2013 (“Hubbell”)
1042	Peters <i>et al.</i> , “Accurate whole-genome sequencing and haplotyping from 10 to 20 human cells,” <i>Nature</i> , 487:190-195 (July 2012) (“Peters”)
1043	International Patent Application Publication No. WO 2010/091107 A1 to Kermani <i>et al.</i> , published August 12, 2010
1044	U.S. Patent Application Publication No. 2010/0323348 A1 to Hamady <i>et al.</i> , published December 23, 2010
1045	File History of U.S. Patent No. 10,829,814 (“814 Patent File History”)
1046	Redline Comparison of Fan (EX1005) and Fan-Prov (EX1006)

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1047	Vizgen’s Initial Infringement Claim Chart, <i>10x Genomics, Inc. v. Vizgen, Inc.</i> , Case No. 1:22-cv-00595 (D. Del.), served November 23, 2022 (CONFIDENTIAL – PROTECTIVE ORDER MATERIAL)
1048	Digital CCD Camera C4742-95-12HR Instruction Manual, Version 1.4, Hamamatsu Photonics K.K. (April 2007)
1049	U.S. Patent No. 10,267,808 B2 to Cai, issued April 23, 2019 (“Cai 808”)
1050	International Patent Application No. WO 2014/182528 A2 to Cai <i>et al.</i> , published November 1, 3 2014 (“Cai PCT”)
1051	Revised Scheduling Order, <i>10x Genomics, Inc. v. Vizgen, Inc.</i> , Case No. 1:22-cv-00595 (D. Del.), filed April 11, 2023
1052	Bates <i>et al.</i> , “Stochastic Optical Reconstruction Microscopy (STORM): A Method for Superresolution Fluorescence Imaging,” <i>Cold Spring Harbor Protocols</i> , 498-520 (2013)
1053	U.S. Provisional Application No. 62/142,653 to Zhuang <i>et al.</i> , filed on April 3, 2015 (“653 Application”)
1054	U.S. Provisional Application No. 62/050,636 to Zhuang <i>et al.</i> , filed September 15, 2014 (“636 Application”)
1055	U.S. Provisional Application No. 62/031,062 to Zhuang <i>et al.</i> , filed July 30, 2014 (“062 Application”)
1056	Mujumdar <i>et al.</i> , “Cyanine Dye Labeling Reagents: Sulfoindocyanine Succinimidyl Esters,” <i>Bioconjugate Chemistry</i> , 4(2):105-111 (March 1993) (“Majumdar”)
1057	Arhel <i>et al.</i> , “Quantitative four-dimensional tracking of cytoplasmic and nuclear HIV-1 complexes,” <i>Nature Methods</i> , 3(10):817-824 (October 2006) (“Arhel”)
1058	Schermelleh <i>et al.</i> , “A guide to super-resolution fluorescence microscopy,” <i>The Journal of Cell Biology</i> , 190(2):165-175 (July 2010) (“Schermelleh”)
1059	Vizgen’s Answer, Defenses, and Counterclaims to Plaintiff’s Second Amended Complaint, <i>10x Genomics, Inc. v. Vizgen, Inc.</i> , Case No. 1:22-cv-00595 (D. Del.), filed April 25, 2023
1060	Exhibit 9 to Vizgen’s Answer, Defenses, and Counterclaims to Plaintiff’s Second Amended Complaint, <i>10x Genomics, Inc. v. Vizgen, Inc.</i> , Case No. 1:22-cv-00595 (D. Del.), filed April 25, 2023

I. INTRODUCTION

10x Genomics, Inc. requests review of claims 1-12 of U.S. Patent No. 11,098,303 (EX1001), which is assigned to President and Fellows of Harvard College (“Harvard”) and exclusively licensed to Vizgen, Inc.

The challenged claims of the 303 patent relate to methods for detecting nucleic acids in a sample using a technique known as “in situ hybridization,” which uses fluorescent probes to interrogate the locations of nucleic acid molecules of interest in the sample.

At its core, the 303 patent relates to the application of a mathematical concept—known as *error correction*—to an assay for *sequential* in situ hybridization. *Sequential* in situ hybridization is a well-known method of encoding biological analytes using a *sequence* of fluorescent colors. The large number of possible fluorescent signal combinations—referred to as “codewords” in the 303 patent—allows a large number of analytes to be encoded and detected simultaneously. In this fashion, a thousand known analytes can be encoded by a “codebook” of a thousand such combinations that are known in advance of the assay. These predetermined combinations in the codebook corresponding to known genes are referred to as “valid codewords” in the 303 patent. During the assay, a sequence of signals is detected at each location corresponding to an analyte, and

that analyte's identity can be ascertained by matching the detected sequence with the valid codewords in the codebook.

The 303 patent claims methods of “applying an error detection or correction system” if the observed codeword does not match a valid codeword in the codebook, providing examples of well-known error detection and correction techniques, such as Hamming and Golay codes. EX1001, 11:12-16.

However, the 303 patent's claimed methods were already disclosed in a prior art patent, Fan. Fan describes using sequential in situ hybridization to generate a color combination to identify a nucleic acid target of interest, and also applies the error-detection and correction technique of Gunderson, which Fan incorporates by reference. As shown herein, Fan anticipates claims 1-12 and/or renders them obvious.

The 303 patent's claimed methods were also disclosed in Curry, a patent by Agilent Technologies. EX1009. Curry taught sequential in situ hybridization using a sequence of signals for each target, and applied art-recognized error-detection and error correction techniques to identify the molecules. As shown herein, claims 1-12 would have been obvious over the combination of Fan and Curry.

For these reasons, claims 1-12 of the 303 patent should be canceled.

II. GROUNDS FOR STANDING (37 C.F.R. § 42.104(A))

10x Genomics, Inc. certifies that the 303 patent; EX1001) is IPR-eligible and 10x is not barred or estopped from requesting IPR of any 303 patent claim.

III. PRECISE RELIEF REQUESTED (37 C.F.R. §42.22(A))

10x petitions for IPR, requesting cancelation of claims 1-12 of the 303 patent assigned to President and Fellows of Harvard College. The Petition is supported by the declaration of Paul Spellman, Ph.D. (EX1002), Professor of Medicine and Human Genetics. EX1002, ¶¶11-22.

IV. IDENTIFICATION OF THE CHALLENGE (37 C.F.R § 42.104(B))

10x requests IPR based on the grounds below.

Ground	35 U.S.C. Section (AIA)	Claims	References
1	§ 102	1-12	Fan
2	§ 103	1, 3, and 6-12	Fan and Gunderson
3	§ 103	2, 4, and 5	Fan, Gunderson, and Larsson
4	§ 103	1-12	Fan and Curry

The asserted references are prior art for at least the following reasons. U.S. Patent No. 10,829,814 (“Fan”; EX1005) names different inventors from the 303 patent and was filed as U.S. Patent Application No. 14/742,027 on June 17, 2015, which claims priority to U.S. Provisional Application No. 62/014,538 (“Fan-

Provisional”; EX1006) filed on June 19, 2014—which is before the 303 patent’s July 30, 2014 claimed priority date. Thus, Fan is prior art under AIA 35 U.S.C. § 102(a)(2).

Gunderson *et al.*, *Genome Research* 14:870-877 (2004) (“Gunderson”; EX1007), is a printed publication that published in April 2004, which is before the 303 patent’s claimed July 30, 2014 priority date. Thus, Gunderson is prior art under AIA 35 § 102(a)(1).

Larsson *et al.*, *Nat. Methods* 7(5):395-397 (2010) (“Larsson”; EX1008), is a printed publication that published in May 2010, which is before the 303 patent’s claimed July 30, 2014 priority date. Thus, Larsson is prior art under 35 U.S.C. § 102(a)(1).

U.S. Patent No. 8,765,642 (“Curry”; EX1009) names different inventors from the 303 patent, was filed as U.S. Patent Application No. 13/448,985 on April 17, 2012, and issued on July 1, 2014—both of which are before July 30, 2014. Thus, Curry is prior art under AIA §§ 102(a)(1) and 102(a)(2).

All four Grounds are presented under Vizgen’s and Harvard’s (collectively, “Co-Plaintiffs”) interpretation of the challenged claims in district court.

V. THE 303 PATENT

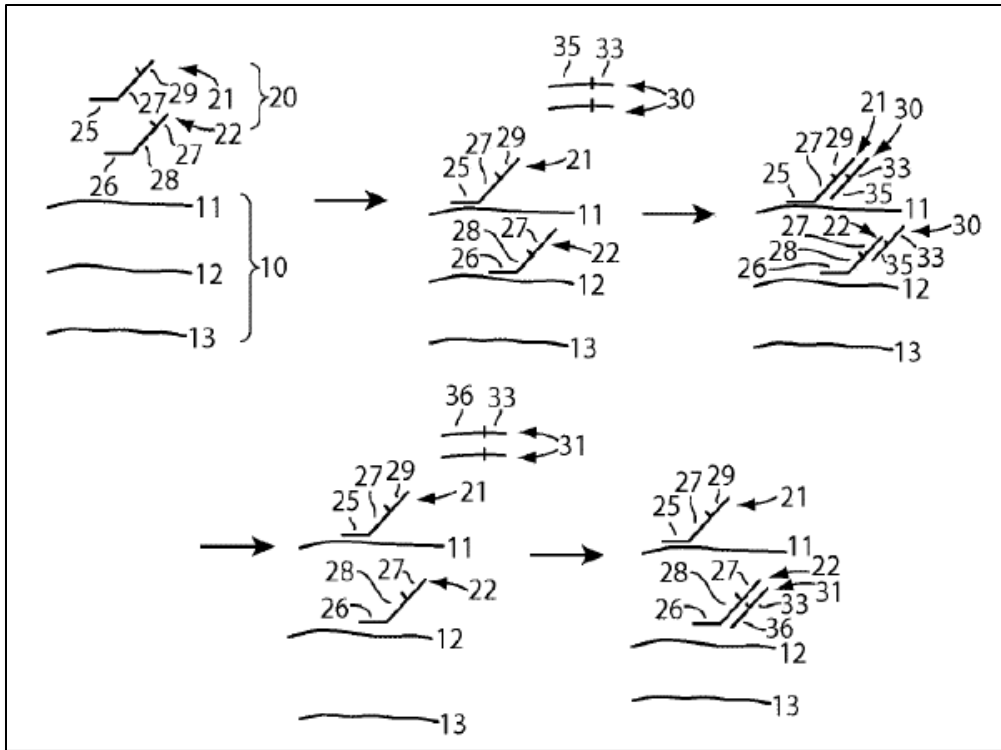
The 303 patent (EX1001) issued on August 24, 2021, from U.S. Patent Application No. 15/326,683, which is a national stage entry of PCT Application

No. PCT/US2015/042556, filed on July 29, 2015, and claims priority to U.S.

Provisional Application Nos. 62/031,062 filed on July 30, 2014; 62/050,636, filed on September 15, 2014; and No. 62/142,653, filed on April 3, 2015.

The patent's method purports to involve modifications to single-molecule fluorescence in situ hybridization (smFISH) techniques to locate nucleic acids within a biological sample. EX1001, Abstract, 1:28-49; EX1002, ¶¶39-55. The patent's method uses multiple primary and secondary probes, with fluorescent labels ("readouts") on the secondary probes, to generate binary "codewords," compare them to codewords in a codebook, and then use well-known algorithms such as Hamming codes to detect and/or correct errors when there is no codeword match. EX1001, 8:58-10:11; EX1002, ¶¶39-55.

The 303 patent methods begin with adding primary probes ("encoding nucleic acid probes") to the biological sample, where they bind a target nucleic acid in the sample. *Id.* This is shown in, *e.g.*, Figure 3A, which depicts primary "encoding nucleic probes" 21 and 22 binding to their respective target nucleic acids 11 and 12 (top row, left and middle):

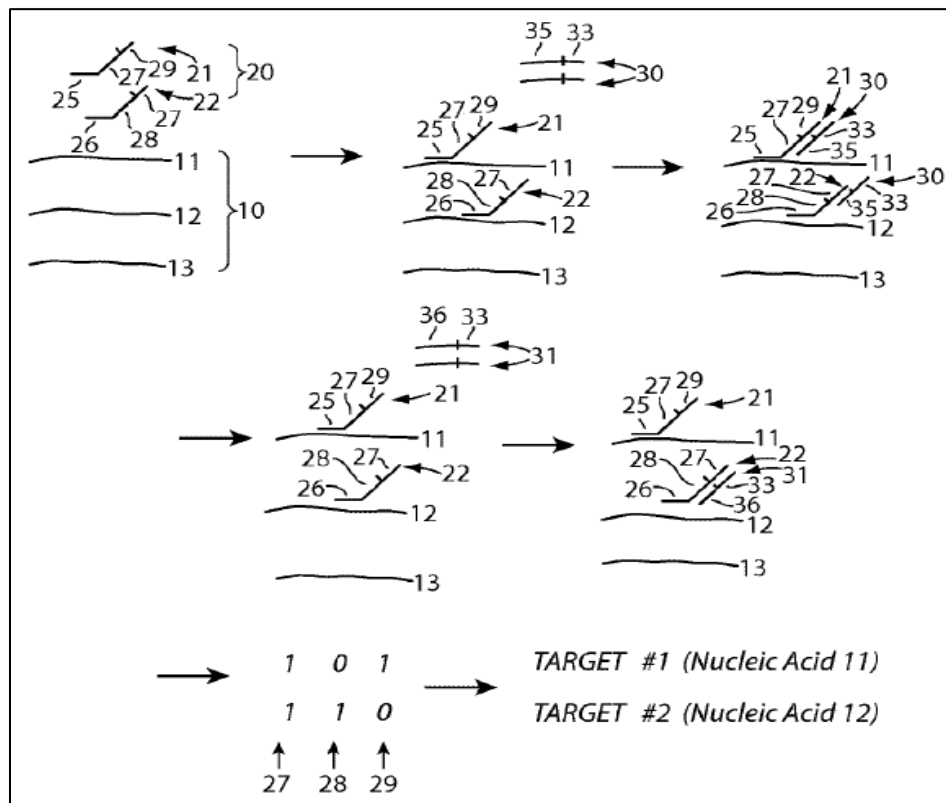


EX1001, FIG. 3A, 8:58-9:25; EX1002, ¶¶40-44.

Next, different sets of secondary probes (called “readout” probes) are added to the sample in sequential fashion, where the secondary probes bind to one or more primary probes. *Id.* In the figure above, the initial set of secondary probes is labeled as 30, and each secondary probe 30 binds to primary probes 27 and 29. *Id.* (top row, middle and right). The secondary probes contain “signaling entities” (e.g., a fluorescent tag) that are used to visualize their location in the sample. EX1001, 9:39-65; EX1002, ¶¶45-46. Visualization can be performed using fluorescence microscopy. *Id.*

After the initial set of secondary probes is detected, they are removed and the process repeated with a different set of secondary probes (depicted as

secondary probes 31 in the figure above), which also bind to one or more primary probes. *Id.* The process is repeated multiple times, which generates a “pattern of binding or hybridization” that is “converted into a ‘codeword.’” EX1001, 10:41-58; EX1002, ¶¶46-51. In Figure 3A, the codewords are 101 and 110 for primary probes 21 and 22, respectively, where “1” represents binding and “0” represents no binding:



EX1001, 10:41-58, FIG. 3A; EX1002, ¶¶46-51.

The 303 patent methods then compare the sample’s binary codeword with codewords in a “codebook” to identify the target. The patent’s codebook is constructed using well-known error detection and correction schemes such as

Hamming codes to detect and correct potential errors. EX1001, 11:8-28; EX1002, ¶¶52-55.

During prosecution, the examiner issued multiple office actions repeatedly rejecting the claims for being indefinite, lacking enablement, and claiming non-statutory subject matter. EX1004, 1462-1469, 1925-1936, 2194-2205. No prior art rejections were raised before the Office allowed the claims. *Id.*, 2382-2394.

After issuance, a Certificate of Correction modified the language in claim 1(a)(ii) to recite, “encoding nucleic acid probes located in its different locations.” *Id.*, 2642.

VI. STATE OF THE ART PRE-DATING THE 303 PATENT

As shown below and explained by Dr. Spellman, the prior art taught methods of detecting and imaging nucleic acids using workflows comprising two basic components: an *assay component*¹ comprising sequential rounds of in situ hybridization and imaging; and an *analysis component* comprising binary coding and error-correction systems. EX1002, ¶¶65-82. The 303 patent claims merely combine these well-established prior art methods.

¹ Emphasis is added throughout, except where otherwise indicated.

A. The art taught sequential in situ hybridization

Fan taught in April 2014 methods useful for “multiplex generation of sequence information from an individual cell.” EX1005, 1:60-62. In Fan’s methods, nucleic acids of interest are first amplified within the cell (in situ) to produce nucleic acid targets (e.g., cDNA colonies). *Id.*, 1:63-2:1, 9:1-22, FIG. 1; EX1002, ¶¶61, 66-71.

In Fan, nucleic acid targets are probed with primary nucleic acid probes that hybridize (bind) to the target, followed by fluorescently labeled secondary probes that hybridize to the primary probes. EX1005, 5:17-20, 9:1-22, 8:30-67, FIG. 1; EX1002, ¶¶66-71. This approach was routine in the art. EX1015, 836-837, FIGs. 1A-1B; EX1025, 66; EX1013, Abstract; EX1026, 3:37-42; EX1020, 4:35-48; EX1009, 6:17-7:13; EX1019, FIG. 1.

Removing or inactivating the signal in between hybridization rounds was also routine, including methods such as “washing, heating, photo-bleaching, displacement..., cleavage, enzymatic digestion, quenching, chemical degradation, bleaching, oxidation, or any combinations thereof.” EX1021, ¶[0016]; EX1002, ¶71; EX1020, FIG. 2a; EX1005, 2:56-58, FIG. 1; EX1007, 875.

B. The art taught routine detection and imaging methods

Fan taught that its sequential hybridization methods produced a series of fluorescent signal patterns that could be detected using routine and commercially

available systems in the art. EX1005, 11:11-15; EX1002, ¶¶72-73. Fan incorporates by reference Larsson et al., which provides an example of using “epifluorescence microscopy.” EX1008, Title, 0005, FIG. 1a; EX1002, ¶¶63, 72-73. POSAs knew that imaging technologies such as Larsson’s could provide the desired resolution to identify nucleic acids in cells, as well as other routine techniques. *Id.*; EX1029, 323; EX1028, 968; EX1030; EX1032, 1103; EX1020, 21:16-45; EX1005, 11:11-19; EX1008, 0004; EX1033, 2227; EX1002, ¶¶72-73.

C. The art taught error-checking and correcting systems

The analytical aspect of binary codes and error-checking in sequential hybridization methods was well established before July 30, 2014. EX1002, ¶¶74-82. In Fan’s method, the fluorescent signals generated during sequential hybridization produced a decodable “code” to identify the different nucleic acid targets. EX1005, 8:30-67, 8:53-55, 14:28-47; EX1002, ¶¶74-82. Fan incorporates by reference Gunderson et al., which taught sequential hybridization methods for decoding and error-checking, using codewords of multiple binary values 1 and 0 for a given detected fluorescent signal. EX1005, 3:36-40, 8:53-59, 14:31-34; EX1007, 871-872; EX1002, ¶¶62, 74-79. Gunderson taught using sequential hybridization stages to generate a spectral pattern, or code, of fluorescent signals. EX1007, 871-872, FIG. 2; EX1002, ¶¶74-79. Gunderson taught a value of “1” when a fluorescent signal is detected, and a value of “0” when that same

fluorescent signal is not detected in another stage. EX1007, 871-872, FIG. 2; EX1002, ¶¶74-79.

Gunderson also taught methods for error-checking, based “on algorithms from digital information theory.” EX1007, 871; EX1035-1037. Gunderson’s scheme used “valid codes” (e.g., a codebook) to cross-reference the codewords generated from sequential hybridization and confirm the sequence was valid (a match to the codebook) or an error (no match). EX1007, 871-872, FIGs. 2-3, Table 1; EX1002, ¶¶74-79.

Similar to Gunderson, Curry taught a method of sequential hybridization in which “the identities of the targets in the sample can be determined” using a “lookup table,” which contains a binary code of “1” for signal detected and “0” for no signal detected. EX1009, 11:46-60, 6:17-7:23; EX1002, ¶¶64, 80-82.

VII. PERSON OF ORDINARY SKILL IN THE ART

A POSA is a hypothetical person who is presumed to be aware of all pertinent art, equipped with conventional wisdom and ordinary creativity. Here, a POSA would have possessed a strong understanding in the use and development of genetics and genomics approaches for detecting or monitoring human diseases, such as detection of a target analyte (*e.g.*, mRNA or DNA) in an individual cell of a biological sample. EX1002, ¶24. A POSA would typically have a Ph.D. in a

relevant field (*e.g.*, molecular biology, genetics, and bioinformatics) with two years of experience. *Id.*

VIII. CLAIM CONSTRUCTION

Solely for the purposes of this IPR, 10x applies Co-Plaintiffs’ interpretations of the 303 patent claims from the concurrent litigation. *See* EX1047; EX1059; EX1060. 10x describes in detail Co-Plaintiffs’ interpretation of the claims where relevant in the Grounds below.

Co-Plaintiffs’ statements before a Federal court in which it “took a position on the scope” of its patent claims are properly cited here under 35 U.S.C. §§ 301(a)(2) and 301(d) and appropriate for consideration by the Board. *10x Genomics, Inc. v. Bio-Rad Lab’s, Inc.*, IPR2020-00086, Paper 8, at 9-17 (P.T.A.B. Apr. 27, 2020) (adopting claim constructions that the Board deemed “consistent with Patent Owner’s infringement contentions.”).

IX. GROUND 1: FAN ANTICIPATES CLAIMS 1-12

As shown below, under Co-Plaintiffs’ interpretation, Fan anticipates claims 1-12. EX1002, ¶¶84-210.

A. Fan is prior art to the 303 patent

Fan was filed as Appl. No. 14/742,027 (“the 027 application”) on June 17, 2015. EX1045, 1-48; EX1005, face. The 027 application claims priority under 35 U.S.C. § 119 to U.S. Patent Application No. 62/014,538 (“Fan-Provisional”), filed

on June 19, 2014. EX1045, 53; EX1006, 1-47; EX1005, face. Fan is prior art to the 303 patent because Fan meets the ministerial requirements of § 119 and thus is entitled to claim a right of priority to the June 19, 2014, filing date of Fan-Provisional. 35 U.S.C. § 119. The 027 application properly claims benefit to Fan-Provisional because the 027 application was filed co-pending with Fan-Provisional, and the 027 application shares common inventors (Fan and Zhang) with Fan-Provisional. EX1045, 53; EX1006, 48; 35 U.S.C. § 119.

Additionally, the specifications of Fan and Fan-Provisional are substantially the same. *Compare* EX1045, 1-31, *with* EX1006, 4-33; EX1005; EX1002, ¶¶84-88; EX1046. As Dr. Spellman demonstrates, all of the Petition’s relied-upon disclosures in Fan are carried forward from Fan-Provisional. EX1002, ¶¶84-88, Appx. B. “[U]nder the AIA, a reference patent document need only meet the ‘ministerial requirements’ of § 119, and the provisional or other earlier application(s) to which the reference patent document claims a right of priority must ‘describe[] the subject matter’ relied upon in the reference patent document as prior art.” *Apple Inc. v. Telefonaktiebolaget LM Ericsson*, IPR2022-00341, Paper 10, at 19 (P.T.A.B. Sept. 14, 2022) (citing 35 U.S.C. § 102(d)(2)); *see also id.*, 15. Fan is therefore prior art under §§ 102(a)(2) and 102(d).

Moreover, at least one of Fan’s issued claims has § 112 written description support in Fan-Provisional. EX1002, ¶¶84-88, Appx. C; *Dynamic Drinkware, LLC*

v. National Graphics, Inc., 800 F.3d 1375, 1378 (Fed. Cir. 2015). As Dr. Spellman explains and demonstrates in Appendix C, all the elements of Fan’s claim 1 are described in Fan-Provisional. *Id.* The Petition Grounds provide parallel citations to Fan (EX1005) and Fan-Provisional (EX1006) throughout.

B. Claim 1

1. Preamble: “A method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization, comprising...”

Fan teaches a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization, as claimed. EX1002, ¶¶90-95. Specifically, Fan discloses that its “methods allow for multiplex detection of a plurality of nucleotide species of interest,” including “*simultaneous* detection of 2 ... or more than 10,000 unique nucleic acid molecules.” EX1005, 9:49-59; EX1006, 14:15-22. In particular, Fan teaches a method for “obtaining sequence information regarding a plurality of nucleic acid molecules from an individual cell” (*i.e.*, detecting a plurality of different nucleic acid targets within a sample, as claimed). EX1005, 2:21-36; EX1006, 2:20-30; EX1002, ¶¶90-95.

Fan also discloses that its method utilizes *in situ* hybridization as claimed because Fan’s method comprises “amplifying a plurality of nucleic acid molecules *in situ* within a cell” and “annealing *in situ* a barcode probe” to the amplified nucleic acid molecules. EX1005, 2:21-36; *see also id.*, 1:63-2:1, 4:9-19, 9:1-22,

14:3-48, FIG. 1, Claim 1; EX1006, 2:20-30; *see also id.*, 1:30-2:6, 5:17-25, 13:12-27, 21:15-22:16, FIG. 1, Claims 1-2; EX1002, ¶¶90-95. Fan further explains that in its method, “cDNA is generated *in situ*,” amplified, and the “amplified nucleic acids can then be *hybridized* with one or more unique *padlock probes* with unique molecular barcodes.” EX1005, 9:4-11, FIG. 1, 14:34-36 (“eight rounds of *hybridization* decoding are performed”); EX1006, 13:17-18, FIG. 1, 22:8; EX1002, ¶¶90-95. Thus, Fan discloses claim 1’s preamble.

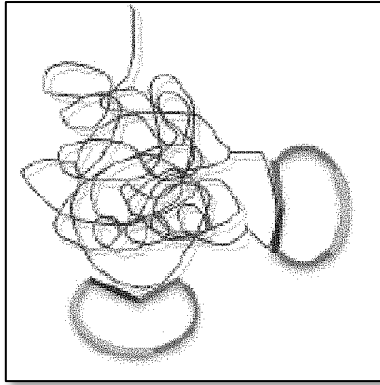
2. “(a) producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes”

Fan discloses Step 1(a) because Fan’s method entails exposing a sample to a plurality of “barcode probes,” which hybridize to a plurality of different nucleic acid targets within a cell, producing a plurality of hybridized complexes within a sample as claimed. EX1005, 2:21-36, 1:63-2:1, 4:9-19, 9:1-22; EX1006, 2:20-30, 1:30-2:6, 5:17-25, 13:12-26, EX002, ¶¶96-102. Specifically, Fan’s method amplifies a “*plurality* of nucleic acid molecules *in situ* within a cell” (*i.e.*, a sample containing a plurality of different nucleic acid targets, as claimed), and then “anneal[s] *in situ* a barcode probe ... to the amplified nucleic acid molecule” (*i.e.*, produces a plurality of first *hybridized complexes* within a sample). EX1005, 2:21-36; *see also id.*, 1:63-2:1, 4:9-19, 9:1-22, 14:3-48, FIG. 1, Claim 1; EX1006, 2:20-

30; *see also id.*, 1:30-2:6, 5:17-25, 13:12-26, 21:17-22:16, FIG. 1, Claims 1-2; EX1002, ¶¶96-102.

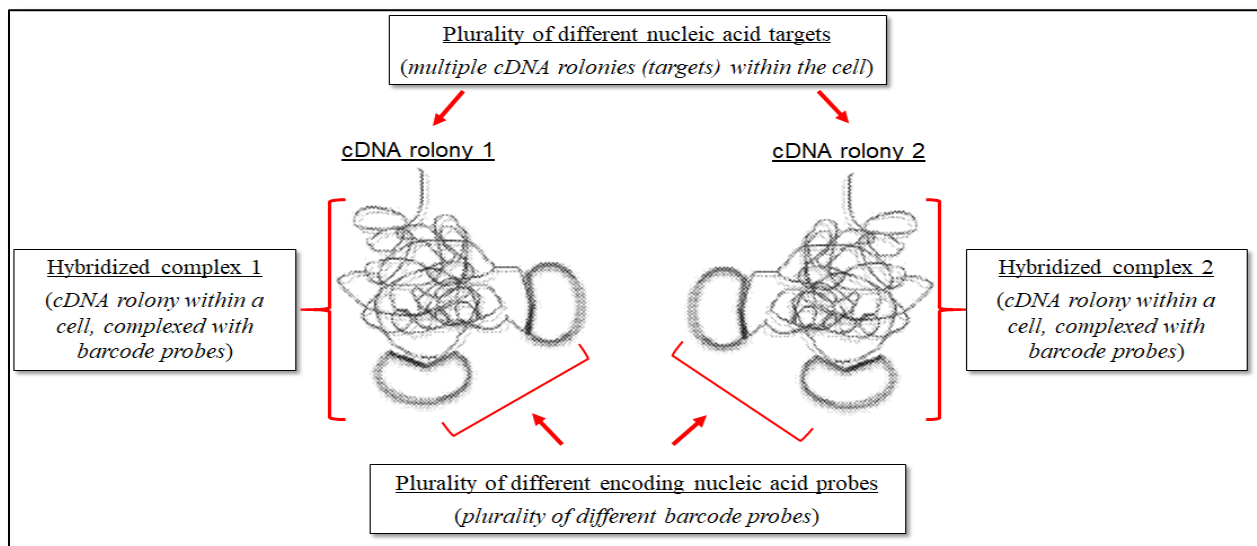
When describing Figure 1, Fan discloses amplifying the nucleic acids (cDNAs generated from mRNAs) to produce cDNA colonies, and then “hybridiz[ing]” the amplified DNA “with one or more unique padlock probes with unique molecular barcodes [*i.e.*, barcode probes].” EX1005, 9:8-11, 7:36-37 (“Barcode probes ... can include padlock probes.”); EX1006, 13:17-18, 11:2. Fan also discloses that its method tags a “subset of genes of interest” with “gene specific barcodes using a library of padlock probes.” EX1005, 14:15-18; EX1006, 21:23-25; EX1002, ¶¶96-102. In other words, Fan’s method generates a plurality of nucleic acid targets (*e.g.*, cDNA colonies) within a cell, which are probed with a plurality of different barcode probes to produce a plurality of first hybridized complexes. *Id.*

Fan’s Figure 1 illustrates one such target nucleic acid-barcode probe complex (*i.e.*, first hybridized complex) within a sample produced by exposing the sample to barcode probes (*i.e.*, encoding nucleic acid probes):



EX1005, FIG. 1.

Although Fan's Figure 1 depicts a single cDNA rolon complexed with a barcode probes, Fan's method produces a plurality of such complexes. EX1002, ¶¶96-102. Dr. Spellman's Figure A below illustrates two such hybridized complexes in a sample:



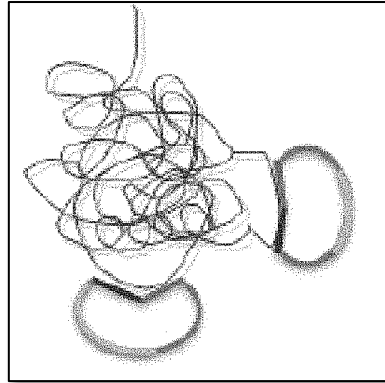
EX1002, ¶100, FIG. A.

The 303 patent admits that "nucleic acids to be determined may be, for example, DNA, RNA, or *other nucleic acids that are present within a cell (or*

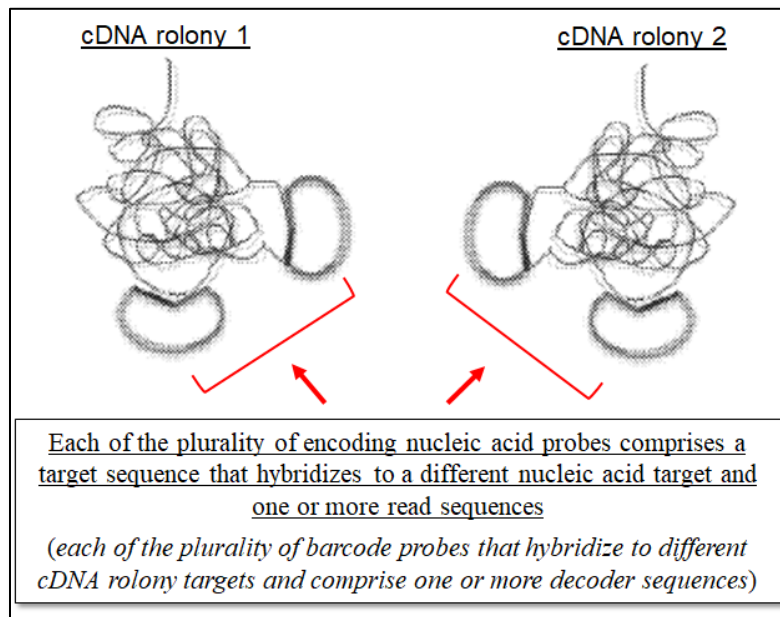
other sample).” EX1001, 13:19-21. Fan’s cDNA colonies are “nucleic acids ... present within a cell (or other sample)” and thus are target nucleic acids as described in the 303 patent. *Id.*; EX1002, ¶¶101-102.

“wherein each of the plurality of encoding nucleic acid probes comprises (i) a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid target of the plurality of different nucleic acid targets and (ii) one or more read sequences”

Fan’s barcode probes meet this limitation. EX1002, ¶¶103-108. Fan’s “barcode probe” is “a nucleic acid molecule which can hybridize specifically to a nucleic acid [target] of interest.” EX1005, 5:17-20, 3:32-35; EX1006, 7:13-15, 4:16-18; EX1002, ¶¶103-108. Fan’s method of Example 1 and Figure 1 uses padlock probes as barcode probes to tag the target nucleic acids (cDNA colonies) in a “gene specific manner.” EX1005, 3:33-36; EX1006, 4:16-19; EX1002, ¶¶103-108. Fan states that “each [padlock] probe has two gene-specific ... capturing arms” and it “specifically hybridizes to a sequence of interest.” EX1005, 3:40-42, 9:11-12; EX1006, 4:22-23, 13:18-19; EX1002, ¶¶103-108. Thus, Fan’s barcode probes comprise a target sequence that specifically hybridizes to a different nucleic acid target, as claimed. *Id.* This is shown below in Fan’s Figure 1 and Dr. Spellman’s Figure B:



EX1005, FIG. 1.



EX1002, ¶105, FIG. B.

Fan's barcode probes also comprise one or more read sequences, as claimed. According to the 303 patent, a "read sequence" is a sequence on its primary probes (encoding nucleic acid probes) that "bind[s] to or hybridize[s] with" a "recognition sequence" present in the secondary probe (readout probes). EX1001, 18:12-14; EX1002, ¶103-108. Thus, a read sequence is a binding site on a primary probe for

a secondary probe. *Id.*

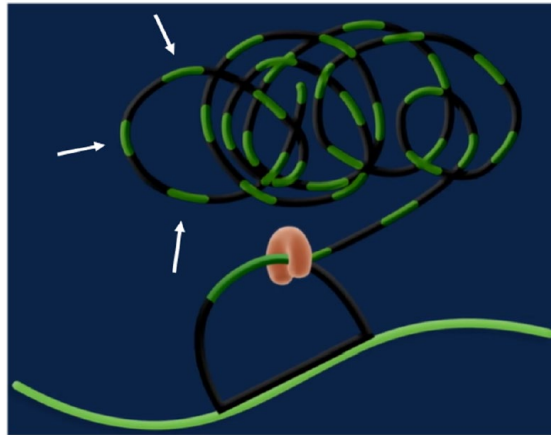
Fan's barcode probes comprise "at least a first decoder sequence and a second decoder sequence." EX1005, 2:21-36; *see also id.*, 1:63-2:1, 4:9-19, 9:1-22, 14:3-48, FIG. 1, Claim 1; EX1006, 2:20-30; *see also id.*, 1:30-2:6, 5:17-25, 13:12-26, 21:17-22:16, FIG. 1, Claims 1-2. Fan's "decoder sequences" are "a plurality of unique sequences [] designed to each specifically hybridize to a labeled decoder probe," (*i.e.*, Fan's secondary probe). EX1005, 8:49-55; *see also id.*, 5:26-29, 8:30-67, FIG. 1; EX1006, 12:28-13:3; *see also id.*, 7:19-21, 12:16-13:11, FIG. 1; EX1002, ¶103-108. In other words, Fan's barcode probes comprise at least a first and a second binding site (decoder sequences) on its primary probes (barcode probes) for its secondary probes (decoder probes). *Id.*

Accordingly, Fan's method uses barcode probes (*i.e.*, encoding nucleic acid probes) comprising (i) a target sequence that specifically hybridizes to a different nucleic acid target and (ii) one or more decoder sequences (*i.e.*, read sequences), as claimed. EX1002, *Id.*

"such that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes of the different encoding nucleic acid probes located in its different locations"

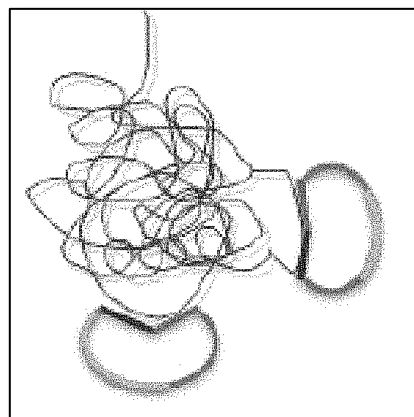
Fan's method discloses this limitation under Co-Plaintiffs' interpretation in the concurrent district court litigation. As evident from the figure below, the Co-Plaintiffs interpret this limitation as broad enough to encompass a method in which

each of the plurality of first hybridized complexes are created by hybridizing multiple copies of the same encoding nucleic acid probe (*i.e.*, barcode probe) at different locations of an identical nucleic acid target:

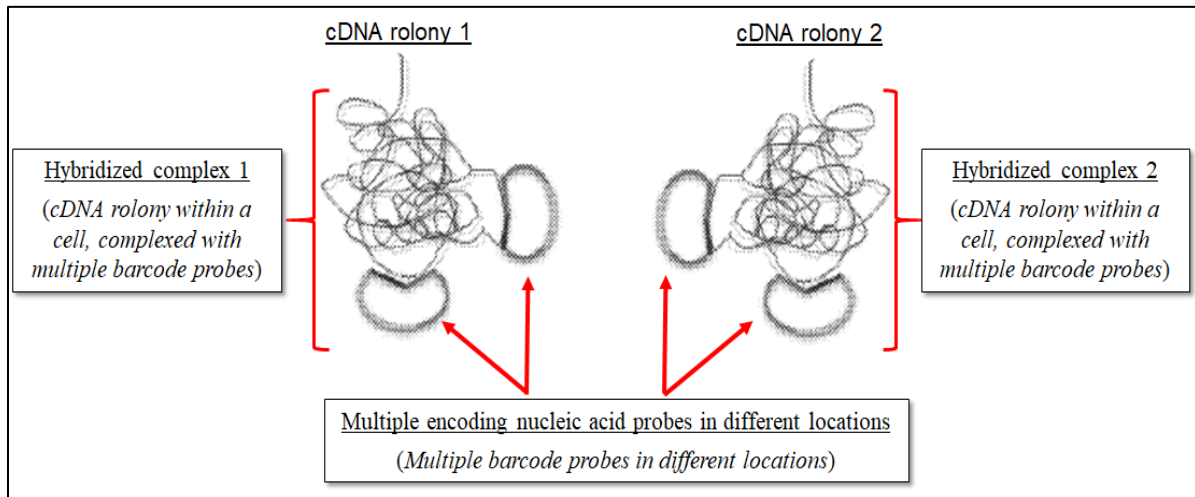


EX1047, 0044; EX1002, ¶¶109-112.

Fan's method discloses hybridizing multiple copies of the same barcode probe (encoding nucleic acid probes) specifically to multiple locations of a nucleic acid target (*e.g.*, cDNA colony). EX1005, 2:21-36, 9:1-22, FIG. 1, 14:1-48; EX1006, 2:20-30, 13:12-26, FIG. 1, 21:17-22:16; EX1002, ¶¶109-112. This is shown below in Fan's Figure 1B and Dr. Spellman's Figure C:



EX1005, FIG. 1B.



EX1002, ¶110, FIG. C.

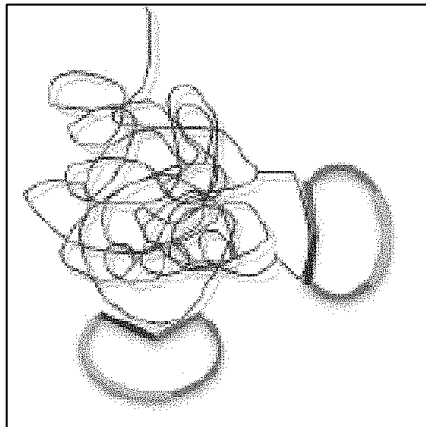
Fan also discloses that the multiple barcode probes (encoding nucleic acid probes) hybridized to multiple locations of a nucleic acid target can be *different barcode probes*. EX1005, 9:8-11 (“The amplified nucleic acids can then be hybridized with *one or more unique padlock probes* with unique molecular barcodes.”); EX1006, 13:17-18; EX1002, ¶¶109-112.

Accordingly, Fan’s method discloses the “such that” clause under Co-Plaintiffs’ interpretation. EX1002, ¶¶109-112. Thus, Fan teaches claim 1(a).

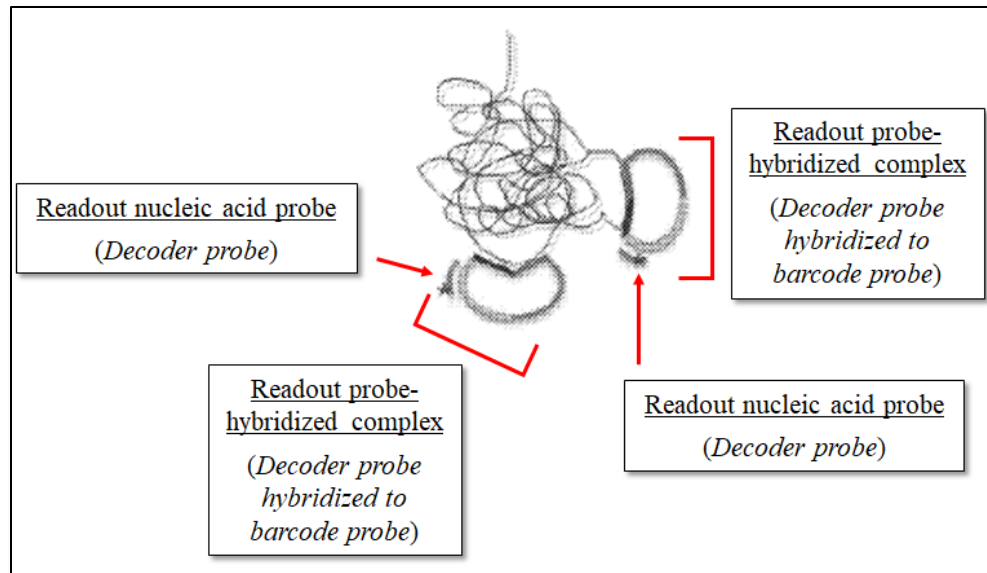
3. “(b) producing a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe”

Fan discloses Step 1(b). EX1005, 9:1-22, FIG. 1, 14:1-48; EX1006, 13:12-26, FIG. 1, 21:17-22:16; EX1002, ¶¶113-117. According to the 303 patent, a

“readout probe” is synonymous with a “secondary probe.” EX1001, 2:28-29 (“secondary nucleic acid probes (*also called readout probes*)”), 8:3 (“secondary probes (*also called readout probes*)”), 35:58-60 (similar), 45:7-8 (similar); EX1002, ¶¶113-117. Fan discloses exposing its barcode probes to secondary probes called “decoder probes.” After hybridizing the barcode probes to the target nucleic acids, Fan teaches hybridizing a “first labeled decoder probe to the first decoder sequence” of the barcode probes. EX1005, 2:21-36; *see also id.*, 8:49-52 (“decoder sequence ... specifically hybridize[s] to a labeled decoder probe”), Claim 1; EX1006, 2:20-30; *see also id.*, 12:28-13:1; Claims 1-2; EX1002, ¶¶113-117. Fan’s hybridized barcode probe/decoder probe complexes are shown below in Fan’s Figure 1 and Dr. Spellman’s Figure D:



EX1005, FIG. 1B.



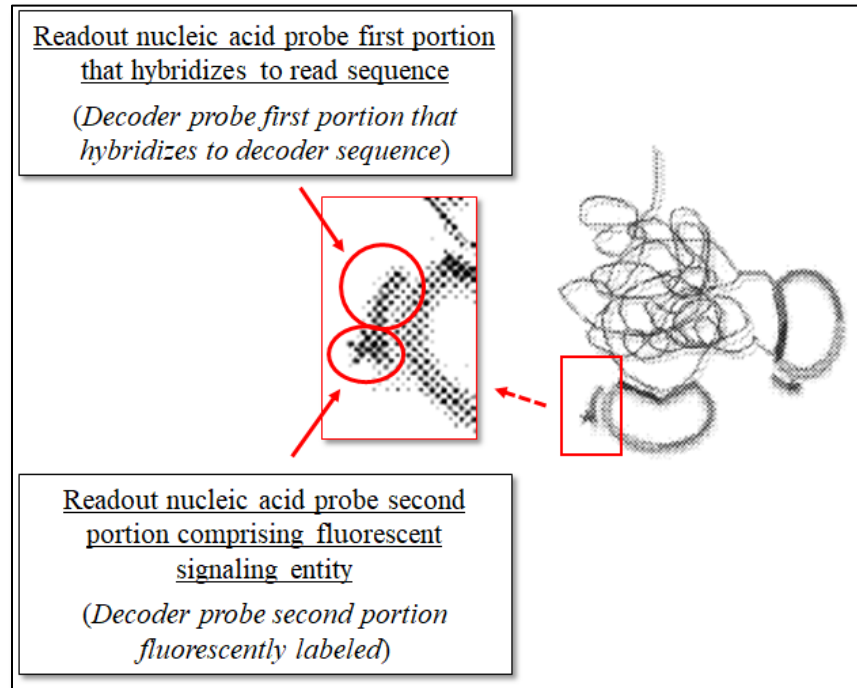
EX1002, ¶115, FIG. D.

Fan's exemplary decoding method used "eight rounds of sequential hybridization with *fluorescently labeled oligos*." EX1005, 9:1-22, FIG. 1, 14:3-48, Claim 1; EX1006, 13:12-26, FIG. 1, 21:17-22:16, Claims 1-2; EX1002, ¶¶116-117. Fan's "fluorescently labeled oligos" (decoder probes) are secondary probes—*i.e.*, readout probes. *Id.*

"wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal"

Fan's decoder probes meet this limitation because they comprise a first portion that specifically hybridizes to the "decoder sequence" in the barcode probes. EX1005, 2:21-36; *see also id.*, 8:49-55 ("decoder sequence ... specifically

hybridize[s] to a labeled decoder probe”); EX1006, 2:20-30; *see also id.*, 12:28-13:1; EX1002, ¶¶118-122. Fan explains that, “[d]uring decoding, labeled decoder probe oligonucleotides are sequentially hybridized to the decoder sequences” in the barcode probes. *Id.* Thus, Fan’s decoder probes act as secondary probes and bind the barcode probes, which are bound to the target nucleic acid. *Id.* Fan’s decoder probes also comprise a second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal because Fan’s “*labeled* decoder probes” are *fluorescently* labeled. EX1005, 9:1-22, FIG. 1, 14:3-48, Claim 1; EX1006, 13:12-26, FIG. 1, 21:17-22:16, Claims 1-2; EX1002, ¶¶118-122. This is shown below in Dr. Spellman’s Figure E:



EX1005, FIG. 1; EX1002, ¶120, FIG. E.

Accordingly, Fan discloses claim 1(b).

4. “(c) detecting a fluorescent signal from each of the plurality of readout probe-hybridized complexes”

Fan discloses Step 1(c) because Fan’s method detects fluorescently labeled decoder probes that are hybridized to the barcode probes. EX1002, ¶¶123-125. Specifically, Fan’s method comprises “*detecting* a plurality of *signals* indicative of the hybridizing” of the decoder probes to the barcode probes. EX1005, 2:21-36; EX1006, 2:20-30; EX1002, ¶¶123-125. Fan shows in Figure 1 that “decoding takes place ... by eight rounds of sequential hybridization with *fluorescently labeled oligos* and imaging of *fluorescent* images.” EX1005, 9:1-22, FIG. 1; *see also id.*, 9:23-43, 11:11-15, 14:3-48, Claim 1; EX1006, 13:12-26, FIG. 1; *see also id.*, 13:27-14:10, 16:29-17:2, 21:17-22:16, Claims 1-2; EX1002, ¶¶123-125. Thus, Fan discloses claim 1(c).

5. “(d) removing the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes”

Fan discloses Step 1(d) under the Co-Plaintiffs’ interpretation. The Co-Plaintiffs interpret this limitation as broad enough to encompass a method in which the fluorescently labeled probe is removed. EX1047, 0072-0074 (“the sample then undergoes successive rounds of fluorescent *probe* hybridization, imaging, and *removal*”), (“sample underwent 15 rounds of fluorescent probe hybridization,

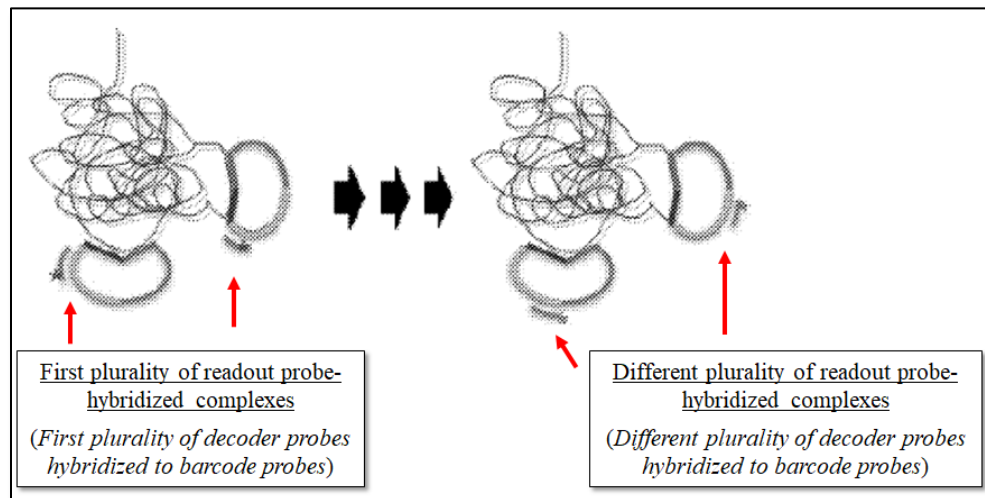
imaging, and *probe removal*"); EX1002, ¶¶126-132. Fan's methods apply "decoding strategies that entail *sequential hybridizations of labeled decoder probes* to decoder sequences found in immobilized nucleic acids within an individual cell." EX1005, 8:34-39, 8:53-59; EX1006, 12:18-21, 13:1-5; EX1002, ¶¶126-132. Fan teaches that "the first labeled decoder probe is *removed* prior to hybridizing the second labeled decoder probe." EX1005, 2:56-58; *see also id.*, 9:25-27 (noting that Fan's "decoding process ... involves hybridization and washing of oligo pools at room temperature"); Claim 1 ("de-hybridizing and removing the first fluorescently labeled decoder probe" before hybridizing a second fluorescently labeled decoder probe); EX1006, 3:14-15; *see also id.*, 13:28-30, Claims 1-3; EX1002, ¶¶126-132.

Accordingly, Fan's methods comprise removing the fluorescent signaling entity from the hybridized complexes by removing the fluorescently labeled decoder probes. EX1002, ¶¶126-132. The 303 patent confirms that "removal of the signaling entity" includes removing it "*from the sample*, or from the nucleic acid probe, *etc.*" and includes using "*washing*," just like Fan. EX1001, 23:57-24:3; EX1002, ¶¶126-132. The patent further admits that "the secondary nucleic acid probes are inactivated *or removed*." EX1001, 8:49-51, 9:43-51, FIG. 3A; EX1002, ¶¶126-132. Thus, Fan discloses claim 1(d) under Co-Plaintiff's interpretation.

6. “(e) after step (d), producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe,

wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal”

Fan discloses Step 1(e). EX1002, ¶¶133-137. After removing the *first* set of decoder probes (*i.e.*, readout probes), Fan teaches “hybridizing a *second* labeled decoder probe to the *second* decoder sequence.” EX1005, 2:21-36, 9:1-22, 14:3-48, FIG. 1, Claim 1; EX1006, 2:20-30, 13:12-26, 21:17-22:16, FIG. 1, Claims 1-2; EX1002, ¶¶133-137. Thus, Fan’s method uses decoder probes in the second hybridization different from the decoder probes in the first hybridization. EX1002, ¶¶133-137. This is shown below in Dr. Spellman’s Figure F:



EX1005, FIG. 1; EX1002, ¶135, FIG. F.

As discussed above, Fan's decoder probes comprise (1) a first portion comprising a sequence that specifically hybridizes to one of the decoder sequences (*i.e.*, read sequences) of the barcode probes (*i.e.*, encoding nucleic acid probes), and (2) a second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. EX1005, 8:49-55, 9:18-22; EX1006, 12:28-13:3, 13:23-26; EX1002, ¶¶133-137. Fan shows in Figure 1 that "decoding takes place ... by *eight rounds* of sequential hybridization with *fluorescently labeled oligos* and imaging of fluorescent images." EX1005, 9:1-22, FIG. 1; EX1006, 13:12-26, FIG. 1, EX1002, ¶¶133-137. Thus, Fan discloses claim 1(e).

7. "(f) detecting a fluorescent signal from each of the different plurality of readout probe hybridized complexes"

Fan discloses Step 1(f). EX1002, ¶¶138-141. After hybridizing the second set of fluorescently labeled decoder probes (*i.e.*, readout probes), Fan teaches "*detecting a plurality of signals* indicative of the hybridizing" of the second decoder probes. EX1005, 2:21-36; EX1006, 2:20-30; EX1002, ¶¶138-141. Fan shows in Figure 1 that "decoding takes place ... by eight rounds of sequential hybridization with *fluorescently labeled oligos* and imaging of *fluorescent* images." EX1005, 9:1-22; *see also id.*, 9:23-43, 11:11-15, 14:3-48, Claim 1; EX1006, 13:12-26; *see also id.*, 13:27-14:10, 16:29-17:2, 21:17-22:16, Claims 1-2; EX1002, ¶¶138-141. Fan discloses detecting each of the plurality of readout probe hybridized complexes because it specifies separate steps of "detecting a plurality of

signals indicative of the hybridizing” after “hybridizing a first labeled decoder probe to the first decoder sequence” and after “hybridizing a second labeled decoder probe to the second decoder sequence.” EX1005, 2:21-36; Claim 1; EX1006, 2:20-30, Claims 1-2; EX1002, ¶¶138-141. Thus, Fan discloses claim 1(f).

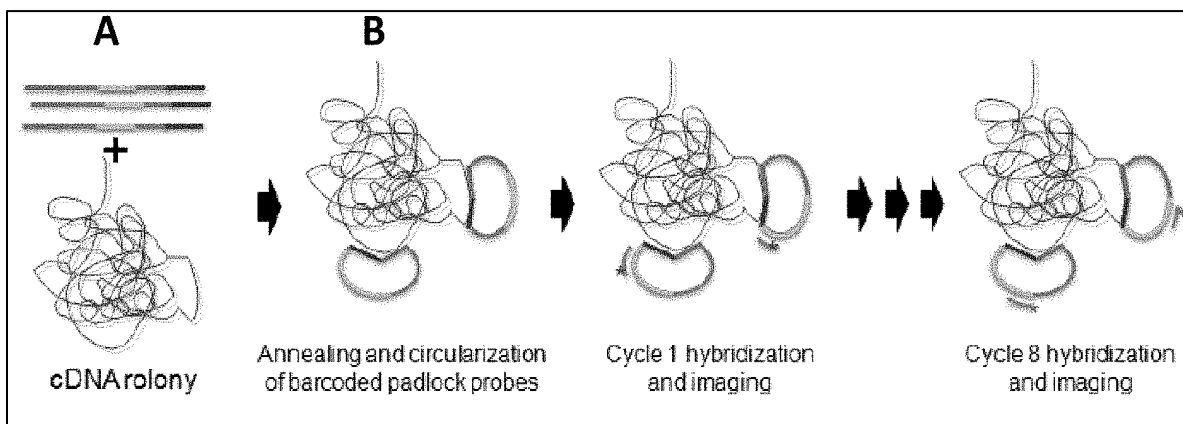
8. “(g) removing the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes”

For the reasons discussed above for Step 1(d), Fan also discloses Step 1(g) under Co-Plaintiff’s interpretation. EX1002, ¶¶142-146. Fan’s method depicted in Figure 1 decodes by “eight rounds of *sequential hybridization* with fluorescently labeled oligos and imaging of fluorescent images.” EX1005, 9:13-22, FIG. 1, 8:34-39, 8:53-59; EX1006, 13:20-26, FIG. 1, 12:18-21, 13:1-5; EX1002, ¶¶142-146. As discussed above in Step 1(g), Fan’s method removes the previous decoder probes before hybridizing the next round. EX1005, 2:56-58, 9:25-27, Claim 1; EX1006, 3:14-15, 13:28-30, Claims 1-3; EX1002, ¶¶142-146. Accordingly, Fan’s method comprises removing the fluorescent signaling entity from the second hybridized complexes between the barcode probes and decoder probes (*i.e.*, second readout probe-hybridized complexes) by removing the fluorescently labeled decoder probes. EX1002, ¶¶142-146. The 303 patent confirms that Fan’s method of removal is a “removal of the signaling entity,” as claimed. EX1001, 8:49-51, 9:43-

51, 23:57-24:3, FIG. 3A; EX1002, ¶¶145; Section IX.B.5, *supra*. Thus, Fan discloses claim 1(g) under Co-Plaintiff's interpretation.

9. **“(h) repeating steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal”**

Fan discloses Step 1(h). EX1002, ¶¶147-150. Fan's methods apply “decoding strategies that entail *sequential hybridizations of labeled decoder probes* to decoder sequences found in immobilized nucleic acids within an individual cell.” EX1005, 8:34-39, 8:53-59; EX1006, 12:18-21, 13:1-5; EX1002, ¶¶147-150. Indeed, Fan's method depicted in Figure 1 performs “*eight rounds of sequential hybridization with fluorescently labeled oligos and imaging of fluorescent images.*” EX1005, 9:1-22, FIG. 1; EX1006, 13:12-26, FIG. 1; EX1002, ¶¶147-150. Fan refers to this as “cycle 1” through “cycle 8” below:



Id.

Fan's methods remove the fluorescent signal between each round of sequential hybridizations by removing the decoder probes. EX1005, 2:56-58, 9:25-27, Claim 1; EX1006, 3:14-15, 13:28-30, Claims 1-3; EX1002, ¶¶147-150.

Accordingly, Fan's method includes repeating one or more times (i) hybridizing with fluorescently labeled decoder probes, (ii) detecting the fluorescent signal, and (iii) removing the fluorescent signal (*i.e.*, Steps 1(e)-1(g), as claimed); EX1002, ¶¶147-150. Thus, Fan discloses claim 1(h).

10. “(i) producing codewords representing the plurality of different nucleic acid targets at locations within the sample,

wherein each of the codeword represents one of the plurality of different nucleic acid targets and comprises multiple binary values 1 and 0,

wherein a value of 1 is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-hybridized complexes at a respective location within the sample while a value of 0 is obtained when the fluorescent signal is not detected from one of the plurality of readout probe-hybridized complexes or one of the different plurality of readout probe-hybridized complexes at the respective location within the sample”

Fan discloses Step 1(i) through its citation to and incorporation by reference of Gunderson (EX1007). EX1002, ¶¶151-161. Fan's method identifies the nucleic acid targets by “decoding the barcode probes.” EX1005, 1:60-2:1, 4:6-19, 9:1-22, Claim 1; EX1006, 1:30-2:6, 5:16-25, 13:12-26, Claims 1-2; EX1002, ¶¶151-161.

When discussing its decoding methods, Fan expressly incorporates by reference in its entirety the decoding and error-checking schemes of Gunderson. EX1005, 3:36-40, 8:53-59, 14:29-34; EX1006, 4:19-21, 13:1-5, 22:3-7; EX1007; EX1002, ¶¶151-161.

“A prior art document may anticipate a claim if it describes every element of the claimed invention, either expressly or inherently.” *Husky Injection Molding Systems Ltd. v. Athena Automation Ltd.*, 838 F.3d 1236, 1248 (Fed. Cir. 2016) (citation omitted). “Other material may be considered as part of that prior art document if it is incorporated by reference.” *Id.* “A host document incorporates material by reference if it identifies with detailed particularity what specific material it incorporates and clearly indicates where that material is found in the various documents.” *Id.*

Fan identifies with detailed particularity the decoding and error-checking schemes of Gunderson. When discussing decoding, Fan teaches that the “*sequential hybridization* of decoder probes to decoder sequences [that] is *described in Gunderson et al.* (Genome Res. (2004) 14:870-877), the content of which is incorporated by reference in its entirety.” EX1005, 8:53-59, 3:32-40 (“barcodes can be *decoded by hybridization*, similar to the *decoding of ... Gunderson et al.*”), 8:34-44, 9:1-22, 14:31-36; EX1006, 13:1-5, 4:16-21, 12:18-21, 12:18-25; 13:12-26, 22:3-8; EX1002, ¶¶153-154. Indeed, Fan’s “design of the

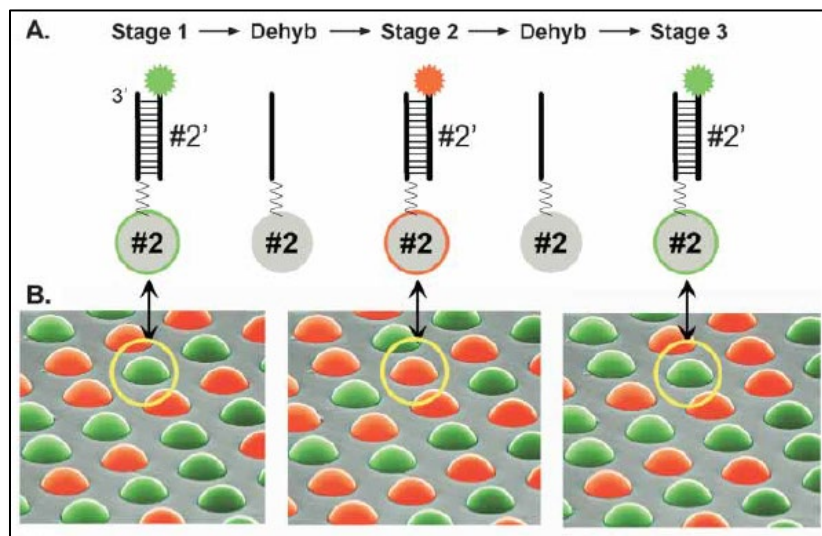
barcoding region *incorporates the error-checking scheme described in the incorporated materials of Gunderson*” and generates a “valid barcode for each gene.” EX1005, 14:31-34; EX1006, 22:5-7; EX1007; EX1002, ¶¶153-154.

Example 1 further specifies using Gunderson’s “three-state, eight-stage decoding scheme” with “two fluorescent (FAM and Cy3) ‘ON’ states and one nonfluorescent OFF state” to decode the nucleic acid targets. EX1005, 14:34-48; EX1006, 22:7-16; EX1007, 871-873; EX1002, ¶¶153-154. Thus, Fan ’s method identifies with particularity the decoding and error-checking schemes of Gunderson and expressly incorporates them by reference. *Husky*, 838 F.3d at 1248.

Gunderson teaches an “algorithm to identify each member of a large collection of DNA-linked objects through the use of hybridization.” EX1007, Abstract. Gunderson’s “[a]lgorithm uses sequential hybridizations of dye-labeled oligonucleotides, or decoders... to create a combinatorial decoding scheme.” *Id.*, 870. The rounds of sequential hybridization in Gunderson produce a “code” (codeword) for each nucleic acid target on the array. EX1002, ¶¶155-161.

The binary code element of claim 1(i) requires “a value of 1 ... when *the fluorescent signal* is detected” and “a value of 0 ... when *the fluorescent signal* is not detected.” EX1001, 86:59-87:6. Gunderson teaches decoding schemes using codewords comprising binary values of 1 and 0, wherein 1 represents detecting red fluorescence and 0 represents not detecting red fluorescence, satisfying this claim

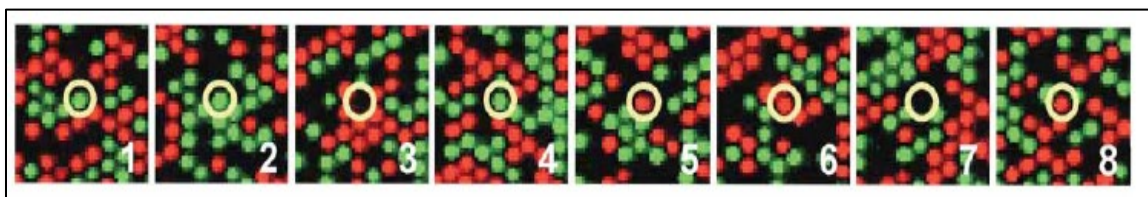
limitation. EX1002, ¶¶155-161. Gunderson depicts this binary “[d]ecoding process” in Figure 2 using a value of 1 for ON (the fluorescent red signal is detected) and a value of 0 for OFF (the fluorescent red signal is *not* detected). EX1007, 872, FIG. 2, 871; EX1002, ¶¶155-161. Gunderson teaches reading the fluorescent signal after each hybridization round to reveal a “*combinatorial code* for each bead” (*i.e.*, codeword) that “identifies each bead type,” wherein 0 represents detecting a red signal and 1 represents not detecting a red signal (a green signal is detected instead). *Id.*, FIG. 2; EX1002, ¶¶155-161. In Gunderson’s method, each bead (*i.e.*, location) on the array is assigned a unique code. *Id.* For example, the “bead circled in Figure 2B has the color signature (GRG), or 010 code in binary representation, in which G=0 and R=1”:



EX1007, FIG. 2, 871; EX1002, ¶¶155-161. Put another way, this exemplary bead has a color signature of red-signal-*not-detected* / red-signal-*detected* / red-signal-

not-detected (code “010”). *Id.*

Gunderson also teaches a coding scheme using “two fluorescent ‘ON’ states (FAM and CY3 fluorescent labels) and one nonfluorescent ‘OFF’ state.” EX1007, 871-872; EX1005, 14:34-41; EX1006, 22:7-16; EX1002, ¶¶155-161. This Gunderson scheme also meets the claim limitation under Co-Plaintiffs’ interpretation. The Co-Plaintiffs interpret this limitation as broad enough to encompass a method in which *multiple different colors* of fluorescent signals are detected and the codeword is made up of different values for each “on” color (A, B, C, and D representing four possible “on” color values in Co-Plaintiffs’ contentions) and another value is assigned for “off” (no detected fluorescence (E in Co-Plaintiffs’ contentions)). EX1047, 0106-0110; EX1002, ¶¶155-161. Under this Gunderson decoding scheme, detection of the green (FAM) or Red (Cy3) fluorescence is mapped to two different “ON” values (1 and 2 respectively) while detection of no fluorescence “OFF” is mapped to 0. EX1007, 871-873; EX1002, ¶¶155-161. Gunderson depicts this code system in Figure 3, showing 8 cycles of detection that produce a code of “11012202” (or On-On-Off-On-On-On-Off-On under Co-Plaintiffs’ contentions):



EX1007, FIG. 3A. Gunderson describes “clear separation between the two modes in the histogram indicat[ing] that most beads can be classified unambiguously as OFF or ON.” EX1007, 872, 873 (“[t]he low intensity peak includes beads in the *OFF state* and empty wells. The higher intensity peak includes beads in the *ON state*.”), 876 (“[t]he histogram is *bimodal* because each stage and color channel will have beads that are in the *ON state* and beads that are in the *OFF state*.”); EX1002, ¶¶155-161.

Accordingly, Fan (incorporating Gunderson) teaches claim 1(i)’s “producing codewords” step.

11. “(j) matching the codewords with valid codewords in a codebook by comparing the codewords to the valid codewords in the codebook,

and if one of the codewords is not matched with one of the valid codewords in the codebook, applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook, or discarding the one of the codewords,

wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets”

Fan (incorporating Gunderson) discloses Step 1(j) via Gunderson’s error detection and error correction code scheme under at least Co-Plaintiffs’ interpretation. EX1002, ¶¶162-170. Gunderson teaches that its “error-checking concepts” are “based on algorithms from digital information theory,” which are “well known in other fields,” citing Shannon (1948) and a Hamming (1986), which

describes Hamming distance and the use of Hamming error detecting and error correcting code systems. EX1007, 871 (citing EX1035-EX1037); EX1002, ¶¶162-170. Gunderson applies these error-checking concepts “to DNA hybridization.” EX1007, 871 (citing EX1035-EX1037); EX1002, ¶¶162-170.

Gunderson teaches a binary code system that compares codewords to valid codewords in the codebook and identifies codewords that match (“valid codes”) or don’t match (“invalid codes”) those in the codebook. EX1007, 871-872; EX1002, ¶¶162-170. Gunderson teaches decoding a sequence for each bead on the array by “referring to the *color-lookup table* in Figure 2C.” EX1007, 871, 872, FIG. 2; EX1002, ¶¶162-170. Gunderson’s “color-lookup table” in Figure 2C (shown below) is a codebook because it contains the list of valid codewords for the sequences (*i.e.*, valid codewords of the plurality of nucleic acid targets, as claimed). *Id.*

Sequences		Stage 1	Stage 2	Stage 3	Signature	Code	Parity Code
	0				GGG	000	0000
	1				GGR	001	0011
	2				GRG	010	0101
	3				GRR	011	0110
	4				RGG	100	1001
	5				RGR	101	1010
	6				RRG	110	1100
	7				RRR	111	1111

EX1007, 871, FIG. 2C; EX1002, ¶¶164-165.

Gunderson’s method uses the color-lookup table (codebook) to identify

sequences of beads on the array and determine a “match” by comparing and determining whether each bit of a produced codeword is the same as each bit in the corresponding position of a valid codeword in the codebook. *Id.* For example, Gunderson teaches that the “sequence” of the bead circled in yellow in Figure 2B (with color signature GRG and binary code 010) “can be identified as sequence 2 by referring to the color-lookup table.” *Id.*

Co-Plaintiffs’ interpretation of the “and if one of the codewords is not matched...” step is broad enough to include a method that applies “an error detection or correction” system that can detect errors and discard erroneous/unmatched codewords even if that system is not also capable of correcting an error to assign an erroneous/unmatched codeword to a corrected “matched” valid codeword. EX1047, 0114 (“an error detection or correction system is applied (e.g., the decoding algorithm) to either match the decoded transcript to a valid codeword or discard the one of the codewords”); EX1002, ¶166.

Gunderson also teaches performing an error detection (“error-checking”) step when a codeword from a bead does *not* match the list of valid codewords in the color-lookup table. EX1007, 871; EX1002, ¶¶167-170. Gunderson explains that “[t]he simplest form of *error checking* we have used has a parity bit and is illustrated in Figure 2C.” *Id.* In Gunderson’s error-checking scheme, “the assigned

four-bit codes all have an even parity bit sum and are termed *valid codes*,” while “[a]n error in a single decode stage is a one-bit error that creates an odd parity, or *invalid code*.” *Id.*

Similarly, for Figure 3’s decoding scheme, Gunderson describes a codebook for detecting errors and eliminating “the most common error type.” EX1007, 871-72, 875; EX1002, ¶¶168-170. Here, Gunderson teaches “designing the decode stages so that every valid code has a fixed number of OFF states” (exactly 6 ON and 2 OFF states at 871-872; and either exactly 6 ON and 2 OFF states or exactly 3 ON and 5 OFF states at 875). *Id.* Gunderson teaches that errors are further reduced by removing from the set of valid codes “all codes ending in three or more OFF states” and “any codes that do not have at least one ON state in each of the color channels.” *Id.*, 875; EX1002, ¶¶168-170. Gunderson also uses an additional eighth detection cycle as an error checking parity bit. *Id.*, 871; EX1002, ¶¶168-170. Thus Gunderson’s coding system detects errors at least anytime a detected codeword fails the parity bit check and any time a detected codeword does not contain the predefined required number of ON and OFF states. EX1002, ¶¶168-170.

Gunderson teaches that this technique eliminates the most common error type: “With this scheme, it is theoretically impossible to misclassify a code through any number of occurrences of the most common error type: a transition from an ON state to an OFF state.” EX1007, 871; EX1002, ¶¶168-170. Using Gunderson’s

code system, Gunderson was able to detect and calculate a median error rate and determined that Gunderson's system reduced the average misclassification rate by "86-fold" compared to a system without his error detection and prevention rules. *Id.*, 872, Table 1, 873; EX1002, ¶¶169-170.

Thus, Fan (incorporating Gunderson) discloses claim 1(j) under at least Co-Plaintiffs' interpretation.

12. "(k) determining the plurality of nucleic acid targets in the sample based on results from the matching step."

Fan discloses Step 1(k). EX1002, ¶¶171-174. Fan's method determines the "identity of the amplified nucleic acid molecules" by "decoding of the barcode probes." EX1005, 1:60-2:1, 4:6-19, 9:1-22; EX1006, 1:30-2:6, 5:16-25, 13:12-26; EX1002, ¶¶171-174. As discussed above, Fan performs decoding by using Gunderson's error-checking schemes that include the matching step. EX1005, 8:53-59 (citing and incorporating by reference EX1007), 3:32-40, 8:34-44, 9:1-22, 14:31-36; EX1006, 13:1-5, 4:16-21, 12:18-25, 13:12-26, 22:5-8; EX1002, ¶¶171-174; Section IX.B.11. Accordingly, Fan's method determines the plurality of nucleic acid targets in the sample based on the results of the matching step taught in Gunderson's decoding and error-checking schemes. EX1002, ¶¶171-174. Thus, Fan (incorporating Gunderson) discloses claim 1(k).

In sum, Fan (incorporating Gunderson) teaches every limitation of claim 1 under Co-Plaintiffs' interpretation, arranged as claimed. Fan's methods are

presumed enabled. *In re Antor Media Corp.*, 689 F.3d 1282, 1290-91 (Fed. Cir. 2012). Accordingly, Fan anticipates claim 1.

C. Claim 2

Claim 2 depends from claim 1 and requires that “the fluorescent signal in step (c) and/or step (f) is detected at a resolution better than 300 nm.” EX1001, 87:19-21. Fan teaches this limitation under Co-Plaintiffs’ interpretation. EX1002, ¶¶175-181.

Under Co-Plaintiffs’ interpretation, claim 2 is broad enough to include use of a system with a per-pixel resolution of less than 300 nm—an “imager [that] uses a high numerical aperture and a fast area scan camera with a low read noise sensor to achieve ~200 nm per-pixel resolution.” EX1047, 0135. The “per-pixel resolution” is a function of the pixel size of the sensor and the optical magnification of the sample. EX1002, ¶175-181. For example, a camera with a per-pixel size of 4.25 μm (e.g., 4.25 x 4.25 μm square) magnified at 20x has a 0.212 μm (212.5 nm) per-pixel resolution. EX1002, ¶175-181.

Fan discloses that the fluorescent signal during decoding “can be detected using any suitable imaging and detection technologies which allow for detection of individual amplicons in situ ... as exemplified by Larsson et al., Nat. Methods (2010) 7:395-397 and associated supplemental materials, the entire content of which is incorporated by reference [] in its entirety.” EX1005, 11:11-19; EX1006,

16:29-17:5; EX1008; EX1002, ¶¶175-181. Thus, Fan identifies with particularity Larsson’s imaging and detection methods and expressly incorporates them by reference. *Husky*, 838 F.3d at 1248.

Larsson specifies acquiring images “using an AxioplanII epifluorescence microscope (Zeiss) equipped with ... *a CCD camera (C4742-95, Hamamatsu)*, and a computer-controlled filter wheel with excitation and emission filters for visualization of DAPI, FITC, Cy3, Cy3.5 and Cy5.” EX1008, 0005; EX1002, ¶¶175-181. Larsson further specifies that a “*×20 (Plan-Apocromat, Zeiss)*, *×40 (Plan-Neofluar, Zeiss)* or *×63 (Plan-Neofluar, Zeiss)* objective was used for capturing the images.” *Id.*

Larsson’s Hamamatsu C4742-95 CCD camera is “a high resolution image sensor featuring an effective pixel number of 4000 (Horizontal) x 2624 (Vertical) pixels.” EX1048, 7; EX1002, ¶¶175-181. The effective imaging area of the camera is 23.6 mm x 15.5 mm with each pixel having a size of 5.9 μm (5.9 x 5.9 μm square). EX1048, 35; EX1002, ¶¶175-181. Thus, as Dr. Spellman explains, the resolution of fluorescent signals for the 20x, 40x, and 63x magnifications is 0.295 μm (295 nm), 0.148 μm (148 nm), and 0.094 μm (94 nm), respectively—each of which is better than 300 nm. *Id.*

Thus, Fan (incorporating Larsson) discloses claim 2 under Co-Plaintiffs’ interpretation.

D. Claim 3

Claim 3 depends from claim 1, and requires that “the sample comprises a cell.” EX1001, 87:22-23. Fan teaches this limitation. EX1002, ¶¶182-183.

Fan’s method obtains sequence information for “a plurality of nucleic acid molecules from an individual *cell*.” EX1005, 2:21-36, 1:58-2:1, 4:6-19, 9:1-22, Example 1; EX1006, 2:20-30, 3:15-22, 5:16-25, 13:12-26, Example 1; EX1002, ¶¶182-183. Fan also explains that its “methods and compositions make use of hybridization, amplification, washing and detection within *individual cells*.” EX1005, 10:62-64; EX1006, 16:16-17; EX1002, ¶¶182-183. Accordingly, Fan anticipates claim 3.

E. Claim 4

Claim 4 depends from claim 3, and requires that “the cell is fixed on a coverslip.” EX1001, 87:24-25. Fan teaches this limitation. EX1002, ¶¶185-187.

Fan discloses using “[m]ethods for permeabilization and fixation of cells and tissue samples” that were “well-known in the art, as exemplified by . . . Larsson et al., at. Methods (2010) 7:395-397 [EX1008] and associated supplemental materials, the content of each of which is incorporated herein by reference in its entirety.” EX1005, 10:66-11:6; EX1006, 16:19-23. Thus, Fan again identifies with particularity Larsson—for its methods of permeabilization and fixation of cells—

and again expressly incorporates by reference Larsson's teachings. *Husky*, 838 F.3d at 1248.

Larsson discloses placing frozen sections of mouse embryos on "Superfrost Plus Gold *slides*" and placing breast tissue sections on "Starfrost microscope *slides*." EX1008, 0004; EX1002, ¶¶185-187. Thus, Fan (incorporating Larsson) discloses detecting nucleic acid molecules within cells that were fixed on a slide. EX1002, ¶¶185-187. A POSA would have known a cell fixed on a slide includes a cell fixed on a coverslip. *Id.* Accordingly, Fan (incorporating Larsson) anticipates claims 3 and 4.

F. Claim 5

Claim 5 depends from claim 1 and comprises "determining the fluorescent signal entity by imaging at least a portion of the sample using a light microscope." EX1001, 87:26-28. Fan teaches this limitation. EX1002, ¶¶188-190.

As discussed above for claim 2, Fan incorporates by reference Larsson's "imaging and detection technologies." EX1005, 11:11-19; EX1006, 16:29-17:5; EX1002, ¶¶188-190. Larsson teaches that the "[i]mages of cultured cells were acquired using an AxioplanII *epifluorescence microscope* (Zeiss) . . ." which is a fluorescent *light microscope*. EX1008, 0005; EX1002, ¶¶188-190. Accordingly, Fan (incorporating Larsson) anticipates claim 5. *Id.*

G. Claim 6

Claim 6 depends from claim 1 and comprises “determining an abundance and/or spatial distribution of the different nucleic acid targets at locations within the sample.” EX1001, 88:1-3. Fan teaches this limitation. EX1002, ¶¶191-192.

Fan’s methods “comprise evaluating a signal to determine the presence, *quantity*, frequency, and/or *spatial distribution* of a disease-causing mutation, allele or common variant within a tissue.” EX1005, 3:21-24, 3:61-4:5, 10:42-46, 14:57-61; EX1006, 4:8-10, 5:8-15, 16:1-3, 22:21-24; EX1002, ¶¶191-192.

Similarly, Fan discloses that “the *spatial location* of nucleic acids can be simultaneously *determined* for several individual cells in a tissue or other cell-containing sample.” EX1005, 4:32-34; EX1006, 6:5-7; EX1002, ¶¶191-192.

Accordingly, Fan anticipates claim 6. *Id.*

H. Claim 7

Claim 7 depends from claim 1 and comprises “removing the readout nucleic acid probes unhybridized to each of the plurality of first hybridized complex prior to step (c).” EX1001, 88:4-6. Fan discloses all the elements of claim 7. EX1002, ¶¶193-197.

Fan discloses that its methods “make use of hybridization, amplification, washing and detection within individual cells” (*i.e.*, washing takes place *before* detection). EX1005, 10:62-64, 9:25-27; EX1006, 16:16-17, 13:28-30; EX1002,

¶¶193-197. Gunderson, incorporated in Fan, teaches that “[f]ollowing hybridization, three 1-min washes ... were used to *remove unbound oligonucleotides*.” EX1007, 875. Fan confirms this removing step when claiming that “the first fluorescently labeled decoder probe that is *not hybridized* to said first decoder sequence is *removed* by washing.” EX1005, 27:30-33 (Claim 1(c)); EX1002, ¶¶193-197.

Accordingly, Fan (incorporating Gunderson) anticipates claim 7. *Id.*

I. Claim 8

Claim 8 depends from claim 1 and requires that “the valid codewords form an error-checking or an error-correcting code space.” EX1001, 88:7-8. Fan teaches this limitation. EX1002, ¶¶198-201.

The 303 patent discloses that “codewords may be assigned within a *code space* ... using *an error-detection or an error-correcting system*, such as a Hamming system...” EX1001, 12:46-51; *see also id.*, 20:51-56. Fan teaches this limitation through its incorporation of Gunderson. *Id.*

As discussed above for claim 1(i)-1(j), Fan’s method incorporates Gunderson’s (EX1007) error-checking scheme. *See supra*, Section IX.B; EX1002, ¶¶198-201. Gunderson teaches that its “*error-checking* concepts ... are based on algorithms from digital information theory” and cites Shannon 1948 and Hamming 1986. EX1007, 871; EX1002, ¶¶198-201. That is, Gunderson teaches using the

same error-checking systems disclosed in the 303 patent. *Id.* Gunderson further discloses that its valid codewords are used for error-checking (*i.e.*, they form an “error-checking codespace”) because Gunderson teaches adding “additional stages ... for error checking purposes” to its color-lookup table. *Id.* Gunderson further explains that it’s “assigned four-bit codes all have an even parity bit sum and are termed *valid codes*,” while “[a]n error in a single decode stage is a one-bit *error* that creates an odd parity, or *invalid code*.” *Id.*; EX1002, ¶¶198-201. Accordingly, Fan (incorporating Gunderson) anticipates claim 8 under Co-Plaintiff’s interpretation.

J. Claims 9-11

Claims 9-11 each depends from claim 1 and requires that “in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one or more errors” (claim 9), or “has one error” (claim 10), or “has two errors” (claim 11). EX1001, 88:9-23. Fan discloses all the elements of claims 9-11. EX1002, ¶¶202-205.

As discussed above for claim 1, Fan incorporates by reference Gunderson’s decoding and error-checking schemes. *See* Section IX.B; EX1002, ¶¶202-205. Gunderson teaches an error-checking scheme that uses a “color-lookup table” (codebook) containing a list of valid codewords, and identifies sequences by

comparing and matching a bead's color signature/codeword with valid codewords in the color-lookup table. EX1007, 871. Gunderson discloses that, if a codeword from a bead does *not* match one of the valid codewords in the lookup table, there must be *at least one error*. *Id.* (“[a]n error in a single decode stage is a one-bit error that creates an odd parity, or invalid code.”), FIG. 2C; EX1002, ¶¶202-205. Indeed, Gunderson discloses that one of the “[p]urpose[s]” of the “[i]nvalid codes” in Table 1 is to “[d]etect *single* stage errors.” EX1007, 872; EX1002, ¶¶202-205. Thus, Gunderson teaches that one of the codewords is not matched with one of the valid codewords in the codebook when one of the codewords has one error (claims 9-10) or two errors (claim 11). EX1002, ¶¶202-205. Gunderson also teaches detecting an error and discarding the erroneous codeword when a detected codeword: does not have exactly the prescribed number of ON and OFF cycles; ends in three or more OFF states; or does not have at least one ON state in each of the color channels (any of which can result from either one error or two errors). *Id.*; *see* claim 1(i)-1(j) above; EX1002, ¶¶202-205.

Fan (incorporating Gunderson) thus anticipates claims 9-11 for the reasons discussed here and in claim 1(i)-1(j) above.

K. Claim 12

Claim 12 depends from claim 1 and requires wherein “said comparing the codewords to the valid codewords in the codebook in step (j) comprises comparing

the values at each of the positions of the codewords.” EX1001, 88:24-27. Fan discloses all the elements of claim 12. EX1002, ¶¶206-210.

As discussed above for claim 1, Fan incorporates by reference Gunderson’s decoding and error-checking schemes. Gunderson refers to Figure 2C’s “color-lookup table” for reference (or valid) codewords for error-checking. EX1007, 871-872, FIG. 2C; EX1002, ¶¶206-210. Gunderson uses this table as a codebook. *Id.* To check for errors, Gunderson teaches that the values of *each of the positions* of a generated codeword must be compared with that of the valid codewords in the codebook. *Id.* Thus, Gunderson’s error-checking scheme inherently compares the values at each position in the codewords for error-checking. EX1002, ¶¶206-210. “[A] prior art reference may anticipate without disclosing a feature of the claimed invention if that missing characteristic is necessarily present, or inherent, in the single anticipating reference.” *Schering Corp. v. Geneva Pharm., Inc.*, 339 F.3d 1373, 1377 (Fed. Cir. 2003). Accordingly, Fan (incorporating Gunderson) anticipates claim 12.

X. GROUND 2: CLAIMS 1, 3, AND 6-12 WOULD HAVE BEEN OBVIOUS OVER FAN AND GUNDERSON

Fan’s express incorporation by reference of Gunderson is operative to bring the entire disclosure of Gunderson within Fan “as if it were explicitly contained therein.” *Paice LLC v. Ford Motor Co.*, 881 F.3d 894, 906 (Fed. Cir. 2018). Even if Fan and Gunderson were not considered a single reference, combining Fan and

Gunderson would have been obvious. EX1002, ¶¶211-214. Incorporation by reference, an overlapping researcher (*e.g.*, Jian-Bing Fan), overlapping research groups from Illumina Inc., and Fan building on and expressly endorsing Gunderson, reflect a POSA's motivation to combine Fan and Gunderson. *Bayer Healthcare Pharms., Inc. v. Watson Pharms., Inc.*, 713 F.3d 1369, 1374-75 (Fed. Cir. 2013) (obvious to combine references when one "refers expressly" to the other). A POSA would have also reasonably expected to combine Fan and Gunderson with success because, as explained above, Fan specifically incorporates by reference Gunderson for its decoding and error-checking schemes and successfully uses the schemes in its methods. EX1005, 3:36-40, 8:53-59, 14:31-48; EX1006, 4:19-21, 13:1-5, 22:5-16; EX1007; EX1002, ¶¶211-214. For example, Fan's method in Example 1 uses Gunderson's ON/OFF decoding scheme for detecting 1700 target genes with eight rounds of hybridization decoding. EX1005, 14:31-48; EX1006, 22:5-16; EX1007, 872-873, FIG. 3; EX1002, ¶¶211-214.

Harvard did not rely on objective indicia during prosecution (EX1004), nor is 10x aware of any objective indicia with a nexus to the challenged claims.

Accordingly, claims 1, 3, and 6-12 would have been obvious over Fan and Gunderson under Co-Plaintiffs' interpretation for the reasons discussed above and the reasons and disclosures discussed for Ground 1.

XI. GROUND 3: CLAIMS 2, 4, AND 5 WOULD HAVE BEEN OBVIOUS OVER FAN, GUNDERSON, AND LARSSON

Fan's express incorporation by reference of Gunderson and Larsson is brings the entire disclosure of Gunderson and Larsson within Fan. *Paice*, 881 F.3d at 906. Even if Fan, Gunderson, and Larsson were not considered a single reference, combining Fan, Gunderson, and Larsson would have been obvious. EX1002, ¶¶215-219. A POSA would have been motivated to combine Fan, Gunderson, and Larsson at least because Fan incorporates by reference both Gunderson and Larsson, and endorses each reference's teachings as compatible with Fan's methods. EX1002, ¶¶215-219; *Bayer*, 713 F.3d at 1374-75. A POSA would have also reasonably expected to combine Fan, Gunderson, and Larsson with success because Fan specifically incorporates (i) Gunderson for its decoding and error-checking schemes and successfully uses the schemes in its methods, and (ii) Larsson for its imaging and detection methods. EX1005, 3:36-40, 8:53-59, 11:11-19, 14:31-48; EX1006, 4:19-21, 13:1-5, 22:5-16; EX1008; EX1002, ¶¶215-219 (EX1005; EX1007; EX1008). Fan's Example 1 uses Gunderson's ON/OFF decoding scheme for detecting 1700 target genes with eight rounds of hybridization decoding. EX1005, 14:31-48; EX1006, 22:5-16; EX1007, 872-873, FIG. 3; EX1002, ¶¶215-219. Similarly, Larsson teaches methods for "Image acquisition and analysis" and specifies acquiring images using an epifluorescence

microscope and a computer-controlled filter wheel with excitation and emission filters for visualization of fluorophores. EX1008, 0005; EX1002, ¶215-219.

Moreover, as discussed above in Section VI, the POSA's general knowledge in the art included a host of common microscopy techniques, such as superresolution microscopy, with increased resolution of images—including resolution better than 300 nm. EX1002, ¶¶73, 215-219; *see also* EX1029, 323; EX1028, 968; EX1030, 16; EX1005, 11:11-19; EX1006, 16:29-17:2; EX1031, 76; EX1032, 1103; EX1020, 21:16-45; EX1008, 0004; EX1033, 2227. The POSA's general knowledge in the art also included a variety of routine, cell-fixation techniques, including fixing cells on commercially available glass coverslips for imaging under a fluorescent microscope. EX1002, ¶¶215-219; EX1020, 38:39-52; EX1049, 26:59-61, 27:60-65.

Again, Harvard did not assert any objective indicia during prosecution, nor is 10x aware of any objective indicia with a nexus to the challenged claims. EX1004.

Accordingly, claims 2, 4, and 5 would have been obvious over Fan, Gunderson, and Larsson under Co-Plaintiffs' interpretation for the above reasons and the reasons and disclosures addressed in Ground 1.

XII. GROUND 4: CLAIMS 1-12 WOULD HAVE BEEN OBVIOUS OVER FAN AND CURRY

As discussed above in Ground 1, Fan anticipates claims 1-12 under Co-Plaintiffs' interpretation. To the extent it is argued that Fan does not anticipate any of claims 1-12, any differences between Fan and the challenged claims would have been obvious to a POSA in view of Fan alone (incorporating Gunderson and Larsson) (see Grounds 2-3 above) or in view of Fan and a POSA's knowledge and further in view of Curry (Ground 4). As addressed below, the combination of Fan's method with Curry's teachings for generating "combinatorial probe libraries" (similar to Fan's) that include multiple different probes that target different sequences within the same target analyte (and Curry's accompanying coding/decoding system for Curry's probe libraries) provides additional disclosures relevant to the challenged claims including but not limited to step 1(a), the "removing" steps 1(d) and 1(g), the decoding steps 1(i) and 1(j), and certain of the dependent claims. EX1002, ¶¶220-281. Accordingly, as demonstrated below, claims 1-12 would have been obvious over Fan and Curry. *Id.*

A. The Fan+Curry Combination

Fan and Curry address the same general concept, problem, and field (multiplexed detection of target molecules in situ) and share many similarities. For example, both are directed towards detecting a plurality of nucleic acid targets in a biological sample by sequentially hybridizing labeled probes to the sample and

determining the identity of an individual target based on the pattern of sequential hybridization. EX1005, 8:31-44, 8:49-59, 9:13-22; EX1006, 12:16-25, 12:28-12:35, 13:20-26; EX1009, Abstract, 6:17-7:13, 8:19-21, 8:46-9:9, 9:62-10:5, 11:46-60, 12:40-13:6; EX1002, ¶222. Both also apply decoding schemes that generate codewords with on/off values and error detection or correction schemes that are based on comparing a codeword with valid codewords in a codebook. EX1005, 8:53-59, 3:36-40, 14:31-48; EX1006, 13:1-5, 4:19-21; EX1009, 12:40-51; EX1002, ¶222.

1. Motivation to combine

As discussed above, Fan already discloses binding multiple barcode (primary) probes to a target nucleic acid. *See* Ground 1, claim 1(a). Fan also discloses that the multiple barcode probes hybridized to multiple locations of a nucleic acid target can be different barcode probes. EX1005, 9:8-11 (“one *or more* unique padlock probes.”); *see also id.*, 2:1-7; EX1006, 13:17-18, 2:6-10; EX1002, ¶¶223-228.

A POSA would have known of multiple advantages (detailed below) that result from using multiple different probes to target different target regions of the same target nucleic acid. In view of Fan’s teachings to use multiple probes (including multiple different probes) and in further view of these art-recognized advantages, a POSA would have been motivated to look to Curry because Curry

details a detection probe design compatible with Fan that uses multiple different probes to target multiple different target locations within the same target nucleic acid analyte. EX1002, ¶¶223-228.

POSAs knew that having multiple binding sites in a target reduces the effect of off-target binding and increases binding specificity. EX1002, ¶¶223-228. A nucleic acid probe might hybridize off-target to sequences in other molecules that are sufficiently similar to or overlap with the intended target sequence, resulting in false-positive signals. *Id.* A POSA knew that by binding multiple probes to different sequences of the same target, even if one or two probes bind off-target, other probes will bind correctly, producing a positive signal to identify the target. *Id.* In contrast, using a single in each target increases the risk of off-target binding. EX1024, 82, 90-91; EX1002, ¶¶223-228.

Different probes require different optimal hybridization conditions (including temperature), such that having multiple different probes that target different target sequences of the same analyte all with the same predicted optimal hybridization conditions (e.g., temperature) increases the chances of successful hybridization of at least some of those probes at those conditions. EX1024, 92; EX1002, ¶¶226-228.

In a highly multiplexed detection method (such as in Fan), in which all probes are hybridized simultaneously under the same conditions, some probes will

fail to bind. EX1024, 82; EX1002, ¶¶223-228. A POSA would have known that using probes that bind to multiple different sequences of each target increases the likelihood that at least some of the probe will bind correctly to that target, reducing the chance of off-target binding or no binding at all. *Id.*

Having looked to Curry for its teachings on using multiple probes targeting different target sites per analyte to incorporate into Fan's method, a POSA would also have been motivated to use Curry's coding/decoding methods for those same Curry probes. EX1002, ¶¶223-228. As Curry teaches, Curry's Hamming code-based error detection and correction system that uses three parity bits has benefits over Gunderson's disclosed coding schemes—namely, Curry's system allows for the correction of unmatched codewords containing only a single error and the detection of unmatched codewords that contain two errors. EX1009, 12:42-49; EX1002, ¶¶223-228. These Hamming distance code systems were well known in the art, as were the benefits of being able to correct some erroneous detected codewords. EX1002, ¶¶223-228; EX1007, 871; EX1001, 20:18-24; EX1044, ¶[0106].

2. Reasonable expectation of success

A POSA also would have had a reasonable expectation of success in adapting Fan's primary probes (such as padlock probes) in view of Curry's teaching to create libraries of primary probes that include multiple different probes

that bind to different sites within the same target analyte. EX1002, ¶¶229-231. A POSA knew of many examples in the art successfully applying multiple primary probes to different target sequences in the same target nucleic acid. *See, e.g.*, EX1012; EX1015, 836-837, FIG. 1A-1B; EX1025, 66; EX1013, Abstract; EX1026, 3:37-42; EX1020, 4:35-48; EX1009, 6:17-7:13; EX1019, FIG. 1; EX1002, ¶¶229-231. Fan's teachings already disclose (at least) the use of multiple barcode probes (such as padlock probes) per target analyte (*see* Ground 1, claim 1(a)). Fan also discloses that its methods are compatible with the use of multiple different probes per target. EX1005, 9:8-11; *see also id.*, EX1006, 13:17-18, 2:6-10; 2:1-7; EX1002, ¶¶229-231. And as Dr. Spellman explains, these art-recognized techniques required no more than ordinary skill and routine methods to implement into Fan's method. EX1002, ¶¶229-231.

Before July 30, 2014, POSAs knew how to and had designed multiple padlock probes that hybridize to different target regions of a target nucleic acid. For example, Fan expressly cites and incorporates Deng when discussing "examples of padlock probes useful for the methods provided herein." EX1005, 8:1-4; EX1006, 11:24-26. Deng describes the use of padlock probes and teaches that each target "was covered by *multiple probes* targeting partially *overlapping DNA fragments*." EX1027, 355; EX1002, ¶230.

A POSA would also have a reasonable expectation of success in combining Curry's error detection or correction system with Fan's disclosures. EX1002, ¶¶229-231. Parity-based error detection or correction schemes such as the Hamming code were conventional and widely used. *Id.*; Section VI.C. Gunderson itself taught that its coding system was "based on algorithms from" Hamming. EX1007, 871. Implementing Curry's Hamming-based coding scheme using three parity bits instead of Gunderson's one parity bit would have involved only the application of routine and conventional knowledge and would have produced the known and expected benefits of being able to correct single-bit errors while detecting two-bit errors. EX1002, ¶¶229-231.

B. Claim 1

The combination of Fan with Curry's multiple different probes per analyte and related coding scheme discloses the preamble and claim elements 1(b)-1(c), 1(e)-1(f), 1(h), and 1(k) based on the reasoning and disclosures addressed for Grounds 1-3. EX1002, ¶¶232-253; Section IX-XI, *supra*.

1. Step 1(a)

In addition to Fan's teachings discussed for step 1(a) in Grounds 1-3, the Fan+Curry combination also discloses this step. Fan+Curry discloses a method in which each of the plurality of first hybridized complexes are created by hybridizing multiple encoding nucleic acid probes (*i.e.*, Fan's barcode probes) at

different locations of an identical nucleic acid target (where those locations have different *sequences* that are targeted by the different encoding probes).

Fan discloses hybridizing its multiple barcode probes to different sequences of the same nucleic acid target. EX1005, 9:8-11; EX1006, 13:17-18; EX1002, ¶¶229-231. Curry also discloses hybridizing multiple primary probes to different sequences of the same nucleic acid target. EX1002, ¶¶229-231.

Specifically, Curry identifies nucleic acid targets in a biological sample by sequentially hybridizing fluorescently labeled probes to the targets and using the hybridization pattern for each target to decode its identity. *See, e.g.*, EX1009, Abstract, 6:17-7:13, 8:19-21, 8:46-9:9, 9:62-10:5, 11:46-60, 12:40-13:6; EX1002, ¶235. Curry identifies “targets (e.g. [] RNA molecules [])” by “sequentially” hybridizing different libraries of primary probes with a “sample containing a plurality of spatially separated targets.” EX1009, 6:17-35, 10:10-11 (“hybridizing labeled libraries to RNA or DNA molecules contained within the fixed sample”), 11:3-7, Abstract, 1:26-29, 5:8-10, 5:25-31, 9:10-11, 9:21-23; EX1002, ¶235. Curry then identifies a target “by identifying which combination of the multiple libraries hybridize[s] to that target.” EX1009, 6:30-35, 6:22-23 (“The identity of an individual target may be determined by determining which of the libraries hybridize to the target.”), Abstract, 5:8-10, 11:46-50; EX1002, ¶235.

Each of Curry's libraries comprises "probe sets," with multiple "polynucleotide probes that all specifically hybridize to the same target." EX1009, 5:22-24, 6:23-41, 7:46-47; EX1002, ¶¶236-231. Each of Curry's probe sets comprises primary probes that are "*specific for a target*" and "*specifically bind to a particular molecule*," though they "hybridize to different sequences in a target" or "overlap with each other" on a target (so that they are tiled). EX1009, 6:17-31, 7:24-28, 7:32-45; EX1002, ¶¶236-231. Each probe set may contain "up to 1,000 or 10,000 or more probes." EX1009, 7:24-28. Curry's libraries comprise a "different combination of the target-specific probe sets." EX1009, 6:36-41. Thus, each of Curry's libraries comprises different probe sets of primary probes that specifically hybridize to different sequences (*i.e.*, different locations) on the same target. *Id.*

Thus, Fan+Curry discloses producing a plurality of first hybridized complexes (Fan's target-barcode probe complexes modified as in Curry to include probes targeting multiple sequences within a given analyte) within a sample containing a plurality of different nucleic acid targets (Fan's amplified nucleic acids, such as cDNA colonies) by exposing the sample to a plurality of different encoding nucleic acid probes (Fan's barcode probes), such that each of complexes comprises multiple probes located in its different locations. EX1002, ¶¶233-231.

a. Steps (d) and (g)

Under Co-Plaintiffs' interpretation, steps 1(d) and 1(g) are broad enough to encompass a method in which the fluorescently labeled probe is removed. EX1047, 0072-0074. As discussed above under Ground 1, Fan's method removes the fluorescent signaling entity from each of its barcode probe-decoder probe complexes washing before the next round of decoder probe hybridization. EX1005, 2:56-58, 9:25-27, Claim 1; EX1006, 3:14-15, 13:28-30, Claims 1-3; EX1007, 875; EX1002, ¶¶126-132, 142-146. Curry also discloses that its "libraries [of probes] are hybridized successively, measured, then *washed* away to allow the sample to be hybridized to the next library." EX1009, 9:33-35. Thus, Fan+Curry teaches steps 1(d) and 1(g) under Co-Plaintiffs' interpretation. EX1002, ¶¶240-243.

To the extent it is argued that the washing and removal in Fan (and Fan+Curry) do not disclose these "removing" steps, these steps were well known based on a POSA's general knowledge in the art. As confirmed by Dr. Spellman, a POSA's general knowledge in the art included a variety of routine methods for removing or inactivating the fluorescent signal between rounds of sequential hybridization techniques like those in Fan and Fan+Curry, including: "washing, heating, photo-bleaching, displacement..., cleavage, enzymatic digestion, quenching, chemical degradation, bleaching, oxidation, or any combinations thereof." EX1021, ¶[0016]; EX1050, ¶¶[0011], [0097], [00120], [00121], [00164],

[00172], [00180]; EX1002, ¶¶240-243. Photo-bleaching, for example, was known to be fast and effective in inactivating fluorescent signals. EX1050, [0042].

Removing or inactivating fluorescent signals is especially suitable for Fan's probe design because Fan's barcode probes comprise more than one decoder sequence, and decoder probes from the previous hybridization round need not be physically removed before the next hybridization round is performed. EX1005, 8:47-49; EX1006, 12:27-28; EX1002, ¶¶240-243. Thus, Fan+Curry and a POSA's knowledge render obvious inactivating the fluorescent signaling entity from each of Fan's barcode probe-decoder probe complexes by photo-bleaching before the next round of decoder probe hybridization. EX1002, ¶¶240-243.

b. Steps (i) and (j)

As discussed above under Ground 1, Fan (incorporating Gunderson) discloses decoding the identities of the nucleic acid targets by sequential hybridization of labeled decoder probes (*i.e.*, secondary probes) to the barcode probes (*i.e.*, primary probes) hybridized to the targets according to a binary decoding scheme with ON/OFF states, such as the decoding and error checking scheme of Gunderson. EX1005, 8:53-59, 3:36-40, 14:31-48; EX1006, 13:1-5, 4:19-21, 22:5-16; EX1002, ¶¶151-170. To the extent it is argued that Fan (incorporating Gunderson) does not disclose these steps under Grounds 1-3 (whether under Co-Plaintiffs' interpretation or some other construction), the

Fan+Curry combination also discloses this step and renders obvious the challenged claims. EX1002, ¶¶244-253.

Similar to Fan, Curry teaches (1) sequentially hybridizing labeled probes to targets, (2) generating a binary code (codeword) with 1 representing fluorescent signal and 0 representing absence of fluorescent signal, (3) using a “look-up table” to match detected codewords against valid codewords to determine the identity of the target molecules, and (4) applying an error-detecting and correcting Hamming code system to unmatched codewords in order to detect errors, correct certain correctable errors, and discard uncorrectable codewords. EX1009, 11:48-60, Abstract, 6:17-7:13, 5:8-10, 5:17-21 12:40-51; EX1002, ¶¶244-253.

Curry uses a “look-up table” that acts as a codebook listing valid binary codes (*i.e.*, codewords) for each target, in which 1 represents detecting a fluorescent signal from a labeled probe and 0 represents *not* detecting a fluorescent signal. EX1009, 11:50-60, 4:47-53, 14:29-34, Table 1; EX1002, ¶¶246-253.

Curry teaches that after determining “which libraries hybridize to a target... the identity of the target may be determined matching a ‘code’ ... with a code in a look-up table.” EX1009, 4:48-53. Thus, Curry’s method performs a step of matching each of the detected codewords against the set of valid codewords in the codebook and determines a “match” by comparing and determining whether each bit of a produced codeword is the same as each bit in the corresponding position of

a valid codeword in the codebook (look-up table). EX1002, ¶¶246-253. When there is a match (or when an unmatched codeword can be corrected), the Fan+Curry system identifies a target analyte based on the analyte identity assigned to that valid codeword in the codebook (look-up table). EX1002, ¶¶246-253.

Fan+Curry also discloses an error detection or correction system based on Hamming code. Curry describes “an error correction system so that if any one hybridization fails, useful data can still be obtained.” EX1009, 12:40-44. Curry further teaches that the method includes an “an error correcting (7,4) Hamming code, for example.” EX1009, 12:43-44. Hamming code is well-known algorithm from digital information theory that has been applied in other fields. *See, e.g.*, EX1007, 871; EX1002, ¶¶249-253. Curry provides an exemplary scheme in Table 2.

TABLE 2							
	L1p	L2p	L3	L4p	L5	L6	L7
P1	1	1	0	1	0	0	1
P2	0	1	0	1	0	1	0
P3	1	0	0	0	0	1	1
P4	1	0	0	1	1	0	0
P5	0	1	0	0	1	0	1
P6	1	1	0	0	1	1	0
P7	0	0	0	1	1	1	1
P8	1	1	1	0	0	0	0
P9	0	0	1	1	0	0	1
P10	1	0	1	1	0	1	0
P11	0	1	1	0	0	1	1
P12	0	1	1	1	1	0	0
P13	1	0	1	0	1	0	1
TABLE 2-continued							
	L1p	L2p	L3	L4p	L5	L6	L7
P14	0	0	1	0	1	1	0
P15	1	1	1	1	1	1	1

EX1009, Table 2, 12:40-13:6; EX1002, ¶249.

In the above table, libraries L3, L5, L6, and L7 encode the data, and libraries L1p, L2p, L4p encode parity bits for error detection and error correction. *Id.* Curry further teaches that using Curry's Hamming system with three parity bits "[a]ny single FP or FN error can be corrected using this scheme, and the occurrence of two errors can be detected." EX1009, 12:46-49. As Curry teaches, if only one error occurs for a particular detected analyte ("if any one hybridization fails") then this error detection or correction system means "useful data can still be obtained" because single-error detected codewords can be corrected to the one (and only one)

closest matching valid codeword. EX1009, 12:40-49; EX1002, ¶¶250-253. Where two errors occur, this system can still detect the error. *Id.* A POSA would have recognized that in this Fan+Curry system, detecting two errors in an unmatched codeword means the codeword is not correctable and should be discarded.

EX1002, ¶¶250-253. The Fan+Curry system is thus an extended Hamming-based “SECDED” (Single-Error Correction, Double-Error Detection) error detection or correction system of the types disclosed by the 303 Patent. EX1001, 11:12-20.

Thus, Fan+Curry discloses all the elements of claim 1 and renders it obvious. As discussed above in Grounds 2-3, 10x is not aware of objective indicia sharing a nexus with the challenged claims.

C. Claim 2

Fan+Curry renders obvious Claim 2 under Co-Plaintiff’s interpretation based on the same Fan (incorporating Larsson) disclosures addressed above for Grounds 1-3.

Claim 2 is further obvious over Fan+Curry and a POSAs general knowledge in the art. EX1002, ¶¶254-258. Fan’s method detects individual nucleic acid molecules in a cell. EX1005, 4:6-9 (“presented herein are methods ... useful for obtaining sequence information at the level of *individual* nucleic acid molecules”), 9:59-64 (“the methods presented herein have the advantage of virtually limitless numbers of *individually* detected molecules in parallel and in situ”); EX1006, 5:16-

18, 14:22-26. Fan discloses detecting the fluorescent signals generated in its methods in situ by “using *any suitable imaging and detection technologies* which allow for detection of individual amplicons in situ.” EX1005, 11:11-15; EX1006, 16:29-17:2. A POSA would have known that fluorescence microscopy can achieve a resolution of about 200 nm (better than 300 nm resolution). EX1028, 967. Thus, Fan’s teaching of using “any suitable imaging and detection technolog[y],” which includes fluorescence microscopy, suggests using an imaging technology with a resolution “better than 300 nm,” as claimed. EX1002, ¶¶254-258. *Beckson Marine, Inc. v. Nfm, Inc.*, 292 F.3d 718, 727 (Fed. Cir. 2002) (“obviousness does not require the prior art to reach expressly each limitation exactly. Rather, obviousness may render a claimed invention invalid where the record contains a suggestion or motivation to modify the prior art teaching to obtain the claimed invention.”). Moreover, the POSA’s general knowledge included well-known microscopy and imaging techniques, like super-resolution microscopy, which achieved resolutions better than 300 nm. EX1002, ¶¶72-73, 254-258; EX1028, 967; EX1031, 76; EX1029, 322-323; EX1052, Abstract, 498; EX1032, 1104; Section VI.B.

A POSA combining Fan+Curry would have had a reasonable expectation of successfully detecting a fluorescent signal at “a resolution better than 300 nm” because Fan teaches using art-recognized detection and imaging techniques, which include superresolution microscopy. EX1002, ¶¶254-258; EX1049, 24:21-37,

26:9-12, 27:5-9; EX1002, ¶¶254-258. Accordingly, claim 2 would have been obvious over Fan+Curry under Co-Plaintiffs' interpretation.

D. Claims 3 and 6

Fan discloses all of the limitations of claims 3 and 6. *See* Section IX.D, G. A POSA would have been motivated to combine Fan and Curry with a reasonable expectation of success for at least the same reasons discussed above for claim 1 (Section XII.A). EX1002, ¶¶223-231, 259. Accordingly, Fan+Curry render obvious claims 3 and 6. *Id*

E. Claim 4

Fan+Curry renders obvious Claim 4 based on the same Fan (incorporating Larsson) disclosures addressed above for Grounds 1-3.

If it is argued that Fan (incorporating Larsson) or Fan+Curry do not render obvious this claim based on those disclosures, then this claim was also obvious in view of Fan+Curry and a POSA's general knowledge. EX1002, ¶¶260-262; *Beckson Marine*, 292 F.3d at 727.

Fan discloses using “[m]ethods for permeablization and fixation of cells and tissue samples” that were “well-known in the art.” EX1005, 10:66-11:6; EX1006, 16:19-23. Biological samples (*e.g.*, cells) were routinely fixed on slides or coverslips before they could be visualized under a microscope. EX1002, ¶¶260-262; EX1008, 0004 (placing tissue sections on “Starfrost microscope *slides*”);

EX1050, ¶¶[00167]-[00168], [00208] (“cells and tissues fixed on the bottom *coverslip*”). Moreover, as discussed above in Section VI, the POSA would have generally known a variety of routine methods for fixing cells on glass substrates, including glass slides and coverslips. EX1002, ¶¶260-262; EX1020, 38:30-52; EX1049, 26:59-61. Thus, using a coverslip in lieu of a glass slide is nothing more than “the simple substitution of one known element for another.” *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 415-421 (2007). Accordingly, claim 4 would have been obvious over Fan+Curry. EX1002, ¶¶260-262.

F. Claim 5

Fan+Curry renders obvious Claim 5 based on the same Fan (incorporating Larsson) disclosures addressed above for Grounds 1-3.

As discussed above for claim 2 (Ground 4), a POSA would have been motivated to use superresolution microscopy to detect the fluorescent signal at a resolution better than 300 nm with a reasonable expectation of success. STORM and (F)PALM each uses a light microscope. EX1002, ¶¶263-264. Accordingly, claim 5 would have been obvious over Fan+Curry. *Id.*

G. Claim 7

Fan+Curry renders obvious Claim 7 based on the same Fan disclosures addressed above for Grounds 1-3.

If it is argued that Fan (incorporating Larsson) or Fan+Curry do not render obvious this claim based on those disclosures, then this claim was also obvious in view of Fan+Curry and a POSA's knowledge. EX1002, ¶¶265-268.

As discussed above, Fan discloses its methods “make use of hybridization, amplification, washing and detection within individual cells” (*i.e.*, washing takes place *before* detection). EX1005, 10:62-64, 9:25-27, 27:21-37; EX1006, 16:16-17, 13:28-30; EX1002, ¶¶265-268; EX1024, 81. Similarly, Curry discloses that detection assays involve “contacting a labeled target-specific probe ... with a sample” and after “unbound probe is removed, e.g., by washing, the signal emanating from [the] probe's label is detected.” EX1009, 1:5-10. *See also id.*, 10:8-21; EX1007, 875. Thus, a POSA would have been motivated to remove the unhybridized readout nucleic acid probes prior to detecting a fluorescent signal in step (c). Accordingly, Fan+Curry render obvious claim 7. EX1002, ¶¶265-268.

H. Claim 8

Fan+Curry also render obvious claim 8. EX1002, ¶¶269-274.

The 303 patent discloses that “codewords may be assigned within a *code space* ... using *an error-detection or an error-correcting system*, such as a Hamming system ... or an extended Hamming system or a SECDED system (single error correction, double error detection.)” EX1001, 12:46-51.

As discussed above for claim 1 (Ground 4), Curry discloses using a Hamming system, including the same extended Hamming-based “SECDED” error detection or correction system disclosed in the 303 Patent. EX1009, 12:40-42; EX1002, ¶¶244-253, 269-274. Curry thus discloses that its valid codewords form an error-checking or an error-correcting code space. EX1002, ¶¶269-274.

I. Claims 9-11

Fan+Curry also render obvious claims 9-11. EX1002, ¶¶275-277.

As discussed above for claim 1 (Ground 4), Fan+Curry discloses using a Hamming system, including using an extended Hamming-based “SECDED” error detection or correction system of the same type disclosed by the 303 Patent. EX1009, 12:40-42; EX1002, ¶¶244-253. Curry states that “[a]ny single FP [(i.e., false positive)] or FN [(i.e., false negative)] error can be corrected using this scheme, and the occurrence of two errors can be detected.” EX1009, 12:46-48; EX1002, ¶¶275-277. Thus, Curry teaches that one of the codewords is not matched with one of the valid codewords in the codebook when one of the codewords has one error (claims 9-10) or two errors (claim 11).

J. Claim 12

Fan+Curry render obvious claim 12. EX1002, ¶¶278-281.

As discussed above for claim 1, Curry discloses decoding and error-checking schemes using a “look-up table.” EX1009, Table 1-2; EX1002, ¶¶244-

253, 278-281. Curry uses Table 1 as a codebook of valid codes (codewords) for error-checking. *Id.* Curry discloses that “the identity of the target may be determined matching a ‘code’ which indicates which libraries hybridized to the target (e.g., 1010), with a code in a look-up table.” EX1009, 4:47-53. As such, to check for errors, Curry compares values of *each of the positions* of a generated code with that of the valid code in the look-up table. *Id.* Thus, a POSA would have been motivated to compare the values at each of the positions of the codewords (codes) generated by sequential hybridization to each of the positions of the valid codewords (codes) in the look-up table in Fan and Curry’s method with a reasonable expectation of success. *Id.*

XIII. DISCRETIONARY DENIAL IS NOT APPROPRIATE HERE

A. The Petition satisfies 35 U.S.C. § 325(d)

The Board’s two-part framework set forth in *Advanced Bionics* does not apply here because neither Fan, Larsson, nor Curry was previously presented to the Office by the Applicant, let alone asserted by the Examiner. EX1004; *Advanced Bionics, LLC v. Med-El Elektromedizinische Geräte GmbH*, IPR2019-01469, Paper 6, at 8-9 (P.T.A.B. Feb. 13, 2020) (precedential). In fact, there were no art-based rejections. Thus, this Petition satisfies 35 U.S.C. § 325(d).

B. *Fintiv* does not support discretionary denial under 35 U.S.C. § 314(a)

The *Fintiv* factors do not support discretionary denial. *Commscope Technologies LLC v. Dali Wireless, Inc.*, IPR2022-01242, Paper 23 (P.T.A.B. Feb. 27, 2023) (precedential); *Apple Inc. v. Fintiv, Inc.*, IPR2020-0019, Paper 11, at 6 (P.T.A.B. Mar. 20, 2020) (precedential).

Here, the 303 patent district court litigation is still in the early stages and the parties have not significantly invested in the litigation. The trial date for the 303 patent is currently projected to be November 12, 2024—approximately four months before a Final Written Decision would be reached in this IPR. *See* EX1051, 3. Thus, much of the substantive briefing and discovery in this IPR may be complete before the projected trial date.

Moreover, the Petition demonstrates compelling unpatentability bases for multiple independent reasons. “[W]hen determining whether there is a compelling unpatentability challenge, the Board evaluates whether the Petition presents challenges ‘in which the evidence, if unrebutted in trial, would plainly lead to a conclusion that one or more claims are unpatentable by a preponderance of the evidence.’” *Commscope* at 3; EX1029, 4. As shown herein, under Co-Plaintiffs’ interpretations, all challenged claims are “highly likely” anticipated and/or obvious over the prior art. *Commscope* at 4.

XIV. MANDATORY NOTICES (37 C.F.R. § 42.8(A)(1))

A. Real party-in-interest 37 C.F.R. § 42.8(b)(1)

Petitioner lists 10x Genomics, Inc. as its real party in interest.

B. Related Matters (37 C.F.R. § 42.8(b))

The 303 patent was asserted in a counterclaim in *10x Genomics, Inc. and President and Fellows of Harvard College v. Vizgen, Inc.*, Case No. 1:22-cv-00595 (D.Del.), filed on August 30, 2022.

C. Lead and back-up counsel (37 C.F.R. § 42.8(b))

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D. Notice of service information (37 C.F.R. §42.8(b))

Please direct all correspondence regarding this Petition to counsel at the above addresses and PTAB@sternekessler.com. 10x consents to service by email at the addresses above.

E. Procedural statements

This Petition is filed in accordance with 37 C.F.R. §42.106(a). Concurrently filed herewith are a Power of Attorney and Exhibit List under 37 C.F.R. §42.10(b), respectively. The required fee is paid through Deposit Acct. No. 19-0036 (Customer ID No. 45324). The Office is authorized to charge any fee deficiency, or credit any overpayment, to Deposit Acct. No. 19-0036 (Customer ID No. 45324).

XV. CONCLUSION

Claims 1-12 should be canceled.

Respectfully submitted,

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CERTIFICATE OF WORD COUNT (37 C.F.R. § 42.24(d))

1. This Petition for *Inter Partes* Review complies with the type-volume limitation of 14,000 words, comprising 13,981 words, excluding the parts exempted by 37 C.F.R. § 42.24(a)(1).

2. This Petition for *Inter Partes* Review complies with the general format requirements of 37 C.F.R. § 42.6(a) and has been prepared using Microsoft® Word 2016 in 14-point Times New Roman font.

Respectfully submitted,

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CERTIFICATE OF SERVICE (37 C.F.R. §§ 42.6(e), 42.105(a))

I certify that the above-captioned **PETITION FOR *INTER PARTES***
REVIEW FOR U.S. PATENT NO. 11,098,303 and associated Exhibits 1001-
1060 were served in their entireties upon the Patent Owner on August 30, 2023, via
FedEx® Express at the following address:

86110 - Wolf, Greenfield & Sacks, P.C. (Harvard)
600 Atlantic Avenue
Boston, MA 02210-2206
UNITED STATES
Patent Owner's Correspondence Address of Record for U.S. Patent No.
11,098,303

Respectfully submitted,

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