

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10X GENOMICS, INC.
Petitioner,

v.

BRUKER SPATIAL BIOLOGY, INC.
Patent Owner.

Case No. IPR2023-01298
U.S. Patent No. 11,377,689 B2

REPLY DECLARATION OF PAUL T. SPELLMAN, PH.D.

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10x EX1055
10x v. Bruker
IPR2023-01298

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I, Paul T. Spellman, hereby declare as follows:

I. INTRODUCTION

1. I am over the age of 18 and competent to make this declaration.
2. I previously provided a declaration dated August 16, 2023—my first declaration (EX1002)—in this proceeding in support of 10x Genomics, Inc.’s (“10x”) Petition for the above-captioned *inter partes* review (IPR). The opinions I expressed in my first declaration remain unchanged. My professional background and qualifications discussed in ¶¶11-22 of my first declaration also remain unchanged. I am being compensated for my time in connection with this IPR.
3. I understand that this declaration accompanies 10x’s Reply to Bruker Spatial Biology’s (Bruker) Patent Owner Response.¹
4. In preparing this declaration, I have reviewed Dr. Sherman’s declaration and each of the documents cited therein, and each of the documents cited in this declaration in light of general knowledge in the art before February 12, 2018. I have relied upon my experience, education, and knowledge in the relevant art. In formulating my opinions, I have also considered the viewpoint of a person

¹ I understand that Bruker acquired NanoString, and now Bruker is the owner of the 689 patent.

of ordinary skill in the art (“POSA”), which I defined in my first declaration (EX1002, ¶¶24-25) prior to February 12, 2018.

II. LIST OF DOCUMENTS I CONSIDERED IN FORMULATING MY OPINIONS

5. In formulating my opinions, I have considered all the references and documents cited herein and in my first declaration, including those listed below.

Paper/Exhibit No.	Description
14	Decision on Institution (“DOI”)
1001	U.S. Patent No. 11,377,689 to Beechem <i>et al.</i> , issued July 5, 2022 (“689 patent”)
1002	Declaration of Paul Spellman, Ph.D.
1003	<i>Curriculum Vitae</i> of Paul Spellman, Ph.D.
1004	File History of U.S. Patent No. 11,377,689
1005	International Patent Application Publication No. WO 2017/019456 A2 to So <i>et al.</i> , published February 2, 2017 (“So-456”)
1006	International Patent Application Publication No. WO 2021/133849 A1 to Chell <i>et al.</i> , published July 1, 2021 (“Chell”)
1007	“Explore Illumina sequencing technology,” Illumina, Inc., accessible at https://www.illumina.com/science/technology/next-generation-sequencing/plan-experiments/paired-end-vs-single-read.html
1008	“Visium Spatial Gene Expression Reagent Kits for FFPE,” 10x Genomics, Inc., Document No. CG000407, Rev. D, 2022

Paper/Exhibit No.	Description
1009	U.S. Patent Application No. 16/272,487, filed February 11, 2019
1010	U.S. Provisional Application No. 62/771,212, filed November 26, 2018
1011	U.S. Provisional Application No. 62/629,180, filed February 12, 2018
1012	File History of U.S. Patent Application No. 16/272,487
1013	Merritt, <i>et al.</i> , “Multiplex digital spatial profiling of proteins and RNA in fixed tissue,” <i>Nature Biotechnology</i> 38:586-599 (May 2020)
1016	Stahl <i>et al.</i> , “Visualization and analysis of gene expression in tissue selections by spatial transcriptomics,” <i>Science</i> 353(6294):78-82 (July 1, 2016), with attached Supplementary Materials
1017	International Patent Application Publication No. WO 2012/140224 A1 to Frisen <i>et al.</i> , published October 18, 2012
1018	Spatial Transcriptomics Workflow, accessible at https://spatialtranscriptomics.com/workflow/
1019	Kivioja <i>et al.</i> , “Counting absolute numbers of molecules using unique molecular identifiers,” <i>Nature Methods</i> 9(1):72-76 (January 2012), with attached Supplementary Information
1020	NanoString Technologies, Inc.’s Initial Infringement Contentions with Attachment A and Exhibit 1, <i>10x Genomics, Inc. et al. v. NanoString Technologies, Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), filed November 23, 2022
1024	Joint Claim Construction Statement with Exhibits A through C, <i>NanoString Technologies, Inc. v. 10x Genomics, Inc.</i> , Case No. 1:22-cv-01375 (D. Del.), filed August 4, 2023

Paper/Exhibit No.	Description
1025	Duncan <i>et al.</i> , “A method for detecting single mRNA molecules in <i>Arabidopsis thaliana</i> ,” <i>Plant Methods</i> 12:13 (2016)
1026	Lieben, L., “Spatial transcriptomics in plants,” <i>Nature Reviews Genetics</i> 18:394 (2017)
1027	Jemt <i>et al.</i> , “An automated approach to prepare tissue-derived spatially barcoded RNA-sequencing libraries,” <i>Scientific Reports</i> 6:37137 (2016)
1028	Asp <i>et al.</i> , “Spatial detection of fetal marker genes expressed at low level in adult human heart tissue,” <i>Scientific Reports</i> 7:12941 (2017)
1030	Fantini <i>et al.</i> , “Assessment of antibody library diversity through next generation sequencing and technical error compensation,” <i>PLOS One</i> 12(5):e0177574 (2017)
1031	U.S. Patent Application Publication No. 2007/0172873 to Brenner <i>et al.</i> , published July 26, 2007 (“Brenner 873”)
1032	U.S. Patent No. 9,567,645 to Fan <i>et al.</i> , issued February 14, 2017 (“Fan 645”)
1033	U.S. Patent No. 8,835,358 to Fodor <i>et al.</i> , issued September 16, 2014 (“Fodor 358”)
1034	International Patent Application Publication No. WO 2016/138496 A1 to Fodor <i>et al.</i> , published September 1, 2016 (“Fodor 496”)
1035	U.S. Patent No. 9,902,950 to Church <i>et al.</i> , issued February 27, 2018 (“Church 950”)
1036	U.S. Patent Application Publication No. 2016/0122753 to Mikkelsen <i>et al.</i> , published May 5, 2016 (“Mikkelsen 753”)

Paper/Exhibit No.	Description
1037	“An Introduction to Next-Generation Sequencing Technology,” Illumina, Inc. (2017), accessible at https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf
1056	Transcript of the Deposition of David Sherman, Ph.D., October 30, 2024
2006	Declaration of Dr. David H. Sherman, Ph.D.
2007	Transcript from Deposition of Paul Spellman on August 5, 2024
2008	U.S. Patent Application Publication No. US 2016/0194701 A1 (“Application No. 14/946,386” or “’386 Application”)
2009	U.S. Patent Application Publication No. US 2010/0015607 A1 (“’607 Publication”)
2010	U.S. Patent Application Publication No. US 2011/0086774 A1 (“’774 Publication”)
2012	U.S. Patent Application Publication No. US 2011/0245111 A1 (“’111 Publication”)

III. CLAIM CONSTRUCTION

6. In my first declaration, I applied Bruker’s interpretations according to its infringement positions. EX1002, ¶116; EX1022.

7. I understand that in its decision granting institution of this IPR, the Board interpreted the term “nucleic acid probes” to mean “an oligonucleotide comprising a target binding domain that binds to one or more target analytes, and

is modified after collection to comprise a location-identifying nucleic acid sequence for use in identifying the location of the target analyte.” DOI, 23. I understand from Dr. Sherman’s declaration that he agrees with the Board’s construction “[t]o the extent that the Board’s construction refers to the use of an extension reaction to encode spatial information.” EX2006, ¶27. I apply Dr. Sherman’s construction in this declaration. I refer to “a location-identifying nucleic acid sequence for use in identifying the location of the target analyte” as a “spatial barcode” in this declaration.

8. I understand that the Board also interpreted “collecting the nucleic acid probes” to mean “releasing all or a portion of the nucleic acid probe bound to the at least one target analyte through application of a force followed by retrieval of the released probe or portion of the probe.” DOI, 24. I understand that Dr. Sherman agrees with this construction. EX2006, ¶28. I apply the Board’s construction in this declaration.

9. The opinions I provided in my first declaration remain the same under these constructions.

IV. TECHNICAL TERMINOLOGY

10. I am using the same terms as in the manner set forth in my first declaration. EX1002, ¶¶40-43.

V. SO-456 ANTICIPATES CLAIMS 1-8, 11-23, AND 26-30.

11. In my first declaration, I opined that So-456 anticipates claims 1-8, 11-23, and 26-30 because So-456 teaches every element of the claims, arranged as claimed. EX1002, ¶¶117-216. I offer the following additional comments in response to Dr. Sherman's declaration.

12. Dr. Sherman disputes So-456's disclosure arguing that So-456 does not teach a method that (1) treats a tissue sample as required by claim steps 1(a) and 16(a); (2) collects the nucleic acid probes as required by claim steps 1(b)-(c) and 16(c)-(d); (3) includes an extension reaction that incorporates a nucleic acid sequence that identifies a location, as required by claim steps 1(d)-(e) and 16(e)-(f); and (4) forms ligated probes, as required by claim step 16(b). EX2006, ¶35. As I explain below, I disagree with Dr. Sherman's opinions.

A. So-456 teaches a method that includes treating a tissue sample as claimed.

13. I opined in my first declaration that So-456 discloses a method that includes treating a tissue sample to facilitate binding of the nucleic acid probes to the target analytes as required in Steps 1(a) and 16(a) of claims 1 and 16, respectively. EX1002, ¶¶125-127, 164-165.

14. Dr. Sherman, however, argues that So-456's paragraphs 152 and 153 "do not describe a step of treating a tissue sample prior to applying probes for contacting, for the purpose of facilitating binding." EX2006, ¶51. According to Dr.

Sherman, these paragraphs describe a sectioning step (Step 210) and then in situ biochemistry, such as reverse transcription or amplification, for subsequent manipulation (Step 215). EX2006, ¶¶50-52. I disagree with Dr. Sherman.

15. As I explained in my first declaration, So-456's paragraphs [00152] and [00153] describe Figure 2's Steps 210 and 215, respectively, both of which occur *before* the nucleic acid probes bind to the target analytes in the tissue sample. EX1002, ¶¶125-127, 164-165; EX1005, ¶¶[00152], [00153], FIG. 2. Thus, the disclosures in paragraphs [00152] and [00153] describe treatments that facilitate binding of the nucleic acid probes to the target analytes. EX1002, ¶¶125-127, 164-165.

16. Further, as I previously discussed, So-456's paragraph 152 teaches that in Step 210 the "tissue sample is prepared for analysis." EX1002, ¶¶125, 164; EX1005, ¶¶[00152], FIG. 2. A POSA would understand that preparing a sample is not limited to just sectioning the tissue samples. Instead, it includes any treatment that prepares a tissue sample for analysis, including a treatment that facilitates binding of the nucleic acid probes to the target analytes. EX1002, ¶¶125-127, 164-165; EX1005, ¶¶[00152]. Paragraph 152 mentions "sectioning" in the following context: "*In some embodiments*, the tissue sample is a FFPE tissue sample that is sectioned onto a slide." EX1005, ¶¶[00152] (emphasis added). A POSA would understand this disclosure to mean that in some instances when a tissue sample is

an FFPE sample, it is sectioned onto a slide. A POSA would further understand that paragraph 125 does not limit sample preparation in Step 210 to only sectioning the tissue samples.

17. I previously also discussed that So-456's paragraph 153 teaches that Step 215 performs "in situ biochemistry" that facilitates binding of the nucleic acid probes to target analytes in a tissue sample. EX1002, ¶¶126, 164; EX1005, ¶[00153], FIG. 2. Paragraph 153 lists a reverse transcriptase reaction, and an amplification reaction as "some embodiments" of *in situ* biochemistry techniques. EX1005, ¶[00153]. A POSA would understand this disclosure in paragraph [00153] to mean that in some instances Step 215 includes a reverse transcription reaction and/or amplification reaction. A POSA would *not* understand paragraph [00153] as precluding other *in situ* biochemistry techniques that facilitate binding of the nucleic acid probes to target analytes.

18. Moreover, because the nucleic acid probes in So-456's method of Figures 8-9 bind to the target analytes and are processed in the subsequent steps, the method *must* treat the tissue sample to facilitate binding of the probes to the target analytes. EX1002, ¶¶125-127, 164-165; EX1005, ¶¶[00182]-[00183], FIGs. 8, 9.

19. Dr. Sherman also argues that So-456 does not teach the claimed treatment because it does not provide a similar disclosure as the 689 patent.

EX2006, ¶¶44-53. The 689 patent's disclosures that Dr. Sherman cites (such as, Figure 21B, Examples 7-9) are limited to an FFPE tissue sample. EX2006, ¶¶46 (“[a]ll three examples analyze an FFPE sample....”), 45 (“the method begins with the use of a formalin-fixed paraffin-embedded (FFPE) block.”). However, claims 1 and 16, as well as So-456's methods, are *not* limited to analyzing only an FFPE tissue sample. EX1001, 145:57-59, 148:1-4. On the contrary, So-456 explains that its methods use tissue samples of any type, including an FFPE tissue sample, a fresh tissue sample, or a frozen tissue sample. EX1005, ¶[00152].

20. Moreover, the treatment used to facilitate probe binding varies depending on the type of tissue sample, sample's prior treatments, and the objective of the experiment. Appropriate treatments include, depending on the tissue sample, prior treatment, and experiment objective, for example, thawing, sectioning, immobilization, de-paraffinization, de-crosslinking, and/or permeabilization. Dr. Sherman agrees that treatments to facilitate binding of the nucleic acid probes with the target analytes depend on the tissue sample type and that permeabilization is one such treatment. EX1056, 50:6-52:2; EX2006, ¶48 (agreeing that permeabilization meets the limitation of treating a tissue sample; “‘there are reagents, for example buffers and other reagents, that are known in the art to be useful in facilitating binding of a protein or a nucleic acid in a sample,’ and so it would be ‘well within the purview of a person of ordinary skill in the art’

to treat a tissue sample.”). Further, the same sample type can be treated in multiple ways.

21. Therefore, I maintain my opinion that So-456 teaches a method that includes treating a tissue sample as required by the claims.

B. So-456 teaches a method that includes forming ligated probes as claimed.

22. As I opined in my first declaration, So-456 teaches a method that includes forming ligated probes by ligating nucleic acid probe pairs that are bound to the identical molecule of the target analyte. EX1002, ¶¶128-130, 168-169. Dr. Sherman argues that So-456 does not teach a method that includes “forming ligated probes, as required by claim 16(b).” EX2006, ¶66. I disagree with Dr. Sherman.

23. Dr. Sherman argues that the term “ligating the probes” in the 689 patent is “directed specifically to ligating *adjacent, nonoverlapping* probes with, for example, a nick repair reaction.” EX2006, ¶68 (emphasis added). Dr. Sherman however ignores the 689 patent’s claim language and specification.

24. The 689 patent claims are not limited to ligating only the probes that are adjacent and non-overlapping. EX1001, 145:51-147:11, 148:5-10. For example, claim 16 only requires ligating nucleic acid probes, without limiting the probes to only those that are adjacent and non-overlapping. EX1001, 147:54-148:11.

25. Similarly, as discussed in my first declaration, the 689 patent specification does not limit ligation of the probe pairs to ligating only the *adjacent*, non-overlapping probes. EX1002, ¶128; EX1001, 25:66-26:6, 6:66-7:4, 7:34-40, 9:48-54, 10:19-24, 30:59-65, 68:66-69:3, 70:40-44. Instead, the specification explicitly discloses that ligation involves performing a gap extension reaction and a nick repair reaction² when the probes are *non-adjacent* and non-overlapping. EX1002, ¶128; EX1001, 25:66-26:6, 6:66-7:4, 7:34-40, 9:48-54, 10:19-24, 30:59-65, 68:66-69:3, 70:40-44. For example, the 689 specification states:

Alternatively, in the aspect in which the first and the second nucleic acid probe hybridize to the identifier oligonucleotide such that the first and the second nucleic acid probes are *not adjacent and are not overlapping*, the method comprises *ligating* the first and the second nucleic acid probes together, for example, *by performing a gap extension reaction and a nick repair reaction, such that the first and the second nucleic acid probes are ligated together*.

EX1001, 25:66-26:6 (emphasis added); EX1002, ¶128.

² Dr. Sherman testified that “nick repair includes the ligation.” EX1056, 56:23-57:3.

26. Thus, I maintain my opinion that ligating probes in the claims includes ligating probe pairs that are not adjacent and not overlapping by extending one probe of the pair using a DNA polymerase enzyme (gap extension) and ligating the extension product to the other probe of the pair using a ligase enzyme (ligation), as the 689 patent so clearly discloses. EX1002, ¶¶128-130, 168-169. Dr. Sherman agrees that gap extension/ nick repair involves extending one probe of the pair using a polymerase followed by ligating the extension product to the other probe of the pair using a ligase. EX1056, 55:9-57:3.

27. As I explained previously, So-456's Figure 9 method includes an "extension/ligation reaction" (Step 815) where a pair of non-adjacent, non-overlapping probes are ligated by performing an "extension" followed by a "ligation." EX1002, ¶¶104, 128-130, 168-169; EX1005, ¶[00182]. In this step, one probe from the probe pair bound to the target analyte is extended by a DNA-dependent DNA polymerase using the target analyte as a template and the resulting extension product is ligated to the second probe from the probe pair by a ligase. Thus, Figure 9's "extension/ligation reaction" used for ligation of the probe pairs bound to the target analytes is a different name for the 689 patent's "gap extension reaction and [] nick repair reaction." Accordingly, Figure 9's method in So-456 includes ligating probe pairs as required in the claims. EX1002, ¶¶128-130, 168-169; EX1005, ¶[00182].

28. Dr. Sherman argues that Figure 9's extension/ligation step (Step 815) does not meet the ligation of probes as claimed because it is "not exclusively a ligation reaction, but rather an extension/ligation reaction" that "does not ligate the probes together." EX2006, ¶70. I disagree with Dr. Sherman. As I explain above (and in my first declaration), the ligation of probes in the claims includes performing an extension reaction followed by a ligation reaction. EX1002, ¶¶128-130, 168-169; EX1005, ¶[00182]. And this is exactly what Figure 9's extension/ligation step does, and accordingly meets the ligation step of the claims. EX1002, ¶¶128-130, 168-169; EX1005, ¶[00182].

29. Dr. Sherman admits in his declaration that Figure 9's method includes an extension/*ligation* reaction, where the extension product is ligated with the probe. EX2006, ¶¶70 ("[S]tep 815 includes an extension reaction that captures the oligo of interest, and does not ligate the probes together. Rather, the referenced ligation is the extension product with the probe."), 71 ("So-456's 'extension/ligation'").

30. But during deposition, Dr. Sherman took a contradictory position that while Figure 9's method at Step 815 includes extension by DNA polymerase, it does not include ligation at all. EX1056, 34:6-35:22, 35:23:36-14 ("There's no ligation. Ligation has a very specific meaning – they use the word 'ligation,' but it's not a ligation because there is no ligase."). Dr. Sherman's new position that

Figure 9's Step 815 does not include ligation is also inconsistent with his admission that the ligated probes (950) captured in Step 825 include segments 935 and 945 of the probe pairs that were bound to the target analytes in Step 810 and underwent extension/ligation in Step 815. EX1056, 44:13-45:5.

31. Thus, Dr. Sherman's new position that there is no ligation in Figure 9's extension/ligation step (Step 815) is not only contradictory to his original opinion, it is also inconsistent with his position that the captured ligated probe in Step 825 contains the same segments as the original ligated probe. And it is also inconsistent with and contradictory to the disclosure of the 689 patent itself. EX1001, 25:66-26:6; EX1002, ¶128. Moreover, Dr. Sherman's new position cannot undermine So-456's express disclosure that Figure 9's extension/ligation step (Step 815) includes ligation as required in the claims. EX1005, ¶[00182], FIG. 9; EX1002, ¶¶128-130, 168-169.

32. Dr. Sherman also attempts to distinguish So-456's "extension/ligation" step from the 689 patent's "gap extension/nick repair" ligation by arguing that the 689 patent's "gap extension is not meant to capture a meaningful extension product, but correct for the possibility that the probes are too far apart to be ligated directly." EX2006, ¶71. Whereas, according to Dr. Sherman, "So-456's extension/ligation is an intentional step designed to create the template for isothermal amplification by specifically capturing the mutation to be studied

with the extension reaction.” EX2006, ¶71. Given the claim scope, this is a meaningless distinction. The claim steps do not require an intent, nor do they exclude creating a ligation product that includes meaningful data. EX1001, 145:51-147:11, 147:54-148:47.

33. Moreover, as I explain above (and in my first declaration), So-456’s extension/ligation reaction and the 689 patent’s gap extension/nick repair reaction involve the same steps: hybridizing non-adjacent probe pairs to a target analyte, extending one of the probes, and ligating the extension product to the other probe. EX1005, ¶¶109, 182, 183; EX1002, ¶¶128-130. Thus, the extension/ligation and gap extension/nick repair are the *same reactions* that use the *same types of extension enzyme* (DNA-dependent DNA polymerase) and the *same types of ligation* (DNA ligation) mediated by DNA ligases. There is no difference in the end product of So-456’s extension/ligation reaction and the 689 patent’s gap extension/ligation reaction.

34. Therefore, I maintain that So-456 teaches a method that includes forming ligated probes by ligating nucleic acid probe pairs that are bound to the identical molecule of the target analyte as claimed. EX1002, ¶¶128-130, 168-169.

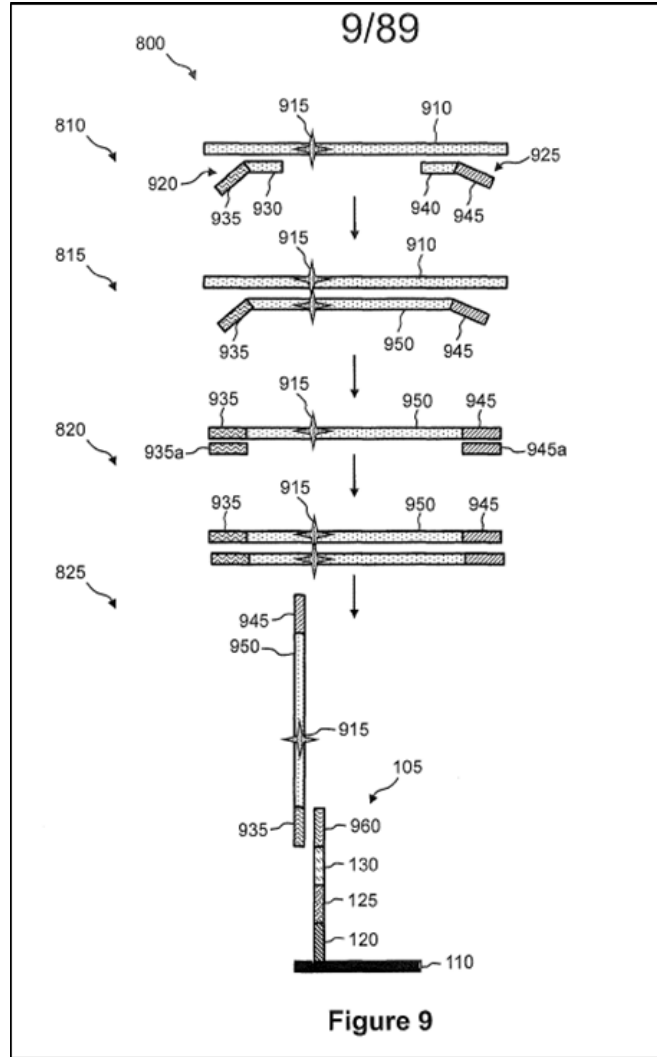
C. So-456 teaches a method that collects nucleic acid probes as claimed under Bruker's construction.

35. I explained in my first declaration that under Bruker's interpretation So-456's method includes collecting the nucleic acid probes as required by claim steps 1(b)-(c) and 16(c)-(d). EX1002, ¶¶131-137, 166-169.

36. Dr. Sherman in response argues that So-456's method does not collect the nucleic acid probes because instead of collecting the original ligated probes (that were bound to the target analytes), So-456's method *only* collects their amplified copies that were never bound to the target analyte. EX2006, ¶¶37-43. I disagree with Dr. Sherman.

37. As I explained in my first declaration, So-456's method collects *both* the original ligated probes and their amplified copies. EX1002, ¶¶131-137, 166-169; EX1005, ¶¶[00182], [00183], FIGs. 8, 9. For example, I discussed in my first declaration that So-456's method of Figures 8-9 includes "capturing *whole* ligated probes (formed by ligating the primary probe pairs)." EX1002, ¶¶137; *see also* EX1002, ¶169 ("So-456's method includes collecting *whole* ligated probes that were previously formed in the ligation"), 104 ("captured ligated products (950)"), 135 ("amplicons (950) (i.e., ligated probes of the claim)"). I also confirmed during my deposition that "the 950 [molecule] that is ligated is amongst the 950s that bind to 960 at the capture oligo." EX2007, 117:5-7.

38. Further, So-456's Figure 9 included in my first declaration—which Dr. Sherman completely ignores when discussing the collecting steps 1(b)-(c) and 16(c)-(d)—makes clear that the original ligated probes are collected along with their amplified copies. EX1002, ¶¶131-137, 166-169; EX1005, FIG. 9. Figure 9 (reproduced below) details Figure 8-9's method and labels both the original ligated probes (produced in Step 815) and their amplified copies (produced in Step 820) as molecule "950."



EX1005, FIG. 9. The original ligated probe 950 and its amplified copies 950 are identical molecules depicted as having the same features (935, 915, and 945).

39. Figure 9 also shows that after Step 815, the original ligated probe (molecule 950) is *released* from the target analyte (molecule 915). EX1005, FIG. 9; EX1002, ¶104. Next, in Step 820, the released original ligated probes (molecule 950) bind to amplification primers 935a and 945a for isothermal amplification and are amplified to generate identical copies (molecule 950). EX1005, ¶¶[00182], [00180]; EX1002, ¶¶104, 134, 169.

40. Following amplification, in Step 825, molecule 950—which is *both* the released original ligated probes and their amplified copies—are captured on the immobilized capture probes. EX1005, ¶¶[00180]-[00182], FIGs. 1A-B, 8, 9. Dr. Sherman also agrees that the molecule 950 captured in Step 825 has the same segments as the original ligated probe 950. EX1056, 44:13-45:5. Thus, So-456's method collects both the original ligated probes and their amplified copies for use in its subsequent steps. EX1002, ¶¶131-137, 166-169. So-456 does not disclose any method for removing the original ligated probes before collection in Step 825, it has no reason to remove the original ligated probes as they are functionally identical to the copies. If there were some reason to remove the original molecules, So-456 would have to explicitly instruct a POSA to use methods to remove the originally ligated probes.

41. Therefore, I maintain that So-456 discloses a method that collects the nucleic acid probes as required by claims 1(b)-(c) and 16(c)-(d) under Bruker's construction. EX1002, ¶¶131-137, 166-169.

D. So-456 teaches a method that includes the extension steps as claimed.

42. I opined in my first declaration that So-456 teaches the extension steps required in Steps 1(d)-1(e) and 16(e)-16(f) of claims 1 and 16. EX1002, ¶¶138-152, 170-184. Specifically, I explained that So-456's method in Figures 8-9 includes an extension reaction that is inherently bidirectional and incorporates a spatial barcode into the collected ligated probes. EX1002, ¶¶138-152, 170-184.

43. I explained in my first declaration that Figure 9's method includes an extension reaction and an amplification reaction after Step 825. EX1002, ¶¶104, 140-152, 170-184. Figure 2 details the overall workflow of So-456's methods, which includes preparing a sequencing library (Step 225), sequencing the library (Step 230), and analyzing the sequence data to decode the spatial information of the target analytes (Step 245). EX1005, FIG. 2, ¶¶[00151]-[00158]; EX1002, ¶¶140-152, 173-184.

44. Following Figure 2's workflow, Figure 9's method after Step 825 includes sequencing library preparation to generate a library of molecules containing sequences of the captured ligated probe (950), spatial barcode (130), and the two primer-binding sites (945, 125) necessary for sequencing. EX1002,

¶¶104, 140-152, 172-184. The library preparation step involves extension of the capture probes and captured ligated probes (950) followed by amplification of the extension products. EX1002, ¶¶104, 140-152, 172-184. For these reasons, I disagree with Dr. Sherman that “there is no evidence of any amplification occurring at the final step.” EX2006, ¶63. Dr. Sherman ignores the full context of So-456’s disclosure from the viewpoint of a POSA.

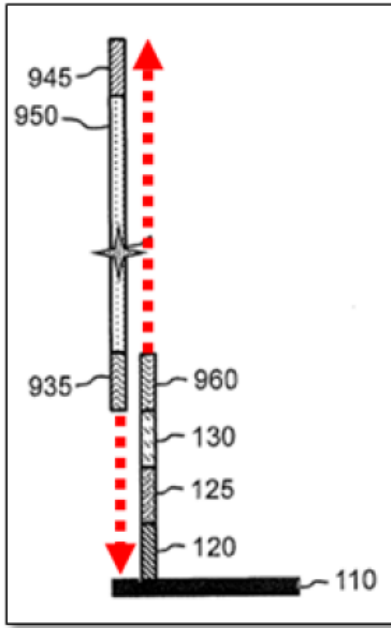
45. I also explained in my first declaration that Figure 9’s method inherently proceeds in both directions, incorporating a spatial barcode into the captured ligated probes. EX1002, ¶¶104, 147-152; 179-184. So-456 discloses that the “[o]ligonucleotides to be used as capture primers . . . can be immobilized such that a 3’-end is available for enzymatic extension.” EX1005, ¶[00109] (emphasis added); EX1002, ¶¶149, 181. So-456 does *not* disclose any blocking or other modifications to the 3’-end of the captured ligated probes in Figure 9’s method³, rendering both the 3’-end of the captured ligated probes and 3’-end of the capture probes available for extension. EX1002, ¶¶104, 147-152, 179-184; EX1005,

³ In contrast, So-456’s other disclosures specify modifying the 3’ end of a nucleic acid, *e.g.*, to prevent 3’-end ligation or to prevent further nucleotide incorporation during sequencing. *See, e.g.*, EX1005, ¶¶[00484], [00491], [00601], FIGs. 48-49.

¶¶[00109], [00182], [00183], FIGs. 8, 9. This is because enzymes, such as DNA polymerase, that are used in the extension indiscriminately extend all available 3'-ends that have template DNA available to them on the other strand. EX1002, ¶¶149, 181. Dr. Sherman does not (and cannot) argue that a DNA polymerase enzyme does not extend all available 3'-ends.

46. Thus, due to the nature of the extension enzymes and the available 3'-ends, Figure 9's extension reaction after Step 825 necessarily proceeds in both directions. EX1005, ¶¶[00109], [00182], [00183], FIG. 9; EX1002 ¶¶104, 147-152, 179-184. Indeed, So-456 confirms that when the capture probe is "used as a primer," the captured "DNA molecule [] is copied," and when the captured DNA molecule is "used as a primer," the "spatial address region 130" is copied. EX1005, ¶[00173], FIG. 6; EX1002 ¶¶149, 181. So-456 confirms that "[b]oth extension products can be used for downstream library generation." EX1005, ¶[00173], FIG. 6; EX1002 ¶¶149, 181.

47. I illustrated this inherent bidirectional extension after Step 825 in Figure 9's method by red arrows in my first declaration (reproduced below).



EX1005, FIG. 9 (annotated); EX1002, ¶¶150-152; 182-184.

48. Thus, Figure 9’s method inherently proceeds in both directions, incorporating a spatial barcode into the captured ligated probes in the reaction depicted by the downward arrow in the annotated Figure 9 above. EX1002, ¶¶104, 138-152, 170-184. Further, Figure 9’s extension reaction forms extension products that comprise the sequences of the captured ligated probe (950), spatial barcode (130), and the two primer-binding sites (945, 125) necessary for sequencing.

EX1005, ¶¶[00141], [00182], FIG. 9; EX1002, ¶¶104, 147-152, 179-184.

1. So-456’s method collects the original ligated probes and extends them.

49. Dr. Sherman argues that because “So-456 does not disclose collecting bound probes,” it “cannot, as a matter of logic, disclose claim limitations 1(d)-(e) nor 16(e)-(f).” EX2006, ¶58. As I explained above, So-456 teaches a method that

collects the original ligated probes that were bound to the target analyte (along with their amplified copies).

2. So-456 discloses that segment 130 is the spatial barcode in its Figure 9 capture probes.

50. Dr. Sherman argues that So-456's Figure 9 does not include a spatial barcode at all, because in Figure 9's capture probes "region 130 is a sequencing by synthesis primer region" and not a spatial barcode. EX2006, ¶¶59-60. Dr. Sherman also alleges that my first declaration "attempt[s] to string together disparate disclosures" by relying on So-456's paragraph 141 to identify segment 130 in the capture probes as a spatial barcode. EX2006, ¶60; *see also* EX2006, ¶56. I disagree with Dr. Sherman.

51. Dr. Sherman fails to consider the full teachings of So-456 from the viewpoint of a POSA. As I previously explained, the capture probes (115) in Figure 9's method must include a spatial barcode for the spatial identification method to be useful. EX1005, ¶¶[0001], [0002], [0085], [0089]; EX1002, ¶¶140-152, 172-184. So-456's methods are based on a "realization that information related to the spatial origin of a nucleic acid in a tissue sample can be *encoded in the nucleic acid[s]* in the process of *preparing* the nucleic acid for sequencing." EX1005, ¶[0089] (emphasis added). Under Dr. Sherman's flawed interpretation that "Figure 9 [] does not include a spatial barcode at all," Figure 9's method could

not determine the target analyte's spatial location in the tissue sample—thus defeating the entire purpose of So-456's methods. EX2006, ¶¶59-60.

52. Figure 9's description in So-456's paragraphs 182-183 does not identify a spatial barcode segment of the capture probes. EX1005, ¶¶[00182]-[00183]. Because So-456's methods must include a spatial barcode, a POSA reading So-456 in its entirety, would rely on the related disclosures of Figure 1, including paragraph 141. EX1002, ¶¶ 143, 175. Figures 8-9 illustrate the same method. EX1002, ¶101; EX1005, ¶[00182] ("Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8."). So-456 details that Figure 8's (and thus Figure 9's) method uses the capture array depicted in Figure 1A. EX1005, ¶[00177] ("capture array 100 of Figure 1A").

53. Figures 1A and 1B illustrate different views of the same capture array, which comprises capture sites (105) with capture probes (115) immobilized on a solid support (110). EX1005, FIGs. 1A, 1B, ¶¶[00141], [00183]. The capture probes (115) of Figure 1B and 9 are immobilized in capture sites (105) on a solid support (110) and have the same design with four segments: cleavable region (120), SBS primer binding site (125), spatial address region (130), and capture region (135 in Figure 1B; 960 in Figure 9).

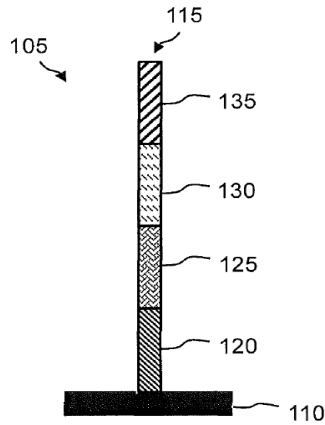


Figure 1B

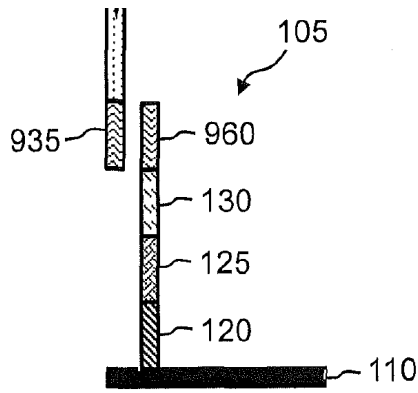


Figure 9

EX1005, FIGs. 1B, 9, ¶¶[00141], [00182], [00183].

54. Thus, So-456's disclosures for Figure 1 (such as, paragraph 141) directly relate to Figure 8-9's method. Accordingly, my first declaration properly relied on paragraph 141 (and other Figure 1's disclosures) for Figure 9's method. EX1002, ¶¶138-152, 170-184.

55. My first declaration also correctly identified segment 130 in Figure 8-9's capture probes as a spatial barcode. EX1002, ¶¶99, 104, 143, 175. So-456's paragraph 141 identifies segment 130 as a "spatial address region," i.e., a spatial barcode. EX1005, ¶[00141]. So-456 also repeatedly identifies segment 130 as a spatial barcode throughout its disclosure, except in Figures 6 and 8-9. EX1005, ¶¶[00141], [00145], [00146], [00173], [00189], [00201], [00202], [00213], [00214], FIGs. 1B, 1C, 3, 4, 6, 7, 8, 9, 11, 13A, 13B, 15B, 15C.

56. While both Figure 6's and Figure 8-9's disclosures identify segment 130 in their capture probes as an "SBS primer region" and "SBS sequencing primer," respectively, it is clear from Figure 6's full disclosure that segment 130's identification as an SBS primer region is a typographical error. EX1005, ¶¶[00172]-[00173], [00182]-[00183]. Figure 6's disclosure identifies segment 130 as *both* an "SBS sequencing primer" and a "spatial address region," while at the same time it identifies segment 125 also as an "SBS primer region." EX1005, ¶¶[00172]-[00173]. Given that So-456 repeatedly describes segment 130 as a spatial barcode (*see, e.g.*, EX1005, ¶¶[00141], [00145], [00146], [00172], [00183], [00189], [00201], [00202], [00213], [00214], FIGs. 1B, 1C, 3, 4, 6, 7, 8, 9, 11, 13A, 13B, 15B, 15C), a POSA would readily recognize that segment 130 should be the spatial barcode and segment 125 should be the SBS primer region in Figure 6's capture probes. A POSA would also readily recognize that Figure 9's disclosure repeats Figure 6 disclosure's error by identifying segment 130 as an SBS primer region. Thus, a POSA would readily know that segment 130 in Figure 9's (and Figure 6's) capture probe is a spatial barcode, and not an SBS primer region.

57. A POSA would also find it logical that segment 130 (and not segment 125) of Figure 9's capture probe is a spatial barcode. A spatial barcode must be downstream (3') of the SBS primer region (125) in order for the spatial information of the target analyte present in the spatial barcode to be copied into the

molecules that are ultimately sequenced for determining the spatial location of the target analyte. Figure 9's capture probe (just like Figure 1B's capture probe) has four segments: 960, 130, 125, and 120. EX1005, FIG. 9. So-456's paragraphs 182-183 discussing Figure 9's capture probes identify segment 960 as a "capture region" and segment 120 as a "cleavable sequence" (consistent with the rest of So-456's disclosure). EX1005, ¶¶[00182]-[00183]. Because segment 130 is downstream (3') of segment 125, they must be the spatial barcode and SBS primer region, respectively.

58. Segments 130 and 125 of Figure 8-9's capture probes cannot be the other way around: segment 125 as a spatial barcode, and segment 130 as an SBS primer region. A POSA would understand this because in this design, the spatial barcode would be 5' of the SBS primer region, and during subsequent amplification reactions the spatial barcode would not be amplified. As a result the molecules that are ultimately sequenced would lack a spatial barcode and no longer contain *any* spatial information—defeating the whole purpose of So-456's method to determine spatial information of the target analytes in a tissue sample. Dr. Sherman also admits that a spatial barcode downstream (3') of the SBS primer region as in this design would not be sequenced. EX1056, 46:19-21. Dr. Sherman is therefore wrong to assert this flawed design (also depicted in his mislabeled

Figure 9), arguing that segment 130 in Figure 9's capture probe is an SBS primer region.

59. Accordingly, for the reasons above and those provided in my first declaration, I maintain my position that segment 130 in the capture probes of Figure 9 is the spatial barcode and that a POSA would readily understand this.

3. So-456's Figure 9 includes an extension reaction that incorporates a spatial barcode as claimed.

60. Dr. Sherman argues that So-456's extension reaction in Figure 9 is only unidirectional and does not incorporate a spatial barcode into the target analyte because, according to Dr. Sherman, So-456 discloses "numerous embodiments that feature uni-directional extension of the capture probe to incorporate the target analyte's sequence." EX2006, ¶¶61-63 (citing So-456's Figures 22, 23A, 24, 25A, 25B, 29A, 39, 40, ¶¶[00297]-[00301], [00307]-[00320], [00332]-[00334], [00391]-[00395], [00410]-[00412]). The fact that So-456 discloses unidirectional extension of the capture probes (depicted by a unidirectional arrow) in other examples, does not change that Figure 9's method includes a bidirectional extension for the reasons explained in my first declaration (and summarized above).

61. Moreover, the other examples that Dr. Sherman cites are different from the extension reaction included in Figure 9's method after Step 825. As I explained in my first declaration, Figure 9's method after Step 825 includes

extension of the capture probes and captured ligated probes (950) followed by amplification of the extension products for sequencing library preparation. EX1002, ¶¶140-152, 173-184; EX1005, ¶¶[00151]-[00158], [00177]-[00183], FIGs. 2, 8, 9. Figure 9's method uses "[b]oth extension products ... for downstream library generation." EX1005, ¶[00173]; EX1002, ¶149, 181. Thus, Figure 9's extension reaction is designed to use a DNA-dependent *DNA polymerase* enzyme—which extends the 3'-ends of both the capture probes (115) and captured ligated probes (950) hybridized to a corresponding DNA template in a bidirectional extension reaction.

62. In contrast, Dr. Sherman's cited reactions in Figures 22-23A (Step 2215), 24-25A (Step 2415), 28-29A (Step 2815), and 38-39 (Step 3810) are designed to synthesize from an mRNA template a "first strand cDNA" for use in the subsequent steps. EX1005, ¶¶[00299], [00309], [00328], [00386], FIGs. 22-23, 24-25, 28-29, 38-39. I have reproduced below So-456's Figures 22, 24, 28, and 38.

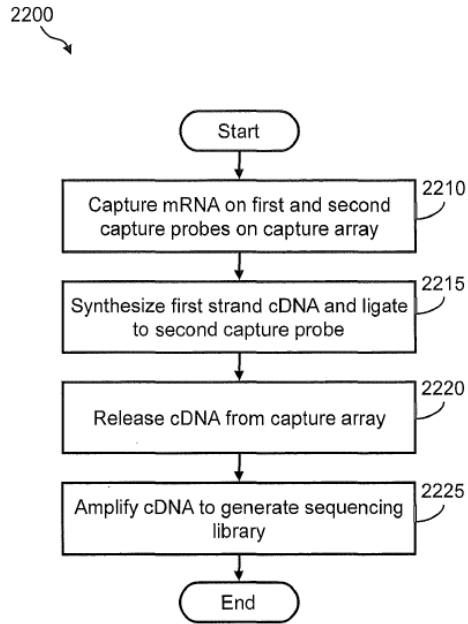


Figure 22

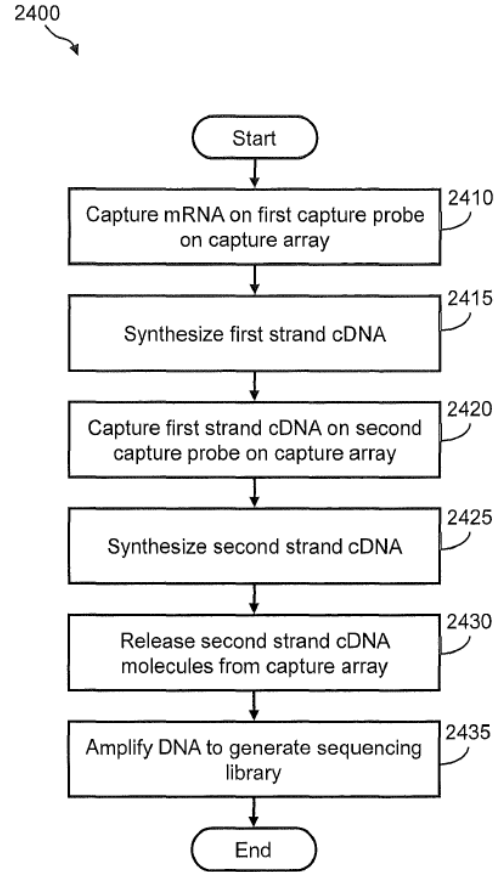


Figure 24

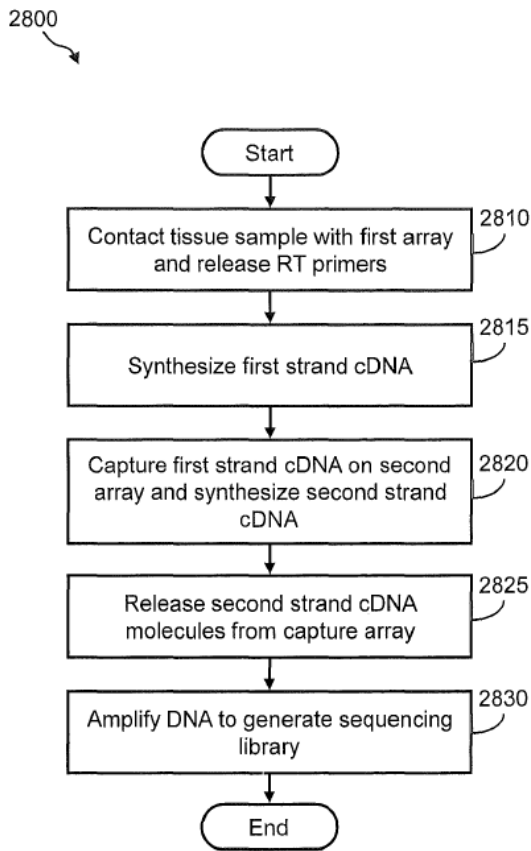


Figure 28

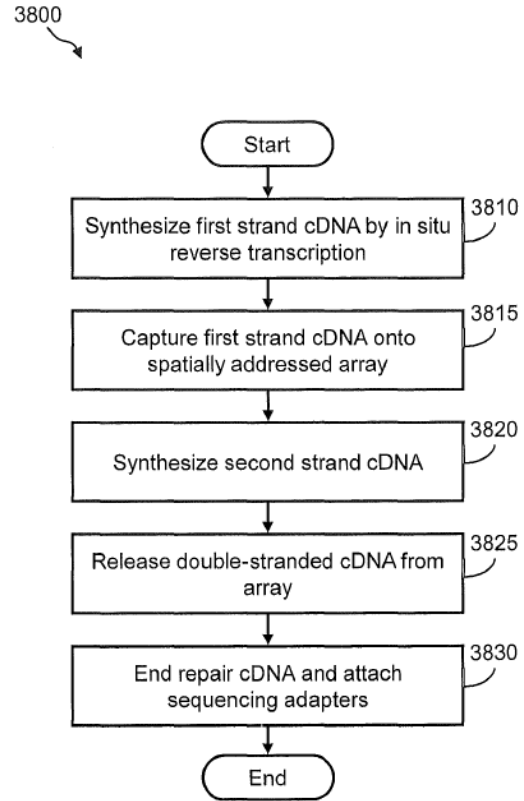


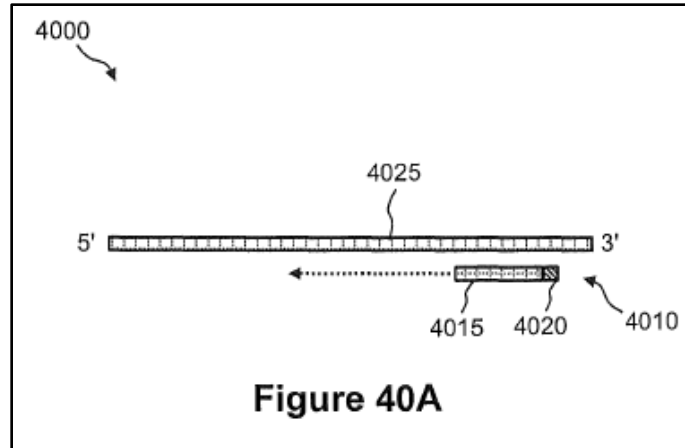
Figure 38

EX1005, FIGs. 22, 24, 28, 38.

63. In each case, the first-strand cDNA is the product generated in the extension reaction depicted by a single arrow. EX1005, FIGs. 23A, 25A, 29A, 39. Thus, Dr. Sherman's cited reactions in Figures 22-23A (Step 2215), 24-25A (Step 2415), 28-29A (Step 2815), and 38-39 (Step 3810) are *not* designed to be bidirectional.

64. Similarly, Dr. Sherman's cited reactions in Figures 24-25B (Step 2425), 28-29A (Step 2820), and 38-39 (Step 3820) are designed to synthesize a "second strand cDNA" for use in the subsequent steps. EX1005, ¶¶[00311], [00329], [00388], FIGs. 22-23, 24-25, 28-29, 38-39. In each case, the second-strand cDNA is the extension product generated in the extension reaction depicted by the single arrow. Thus, Dr. Sherman's cited reactions in Figures 22-23A (Step 2215), 24-25A (Step 2415), 28-29A (Step 2815), and 38-39 (Step 3810) are *not* designed to be bidirectional.

65. Finally, in Dr. Sherman's cited reaction in Figure 40 (reproduced below), the probe (4010) is designed such that it is bound in the interior of the target nucleic acid (4015). The only 3'-end with an available corresponding template is that of the capture probe and is thus the only 3'-end that can be extended (shown by the arrow). The 3'-end of the target molecule is not complementary to the 5'-end of the probe and is therefore not available to extend in a template DNA dependent manner, as there would be no template. EX1005, ¶¶[00410], [00411], FIG. 40A.



EX1005, FIG. 40A.

66. Thus, all of Dr. Sherman's cited So-456 embodiments include an extension reaction that is designed to be unidirectional, unlike So-456 Figure 9's extension reaction after Step 825.

67. Another example with the same design as Figure 9 is Figure 6. EX1005, ¶¶[00172]-[00173], FIG. 6; EX1002, ¶¶149, 181. Like Figure 9, Figure 6 after Step 520 includes extension of the capture probes and captured DNA molecules (950) followed by amplification of the extension products for sequencing library preparation. EX1005, ¶¶[00173]-[00174], [00177]-[00183], FIGs. 2, 6, 9. Figure 6's description explicitly states that "both capture region 660 and universal capture region 630 can also function as primers for an extension reaction" and "[b]oth extension products can be used for downstream library generation." EX1005, ¶[00173]; EX1002, ¶¶149, 181. Thus, unlike Dr. Sherman's

cited embodiments, Figure 8-9's and Figure 6's extension reaction proceeds in both directions.

68. Dr. Sherman also argues that a POSA "would have recognized challenges with bidirectional extension." EX2006 ¶64. I disagree with Dr. Sherman. As discussed above, So-456 teaches bidirectional extension using both the capture probes and captured ligated probes as primers. EX1005, ¶[00173]; EX1002, ¶¶149-152, 180-184. Further, a POSA would *not* have experienced challenges with bidirectional extension because such extensions were common in the prior art. *See, e.g.*, EX1005.

69. Therefore, I maintain my position that So-456's Figure 9 method includes an extension reaction that inherently proceeds in both directions and incorporates a spatial barcode into the captured ligated probes as required by the claims.

VI. CLAIMS 1-30 WOULD HAVE BEEN OBVIOUS OVER SO-456, AND CLAIMS 9, 10, 24, AND 25 WOULD HAVE BEEN OBVIOUS OVER SO-456 AND STAHL.

70. My first declaration explained why claims 1-30 would have been obvious over So-456 and claims 9, 10, 24, and 25 would have been obvious over So-456 and Stahl. EX1002, ¶¶217-268. I reviewed Dr. Sherman's declaration, and my opinions remain the same.

A. A POSA would have been able to bridge any alleged gap between one or more disclosures in So-456 and the challenged claims.

71. Even if So-456 were found to not teach all of the limitations of the challenged claims, a POSA would have been able to easily bridge any gaps between So-456's disclosures and elements of the claims. As I explained in my first declaration, NanoString (now Bruker) stated during prosecution that "the skill level of a [POSA] in the context of the instant application is high, at least that of a PhD in biology, if not higher." EX1002, ¶25; EX1004, 268. A POSA armed with such skills and reading So-456 would have easily practiced the claimed methods, and been motivated to do so.

1. Treating a tissue sample to facilitate probe binding would have been obvious.

72. So-456 discloses treating a tissue sample to facilitate probe binding, as claimed in the 689 patent. EX1002, ¶¶125-127, 164-165. Even if it did not, this step would have been obvious to a POSA. I understand that the Board stated in its Decision on Institution that a POSA "would have known how to prepare tissue for in situ histochemistry." DOI, 31. I agree with this statement. Dr. Sherman also quotes a NanoString (now Bruker) statement made to the Examiner during prosecution of the 689 patent, stating that treating a tissue sample is "well within the purview of a [POSA]." EX2006, ¶48 (quoting EX1012, 1560). Below I provide NanoString's full statement to the Examiner:

Applicant submits that it would be *well within the purview of a person of ordinary skill in the art* (i.e. a person with at least a PhD in biology) *to treat a tissue sample* such that at least one probe can bind to at least one target analyte in at least one cell in a tissue sample, as *such a step is a routine laboratory procedure* that is *commonly performed in both research and industrial labs* within the field of the present disclosure.

EX1012, 1560 (emphasis added).

73. I also understand from Dr. Sherman's deposition transcript that he testified that there "would have been literature on, you know, the basics, how you create the sample, what sort of buffers you use, what sort of -- you know, how to maintain that sample in a form that would be stable and represent what you're interested in eventually investigating." EX1056, 50:6-15; *see also* 51:6-12 ("There would be a set of best practices for handling that type of tissue sample to maintain its integrity in terms of buffers and timing and pH.").

74. Accordingly, a POSA following So-456's method would have found it routine and obvious to treat a tissue sample to facilitate hybridization of probes to a target analyte.

2. Ligating nucleic acids would have been obvious.

75. Dr. Sherman testified in his declaration that "[l]igation is a basic skill well within the competency of a POSA." EX2006, ¶119. I agree that ligation

generally is a basic skill within a POSA's skillset. I understand Dr. Sherman testified at deposition that So-456's Figure 9 discloses "no ligation" because the word "ligase" is not used in the Figure. EX1056, 36:2-37:18. I disagree with Dr. Sherman's deposition statement. First, So-456 explicitly discloses that an "extension/*ligation*" reaction occurs in the method of Figure 9. EX1005, ¶[00182], FIG. 9. Second, given that ligation was a routine skill possessed by a POSA, the POSA would have easily interpreted So-456's method as teaching a ligation step between the two probes in Figure 9. And third, I understand from Dr. Sherman's declaration testimony that he agrees there is indeed a ligation depicted in So-456's Figure 9. EX2006, ¶70 ("the referenced *ligation* is the extension product with the probe ... This *ligated molecule* is then used ...").

76. Accordingly, a POSA following So-456's method would have found it obvious to ligate So-456's probes depicted in Figure 9, because that is exactly what So-456 discloses doing.

3. Adding a spatial barcode by extension would have been obvious.

77. Dr. Sherman testified in his declaration that "the process of adding a spatial barcode by extension is, in my opinion, an inventive step that was a nonobvious improvement over the prior art." EX2006, ¶75. I disagree with Dr. Sherman's opinion, as this, too, would have been obvious to a POSA.

78. As I explained in my first declaration and reiterate above in Section V.C, a POSA would have collected all of the molecules So-456 labeled as “950” in Figure 9—including the original ligated probe bound to the target DNA and the amplicons of the original ligated probe. EX1002, ¶¶131-137, 166-169. The POSA would have been motivated to include a spatial barcode in the capture probe of So-456’s method (segment 130) because So-456 teaches this design. EX1005, ¶¶[00182]-[00183]. As explained above, a POSA would have immediately recognized the typographical error in So-456’s description of segment 130 in Figure 9 and known that So-456 teaches segment 130 is a spatial barcode, not an SBS primer sequence. *See* Section V.D.2; EX1005, ¶¶[00141], [00145], [00146], [00173], [00189], [00201], [00202], [00213], [00214], FIGs. 1B, 1C, 3, 4, 6, 7, 8, 9, 11, 13A, 13B, 15B, 15C.

79. A POSA would have been motivated to use So-456’s method in spatial detection applications. EX1002, ¶¶234-236. And given this design taught in So-456’s method, the POSA would have reasonably expected the extension reaction on the array to proceed bidirectionally, which would “add[] a spatial barcode by extension,” as Dr. Sherman puts it. EX2006, ¶75; EX1002, ¶¶237-240. The POSA would have been motivated to use both extension products for library generation because So-456 expressly teaches that “[b]oth extension products” from

a bidirectional extension “can be used for downstream library generation.”

EX1005, ¶[00173]; EX1002, ¶229.

80. Dr. Sherman argues that DNA synthesis only occurs in a 5’ to 3’ direction and is thus “unidirectional,” and that my depiction of bidirectional extension necessarily occurring in So-456’s Figure 9 “confusingly showcases bidirectional extension happening in opposite directions, as opposed to the 5’ to 3’ directions.” EX2006, ¶78. It was known since at least 1990s that DNA synthesis by DNA polymerases proceeds in a 5’ to 3’ direction, and Dr. Sherman wildly mischaracterizes my opinions regarding the bidirectional extension depicted in So-456’s Figure 9. Figure 9 discloses a bidirectional extension because the extension in the upward direction uses the capture probe as the primer and the captured ligated probe (molecule 950) as the template (i.e., an extension in the 5’ to 3’ direction; and the extension in the downward direction uses the captured ligated probe (molecule 950) as the primer and the capture probe as the template (i.e., *also* an extension in the 5’ to 3’ direction). The bidirectional extension occurs because both strands are being extended in opposite directions (but both in the 5’ to 3’ direction). So-456 expressly discloses this concept when explaining that “[b]oth *extension products* can be used for downstream library generation.” EX1005, ¶[00173]; EX1002, ¶229.

81. Accordingly, Dr. Sherman's opinions do not undermine or alter my opinions set forth in my first declaration. Claims 1-30 would have been obvious over So-456. EX1002, ¶¶217-254.

4. Claims 9, 10, 24, and 25 would have been obvious over So-456 and Stahl.

82. I explained in my first declaration that claims 9-10 and 24-25 would have further been obvious over the combination of So-456 and Stahl. EX1002, ¶¶225-268. Dr. Sherman does not independently address this combination of art. EX2006, ¶73. I maintain my opinions set forth in my first declaration, and Dr. Sherman's opinions do not undermine or alter them in any way.

VII. CLAIMS 1-30 ARE NOT ENTITLED TO A PRIORITY DATE EARLIER THAN SEPTEMBER 16, 2021, AND ARE ANTICIPATED BY CHELL.

83. As I explained in my first declaration, claims 1-30 of the 689 patent are not entitled to a priority date earlier than September 16, 2021, because the 689 patent's priority applications (including the 487 application) do not provide written description support for (1) collecting whole probes or (2) "on-analyte" ligation in its disclosed methods. EX1002, ¶¶270-293. I also explained that Chell, which was filed and published before September 16, 2021, anticipates the 689 patent claims. EX1002, ¶¶269-378.

84. Dr. Sherman does not rebut Chell's teachings as to the 689 patent claims. EX2006, ¶¶84-122. Instead, Dr. Sherman only argues that Chell cannot be

used as a prior-art reference against the 689 patent claims because the 487 application provides written description support for the 689 patent claims. EX2006, ¶¶88-122. Dr. Sherman however goes outside of the 487 application's specification to create hypothetical scenarios that are not described in the specification, many of which are incompatible with the claimed methods. EX2006, ¶¶88-122.

A. The 487 application does not teach collecting the whole probes.

85. As I explained in my first declaration, the 689 patent claims encompass collecting *whole* probes containing a target-binding domain that had hybridized to the target analyte for use in the subsequent steps of the claimed method. EX1002, ¶¶133, 287. However, the 689 patent's priority applications, including the 487 application, do not describe collecting *whole* probes containing a target-binding domain that had hybridized to a target analyte as claimed. EX1002, ¶¶288-293.

86. In response, Dr. Sherman argues that the 487 application adequately describes collecting whole probes because it (1) requires cleavage to release the probe and the cleavable probes are a “non-limiting example”; (2) teaches releasing whole probes by denaturation; (3) teaches collecting whole probes by performing a “negative purification”; (4) includes embodiments where the target-binding domain and identifier oligonucleotide portions of the probes overlap or are the

same sequence; (5) includes “disclosure of releasing ‘whole probes’” in connection with its teaching of indirect hybridization. EX2006, ¶¶88-122. I disagree with Dr. Sherman for the reasons explained below.

1. Dr. Sherman’s cited disclosures still require releasing portions of probes, and not the whole probes, for collection as claimed.

87. Dr. Sherman argues that the 487 application “does not require a portion of a probe to be released by cleaving the probe,” because the embodiments with cleavable probes are non-limiting examples. EX2006, ¶¶92-93 (citing EX1009, ¶¶[00106]-[00107], [00123]-[00127], [00131], [00140]-[00145], [00159], [00176], [00180], [00184], [00200]-[00201], [00211], [00221], [00236], [00246], [00315]-[00317]). However, Dr. Sherman misrepresents these disclosures. As I explain below, each of Dr. Sherman cited disclosures relates to a step that occurs *after* a portion of the probe has already been released and collected, or it relates to the type of force used to release a probe portion or the type of cleavable linker.

88. The disclosures in Dr. Sherman’s cited paragraphs [00106]-[00107], [00123]-[00127], [00143]-[00145], [00159], [00184], [00200]-[00201], [00211], [00221], and [00246] relate to a method that includes providing a force to release the identifier oligonucleotide portion of the probe and collecting the released identifier oligonucleotide. EX1009, ¶¶[00106]-[00107], [00123]-[00127], [00143]-[00145], [00159], [00184], [00200]-[00201], [00211], [00221], [00246]. After the

identifier oligonucleotide portion of the probe is released and collected, these disclosures teach a “non-limiting example” of the *type* of cleavable linker (e.g., photo-cleavable linker, restriction enzyme site, or a cleavable linker) that can be used in the probe or the *type* of force that can be applied (e.g., light of a wavelength capable of cleaving the photo-cleavable linker, UV light, enzymes) to release the identifier oligonucleotide portion. None of these disclosures apply the term “non-limiting example” for releasing a portion of the probe. EX1009, ¶¶[00106]-[00107], [00123]-[00127], [00143]-[00145], [00159], [00184], [00200]-[00201], [00211], [00221], [00246].

89. The disclosures in Dr. Sherman’s cited paragraphs [00131], [00137], [00176], and [00180] use a “non-limiting example” for the steps that occur *after* the released identifier oligonucleotide portion of the probe bound to the target analyte has already been collected. EX1009, ¶¶[00131]-[00137] (ligating the released identifier oligonucleotide to a nucleic acid adaptor having a constant nucleic acid sequence), [00140]-[00142] (ligating the released identifier oligonucleotide to a nucleic acid adaptor having a constant nucleic acid sequence in the bottom panel of Figure 2), [00176] (hybridizing the released identifier oligonucleotide to a pair of secondary probes called “nucleic acid probes”), [00180] (same). Further, the “non-limiting example” cited by Dr. Sherman in paragraphs [00236] and [00315]-[00317] describe an exemplary target analyte

detected in the methods, and likewise does not describe collecting whole probes.

EX1009, ¶[00236] discussing an exemplary probe that binds to target analyte “protein P53”), ¶¶[00315]-[00317] (discussing exemplary “‘probe X’ designed to spatially detect ‘target analyte X’” where the probe comprises “‘target binding domain X’ linked to an ... ‘identifier oligonucleotide X.’”).

90. Dr. Sherman also cites to the disclosures in paragraphs [0014]-[0015], [0018], [00131], and [00199] to argue that a cleavage site can be present on other sequences (such as a “constant nucleic acid sequence”) or in locations other than between the identifier oligonucleotide and target binding domain. EX2006, ¶93; EX1009, ¶¶[0014]-[0015], [0018], [00131], [00199]. However, all of these disclosures also relate to collecting the released identifier oligonucleotide portion of the probes. EX1009, ¶¶[0014]-[0015], [0018], [00131], [00199]. These collected identifier oligonucleotides then either contain the purported cleavage (¶¶[00199]-[00203]) or are ligated to an adaptor containing a sequence with a cleavage site (¶¶[0014]-[0018], [00131]).

91. Thus, none of Dr. Sherman’s cited disclosures from the 487 application describes a method that collects *un-cleaved* whole probes containing a target-binding domain that had hybridized to the target analyte.

2. The 487 application does not describe using denaturation to release the whole probes for collection as claimed.

92. Dr. Sherman argues that the 497 application is not limited to cleavage as a means of releasing a probe from the target analyte. EX2006, ¶94. Instead, according to Dr. Sherman, the 487 application discloses “denaturation,” and a POSA would have known that the whole probe can be released from the target analyte through denaturation. EX2006, ¶¶94-101.

93. *First*, Dr. Sherman cites two instances where the 487 application explicitly discusses “denaturation”: paragraphs [00285] and [00240]. EX2006, ¶95. Dr. Sherman, however, misreads both these disclosures, as neither disclosure describes using denaturation to collect whole probes that had hybridized to the target analytes. Paragraph [00285] of the 487 application describes using denaturation during *the downstream cleanup steps for sequencing* described in paragraphs [00279]-[00287] of the 487 application (i.e., after release and collection of the identifier oligonucleotide portion). EX1009, ¶[00285]. While paragraph [00285] discusses denaturation, it has nothing to do with denaturation of the probes that were hybridized to the target analytes. Instead, it relates to the “process of removing all or a portion of one immobilized strand in a ‘bridged’ double-stranded nucleic acid structure” during sequencing. EX1009, ¶[00281].

94. And, paragraph [00240] of the 487 application relates to “denaturing of the *identifier oligonucleotide*” portion of the probes, and *not* using denaturation

to release the probes from the target analytes. Moreover, paragraph [00240] describes “double-stranded or partially double-stranded” identifier oligonucleotide portions of the probes, but these identifier oligonucleotide portions are still cleaved off from the probe and collected, as disclosed throughout the 487 application.

95. Having a double-stranded identifier oligonucleotide portion of the probe prevents the identifier oligonucleotide from hybridizing non-specifically with the target analyte. This reduces the amount of non-specific hybridization between the identifier oligonucleotide and the target analyte, improving efficient release of the identifier oligonucleotide portion. The released double-stranded identifier oligonucleotide portion can then be separated via denaturation “at lower temperatures.” This is because the double-stranded identifier oligonucleotide will have fewer interactions with other molecules in the system (e.g., other single stranded nucleic acids) than a single-stranded nucleic acid. In some sense, the double stranded identifier is “inert” or will not be subject to off-target hybridization. In fact, the denaturation language here describes double stranded probes that require less latent heat to release, and as a POSA would understand that frozen material cannot be aspirated, so to, would they understand that identifier oligonucleotides that are too sticky will not release. The whole probes are not being released by denaturation.

96. Dr. Sherman also misreads paragraph [00240] of the 487 application as disclosing that “a double-stranded sequence can be formed when an identifier oligonucleotide binds ... to another sequence, such as the target sequence.” EX2006, ¶95. Dr. Sherman is incorrect because if the identifier oligonucleotide were to bind to the target sequence, that would lead to the identifier oligonucleotide remaining bound to the target and thus requiring a higher temperature for releasing it—opposite to the stated purpose of reducing the release temperature.

97. *Second*, Dr. Sherman relies on disclosures in “U.S. Patent Application No. 14/946,386” that Dr. Sherman represents is incorporated by reference into the 487 application in paragraphs [0064], [00216], and [00227]. EX2006, ¶96; EX1009, ¶¶[0064], [00216], [00227]. I note that the 487 application purports to incorporate “US2014946386,” and not U.S. Patent Application No. “14/946,386.” I understand that the 386 application is not effectively incorporated into the 487 application.

98. Even if the 386 application were properly incorporated into the 487 application, it is incorporated for disclosures of the *sequencing* methods—which occur *after* the collection step in the 487 application methods and the claimed methods. EX1009, ¶¶[00216], [00227] (incorporating the “[e]nzyme-free methods of sequencing ... described in, e.g., US2014946386”). Thus, the 487 application

does not incorporate the 386 application's purported disclosures on denaturation for releasing whole probes from the target analytes in the tissue sample for collection.

99. Moreover, the 386 application itself does not describe denaturation for releasing the whole probes from the target analytes for collection, as required in the claims. Instead, the 386 application is directed to enzyme-free sequencing methods. EX2008, Abstract, ¶¶[0004], [0005], [0010]-[0012], [0174]-[0194], FIG. 8A-E; EX2006, ¶¶97-99. For example, the 386 application is titled “enzyme- and amplification-free sequencing” and discloses related compositions and methods. EX2008; *see also* EX2008, ¶[0005] (disclosing sequencing probes), FIGs. 1-6 (depicting sequencing probes). Dr. Sherman also agrees that the denaturation described in the 386 application is during the disclosed sequencing method. EX2006, ¶¶96-99. For example, Dr. Sherman states that “[t]he ’386 Application is...directed to methods for ‘enzyme-free, amplification-free, library-free *nucleic acid sequencing*...’ and that this nucleic acid sequencing method includes “dehybridiz[ing] (e.g., denatur[ing]) the entire sequencing probe...and repeat the process until the desired sequencing coverage has been reached.” EX2006, ¶97.

100. Indeed, Dr. Sherman's cited paragraphs [0010]-[0012], Figures 8A-8D, and Example 1 (at paragraphs [0174]-[0194]) of the 386 application relate to sequencing. EX2006, ¶¶97-99; EX2008, ¶¶[0010]-[0012], [0174]-[0194], FIGs.

8A-8D. For example, paragraph 11 of the 386 application states that “[p]rovided herein is a *method for sequencing* a nucleic acid using one population of probes of the present invention or a plurality of populations of probes of the present invention.” EX2008, ¶[0011] (emphasis added). Example 1 of the 386 application is directed to the following: “The Present Invention’s Method of Sequencing a Target Nucleic Acid.” EX2008, ¶¶[0174]-[0194]. And Dr. Sherman’s cited paragraph [0086] of the 386 application merely provides general guidance on designing probes. EX2006, ¶98 (Probes can be designed “to control the likelihood of hybridization and/or de-hybridization and the rates at which these occur.”).

101. Thus, none Dr. Sherman’s cited disclosures of the 386 application describe using denaturation for collecting whole probes that were bound to the target analytes, as claimed in the 689 patent. And a POSA would not have looked to the 386 application for releasing whole probes from the target analytes using denaturation for spatially identifying of the target analyte.

102. Finally, Dr. Sherman also cites to paragraphs 64 and 259 of the 487 application and argues that a POSA would have applied the 386 application’s teachings for using denaturation to release the probes from the target analytes as required in the claims. EX2006, ¶¶94-100. I disagree with Dr. Sherman. As discussed above, the 386 application disclosures relate to sequencing and have nothing to do with releasing probes from target analytes. Moreover, paragraphs

[0064] and [00259] of the 487 application provide generic statements. Paragraph [0064] of the 487 application instructs that “in the case of *conflict*, the *present Specification, including definitions, will control*.” EX1009, ¶[0064] (emphasis added). And paragraph [00259] of the 487 application generically references that “any probe,” can be used in the 487 application’s methods. EX1009, ¶[00259]. But, because the 487 application incorporates the 386 application for sequencing, following paragraph 64’s instructions, a POSA would not have applied the 386 application’s disclosures for releasing probes from target analytes or collecting the released probes. EX1009, ¶¶[00216], [00227].

103. Thus, even though “as an *abstract concept*, denaturation is well known to a POSA,” it does not alter the fact that 487 application lacks description of denaturation to release whole probes from the target analytes. EX2007, 113:25-114:7. In fact, a POSA would not have any idea how to make denaturation consistent with the methods of the 487 specification. Accordingly, the 487 application does not describe using denaturation to release whole probes from the target analytes as required by the 689 patent claims.

3. The 487 application’s “negative purification” disclosure does not describe collecting the whole probes as claimed.

104. Dr. Sherman argues that the 487 application “contemplates releasing entire probes from a target nucleic acid, which may be by denaturation” because it

discloses in paragraph [00252] “how to perform negative purification to reduce background noise. EX2006, ¶101; EX1009, ¶[00252]. I disagree with Dr. Sherman.

105. Paragraph [00252] of the 487 application states:

Background noise, during nucleic acid detection, can be reduced by performing a *negative purification of the intact probe molecule*. This can be done by conducting an affinity purification of the target binding domain or photo-cleavable linker *after collection of eluate* from a region of interest. Normally, *released signal oligonucleotides* will *not be pulled out of solution*. To assist in the negative purification, a universal purification sequence may be included in a probe, e.g., in the target binding domain.

EX1009, ¶[00252] (emphasis added).

106. Paragraph [00252] discloses “performing a negative purification of the intact probe molecule[s]” to reduce “[b]ackground noise” during nucleic acid detection. EX1009, ¶[00252]. As Dr. Sherman also agrees, a POSA would understand the term “negative purification” to mean removing the molecule being purified. EX1056, 60:17-24 (stating that a POSA would understand the term “negative purification” as “[t]o remove some sort of analyte from the mixture.”). Thus, paragraph [00252] discloses removing the “intact” (i.e., whole or uncleaved) probes from the solution in order to reduce the unwanted background noise resulting from the intact probes during detection. *See also* EX1056, 61:18-62:2.

107. Paragraph [00252] also discloses performing this negative purification *after* the “signal oligonucleotides” (i.e., identifier oligonucleotides) have been released from the probes upon cleavage. EX1009, ¶[00252]. Dr. Sherman agrees, stating that “[a] POSA would [] have understood that this purification step takes place after an identifier oligonucleotide has been released.” EX2006, ¶101. Further, paragraph 252 discloses that—unlike the intact probes that are removed to reduce the background noise—the “*released signal oligonucleotides will not be pulled out of solution.*” EX1009, ¶[00252] (emphasis added). The paragraph provides that negative purification of the intact probes can be performed, for example, by “conducting an affinity purification of the target binding domain or photo-cleavable linker,”—that are present only in the intact probes and not the identifier oligonucleotide released from the probes. EX1009, ¶[00252].

108. Thus, paragraph [00252] teaches performing a “negative purification” process on a solution containing both the intact probes and identifier oligonucleotides portions released from the probes. During the purification process, the released identifier oligonucleotides portions of the probes are retained in solution for use in the subsequent steps of the method. Meanwhile, the unwanted “intact probe[s]” of paragraph [00252] are pulled out and discarded to reduce the background noise. The intact probes are unwanted.

109. Paragraph [00252]’s negative purification disclosure does not describe collecting the whole probes as claimed. The claimed methods (“nucleic acid probes” in claim 1 and “ligated probes” in claim 16) require using the collected intact/whole probes in the subsequent steps (whereas the intact/whole probes subject to negative purification of paragraph [00252] are not used in subsequent steps). EX1001, 146:53-147:4, 148:23-40. For example, the claims recite that the extension reaction “incorporates at least one nucleic acid sequence that identifies the first [and second] location of the tissue sample into each of the nucleic acid [and ligated] *probes, or portions thereof collected in [the preceding steps].*” EX1001, 146:53-147:4, 148:23-40 (emphasis added). Dr. Sherman also admits as much, stating that “an extension, or amplification step, is performed on the collected probes” in the claimed methods. EX2006, ¶38. Whereas the intact/whole probes of paragraph [00252] of the 487 application are *not* collected for extension and analysis, but instead are *discarded*.

110. Thus, the 487 application’s “negative purification” disclosure does not describe collecting the whole probes as a component of the claimed invention.

4. The 487 application does not describe the identifier oligonucleotide and target binding domain as the same or overlapping sequences.

111. Dr. Sherman argues that paragraphs [00106], [00126], [00144], [00158], [00183], and [00199] of the 487 application support that the target

binding domain and the identifier oligonucleotide portions of the probes “can overlap partially or completely” such that “the probe’s sequence serves as both the ‘target binding domain’ and the ‘identifier oligonucleotide’ [that] can be released completely via a technique such as denaturation.” EX2006, ¶102; EX1009, ¶¶[00106], [00126], [00144], [00158], [00183], [00199]. I disagree with Dr. Sherman.

112. The 487 application repeatedly and explicitly identifies the identifier oligonucleotide portion of the probe as distinct from its target binding domain portion. *See, e.g.*, EX1009, ¶¶[0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00107], [00123], [00126]-[00127], [00140], [00144]-[00145], [00158]-[00159], [00174]-[00175], [00178]-[00179], [00183]-[00184], [00199]-[00200], [00210]-[00211], [00220]-[00221], [00228], [00231]-[00232], [00235]-[00236], [00246], [00310]-[00312], [00315], [00317]. Specifically, Dr. Sherman’s cited paragraphs teach that a probe comprising a target binding domain “*further* comprises an identifier oligonucleotide.” EX1009, ¶¶[00106]-[00107], [00126]-[00127], [00144]-[00145], [00158]-[00159], [00183]-[00184], [00199]-[00200] (emphasis added). Moreover, each of Dr. Sherman’s cited paragraphs relates to a method that includes “contacting” a target analyte with “at least one probe,” followed by “providing a force to a location of the sample sufficient to release the identifier

oligonucleotide.” EX1009, ¶¶[00106]-[00107], [00126]-[00127], [00144]-[00145], [00158]-[00159], [00182]-[00183], [00198]-[00199].

113. For example, paragraphs [00106]-[00107] in the 487 specification state:

A two ended-adapter ligation method of the present disclosure can comprise: (1) contacting at least one target analyte in a sample with at least one *probe* of the present disclosure. . . . In the aspect that the at least one target analyte is a target protein, the probe can comprise a *target binding domain* that is a target protein-binding region that can specifically bind to a target protein of interest. In the aspect that the at least one target analyte is a target nucleic acid, the probe can comprise a *target nucleic acid-binding region* that can directly or indirectly hybridize to a target nucleic acid of interest. *The probe further comprises an identifier oligonucleotide.* The identifier oligonucleotide can comprise a unique nucleic acid sequence which identifies the target analyte bound to the target binding domain.

Following contacting the at least on [*sic*] target analyte with the at least one probe, a two-ended adapter ligation method can further comprise: (2) *providing a force to a location of the sample sufficient to release the identifier oligonucleotide.* . . .

EX1009, ¶¶[00106]-[00107] (emphasis added).

114. Accordingly, the 487 application does not describe that the target binding domain and the identifier oligonucleotide portions of the probes are the same or overlapping sequence.

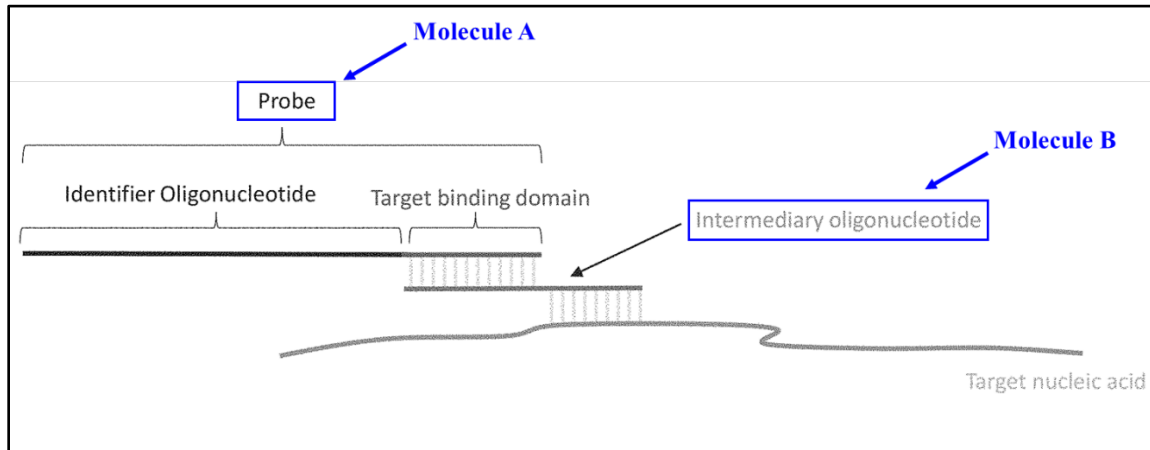
5. The 487 application’s “indirect hybridization” disclosures do not describe releasing the whole probes for collection as claimed.

115. Dr. Sherman argues that the 487 application’s “indirect hybridization” disclosure describes releasing and collecting whole probes. EX2006, ¶103 (citing EX1009, FIG. 14, ¶¶[00106], [00233], and [00310]). According to Dr. Sherman, from the 487 application’s indirect hybridization disclosure, “a POSA would have understood that an ‘intermediary oligonucleotide’ can contain ‘a region which permits the release of an identifier oligonucleotide,’ including ‘a cleavable motif’” such that “it would lead to release of an entire probe.” EX2006, ¶103. I disagree with Dr. Sherman.

116. Dr. Sherman mischaracterizes the 487 application’s indirect hybridization disclosure, e.g., Figure 14 and paragraphs [00106], [00233], and [00310] of the 487 application. EX1009, ¶¶[00106], [00233], [00310], FIG. 14. Dr. Sherman’s imagined indirect hybridization scheme with a cleavable intermediary oligonucleotide is not only missing from the 487 application’s specification, it

would also eliminate the indirect hybridization's advantages stated in the 487 application.

117. To illustrate, I have included below an annotated version of the indirect hybridization schematic of Figure 14.



EX1009, FIG. 14 (annotated in blue).

118. As depicted in the above figure, the 487 application's indirect hybridization includes three molecules: "Target nucleic acid"; "Probe" (Molecule A); and "Intermediary oligonucleotide" (Molecule B). Dr. Sherman argues that Molecule B could hypothetically include a cleavable motif, resulting in the whole Molecule A being released upon cleavage of Molecule B at the cleavable motif. EX2006, ¶103. But, none of the 487 application's indirect hybridization disclosures include a cleavable motif in Molecule B (intermediary oligonucleotide). *See, e.g.*, EX1009, ¶¶[0080], [00106]-[00108], [00126]-[00128], [00144]-[00146], [00158]-[00160], [00174]-[00176], [00178]-[00179], [00183]-

[00185], [00199]-[00202], [00210]-[00212], [00220]-[00222], [00233], [00310], [00420], FIGs. 14, 26, 27. Instead, all of the indirect hybridization disclosures in the 487 application include a cleavable moiety in Molecule A (probe) between its identifier oligonucleotide and target binding domain portions. EX1009, ¶¶[0080], [00106]-[00108], [00126]-[00128], [00144]-[00146], [00158]-[00160], [00174]-[00176], [00178]-[00179], [00183]-[00185], [00199]-[00202], [00210]-[00212], [00220]-[00222], [00233], [00310], [00420], FIGs. 14, 26, 27.

119. Moreover, Dr. Sherman’s hypothetical indirect hybridization scheme (with a cleavable intermediary oligonucleotide) would defeat the benefits that the 487 application highlights for its indirect hybridization schemes. The 487 application emphasizes that indirect hybridization “allows economical and rapid flexibility in an assay design” because the “target[]-specific components of the assay are included in inexpensive and widely-available synthetic DNA oligonucleotides [i.e., Molecule B] rather than the more expensive probes [i.e., Molecule A].” EX1009, ¶¶[00233]. This is critical as manufacturing probes (Molecule A) in large batches that can be reused to measure many different target nucleic acids is achieved by making the intermediary oligonucleotide (Molecule B) cheap and customizable.

120. Under Dr. Sherman’s scheme, the cleavable moiety is included in Molecule B, which is supposed to be the “inexpensive and widely-available

synthetic DNA oligonucleotides” of the indirect hybridization method. But Dr.

Sherman’s scheme makes it the expensive component, increasing the cost of

making Molecule B. EX1009, ¶[00233]. As a result, indirect hybridization under

Dr. Sherman’s scheme would become more expensive and removes the

advantages, defeating the whole purpose of indirect hybridization stated in the 487

application.

121. Furthermore, a cleavable Molecule B would render Molecule A redundant. Molecule B with a cleavable motif can satisfy all the functions of Molecule A: specifically binding to a target analyte, and upon cleavage releasing a portion that identifies the location of the target analyte for collection and use in the subsequent method steps. Thus, there would be no need for an additional probe (Molecule A) that comprises a target binding domain and an identifier oligonucleotide if the method already includes a cleavable Molecule B.

122. Thus, a POSA would not have understood the 487 application’s indirect hybridization disclosures as describing Dr. Sherman’s indirect hybridization scheme with a cleavable Molecule B. Accordingly, the 487 application’s indirect hybridization disclosures do not describe releasing whole probes for collection as claimed.

123. In sum, I maintain my opinion that the 487 application does not provide written description support for the full scope of the 689 patent claims,

which encompass collecting whole probes that had hybridized to the target analyte in the tissue sample.

B. The 487 application does not provide written description support for “on-analyte” ligation.

124. In my first declaration, I explained that none of the priority applications (including the 487 application, EX1009) provides written description support for “on-analyte” ligation of probe pairs, as required in claims 16-30 of the 689 patent. EX1002, ¶¶278-286. In response, Dr. Sherman alleges that written description for on-analyte ligation in the claimed methods is found in a compilation of disparate disclosures taken from numerous other patent applications. EX2006, ¶¶105-122. As I explain in detail below, I disagree with Dr. Sherman’s opinions and neither his testimony nor the evidence he relied upon undermines my opinions that I set forth in my first declaration. EX1002, ¶¶278-286.

1. US 2010/0015607 does not provide written description support for “on-analyte” ligation.

125. Dr. Sherman first states that the 487 application generally discloses “probes that hybridize to a target analyte.” EX2006, ¶109. I agree with this statement to the extent it is limited to *single probes*. I explained in my first declaration, “[o]nly singular ‘probes’ undergo ‘on-target’ hybridization to the

target analyte in the tissue sample in the methods of the priority applications.”

EX1002, ¶280.

126. Because the 487 application does not describe ligating pairs of probes “on-analyte” as part of the 689 patent’s claimed methods, Dr. Sherman then relies on certain disclosures taken from U.S. Patent Application Publication No. 2010/0015607 (“the 607 publication”) (EX2009). EX2006, ¶¶110-111. Dr. Sherman relies on the 607 publication solely for the premise that it discloses “specific examples of probe pairs that bind directly to a target nucleic acid.” EX2006, ¶¶110-111 (citing EX2009, ¶¶[0013], [0021], [0071], and FIGs. 1A-1C).

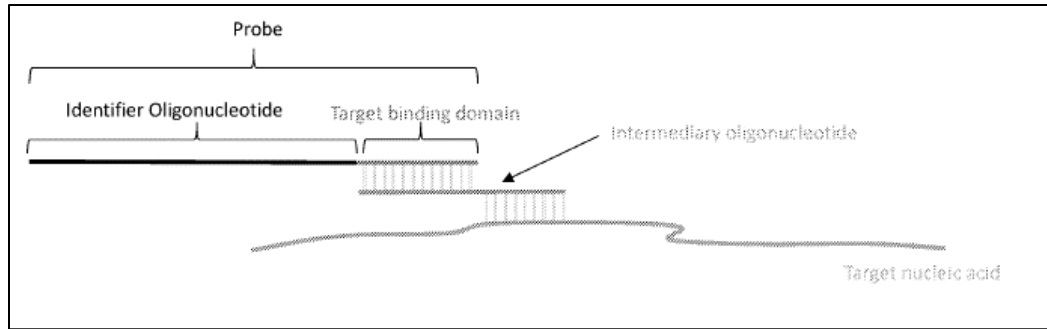
127. The 607 publication relates to nanoreporters and methods of manufacturing and use. EX2009, Title. For example, the 607 publication discloses methods that use a “dual nanoreporter” system comprised of probe pairs, where each probe is a complex comprising a scaffold comprising a label that emits light (“a first signal”) and a second label that emits light (“a second signal”). EX2009, ¶13. In other words, while the 607 publication does, at a high level, disclose “probe pairs” that bind to a target, these probes are light-emitting nanoreporters for an entirely different system that is disconnected and unrelated to the methods and probes for spatial detection of nucleic acids of the 487 application. None of the relied-upon disclosures in the 607 publication describe using the dual

nanoreporters in the claimed on-analyte ligation methods of the 689 patent.

EX2009, ¶¶[0013], [0021], [0071], FIGs. 1A-1C.

128. Next, Dr. Sherman argues that because the 487 application “teaches that probes can hybridize to a target analyte *indirectly* via an indirect target binding domain,” a POSA would have understood that “a *probe pair* can indirectly hybridize to a target nucleic acid via an indirect target binding domain.” EX2006, ¶112 (emphasis added). I disagree with Dr. Sherman’s conclusion on this point because (i) the 487 application does not describe using probe pairs that directly hybridize to the target analyte (“on-analyte”) in its methods (see above discussion of the 607 publication); and (ii) the 487 application does not describe using probe pairs in any of its indirect hybridization embodiments. EX1009, ¶¶[0080], [00106]-[00108], [00126]-[00128], [00144]-[00146], [00158]-[00160], [00174]-[00176], [00178]-[00179], [00183]-[00185], [00199]-[00202], [00210]-[00212], [00220]-[00223], [00310], [00420]. Dr. Sherman’s “indirect hybridization of a probe pair” scheme appears to be completely hypothetical.

129. Dr. Sherman testified in his declaration that “[a]n example of this [indirect hybridization of a probe pair] concept is depicted in Figure 14.” EX2006, ¶112. I provide a copy of the 487 application’s Figure 14 below:



EX1009, FIG. 14. I do not agree with Dr. Sherman that Figure 14 depicts an indirect hybridization of a probe *pair*, because there is *only* one molecule that is labeled a “probe”—not a pair of molecules.

130. I understand that Dr. Sherman further testified during deposition that in his opinion, the “probe pair” depicted in Figure 14 is the “intermediary oligonucleotide” molecule (a single oligonucleotide) because, according to Dr. Sherman, it is “a pair of sequences that have a different function.” EX1056, 64:14-65:13, 66:15-20. I also understand that Dr. Sherman admitted during his deposition that in this hypothetical “probe pair” scheme, there is no ligation of probe pairs because the intermediary oligonucleotide—the molecule Dr. Sherman alleges is a probe pair—is a single molecule:

10	Q.	If this intermediary oligonucleotide is a pair of
11		probes or a probe pair, as you've said, is it
12		subsequently ligated to another probe?
13	A.	In this case, there is no need for that.
14	Q.	So this would be a -- in your view, this is a probe
15		pair that's not ligated together after binding the
16		target nucleic acid?
17	A.	There's no need for it because the probe pair, as you
18		see, is -- comprises this intermediary
19		oligonucleotide with two hybridizing regions, so
20		there's no need for a ligase or a ligation.

EX1056, 68:10-20 (highlighting added), 68:21-69:11. Therefore, regardless of whether the intermediary oligonucleotide is viewed as a “probe pair” or a single oligonucleotide, Dr. Sherman’s “indirect hybridization of a probe pair” embodiment does not include *any ligation* of probe pairs, let alone any “on-analyte” ligation.

131. Dr. Sherman testified that a “POSA would also know how to design oligonucleotides and understand base pairing rules to design probes that hybridize to a nucleic sequence of interest,” and that “a POSA would surely be able to practice the claims in light of the disclosure in the ’487 Application.” EX2006, ¶113. According to Dr. Sherman, any “contrary opinion” from me on this point “defies logic.” EX2006, ¶113. I disagree with Dr. Sherman’s characterization. The 487 application does not describe the claimed method comprising “on-analyte” ligation before collection. It only describes using “off-analyte” ligation after

collection in its methods. EX1002, ¶¶276-286. The notion that a POSA knew how to design oligonucleotides and perform basic hybridization protocols (EX2006, ¶113) does not make up for the lack of written description in the 487 application.

132. Accordingly, in my opinion, Dr. Sherman’s hypothetical “indirect hybridization of a probe pair” scheme in the 487 application and reliance on the light-emitting nanoreporters of the 607 publication cannot and does not provide written description support for on-analyte ligation.

2. US 2011/0086774 does not provide written description support for “on-analyte” ligation

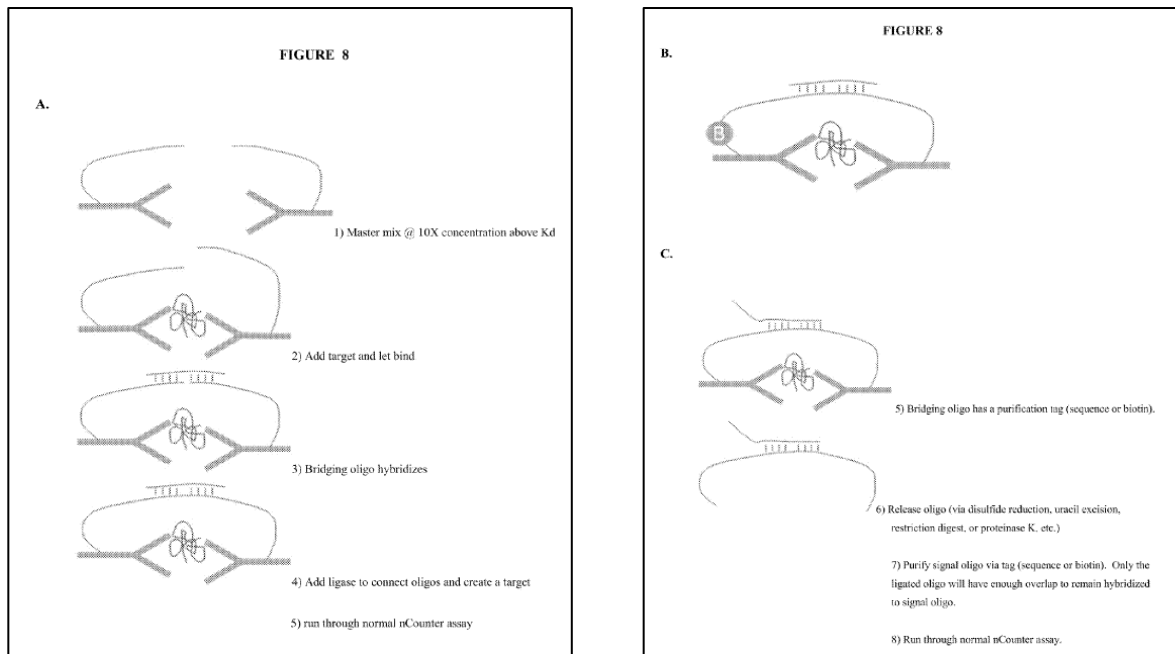
133. Dr. Sherman next relies on disclosures in U.S. Patent Application Publication No. 2011/0086774 (“the 774 publication”) (EX2010), to allege that the 487 application discloses ligation of probe pairs. EX2006, ¶¶114-117. First, Dr. Sherman refers back to his “indirect hybridization of a probe pair” embodiment, and states that “when the probe pair is ligated, a POSA would have understood this to take place while the probe pair is bound to a target analyte.” EX2006, ¶115. However, as I explained above, Dr. Sherman testified at deposition that *there is no ligation of probes* in his “indirect hybridization of a probe pair” embodiment. EX1056, 68:10-20.

134. Dr. Sherman then moves on to selected disclosures in the 774 publication, which he alleges discloses “two nucleic acids that both bind to a third and ligating the two nucleic acids.” EX2006, ¶117. The 774 publication relates to

methods for detecting proteins using protein probes. EX2010, ¶¶[0006]-[0027]. Dr. Sherman's declaration relies exclusively on paragraph 37 and Figure 8A in the 774 publication. EX2006, ¶117. According to Dr. Sherman, paragraph [0037] and Figure 8A describe a method of ligating a "first" and "second oligonucleotide" which are both hybridized to a "third oligonucleotide" called a "bridging oligo," and that "a ligase is subsequently added to ligate the two oligonucleotides together." EX2006, ¶117; EX2010, ¶[0037]. Dr. Sherman then argues that "[a] POSA would have further understood that this is analogous to a probe pair, such that a probe pair can be designed to hybridize to a nucleic acid, the target analyte." EX2006, ¶117. I disagree with Dr. Sherman's opinions for the following reasons.

135. First, the 774 application's disclosure of antibody-based proximity ligation methods for protein detection is disconnected and unrelated to the 689 patent's methods for spatial detection, and would not have conveyed to a POSA that the inventors described "on-analyte" ligation as used in the claimed methods. Dr. Sherman's argument that the 774 publication's antibody-based proximity ligation methods are "analogous" to a probe pair is incorrect because (i) as I discussed above, the 487 application does not describe ligating probe pairs in its claimed methods; and (ii) I understand that a disclosure that renders a claimed invention obvious does not satisfy written description.

136. Second, Dr. Sherman fails to discuss the complete workflow in the 774 publication's antibody-based proximity ligation methods. In particular, Dr. Sherman focuses on the fact that the protein probes of the 774 publication are "ligated," but fails to note the fact that after ligation, the "signal oligo" created from the ligation is *released* from the probe complex, collected, and analyzed. EX2010, ¶86, FIGs. 8A-8C. This is evident in Figure 8C of the 774 publication, which I have copied below along with Figures 8A-8B:



EX2010, FIGs. 8A-8C.

137. Figure 8C shows (step 6) that the signal oligo is released from the antibody. EX2010, ¶[0038] ("FIGs. 8B-D are schematic diagrams depicting methods by which the signal oligo shown in FIG. 8A may be *released and purified*."), FIG. 8C (emphasis added). Dr. Sherman agrees that the signal oligo is

released. EX1056, 74:9-15. Thus, even in the 774 publication's antibody-based proximity ligation method, only portions of probes are collected. Accordingly, if the 774 publication's disclosure of antibody-based proximity ligation were somehow deemed relevant as a description of "on-analyte" ligation before collection in the claimed method (it is not), it would not provide any description of collecting *whole probes*, as required by the claims (see my discussion above in Section VII.A and EX1002, ¶¶287-293).

138. Dr. Sherman also partially quotes my deposition testimony to argue that ligation "is a simple skill" and is "well within the competency of a POSA." EX2006, ¶¶118-120 (citing, e.g., EX2007, 76:11-15). The full context of my deposition statement is shown below:

6	If a skilled artisan was performing the
7	Claim 1 method of the '689 patent, would they have
8	within their basic competency the ability to perform
9	ligation off-analyte for barcoding?
10	MS. KHANDURI: Objection; scope.
11	A. Outside of the claim, a POSA would know
12	how to perform ligation.
13	I don't know how to answer the question
14	beyond that. You've imparted a great deal of extra
15	context and about a simple skill.

EX2007, 76:6-15.

139. As is clear from my testimony, I do not dispute that ligation *generally* is a simple skill. *See also* EX2007, 93:4-10 (“The *abstract concept of ligation*, surely understood to a POSA, yes.”). But whether or not ligation is a simple skill is not the issue here. The issue here is the 487 application’s failure to describe to a POSA the claimed methods comprising “on-analyte” ligation before collection.

140. The 487 application consistently and repeatedly describes collecting identifier oligonucleotides (off-analyte) and *then* ligating probes on those collected identifier oligonucleotides (off-analyte). *See, e.g.*, EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161]-[00170], [00174]-[00177], [00178]-[00181], [00183]-[00196]; EX1002, ¶¶278-286. It is clear from these disclosures, and would have been clear to a POSA, that the 487 application does not describe performing the methods using “on-analyte” ligation before collection, as claimed in the 689 patent. “On-analyte” ligation before collection is simply *not* part of the described methods, and a POSA would have understood this. The fact that the basic principle of ligation was a simple skill in the art does not change this outcome.

141. Accordingly, in my opinion, the 774 publication does not describe “on-analyte” ligation as required by the claims of the 689 patent.

3. US 2011/0245111 does not provide written description support for “on-analyte” ligation.

142. Dr. Sherman relies on disclosures in U.S. Patent Application Publication No. 2011/0245111 (“the 111 publication”) (EX2012), to argue that the

“simple concept of ligating a probe pair hybridized to a target analyte is well within a POSA’s basic knowledge.” EX2006, ¶121. As I explain above, the fact that the basic principle of ligation was a simple skill in the art does not change the fact that the 487 application nowhere describes performing the claimed methods using “on-analyte” ligation before collection. *See also* EX1002, ¶¶278-286.

4. The 689 patent claims require a specific order of steps not described in the 487 application.

143. Dr. Sherman argues that the description in the 487 application “does not require that any of the steps disclosed in any of the embodiments must take place in the order listed.” EX2006, ¶116. According to Dr. Sherman, because the specification allegedly does not “require” a specific order of steps, it must therefore describe ligation of probe pairs “on-analyte” before collection. EX2006, ¶116. I disagree with Dr. Sherman’s opinion.

144. First, claims 16-30 very clearly require a specific order of steps, and specifically require that the ligation of probes takes place “on-analyte,” before collection. EX1001, claim 16. A POSA would have easily understood this because claim 16(b) recites forming ligated probes by ligating probe pairs *bound to target analytes* (on analyte), and then claim 16(c)-(d) recites collecting the ligated probes from step 16(b). EX1001, 147:54-148:47.

145. Dr. Sherman’s argument that the 487 application provides written description of this specifically claimed process because it allegedly does not

“require” the steps to be in a specific order is incorrect. A POSA would have readily understood that every ligation-based embodiment in the 487 application requires the same order of steps: collecting the released identifier oligonucleotides (off analyte); hybridizing secondary probe pairs to the collected identifier oligonucleotides (off analyte); and ligating the secondary probe pairs (off analyte). EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161], [00168]-[00169], [00174]-[00180], [00183]-[00186], [00389]; *see also* EX1002, ¶¶287-292.

VIII. CONCLUSION

146. For the reasons discussed above, I maintain my opinion that each of claims 1-30 of the 689 patent are anticipated or would have been obvious in view of the cited art.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the U.S. Code.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Paul T. Spellman", with a long horizontal flourish extending to the right.

Paul T. Spellman, Ph.D.

Date: November 15, 2024

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