

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10X GENOMICS, INC.
Petitioner,

v.

NANOSTRING TECHNOLOGIES, INC.
Patent Owner.

Case No. IPR2023-01300
U.S. Patent No. 11,473,142

DECLARATION OF PAUL T. SPELLMAN, PH.D.

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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 have been retained as an expert witness on behalf of 10x Genomics, Inc. (“10x”) for the above-captioned *inter partes* review (IPR) against NanoString’s U.S. Patent No. 11,437,142 (“the 142 patent”) (EX1043). I understand that this Declaration accompanies 10x’s Petition for the IPR. I am being compensated for my time in connection with this IPR.
3. I understand that the 142 patent resulted from U.S. Patent Application No. 17/476,712 (“the 712 application”), filed on September 16, 2021, which is a continuation of U.S. Patent Application No. 16/272,487 (“487 application”; EX1009), filed on February 11, 2019, which claims benefit to the following provisional applications:
 - Provisional Appl. No. 62/771,212 (“the 212 application”), filed November 26, 2018; and
 - Provisional Appl. No. 62/629,180 (“the 180 application”), filed February 12, 2018.
4. I understand February 12, 2018, is the 142 patent’s earliest alleged priority date. I refer to this date throughout this Declaration.

5. In preparing this Declaration, I have reviewed the 142 patent (EX1043) and each of the documents cited herein, in light of the general knowledge in the art before February 12, 2018. The documents cited herein are provided as exhibits and each exhibit is a true and correct copy of what it purports to be and contain the types of facts or data an expert in the field would reasonably rely on when forming opinions.

6. In formulating my opinions, 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 of ordinary skill in the art (“POSA”), as defined below in Section IV.a, before February 12, 2018.

II. SUMMARY OF MY OPINIONS

7. I have been asked to consider whether, before February 12, 2018, Stahl *et al.*, “Visualization and analysis of gene expression in tissue selections by spatial transcriptomics,” *Science* 353(6294):78-82 (July 1, 2016) (“Stahl 2016”; EX1016) anticipated the challenged claims of the 142 patent as they are interpreted by NanoString. Stahl 2016 was published before February 12, 2018, and I understand that it qualifies as prior art. For the reasons I explain under Ground 1, in view of the state of the art before February 12, 2018, Stahl 2016 teaches every element of at least claims 1-4 and 6-13, arranged as claimed. I understand that Stahl 2016 is presumed enabled. Thus, for the reasons detailed under Ground 1, Stahl 2016

anticipated claims 1-4 and 6-13 of the 142 patent under NanoString's interpretation.

8. I have been asked to consider whether, before February 12, 2018, Stahl 2016 would have rendered the challenged claims of the 142 patent obvious. For the reasons I explain under Ground 2, in view of the state of the art before February 12, 2018, Stahl 2016 teaches or suggests all elements of the methods of claims 1-13 as interpreted by NanoString. Further, as I explain under Ground 2, a POSA reading Stahl 2016 would have been motivated to perform the claimed methods with a reasonable expectation of success. Thus, for the reasons detailed under Ground 2, Stahl 2016 would have rendered claims 1-13 of the 142 patent obvious under NanoString's interpretation.

9. I have been asked to consider whether, before February 12, 2018, Stahl 2016 in view of WO 2012/140224 ("Frisen") would have rendered claim 5 of the 142 patent obvious. I understand that Frisen was both filed and published before February 12, 2018, and thus it qualifies as prior art. As I explain in Ground 3, Stahl 2016 combined with Frisen teaches all the elements of claim 5. I also explain in Ground 3 that a POSA would have been motivated to modify Stahl 2016's workflow with Frisen's solid-phase second-strand cDNA synthesis methods, and would have had a reasonable expectation of success in so doing. Thus, for the

reasons set forth in Ground 3, claim 5 would have been obvious over the combination of Stahl 2016 and Frisen.

10. I have been asked to consider whether, before February 12, 2018, WO 2017/019456 (“So-456”; EX1005) anticipated the challenged claims of the 142 patent. I understand that So-456 was both filed and published before February 12, 2018, and thus it qualifies as prior art. For the reasons I explain under Ground 4, in view of the state of the art before February 12, 2018, So-456 teaches every element of claims 1-13 as interpreted by NanoString, arranged as claimed. I understand that So-456 is presumed enabled. Thus, for the reasons detailed under Ground 4, So-456 anticipated claims 11-13 of the 142 patent under NanoString’s interpretation.

11. I have been asked to consider whether, before February 12, 2018, So-456 would have rendered the challenged claims of the 142 patent obvious. For the reasons I explain under Ground 5, in view of the state of the art before February 12, 2018, So-456 teaches or suggests all elements of the methods of claims 1-13 as interpreted by NanoString. Further, as I explain under Ground 5, a POSA would have been motivated to perform the claimed methods with a reasonable expectation of success. Thus, for the reasons detailed under Ground 5, claims 1-13 of the 142 patent would have been obvious over So-456 under NanoString’s interpretation.

III. MY BACKGROUND AND QUALIFICATIONS

12. I am a Professor of Medicine and Human Genetics in the David Geffen School of Medicine at the University of California, Los Angeles.

13. I have over 20 years of experience in the field of genetics and genomics, including approaches for detecting or monitoring human diseases, such as spatial detection of target molecules in a biological sample. I also have over 15 years of experience in cancer genomics.

14. My current research focuses on computational biology, cancer biology, and cancer genomics. My lab develops technologies and approaches to understand the process by which cancer develops, monitor disease, and identify therapeutic strategies. Within this broad area of research, I have specific experience in using population genetics to help determine who is at risk for cancer, how to computationally analyze genomic data to identify early changes in cancers, and developing new genomic methodologies including those focused on analyzing single cells and the use of DNA barcodes that help identify individual molecules.

15. I have provided a copy of my *curriculum vitae* as EX1003.

16. I graduated with a Bachelor's degree in Biology from the Massachusetts Institute of Technology in 1995. I then received my Ph.D. in Genetics from Stanford University Medical School in 2000. My Ph.D. thesis topic was "Generating and Analyzing Genome Scale Data."

17. From 2000-2003, I was a postdoctoral fellow in the Department of Molecular and Cellular Biology at the University of California, Berkeley. As a postdoctoral fellow, I was selected for the prestigious National Science Foundation Biocomputing Fellowship, which covered both my stipend and significant research costs.

18. After my postdoctoral fellowship, I became a Staff Scientist at the Life Science Division of Lawrence Berkeley Lab (2007-2011) and served as a Special Assistant to the Deputy Director of the National Cancer Institute (2011). Subsequently, I served as an Associate Professor (2011-2013) and as a Full Professor with Tenure (2013-2023) in the Department of Molecular and Medical Genetics at Oregon Health & Science University. In 2023, I was recruited to the University of California, Los Angeles as a Professor of Medicine and Human Genetics.

19. I have authored more than 115 peer-reviewed publications that discuss methods of DNA manipulation and analysis, including DNA sample preparation for sequencing, amplification (*e.g.*, PCR), methods of DNA sequencing (including NGS and related sequencing methods), and bioinformatics methods for raw data analysis and interpretation.

20. Key among these papers are work on developing the field of gene expression profiling (Spellman *et al.*, *Molecular Biology of The Cell*, 1998; and

Eisen *et al.*, *Proceedings of the National Academy of Sciences*, 1998, which together have been cited in more than 20,000 other works), fabricating DNA microarrays (George, Wooley, and Spellman, *Genome Research*, 2001), ligation-based approaches to detecting and quantifying DNA and DNA methylation (Wang *et al.*, *Genome Biology*, 2008; and Nautiyal *et al.*, *Proceedings of the National Academy of Sciences*, 2011), histopathological classification by gene expression and imaging (*e.g.*, Chang *et al.*, *BMC Bioinformatics*, 2011), and the use of barcodes to identify unique molecules in multiplexed samples (Butler *et al.*, *Cold Spring Harbor Molecular Case Studies*, 2019).

21. In addition, I have presented over 100 invited lectures or conference presentations regarding these topics. I have also supervised five completed Ph.D. theses and have taught graduate-level courses at OHSU in the area of sequencing technologies, computational analysis, and genetics.

22. I have received over \$16 million in federal grants for my research in the area of cancer genomics and cancer precision medicine. I am currently one of 30 principal investigators for the Genome Data Analysis Network, sponsored by the National Cancer Institute. The Genome Data Analysis Network is a consortium of computational researchers from across the United States focused on developing the framework for relating the genomics and outcomes of patients in cancer clinical trials.

23. In 2017, I was named the inaugural holder of the Penny and Phil Knight Endowed Professorship for Cancer Research Innovation. In 2020, I was one of the recipients of the American Association for Cancer Research (AACR) Team Science award for my leadership roles in the Cancer Genome Atlas.

IV. MY UNDERSTANDING OF THE RELEVANT LEGAL PRINCIPLES

24. In formulating my opinions set forth in this Declaration, I have applied the following legal principles.

a. Person of Ordinary Skill in the Art (“POSA”)

25. I understand that patent law analyses are performed from the viewpoint of a POSA. I understand that a POSA is a hypothetical person who is presumed to be aware of all pertinent art, thinks along conventional wisdom in the art, and is a person of ordinary creativity. A POSA, with respect to the 142 patent, would have possessed a strong understanding of genetics and genomics approaches for detecting or monitoring human diseases, such as spatial detection of a target analyte (*e.g.*, mRNA or DNA) in a tissue section. A POSA would typically have a Ph.D. in a relevant field (*e.g.*, molecular biology, genetics, and bioinformatics) with two years of experience in the use and development of genetics and genomics approaches for detecting and monitoring human diseases, such as spatial detection of a target analyte (*e.g.*, mRNA or DNA) in a tissue section.

26. I understand that NanoString stated during prosecution of U.S. Patent No. 11,377,689 (a sibling of the 142 patent) 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.” EX1004, 268.

b. Claim Construction

27. I understand that in an IPR, patent claim terms are given their ordinary and customary meaning as understood by a POSA at the time of the invention, in view of the patent’s specification and its prosecution history. I also understand that when a patent explicitly defines a claim term in the specification, the patent’s definition controls.

28. I understand that claim 1 is an independent claim in the 142 patent, and that claims 2-13 are dependent claims. I understand that claims 2-13 depend directly or indirectly from claim 1. I understand that a dependent claim contains all the elements of the claim from which it depends. Thus, I understand that claims 2-13 contain all the elements of claim 1.

c. Obviousness

29. I understand that an obviousness analysis involves comparing a claim to the prior art to determine whether the claimed subject matter would have been obvious to a POSA in view of the prior art and general knowledge of the prior art. I understand that obviousness must be assessed from the viewpoint of a POSA at the

time of the invention. I further understand that to show obviousness a party must perform the following factual inquiries: (a) determining the scope and content of the prior art; (b) ascertaining the differences between the claimed invention and the prior art; (c) resolving the level of skill in the art; and (d) considering any known objective evidence of non-obviousness.

30. I also understand that, for a finding of obviousness, a POSA needs to have (i) a reason or motivation to modify or combine the prior art to arrive at the claimed subject matter and (ii) a reasonable expectation of doing so successfully.

31. I understand that the reason to combine prior-art references can come from a variety of sources, not just the prior art itself or the specific problem the patentee was trying to solve. And I understand that the references themselves need not provide a specific hint or suggestion of the alteration needed to arrive at the claimed invention; the analysis may include recourse to logic, judgment, and common sense available to a person of ordinary skill that does not need to be explicit in any reference.

32. I understand that a “reasonable expectation of success” is assessed in view of the prior art and general knowledge of the art from the viewpoint of a POSA, and it does not require an absolute certainty of success.

33. I also understand that the objective evidence of non-obviousness can include evidence of commercial success attributable to the claimed invention,

evidence of industry praise for the claimed invention, evidence of a long-felt need that was solved by the claimed subject matter, evidence that others copied the claimed subject matter, or evidence that the claimed subject matter achieved an unexpected, superior result relative to the closest prior art. I understand that such evidence must have a nexus, or causal relationship, to the claimed subject matter beyond what was available in the prior art, and must be commensurate in scope with the patent claim(s) at issue.

d. Anticipation

34. I understand that anticipation analysis involves comparing a claim to a single prior art reference to determine whether the claimed subject matter is novel to a POSA in view of the prior art and general knowledge in the prior art. I understand that anticipation must be assessed from the viewpoint of a POSA at the time of the invention.

35. I understand that anticipation analysis requires comparing the claim language to the prior art on an element-by-element basis. I understand that a prior art reference anticipates a claim if all of the claim's elements are expressly or inherently disclosed in a single prior art reference, arranged as in the claim. I understand that the disclosure in a prior art reference does not have to use the same words as the claim, but all of the requirements of the claim must be expressly or inherently disclosed in the prior art reference.

36. I understand that a prior art reference anticipates a patent claim when the claim element not expressly found in that reference are nonetheless inherent in it. I understand that express disclosure means that the subject matter is clearly described in the prior art reference.

37. I understand that an element or the entire invention is inherent and in the public domain if it is the natural result flowing from the explicit disclosure of the prior art. I understand that for a claimed element to be inherently present within a prior art document, it must be necessarily present. That is, the prior art reference must necessarily function in accordance with or include the claimed element. I understand that inherency may not be established by probabilities or possibilities—the mere fact that a certain thing may result from a given set of circumstances is not sufficient.

38. I understand that in some cases, the inherent property corresponds to a claimed new benefit or characteristic of a purported invention otherwise in the prior art. I understand that in such circumstances, the new realization alone does not render the old invention patentable. I further understand that when a prior art method is at issue, the inherent anticipation examines the natural and inherent results in that method without regard to the full recognition of those benefits or characteristics within the art field at the time of the prior art disclosure.

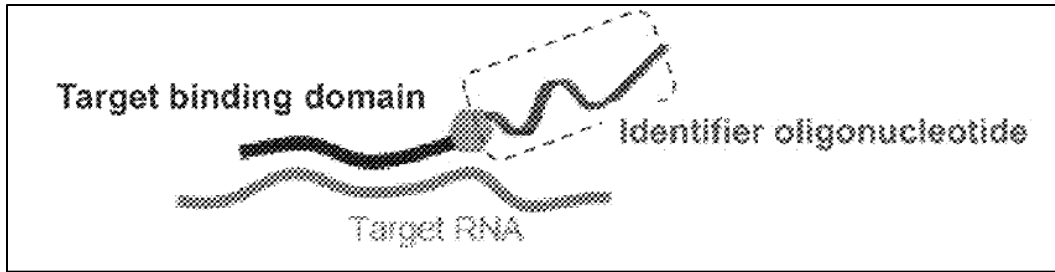
V. THE 142 PATENT AND ITS CLAIMS

a. The 142 patent

39. The 142 patent relates to spatially detecting and quantifying target analytes (*e.g.*, nucleic acids) in a “user-defined region of a tissue, user-defined cell, and/or user-defined subcellular structure within a cell.” EX1043, Abstract.

40. The 142 patent’s methods depend on *cleavable* primary probes (referred to as “probes” in the patent) that bind to target analytes in a tissue sample and are cleaved when a “force” is applied at specific, user-defined locations in the tissue sample. EX1043, 16:51-17:9, 18:43-49, 19:54-62, 21:10-15, 21:63-22:26, 23:16-24, 24:1-24:25, 29:4-29:27, 31:59-67, 32:1-10, 32:32-40, 34:3-28, 34:31-34, 34:60-67, 35:48-55, 35:57-60, 36:26-32, 37:6-8, 39:18-32, 49:19-29, 49:36-62, FIG. 11.

41. The patent’s cleavable “probes” comprise two regions: (1) a “target binding domain” (a portion that binds to the target analyte in the sample), and (2) an “identifier oligonucleotide” (a portion that is cleaved off from the probe when a force is applied and is collected for further processing). EX1043, 37:21-25, 38:36-40, FIG. 11. I have reproduced a schematic depiction of an exemplary cleavable probe against a target nucleic acid (RNA) from Figure 11 of the patent.



EX1043, FIG. 11.

42. Thus, in every disclosure throughout the 142 patent, the methods require the following features:

(1) The methods use cleavable primary probes (referred to as “probes” in the patent) having a “target binding domain” and an “identifier oligonucleotide.”

These cleavable probes hybridize to the target analytes in the tissue sample and are discussed in detail below.

(2) The methods apply a “force” at a user-defined “region of interest” (ROI) in the tissue sample to cleave the “probes” within that ROI and release the “identifier oligonucleotide” portion of the probes, collect the released identifier oligonucleotides separately from each ROI, and tag the collected identifier oligonucleotides with a spatial barcode preassigned to the user-defined ROI for analysis.

See, e.g., EX1043, 1:46-67, 2:3-25, 2:28-54, 3:24-47, 3:50-4:9, 4:36-5:2, 5:5-37, 5:46-6:8, 6:11-43, 6:46-7:9, 7:12-44, 8:20-52, 8:55-9:23, 9:26-58, 9:61-10:29, 11:3-25, 11:28-52, 11:55-12:4, 12:7-28, 12:31-57, 12:60-13:8, 16:52-17:32,

18:43-49, 19:36-20:20, 20:65-21:7, 21:10-15, 22:1-54, 23:15-21, 24:1-52, 27:14-31, 28:7-25, 29:5-27, 29:30-49, 31:37-32:26, 32:33-40, 34:4-39, 34:60-67, 35:29-36:18, 37:2-8, 39:18-32, 49:19-29, 49:52-62, FIGs. 1, 2, 3, 11, 12, 13, 20, 26.

43. In other words, the 142 patent's methods require a user to collect proxies (*i.e.*, the cleaved off identifier oligonucleotides) for the target analytes from within a particular, user-defined region of interest of the tissue sample while maintaining the target analyte's spatial information. *See, e.g.*, EX1043, FIGs. 1, 2, 3, 11, 12, 13, 20, 26, 1:46-67, 2:3-25, 2:28-54, 3:24-47, 3:50-4:9, 4:36-5:2, 5:5-37, 5:46-6:8, 6:11-43, 6:46-7:9, 7:12-44, 8:20-52, 8:55-9:23, 9:26-58, 9:61-10:29, 11:3-25, 11:28-52, 11:55-12:4, 12:7-28, 12:31-57, 12:60-13:8, 16:52-17:32, 19:36-20:20, 20:65-21:7, 21:10-15, 22:1-54, 24:1-52, 27:14-31, 28:7-25, 29:5-27, 29:30-49, 31:37-32:26, 34:4-39, 35:29-36:18, 37:6-17.

44. Below I provide further details of the components and workflow of the patent's methods.

i. Components of the 142 patent's methods

45. **"Probes"**. The patent's methods use "*probes* for the compositions and methods of spatially detecting at least one target analyte in a sample" that comprise "*a target binding domain and an identifier oligonucleotide.*" EX1043, 37:20-22 (emphasis added), 1:46-51 (emphasis added), 2:3-9, 2:28-34, 3:26-32, 3:50-56, 4:36-42, 5:5-11, 5:46-51, 6:11-17, 6:46-52, 7:12-18, 8:20-25, 8:55-61, 9:26-32,

9:61-67, 11:3-11, 11:28-35, 11:55-61, 12:7-13, 12:31-36, 12:60-13:1, 16:52-67, 19:36-53, 22:1-17, 24:1-16, 27:14-27, 28:7-21, 29:4-18, 31:37-58, 34:3-19, 35:29-45. A “target binding domain” is “a region of the probe that specifically binds to at least one target analyte in a sample.” EX1043, 37:23-25. If the target analyte is a nucleic acid (for example, an mRNA), “the target binding domain can be a target nucleic acid-binding region” comprising a nucleotide sequence. EX1043, 37:26-40.

46. **“Identifier oligonucleotide”**. An “identifier oligonucleotide is a nucleic acid molecule that identifies the target analyte bound to the target binding domain.” EX1043, 38:36-40. It “comprises a unique nucleic acid sequence that identifies the target analyte bound to the target binding domain of the probe.” EX1043, 38:36-40. Importantly, the identifier oligonucleotide is separable or releasable from the rest of the probe upon application of a “force” and the released identifier oligonucleotide is collected and analyzed. EX1043, 1:51-67, 2:9-25, 2:34-54, 3:32-47, 3:56-4:9, 4:42-5:2, 5:11-37, 5:52-6:8, 6:17-43, 6:52-7:9, 7:18-44, 8:25-52, 8:61-9:23, 9:32-58, 9:67-10:29, 11:11-25, 11:35-52, 11:61-12:4, 12:14-28, 12:36-57, 13:1-8, 17:3-18:18, 19:56-20:64, 22:20-23:6, 24:19-26:19, 27:14-28:4, 28:7-65, 29:4-31:10, 31:61-32:31, 34:22-52, 35:48-36:18, 39:18-37, 49:8-51, 49:52-62.

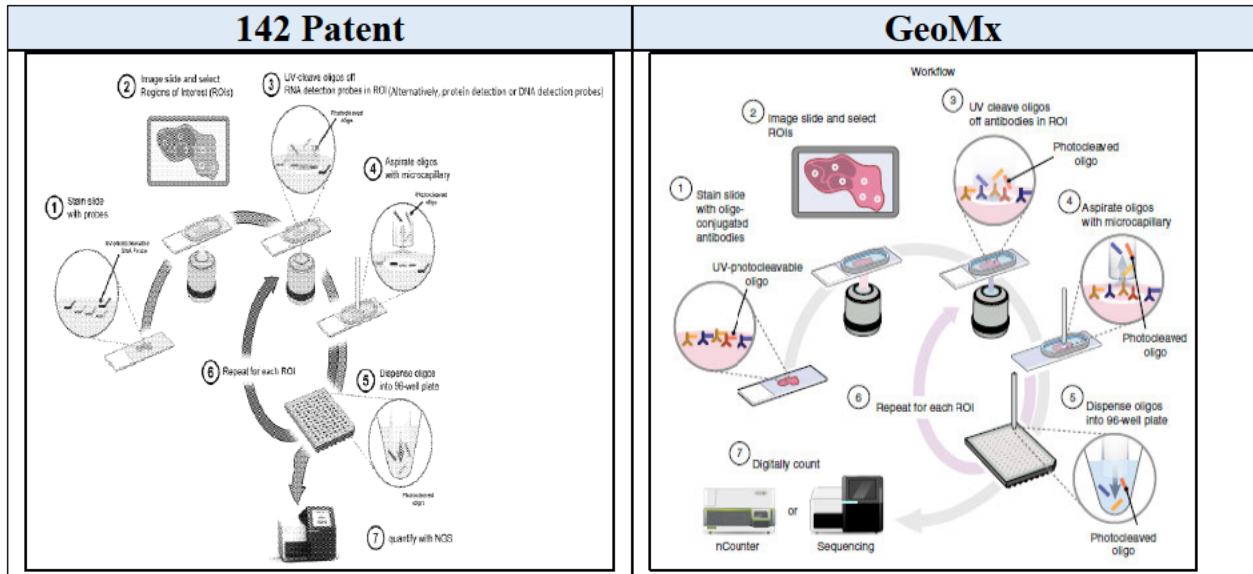
47. The patent’s preferred embodiment of a “force” is ultraviolet light that cleaves a photo-cleavable linker between the target-binding domain and identifier

oligonucleotide. EX1043, 18:45-49, 21:12-15, 23:18-22, 32:36-40, 34:64-67, 36:29-32, 37:6-37:8, 39:32-37, 77:1-3, 77:37-40, 77:60-62, 78:13-16, 81:25-82:1.

48. **“Region of interest” or “ROI”**. A “region of interest” or “ROI” is a “specific location within a sample.” EX1043, 46:58-61. Each ROI has a predetermined spatial barcode sequence assigned to it, allowing identifier oligonucleotides collected from the same ROI to be identified by their shared spatial barcode (*i.e.*, a “nucleic acid sequence which identifies the specific location of the tissue sample from which the identifier oligonucleotide was released”). EX1043, 17:26-32, 18:31-40, 20:65-21:7, 32:16-26, 46:58-61. The collected identifier oligonucleotides are subsequently tagged with a predetermined spatial barcode for their corresponding ROI. EX1043, 17:26-32, 18:31-40, 20:65-21:7, 32:16-26, 46:58-61. Thus, identifier oligonucleotides collected from the same ROI are identified by their shared spatial barcode. EX1043, 17:26-32, 18:31-40, 20:65-21:7, 32:16-26, 46:58-61.

ii. Workflow of the 142 patent’s methods

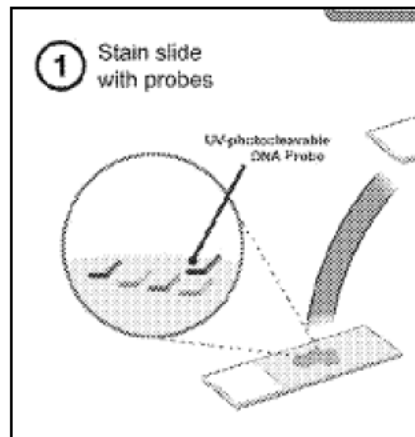
49. The 142 patent’s methods use cleavable “probes” that hybridize to the target analytes (described above) and involve steps that occur on the target analyte (“on-analyte” steps) and off (or away from) the target analyte (“off-analyte” steps). The overall workflow of the 142 patent’s methods is nearly identical to that of NanoString’s GeoMx workflow, as illustrated in the Figures reproduced below.



EX1043, FIG. 20; EX1013, FIG. 1A.

(a) On-analyte steps

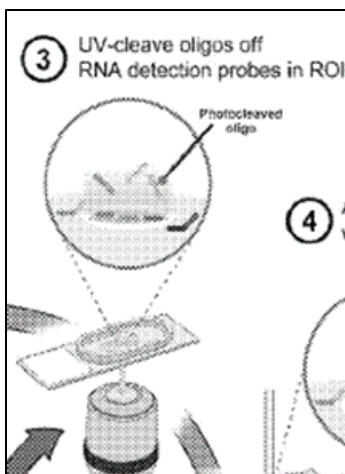
50. The method begins with adding the cleavable primary probes (“probes”) to a slide containing a biological sample (*e.g.*, a tissue sample). The 142 patent refers to this as “stain[ing]” the slide with “UV-photocleavable DNA probe” in its overview Figure 20, Step 1:



EX1043, FIG. 20, 37:1-4. This hybridization step is an on-analyte step, which takes place *on* the target analyte in the tissue sample (*in situ*). EX1043, FIG. 20, 37:1-4.

51. After hybridization of DNA probes (*i.e.*, primary probes), a region of interest (ROI) within the sample is selected (FIG. 20, Step (2)), and a “force” (*e.g.*, UV light) is applied to the ROI to selectively cleave oligonucleotide fragments off the primary probes in the ROIs (FIG. 20, Step (3)). EX1043, FIGs. 16, 18, 20, 18:45-49, 21:12-15, 23:18-22, 32:36-40, 34:64-67, 36:29-32, 37:6-37:18, 39:32-37, 77:1-3, 77:37-40, 77:60-62, 78:13-16, 81:25-82:1.

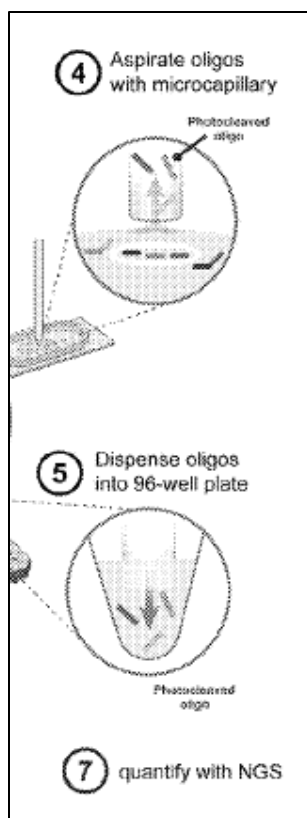
52. The 142 patent’s preferred embodiment of a “force” is UV light that cleaves a photo-cleavable linker between the target binding domain and identifier oligonucleotide, similar to the GeoMx workflow. EX1043, 18:45-49, 21:12-15, 23:18-22, 32:36-40, 34:64-67, 36:29-32, 37:6-37:8, 39:32-37, 77:1-3, 77:37-40, 77:60-62, 78:13-16, 81:25-82:1; EX1013, FIG. 1A. The 142 patent refers to this as “UV-cleave oligos off” in its overview Figure 20, Step 3:



EX1043, FIG. 20.

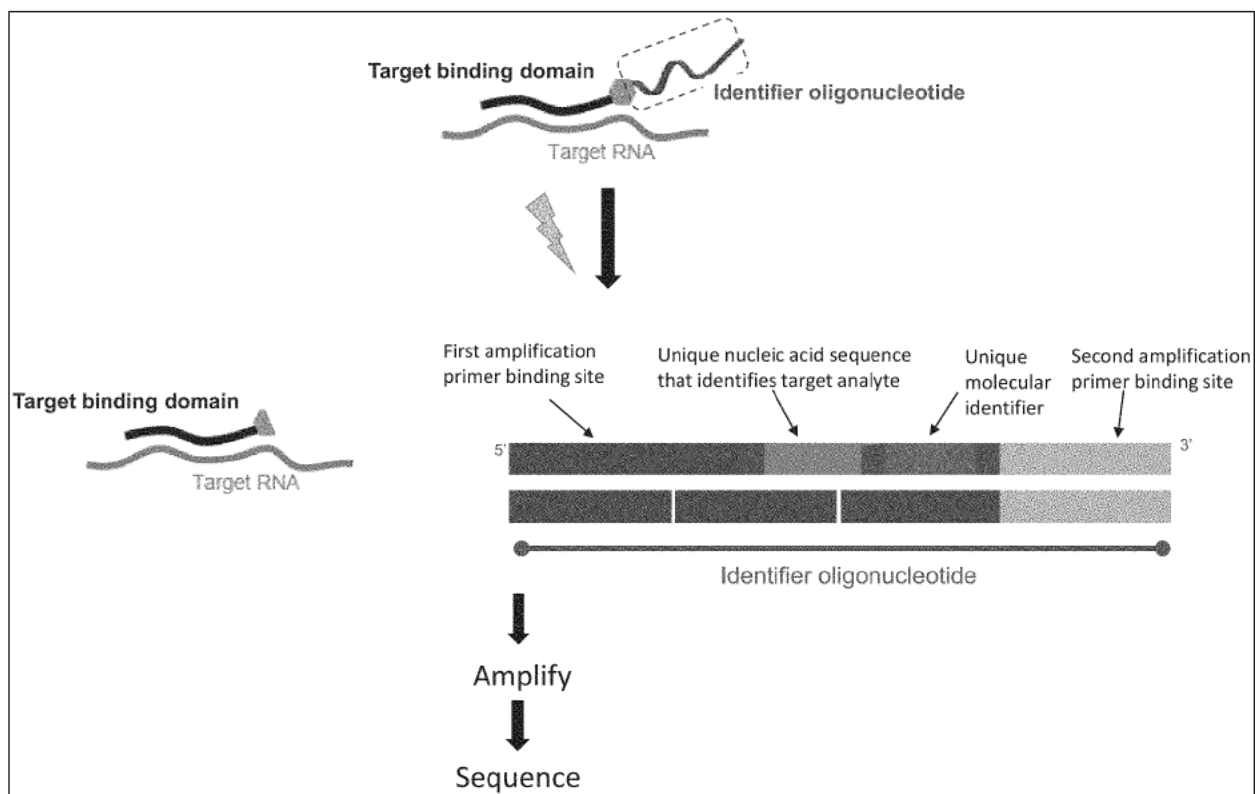
(b) Off-analyte steps

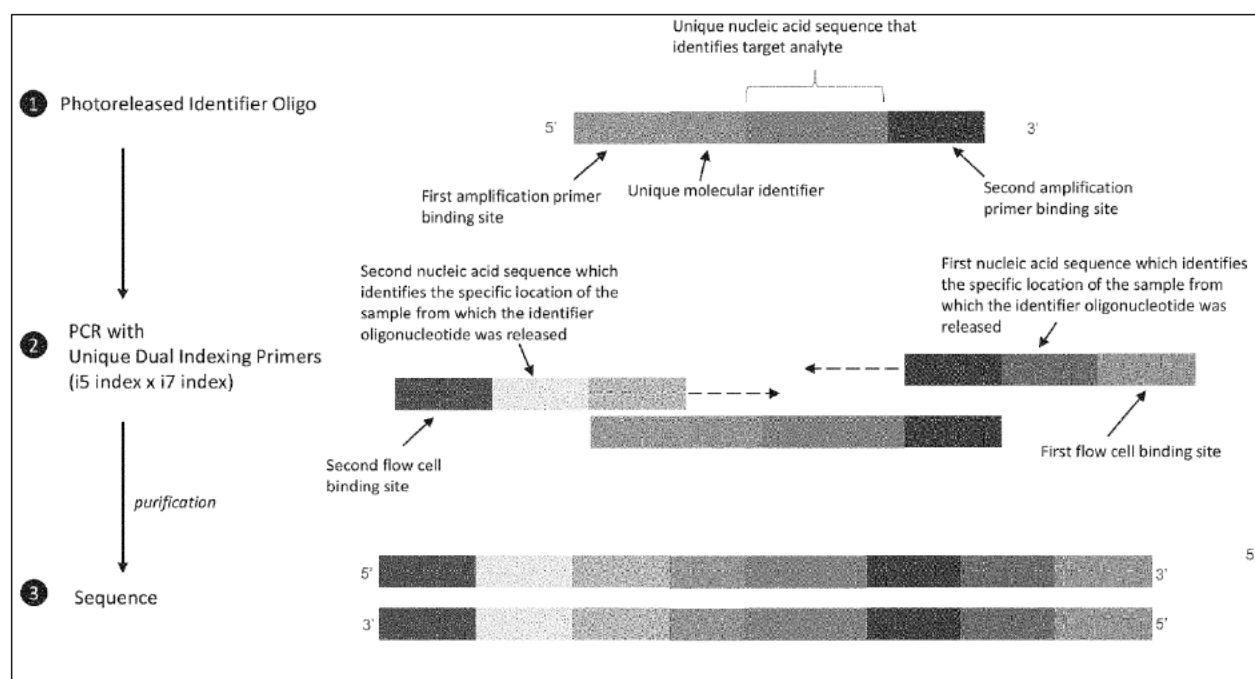
53. The released identifier oligonucleotides are then collected from the selected ROI (step 4, “Aspirate oligos”) and analyzed (steps 5, “Dispense oligos into 96-well plate” and 7, “quantify with NGS”):



EX1043, FIG. 20. These collection and analysis steps are off-analyte steps, taking place *off* the target analyte. The 142 patent does not discuss spatially barcoded arrays for capturing or processing the target analytes or their proxies. *See, e.g.*, EX1043, FIGs. 18, 20.

54. Figures 11 and 19 (reproduced below) exemplify a direct PCR method.

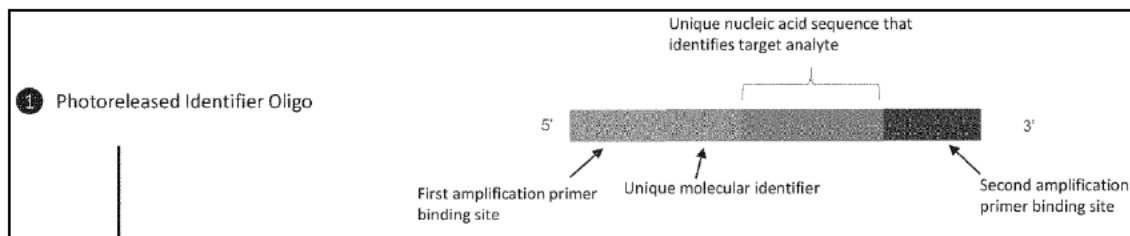




EX1043, FIGs. 11, 19, 32:32-33:38.

55. The direct PCR method begins with an “on-analyte” step that includes, “contacting at least one target analyte in a sample with at least one *probe* of the present disclosure,” comprising a “target binding domain” and an “identifier oligonucleotide.” EX1043, 31:37-49 (emphasis added).

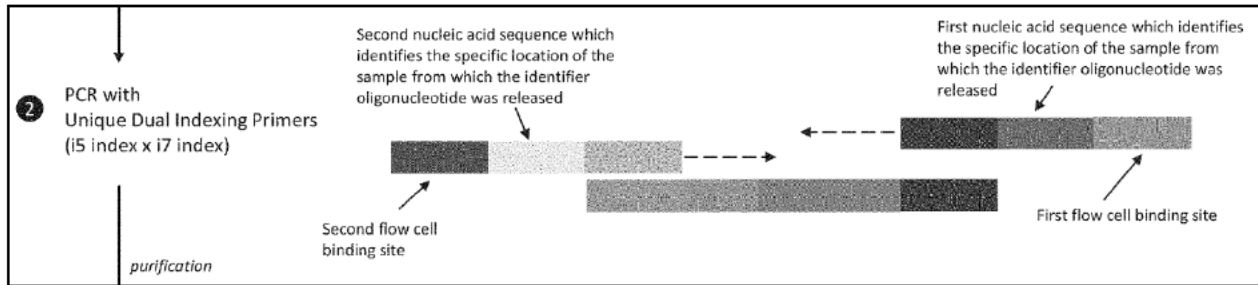
56. Following contacting, the direct PCR method then proceeds by “providing a force to a location of the sample sufficient to release the identifier oligonucleotide” (identified as “[p]hotoreleased identifier Oligo” in Figure 19) and then “collecting the released identifier oligonucleotide.” EX1043, 31:60-32:2, FIGs. 11, 19. The released identifier oligonucleotide contains a first amplification primer binding site at its 5'-end and a second amplification primer binding site at its 3'-end.



EX1043, FIG. 19.

57. After collecting the released identifier oligonucleotides, the method includes a step of “amplifying the released identifier oligonucleotide using a *first amplification primer* capable of binding to the first amplification primer binding site and a *second amplification primer* capable of binding to the second

amplification primer binding site.” EX1043, 32:12-16 (emphasis added), FIGs. 11, 19. The amplification step is depicted in Step 2 of Figure 19 (reproduced below) where the amplification primers hybridize to the released identifier oligonucleotide and amplify the released identifier oligonucleotide in a PCR reaction.



EX1043, FIG. 19.

58. “The amplification primer that hybridizes to the second amplification primer binding site comprises ... a nucleic acid sequence complementary to the second amplification primer binding site,” and “[t]he amplification primer that hybridizes to the first amplification primer binding site comprises ... a nucleic acid sequence complementary to the first amplification primer binding site.” EX1043, 32:67-33:12, FIG. 19. The amplification primers also comprise a first or a second “nucleic acid sequence which identifies the specific location of the sample from which the identifier oligonucleotide was released,” *i.e.*, spatial barcode. EX1043, 32:16-20. The PCR products amplified from the identifier oligonucleotides collected from the same location of the tissue sample are tagged with the same spatial barcode, and the PCR products amplified from the identifier

oligonucleotides collected from different locations of the tissue sample are tagged with different spatial barcodes.

59. Next, the PCR products are processed by “identifying the released oligonucleotide by sequencing the amplified products ... thereby spatially detecting the at least one target analyte in the sample.” EX1043, 32:28-31.

60. Thus, the patent’s direct PCR-based method includes the following general steps:

(1) Contacting a target nucleic acid with a probe comprising a target binding domain and an identifier oligonucleotide;

(2) Providing a force to release the identifier oligonucleotide;

(3) Collecting the released identifier oligonucleotide;

(4) Amplifying the released identifier oligonucleotide using amplification primers that contain a spatial barcode; and

(5) Sequencing the amplification product that contains the spatial barcode to identify the released identifier oligonucleotide and determining the location from which it was derived.

EX1043, 11:3-25, 11:28-52, 12:60-13:8, 31:38-32:31, 32:32-32:56, 32:58-33:16, 33:17-33:38, FIGs. 11, 19.

b. The 142 patent claims

61. I understand that the 142 patent issued with 13 claims. EX1043, 147:33-148:62. I understand that claim 1 is the independent claim, and claims 2-13 depend from claim 1. I understand that dependent claims 2-13 incorporate the elements of the claims from which they depend.

62. The 142 patent claims are drawn to methods for spatially detecting and quantifying oligonucleotides in a tissue sample. Independent claim 1 of the 142 patent recites a method that includes “collecting a plurality of oligonucleotides” from a first and second location of a tissue sample, “synthesizing” a first and second plurality of DNA products that incorporates “at least one nucleic acid sequence that identifies the first [and second] location of the tissue sample,” and “sequencing” the first and second plurality of DNA products to spatially detect the collected oligonucleotides. EX1043, 147:33-61. I have included claim 1 below.

A method comprising:

a) collecting a plurality of oligonucleotides from a first location of a tissue sample under conditions that release the plurality of oligonucleotides from the first location of the tissue sample;

b) collecting a plurality of oligonucleotides from a second location of the tissue sample under conditions that release the plurality of oligonucleotides from the second location of the tissue sample;

c) synthesizing a first plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (a) as templates and incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the first plurality of DNA products;

d) synthesizing a second plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (b) as templates and incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the second plurality of DNA products; and

e) identifying the first plurality of DNA products and the second plurality of DNA products synthesized in step (c) and step (d) by sequencing the first plurality of DNA products and the second plurality of DNA products, thereby spatially detecting the plurality of oligonucleotides collected from the first location of the tissue sample and the plurality of oligonucleotides collected from the second location of the tissue sample.

EX1043, 147:34-61.

63. The dependent claims 2-13 further specify various elements of the independent claim, *e.g.*, the sequencing method and library amplification steps.

EX1043, 147:62-148:62.

c. The 142 patent prosecution history

64. I have reviewed the 142 patent's prosecution history (EX1014).

NanoString filed the 712 application (that led to the 142 patent) on September 16, 2021. EX1014. I understand from the file history that after initially rejecting the claims, the patent examiner eventually allowed the claims—which issued as the 142 patent claims. I understand that the examiner did not raise any rejections against the claims based on prior art. EX1014.

VI. PRIOR ART DISCLOSES THE CLAIMED METHODS

65. The 142 patent's claims relate to spatial analysis technology in life sciences for determining the locations of target oligonucleotides (*e.g.*, DNA or RNA) in biological samples, such as tissue samples. As early as 2011, Stahl *et al.* developed a pioneering spatially barcoded array paradigm for determining the locations of target mRNAs in a biological sample. The scientists disclosed this foundational technology in a patent application filed in 2011 (WO 2012/140224, “Frisen”; EX1017) and subsequently published it in a 2016 article in Stahl *et al.*, “Visualization and analysis of gene expression in tissue selections by spatial transcriptomics,” *Science* 353(6294):78-82 (July 1, 2016), and associated supplementary material (“Stahl 2016”; EX1016). This technology was also applied in the spatial detection products by Spatial Transcriptomics, Inc. (ST). *See, e.g.*, EX1018. Subsequently, numerous publications, such as WO 2017/019456 (“So-

456”), applied Stahl 2016’s spatially barcoded array paradigm for detecting target analytes in biological samples. EX1005, ¶¶[00140]-[00144], [00150]-[00164], [00307]-[00320]; *see also* EX1006; EX1025; EX1026; EX1027; EX1028.

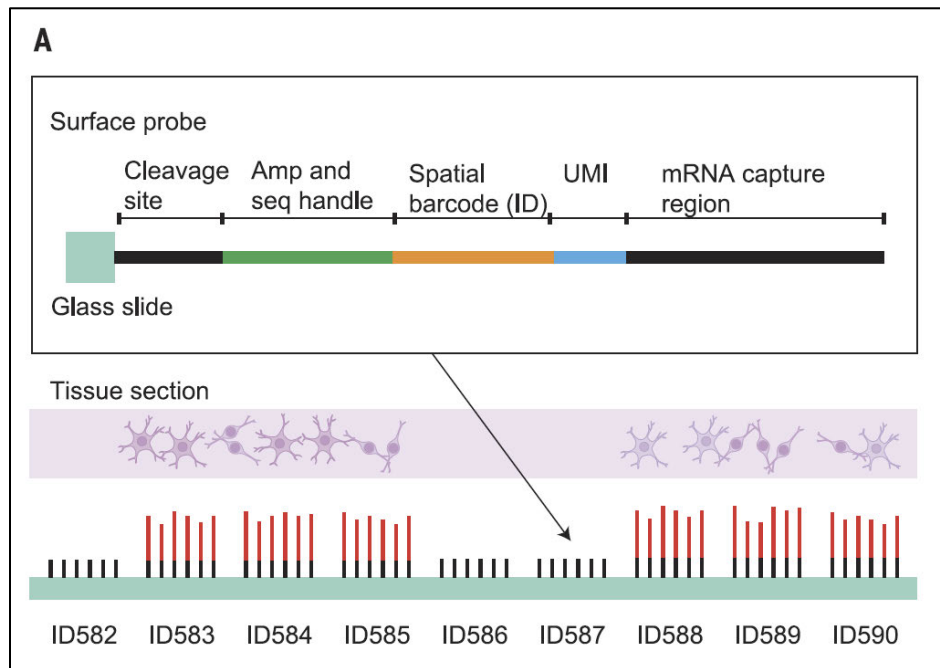
**a. Stahl 2016 disclosed methods using spatially barcoded arrays
before February 12, 2018**

66. Stahl 2016 details a new methodology called “spatial transcriptomics” that allowed for “visualization and quantitative analysis” of the entire transcriptome with “spatial resolution” in individual tissue sections. EX1016, Abstract. A key aspect of this method are unique DNA sequences called “positional barcodes” or “spatial barcodes” that specifically identify the location of a target mRNA molecule in the tissue section. EX1016, Abstract (noting “positioning histological [tissue] sections on arrayed reverse transcription primers with unique positional barcodes”). These spatial barcodes are similar in concept to latitude and longitude, which uniquely identifying a specific location on the map. In Stahl 2016, the spatial barcodes uniquely identify a location on the array that was in contact with a tissue sample.

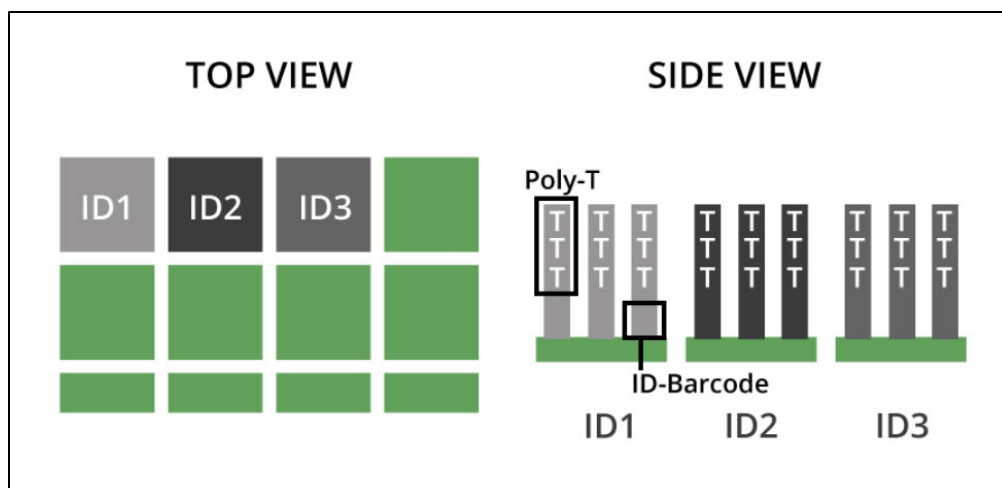
67. Stahl 2016’s method utilizes oligonucleotides (called “surface” or “capture” probes) containing these spatial barcodes, which are immobilized on a solid surface (*e.g.*, a glass slide) in a two-dimensional array of “spots” or “features.” EX1016, 78 (middle col. and right col.), FIG. 2A. Stahl 2016 discloses that its capture probes comprise an “AmC6” (amino modifier C6) conjugate, which

is used to immobilize the capture probes onto the array (e.g., the glass slide).

EX1016, Suppl. Materials, 2-3; EX1039; EX1040, 1. I have reproduced Figure 2A from Stahl 2016 showing an array (green glass slide) containing immobilized array probes (“surface probe”; black vertical lines) and a diagram from ST’s website showing a top- and a side- view of the array.



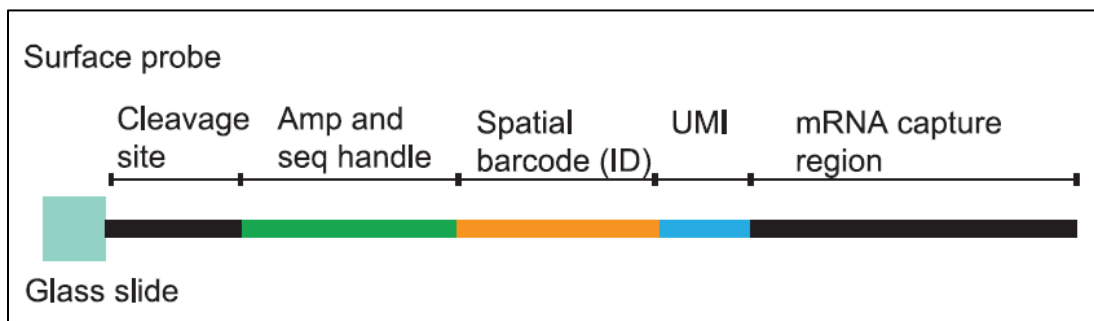
EX1016, FIG. 2A.



EX1018, 3.

68. As depicted above, each spot (identified by a different “ID”) on the solid surface contains a cluster of array probes (vertical rectangular bars in the ST diagram; vertical black lines in Stahl 2016’s Figure 2A) comprising a unique “spatial barcode” (“ID-Barcode”; “ID”) having a nucleic acid sequence specific to that spot. EX1016, Abstract, 78, FIG. 2A. All the array probes in one spot on the array share the same spatial barcode sequence, which is different from the spatial barcode sequence in the array probes in other spots. EX1016, Abstract, 78, FIG. 2A. The spatial barcodes act as “tags” for locations of their corresponding array probes on the solid surface, which were contacted to the locations on the tissue sample and allow one to differentiate biological molecules based on their spatial location on the array surface and therefore in the sample. EX1016, Abstract, 78, FIG. 2A.

69. In addition to the spatial barcode, each array probe contains a cleavage site, an amplification and sequence handle, a spatial barcode, a unique molecular identifier (“UMI”), and an mRNA capture region. EX1016, FIG. 2A. I have reproduced below a figure from Stahl 2016 depicting an array probe.

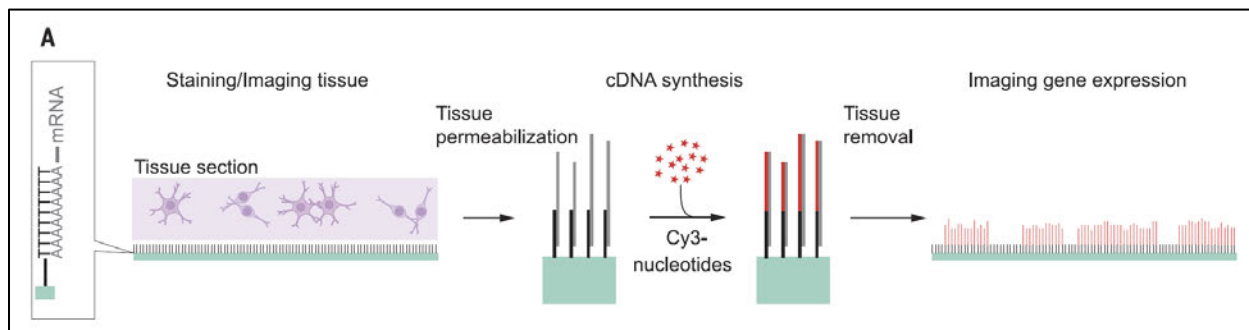


EX1016, FIG. 2A.

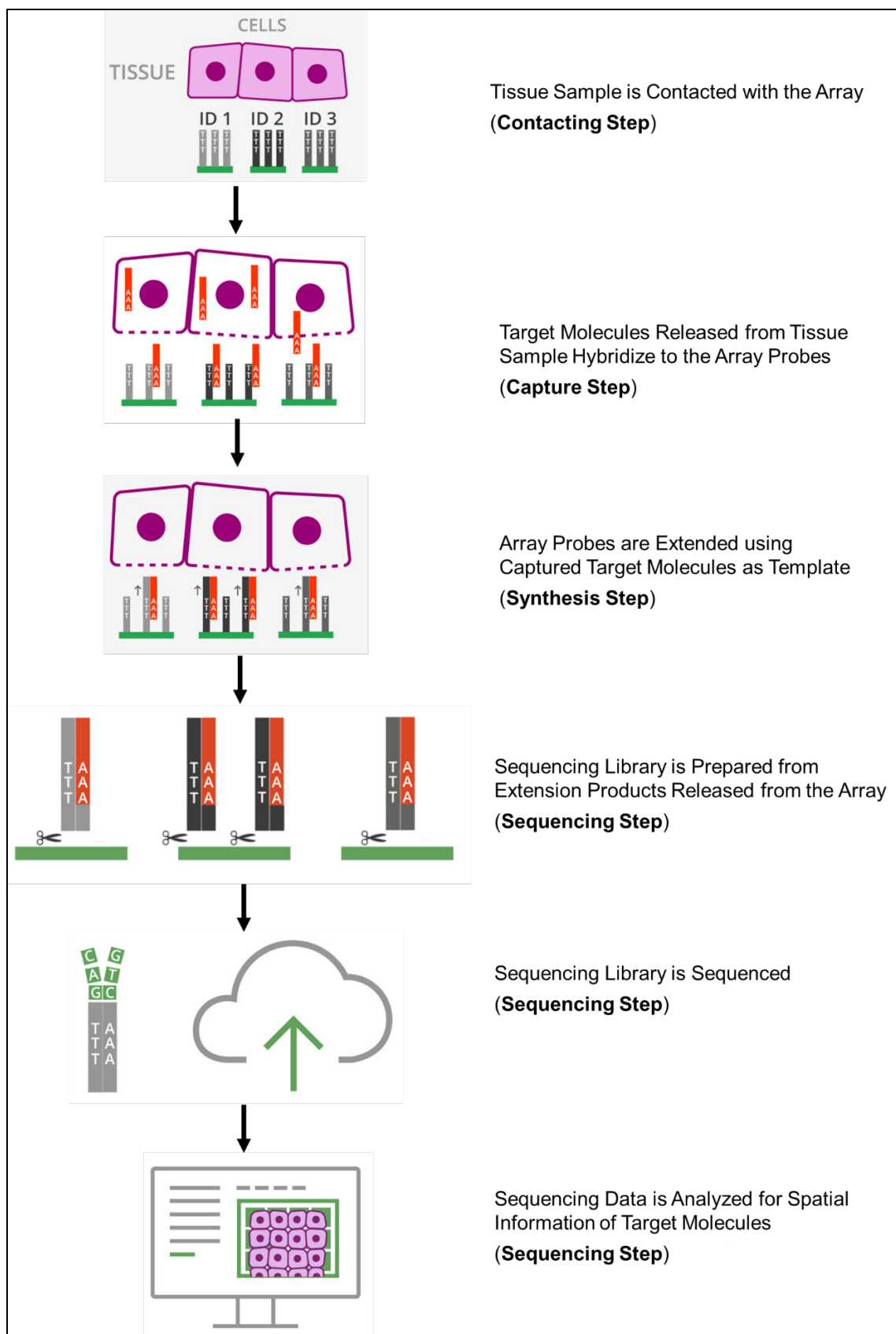
70. The “mRNA capture region” contains a nucleic acid sequence that is complementary to, and hybridizes to, a region of the target mRNA in the tissue sample. For example, an mRNA capture region may contain a poly-T sequence that binds to its complementary poly-A sequence in the target mRNA. EX1016, FIG. 2A, 80. The UMI is a random nucleic acid sequence that is unique to each array probe and is used to identify post-amplification molecules that are copies of the same original molecule. EX1016, FIG. 2A; EX1019, FIG. 1A, 72. UMIs were commonly used in sequencing workflows before 2018 with statistical approaches to provide accurate counts of target molecules. EX1019, FIG. 1A, 72.

71. The above-described barcoded arrays are used in the spatial analysis workflow that ultimately detects the signal by imaging (as shown in Stahl 2016’s

Figure 1A reproduced below) or sequencing (as shown in the figure reproduced below from ST's website, with my annotated text on the right, and discussed in Stahl 2016).



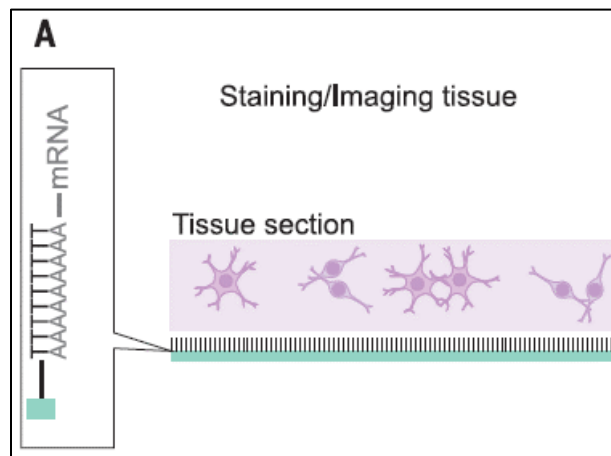
EX1016, FIG. 1A.



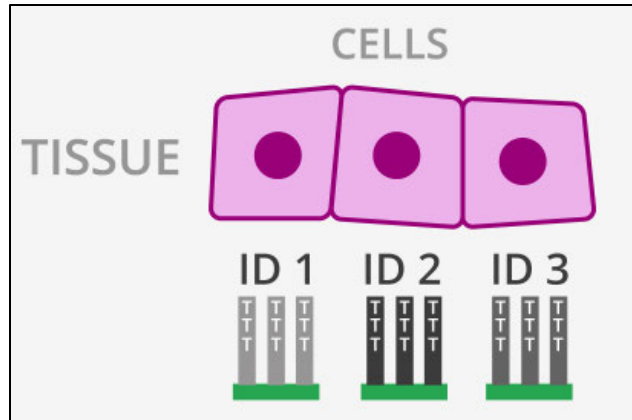
EX1018, 4-9 (annotated).

72. The workflow depicted above includes (1) contacting a tissue section with a barcoded array (contacting), (2) releasing the mRNAs from the tissue section and capturing the released mRNAs via hybridization to the barcoded array probes (release and capture), (3) extending the array probes using the hybridized mRNAs as a template (probe extension), and (4) analyzing the extended probes (imaging or sequencing).

73. **CONTACTING:** A tissue section is placed on the surface of glass slides containing immobilized, barcoded array probes. *See also* EX1016, 78 (middle col. and right col.), FIG. 1A. The figures below show the contacting step.

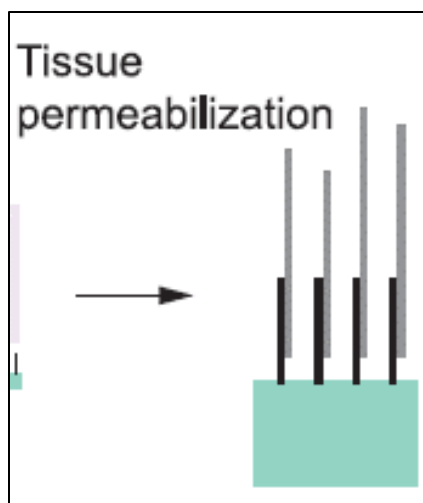


EX1016, FIG. 1A.

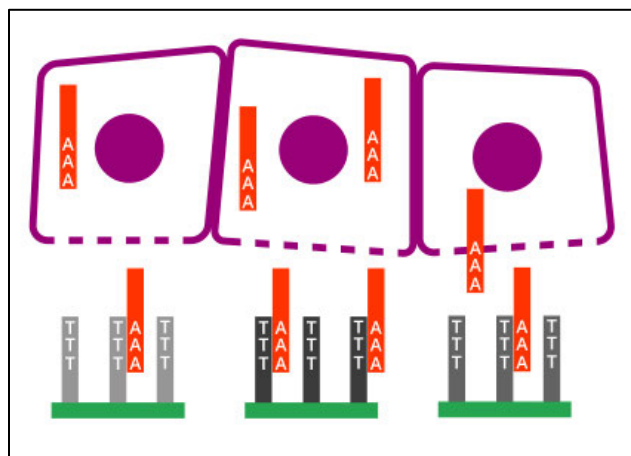


EX1018, 4.

74. **CAPTURE:** The overlaid tissue section is permeabilized, which creates holes in cell membranes and mRNAs diffuse away from the tissue onto the array. EX1016, 78 (middle col.), FIGs. 1A, 2A, Suppl. Materials, 1, 4-5. The mRNAs are then captured via hybridization to the array probes immobilized in spots on the array surface in proximity to where the mRNAs were found in the tissue. EX1016, 78 (middle col.). This localizes the mRNAs from the tissue sample in the spatial position they were found in the cells. The figures below show the target mRNA as grey bars diffusing out of the cell and hybridizing to the array probes on the surface of the glass slide in the capture step.



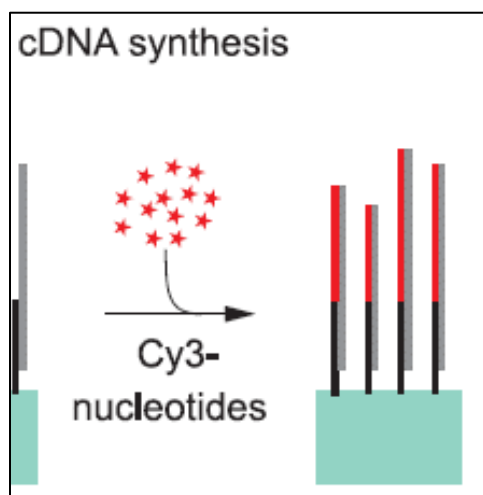
EX1016, FIG. 1A.



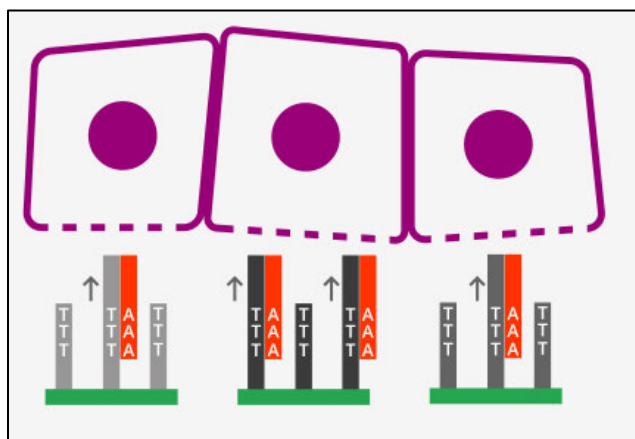
EX1018, 5.

75. **SYNTHESIS**: After capturing the mRNAs, a cDNA strand is synthesized by using the barcoded array probes as primers (which are extended) and the mRNAs hybridized to the array probes as templates. EX1016, 78 (middle col. and right col.), FIG. 1A. EX1018, 6. To achieve this, reverse-transcription reagents are added to the tissue to synthesize complementary DNAs (cDNAs) that are complementary to the target mRNAs hybridized to the array probes and contain

the spatial barcode sequence of the array probes. EX1016, 78 (middle col.). The figures below show the synthesis step.



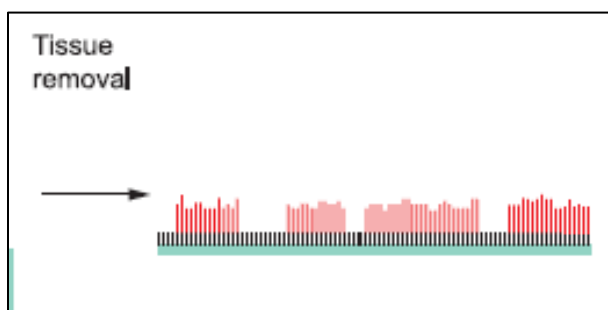
EX1016, FIG. 1A.



EX1018, 6.

76. The resulting cDNA extension product comprises a sequence complementary to the captured mRNA and the sequences of the capture probe's spatial barcode and UMI. EX1016, FIG. 2A.

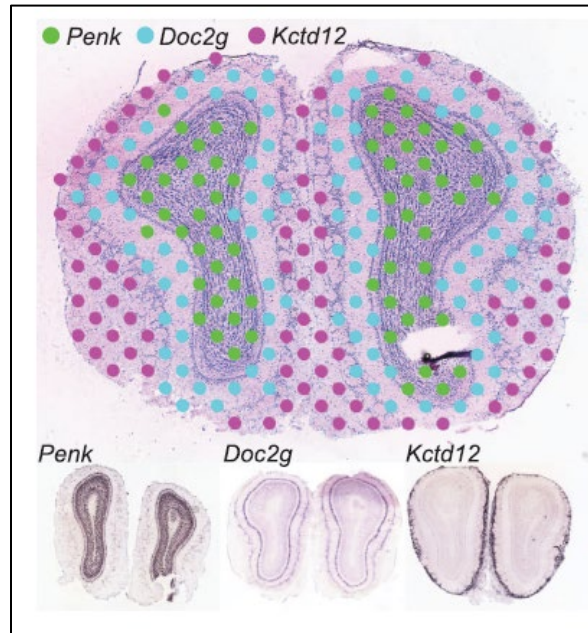
77. **SEQUENCING:** After extension, the tissue section is enzymatically digested, leaving the extended cDNA molecules on the slide, as shown in the figure below. EX1016, 78 (right col.), FIGs. 1A, 2A, Suppl. Materials, 2, 5-6.



EX1016, FIG. 1A.

78. For analysis by sequencing, the extended DNA molecules are processed to generate sequencing libraries that are read out using next-generation sequencing devices. EX1016, 78 (middle col. and right col.), FIGs. 1A, 2A, Suppl. Materials, 2, 5-7. The method next “sort[s] the RNA-seq data to its corresponding array features by using the spatial barcodes and aligned the tissue image with the features of the array, which enabled visualization and analyses.” EX1016, 78 (middle col. and right col.), FIGs. 1A, 2A, Suppl. Materials, 1. The data generated from analyzing the extended DNA molecule captures both the genetic information of the target molecule, as well as the spatial location of the target molecule. EX1016, 78 (middle col. and right col.), FIGs. 1A, 2A, Suppl. Materials, 2, 13-16.

79. For analysis by imaging, Stahl 2016 presents the results in, *e.g.*, Figure 2B, demonstrating visualization of expression of three different genes in a mouse olfactory bulb:

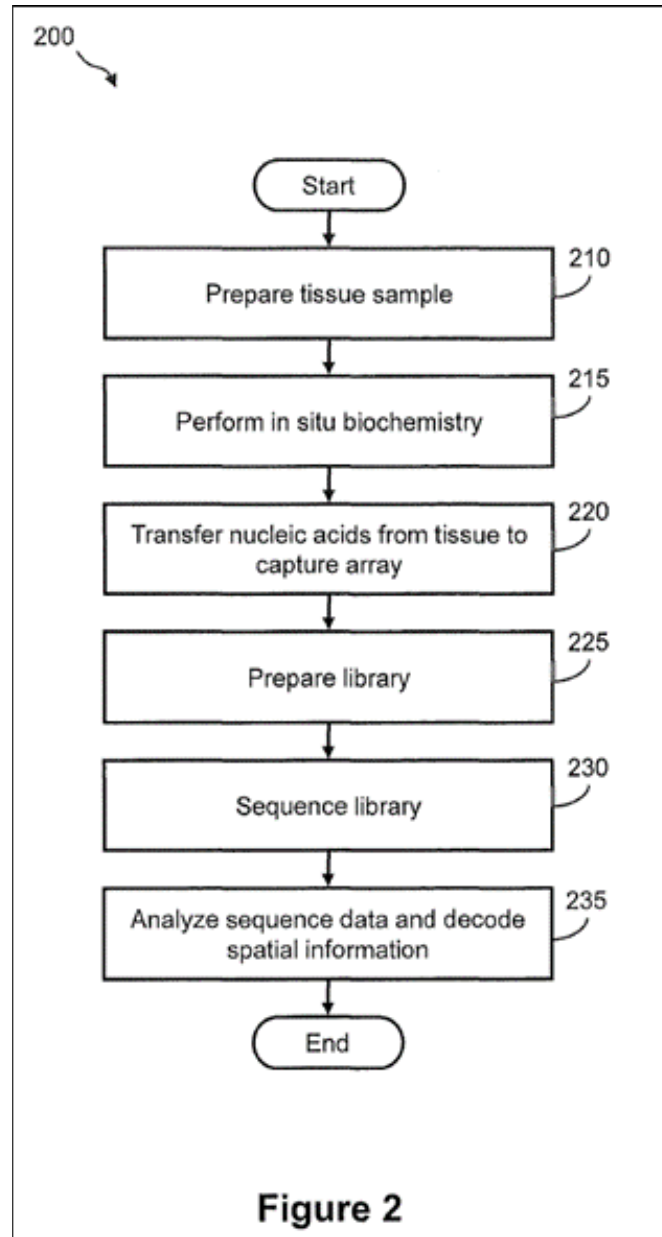


EX1016, FIG. 2B; *see also* EX1016, 81 (left col. and middle col.), FIGs. 3A-3F, S4, S10A-10C.

b. WO 2017/019456 (So-456) disclosed methods using spatially barcoded arrays by 2016

80. I understand that in July 2016, Illumina, Inc., filed a PCT application entitled “Spatial Mapping Of Nucleic Acid Sequence Information,” which published as WO 2017/019456 (“So-456”) on February 2, 2017. EX1005, Face. I understand that So-456 is prior art to the 142 patent because it was filed and published before February 12, 2018.

81. So-456 teaches methods for spatial detection of nucleic acids in a tissue sample that apply Stahl 2016's barcoded array and workflow. EX1005, ¶¶[0034]-[0035], [00307]-[00320], FIGs. 1, 2, 24, 25. So-456's Figure 2 provides overall workflow of its methods, depicting the following steps: preparing a tissue sample, capturing (transferring) the nucleic acids released from the tissue sample onto a spatially barcoded array, preparing a sequencing library, and sequencing the library and analyzing the sequencing data to spatially detect the target nucleic acids:



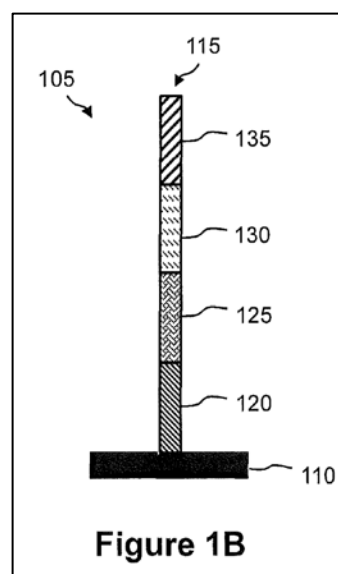
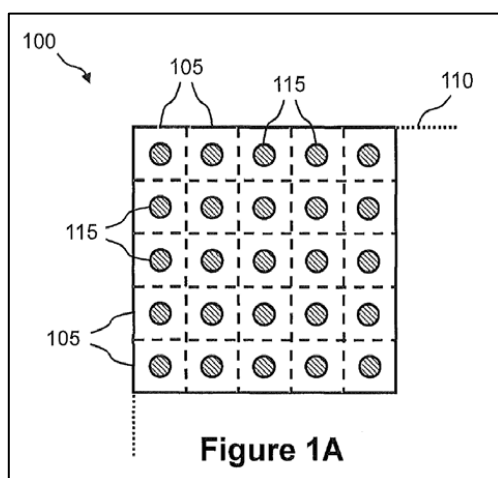
EX1005, FIG. 2, ¶¶[00151]-[00158].

82. In So-456's methods, a tissue sample is prepared (Step 210) and it is mounted on a capture array comprising immobilized, spatially barcoded capture probes. EX1005, ¶[00152]. The tissue sample is permeabilized to release nucleic acids (*e.g.*, mRNAs) from the tissue sample and are transferred onto the array by

hybridization to the immobilized first capture probe (Step 220). EX1005, ¶[00154].

The captured nucleic acids are then processed to generate a sequencing library (Step 225). EX1005, ¶[00156]. The library is then sequenced using any suitable sequencing technique (Step 230), and the sequencing data is subsequently analyzed (Step 235). EX1005, ¶¶[00157]-[00158]. The spatial information is then decoded, to “provide information as to the location of the nucleic acid in the tissue section.” EX1005, ¶[00158].

83. So-456’s methods use a “capture array” (100) that comprises an array of “capture sites” (105), depicted in Figures 1A and 1B (reproduced below). EX1005, ¶[00141].



EX1005, FIGs. 1A, 1B.

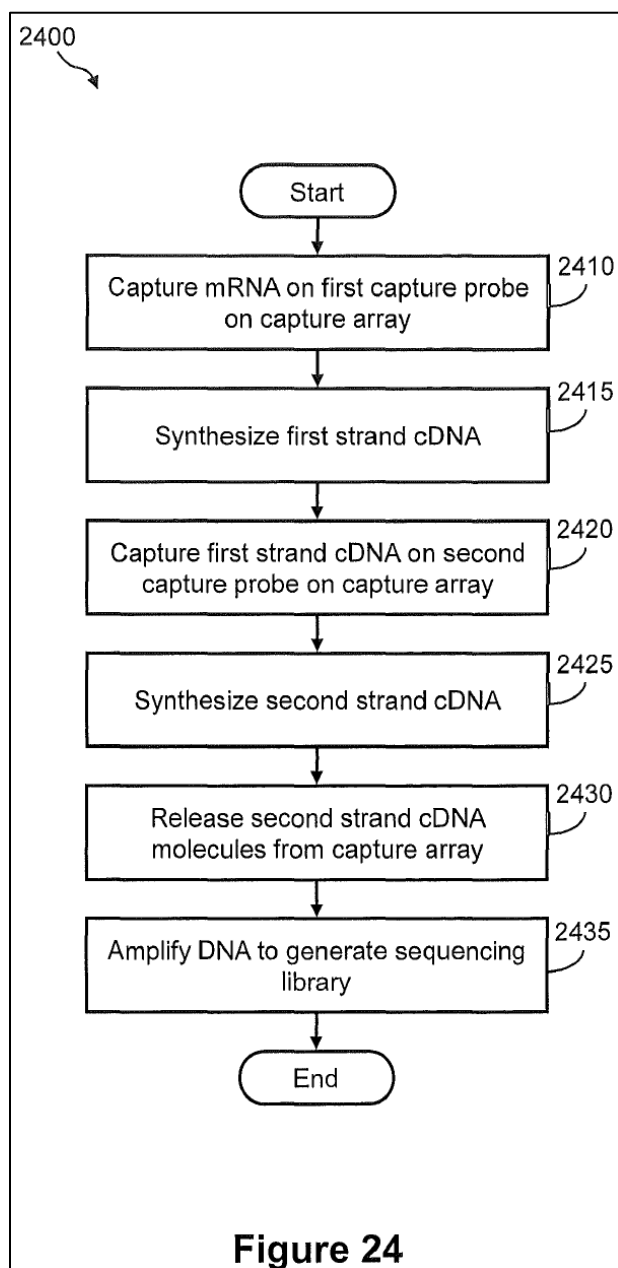
84. So-456’s capture sites (105) comprise at least one “capture probe” (*i.e.*, array probe; 115) for capturing the nucleic acids present in a tissue sample,

wherein the capture probe is immobilized on a solid support (110). EX1005, ¶[00141], FIG. 1B. So-456 explains that any number of capture probes (115) can be immobilized on the solid support at each capture site (105). EX1005, ¶[00141], FIG. 1B. According to So-456, the capture probes (115) comprise the following sequences: “a cleavable sequence in a cleavable region 120, an SBS (Sequencing-by-synthesis) primer sequence in a SBS primer binding site 125, a spatial address sequence in a spatial address region 130, and a capture sequence in a capture region 135.” EX1005, ¶[00141], FIG. 1B.

85. So-456 further teaches that the spatial address region (130) (*i.e.*, the spatial barcode) “corresponds to the position of [the] capture probe [] in [the] capture array,” which in turn “can be correlated with a position in the tissue sample.” EX1005, ¶[00141], FIG. 1B. The SBS primer region (125) is used for sequencing-by-synthesis (SBS) or amplification to generate a sequence library. EX1005, ¶[00141], FIG. 1B. The capture region (135) is used for capturing the nucleic acids released from the tissue sample, which can be a “universal (general) capture region,” such as a poly-T oligonucleotide to capture total mRNA in a tissue sample, or a “gene-specific or target specific capture region” for capturing a specific nucleic acid in a tissue sample. EX1005, ¶¶[00142]-[00143].

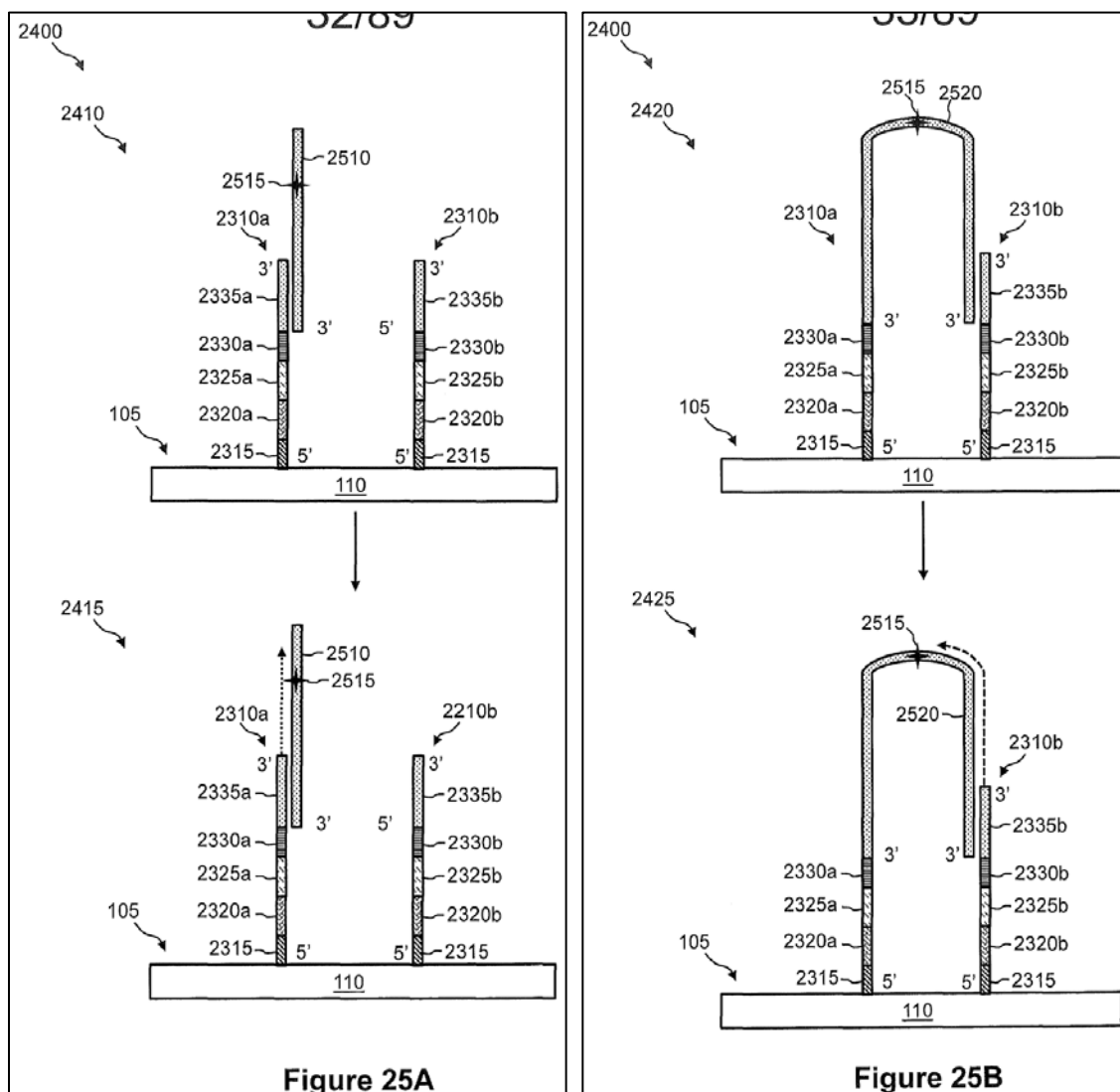
86. The method depicted in So-456’s Figure 24 follows Figure 2’s overall workflow and exemplifies the mRNA-capture spatial detection methods disclosed

in So-456. EX1005, ¶¶[0034], [00307]-[00313], FIG. 24. I have reproduced So-456 Figure 24 below.

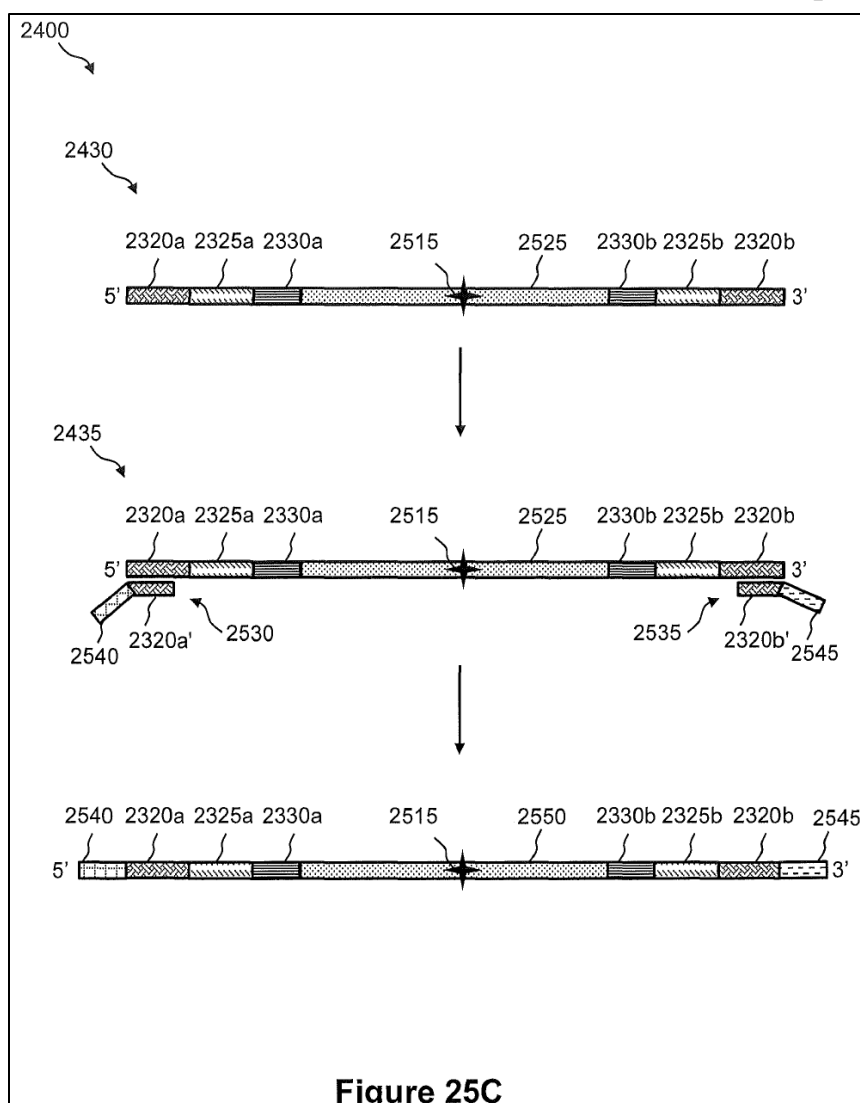


EX1005, FIG. 24.

87. So-456's Figure 25 (reproduced below) further details the steps of the mRNA-capture spatial detection method of Figure 24. EX1005, ¶¶[0035], [00314]-[00320].



EX1005, FIGs. 25A, 25B.



EX1005, FIG. 25C.

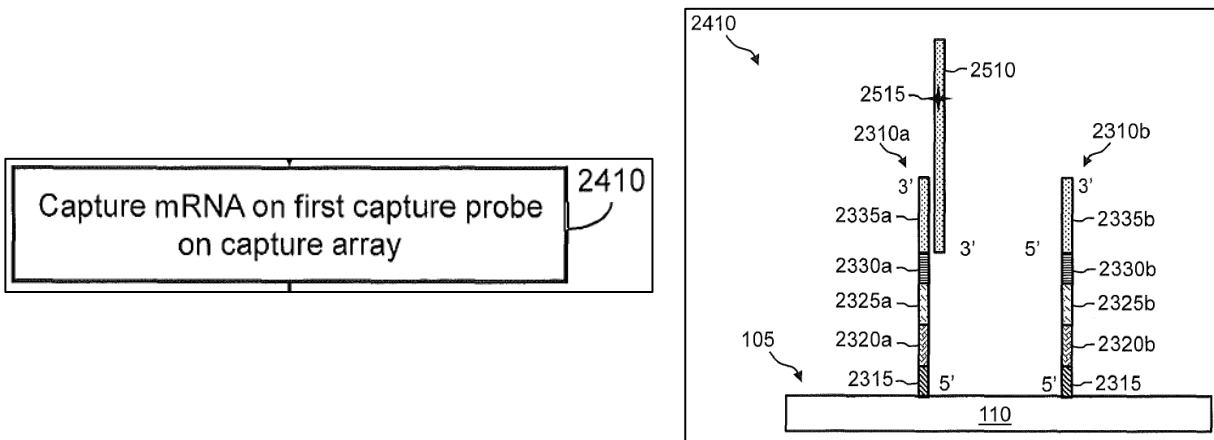
88. As shown above, the method (2400) of Figures 24-25 uses two capture probes for each gene-specific mRNA: (1) a first capture probe (2310a) containing a capture region (2335a) specific for the 3'-end of a mRNA molecule; and (2) a second capture probe (2310b) containing a capture region (2335b) specific for the 3'-end of the corresponding first-strand cDNA molecule, wherein both first and second capture probes contain spatial barcodes (2325a, 2325b), and wherein both

first and second capture probes are immobilized on a glass slide (110). EX1005,

¶¶[00314], [00320].

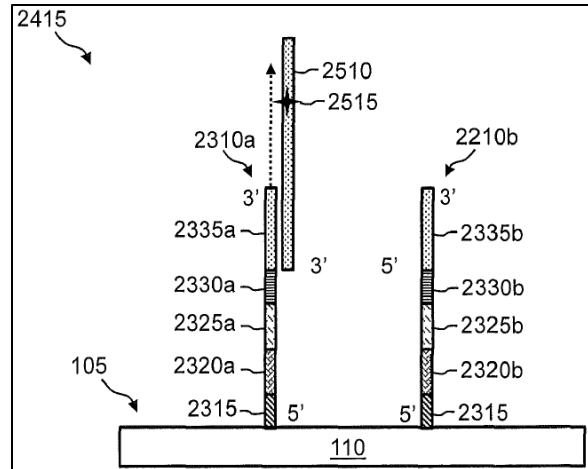
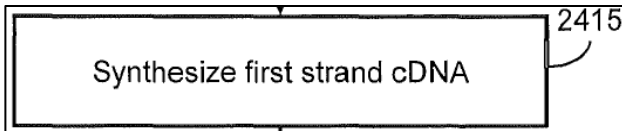
89. So-456's method of Figures 24-25 includes the following steps:

(1) Capturing an mRNA molecule (2510) from a tissue sample on the first capture probe (2310a). The mRNA molecule (2510) is captured at capture site (105) of the array by hybridizing the 3'-end of the mRNA molecule (2510) to the capture region (2335a) of the first capture probe (2310a). EX1005, ¶[00315]. This is shown in Step 2410 of Figures 24 and 25A, reproduced below.



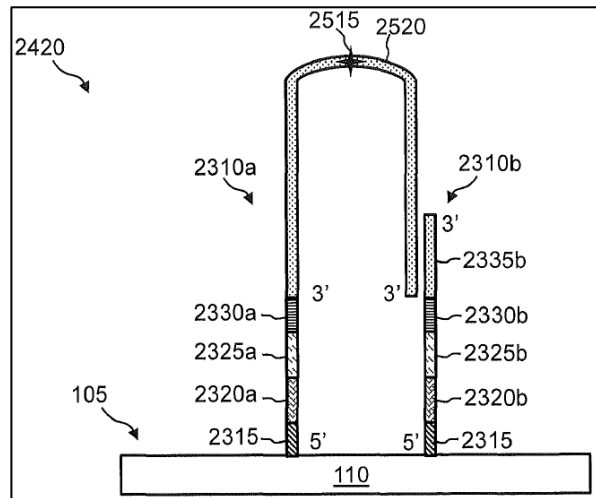
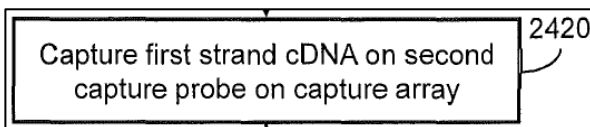
EX1005, FIGs. 24, 25A.

(2) Synthesizing the first-strand cDNA molecule using the capture region (2335a) of the first capture probe (2310a) as a primer and captured mRNA molecule (2510) as a template in a reverse transcription reaction. EX1005, ¶[00316]. This is shown in Step 2415 of Figures 24 and 25A, reproduced below.



EX1005, FIGs. 24, 25A.

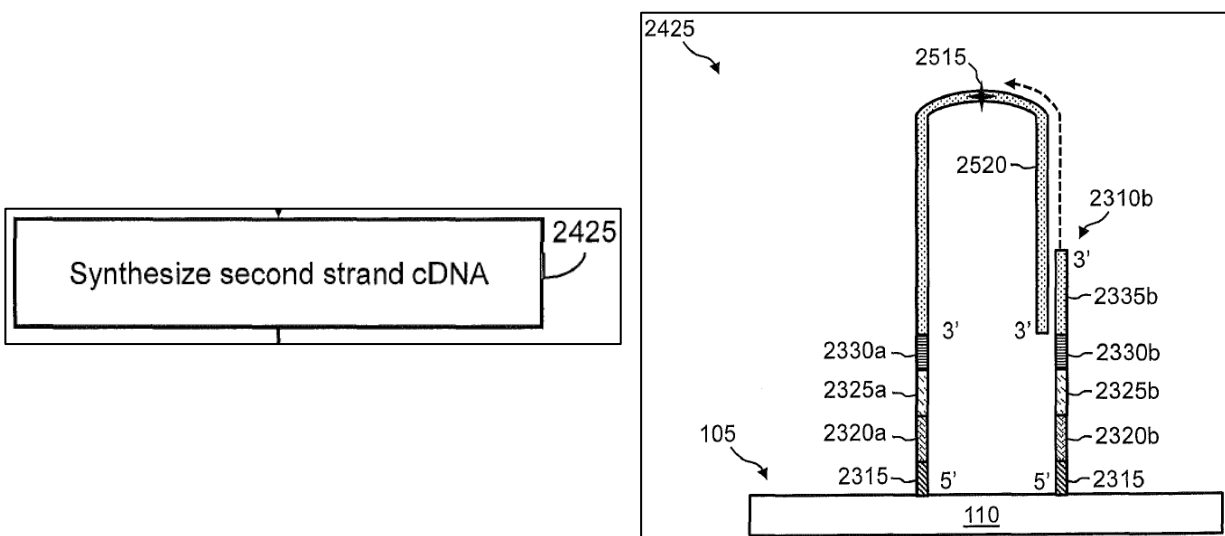
(3) Capturing the first-strand cDNA molecule (2520) on the second capture probe (2310b) by hybridizing the 3'-end of the cDNA molecule (2520) to capture region (2335b) of the second capture probe (2310b). EX1005, ¶[00317]. This is shown in Step 2420 of Figures 24 and 25B, reproduced below.



EX1005, FIGs. 24, 25B.

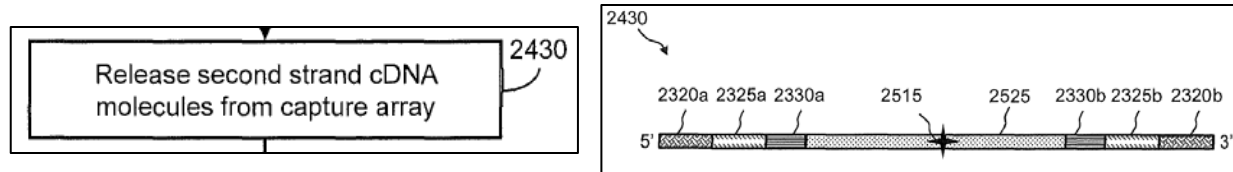
(4) Synthesizing a second-strand cDNA molecule in an extension reaction using the capture region (2335b) of the second capture probe (2310b) as a primer

and the first-strand cDNA molecule (2520) as a template. EX1005, ¶[00318]. This is shown in Step 2425 of Figures 24 and 25B, reproduced below.



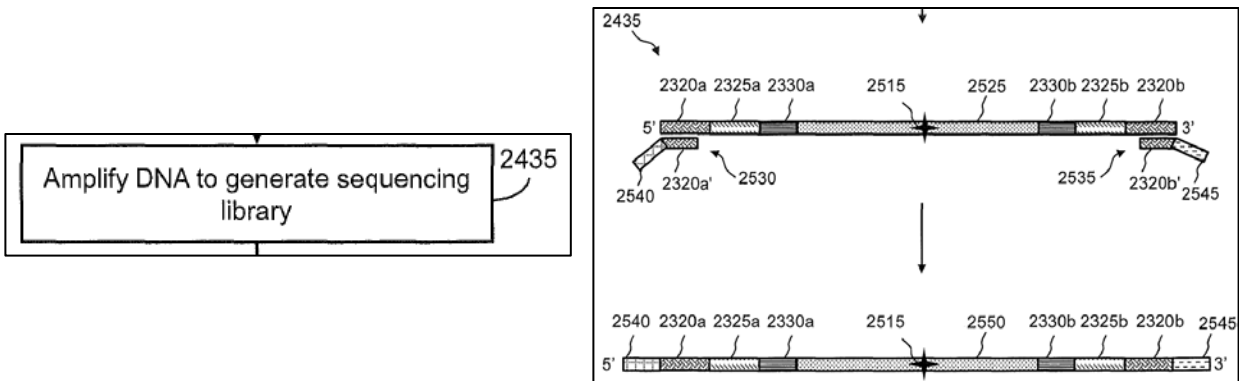
EX1005, FIGs. 24, 25B.

(5) Releasing the cDNA molecule (2525) from the capture site (105) by cleaving the cleavable region (2315) of the capture probes (see Figure 25B above). EX1005, ¶[00319]. The cDNA molecule (2525) (shown in Figure 25C below) includes the SBS primer region 2320a (*i.e.*, SBS3), a first spatial barcode (2325a) (*i.e.*, a unique spatial address for the X dimension), a first UMI region (2330a), a second UMI region (2330b), a second spatial barcode (2325b) (*i.e.*, a unique spatial address for the Y dimension), and the SBS primer region (2320b) (*i.e.*, SBS12). EX1005, ¶[00319]. This is shown in Step 2430 of Figures 24 and 25C, reproduced below.



EX1005, FIGs. 24, 25C.

(6) Amplifying the released cDNA molecule (2525) (shown in Figure 25C above) using a first SBS primer (2530) and a second SBS primer (2535) to generate a library amplicon (2550). EX1005, ¶[00320]. The SBS primer (2530) includes an SBS region (2320a') that is complementary to the SBS primer region (2320a) and a P5 region (2540). EX1005, ¶[00320]. The second SBS primer (2535) includes an SBS region (2320b') that is complementary to SBS primer region (2320b) and a P7 region (2545). EX1005, ¶[00320]. The library amplicon (2550) includes the P5 region (2540), the SBS primer region (2320a) (*i.e.*, SBS3), the first spatial barcode (2325a), the first UMI region (2330a), the second UMI region (2330b), the second spatial barcode (2325b), the SBS primer region (2330b) (*i.e.*, SBS12), and the P7 region (2545). EX1005, ¶[00320]. This is shown in Step 2435 of Figures 24 and 25C, reproduced below.



EX1005, FIGs. 24, 25C.

90. So-456's library is then sequenced (*e.g.*, by SBS), followed by analyzing the sequencing data and decoding the spatial information based on the spatial barcodes to determine the location of mRNA molecules in the tissue sample (*e.g.*, Steps 230-235 of FIG. 2). EX1005, ¶¶[00156]-[00158], FIG. 2.

91. So-456 also discloses that the amplification and library preparation steps can take place before releasing the cDNAs from the array—*i.e.*, on a solid substrate. EX1005, ¶¶[00109], [00156], [00173]. For example, So-456 discloses that the “capture probes” immobilized on the microscope slide can be “*amplification primers ... immobilized such that a 3'-end is available for enzymatic extension.*” EX1005, ¶¶[00109] (emphasis added), [00173]. So-456 further specifies that the “[l]ibrary preparation may be accomplished *on the capture array substrate.*” EX1005, ¶[00156] (emphasis added).

**c. WO 2012/140224 (Frisen) disclosed methods using spatially
barcoded arrays by 2012**

92. I understand that in April 2012, Jonas Frisen, Joakim Lundeberg, and Patrik Stahl—who co-authored the Stahl 2016 Science paper—filed a PCT application entitled “Method and Product for Localised or Spatial Detection of Nucleic Acid in a Tissue Sample,” which published on October 18, 2012, as WO 2012/140224 (“Frisen”). EX1017, Face. I understand that Frisen is prior art to the 142 patent because it was filed and published before February 12, 2018.

93. Frisen discloses methods for “spatial transcriptomics” or “spatial genomics” that spatially detect nucleic acids in a tissue sample using a spatially barcoded array. EX1017, Abstract, 1:12-33. Similar to Stahl 2016, Frisen discloses arrays comprising immobilized capture probes containing a spatial barcode, which Frisen calls a “positional domain,” and a “capture domain,” wherein the capture domain hybridizes to the target nucleic acids. EX1017, Abstract, 8:21-28. The capture probes of Frisen are immobilized to have a free 3’-end to enable the probes to function as a primer for an extension reaction. EX1017, Abstract, 8:21-28.

94. Frisen’s method comprises contacting the array with a tissue sample to allow the position of a capture probe to correlate with a position in the tissue sample. EX1017, 8:29-32. Next, the RNAs (*e.g.*, mRNAs) of the tissue sample are hybridized to the capture domain of the capture probes, “generating cDNA molecules from the captured RNA molecules” using the capture probes as RT

(reverse transcription) primers. EX1017, 8:33-34. This results in the cDNA molecules being tagged with the capture probe's positional domain. Frisen then releases the tagged DNA molecules from the surface of the array, which are processed and subsequently sequenced. EX1017, 9:1-8. The sequence of the tagged DNA molecules is then analyzed to determine the spatial location of the target nucleic acids. EX1017, 9:14-10:5.

95. Frisen's method also discloses generating a complementary strand of tagged DNA (*i.e.*, second-strand cDNA) and/or amplifying the tagged DNA before releasing molecules from the array surface. EX1017, 7:32-8:3, 8:33-9:4, 12:4-11. As Frisen explains, "it will be necessary to enhance, *i.e.*, amplify the amount of secured nucleic acid, *e.g.* synthesized cDNA[,] to yield quantities that are sufficient for DNA sequencing." EX1017, 39:9-11, 42:32-33 ("Thus in a preferred embodiment, the method of the invention may comprise a step of amplifying the DNA, *e.g.* cDNA molecules.").

96. Frisen discloses that the second-strand cDNA synthesis may occur on the array. For example, Frisen states that second-strand cDNA synthesis may occur either as a discrete step of second-strand synthesis or as the initial step of an amplification reaction. EX1017, 12:9-10, 38:20-23, 42:28-43:7. Frisen's Example 11 exemplifies a method for generating a second-strand cDNA together with the first-strand cDNA on a solid surface (chip). EX1017, 103:12-19. As Frisen further

describes, “the 3’ end of the first cDNA strand ... primes the synthesis of the second strand. The library can be readily amplified directly after release of the double-stranded complex from the array surface.” EX1017, 103:12-19.

VII. CLAIM CONSTRUCTION

97. I understand that NanoString has alleged in related litigation that 10x’s Visium Fresh Frozen (FF) product infringes the 142 patent claims. EX1022. Solely for the purposes of this IPR, I have been asked to apply NanoString’s interpretations according to its infringement positions.

VIII. GROUND 1: CLAIMS 1-4 AND 6-13 ARE ANTICIPATED BY STAHL 2016

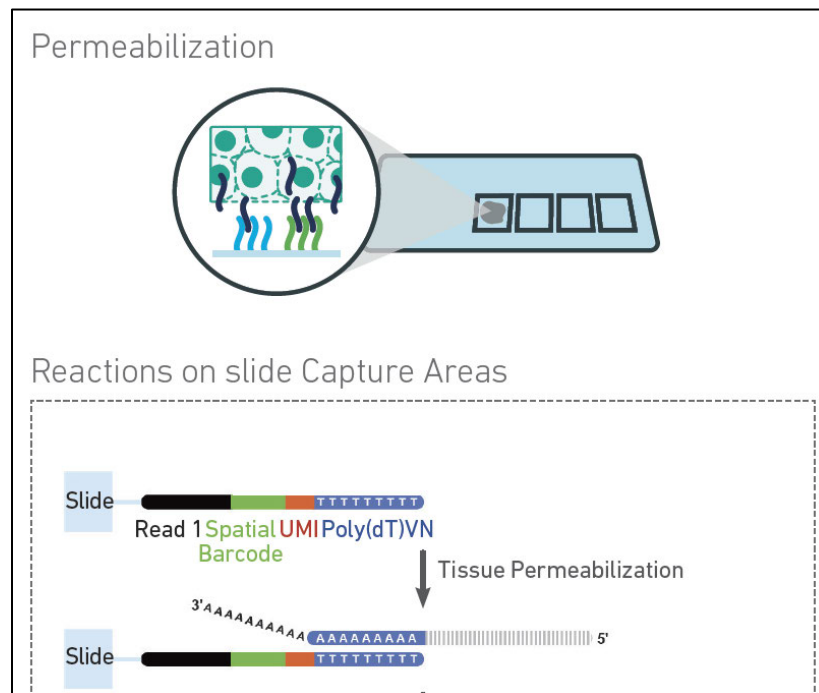
98. In my opinion, claims 1-4 and 6-13 of the 142 patent are anticipated by Stahl 2016 under NanoString’s interpretation of the claims. Specifically, Stahl 2016 discloses a method meeting all the elements of the claimed methods. I understand that Stahl 2016 is presumed enabled. I understand that Stahl 2016 was published before February 12, 2018, and thus it qualifies as prior art to the 142 patent.

a. Claim 1

99. For the reasons I explain below, Stahl 2016 anticipates claim 1. *See also* Appendix A.

- i. **“A method comprising: a) [and (b)] collecting a plurality of oligonucleotides from a first location [and second location] of a tissue sample ...”**

100. In my opinion, Stahl 2016’s method includes the collecting steps (a) and (b) under NanoString’s interpretation. I understand that in related litigation, NanoString alleged that 10x’s Visium FF infringes the 142 patent claims. EX1022. Under NanoString’s interpretation, the collecting steps of claim 1 encompass certain of the steps of 10x’s Visium FF workflow. EX1022, 11-14, 18. I have reviewed the 10x user guide for Visium FF that NanoString identifies in its contentions. EX1015. In Visium FF, mRNAs are diffused from and captured across the *whole* tissue sample, all at the same time. EX1015, 16-17. These steps of Visium FF are depicted in the figure below.

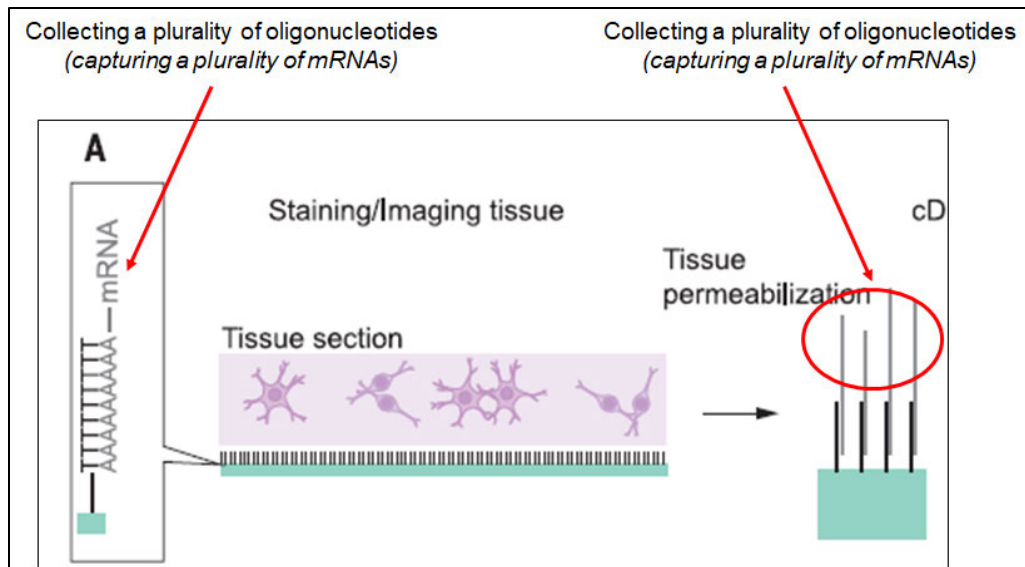


EX1015, 17.

101. Thus, under NanoString’s interpretation, the collecting steps of claim 1 encompass diffusion and capture of mRNAs across the *whole* tissue sample, all at the same time. EX1022, 11-14, 18; EX1015, 16-17.

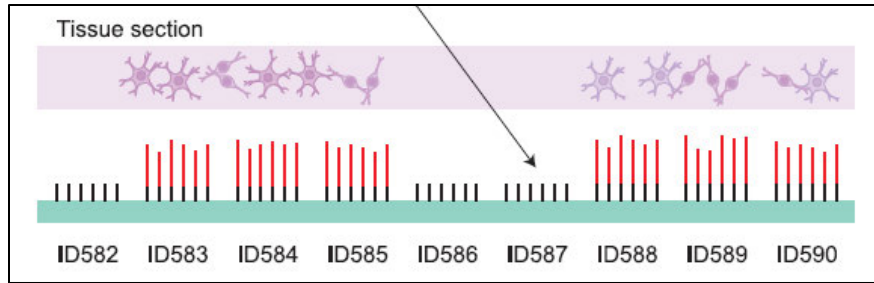
102. Stahl 2016 discloses a “spatial transcriptomics” method that “allows visualization and quantitative analysis of the transcriptome with spatial resolution in individual tissue sections.” EX1016, Abstract, Suppl. Materials, 1 (“[t]he *Spatial Transcriptomics method* follows the following workflow” (emphasis added)).

103. Stahl 2016’s method collects a plurality of oligonucleotides under NanoString’s interpretation. Specifically, Stahl 2016’s method captures (collects) mRNAs (oligonucleotides) from a tissue sample onto an array of immobilized capture probes. EX1016, 78, FIG. 1A. Stahl 2016 teaches that “it is possible to *capture mRNA* in tissue sections,” and further describes generating sequencing libraries “[a]fter *capturing* and reverse-transcribing mRNA” EX1016, 78 (right col.) (emphasis added), 80 (“cDNA ... is generated from *captured mRNA* by reverse transcription.” (emphasis added)). The annotated excerpt of Figure 1A below illustrates that Stahl 2016’s method includes collecting a plurality of oligonucleotides (capturing a plurality of mRNAs).

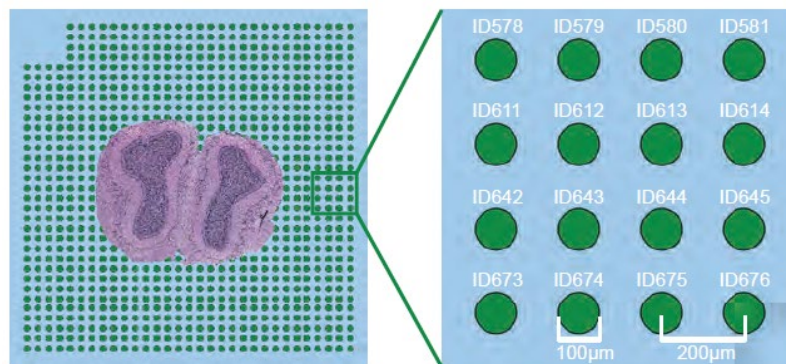


EX1016, FIG. 1A (annotated).

104. In my opinion, Stahl 2016’s method also discloses collecting oligonucleotides (mRNAs) from a first and second location, according to NanoString’s interpretation. EX1022, 11-14, 18; EX1015, 16-17. Specifically, Stahl 2016 discloses that capturing (collecting) the mRNAs on the array is achieved with “minimal diffusion” while “maintain[ing] positional representation” from within the tissue. EX1016, 78 (right col.). To maintain positional representation, Stahl 2016’s method collects mRNAs from a first location in the tissue in one spot on the array, and collects mRNAs from a second location in a different spot on the array, as depicted in, *e.g.*, Figures 2A and S2 (reproduced below):



EX1016, FIG. 2A.



EX1016, FIG. S2. As shown in both figures above, different locations (“spots”) are depicted as ID583, ID584, etc. (FIG. 2A) or ID578, ID579, etc. (FIG. S2).

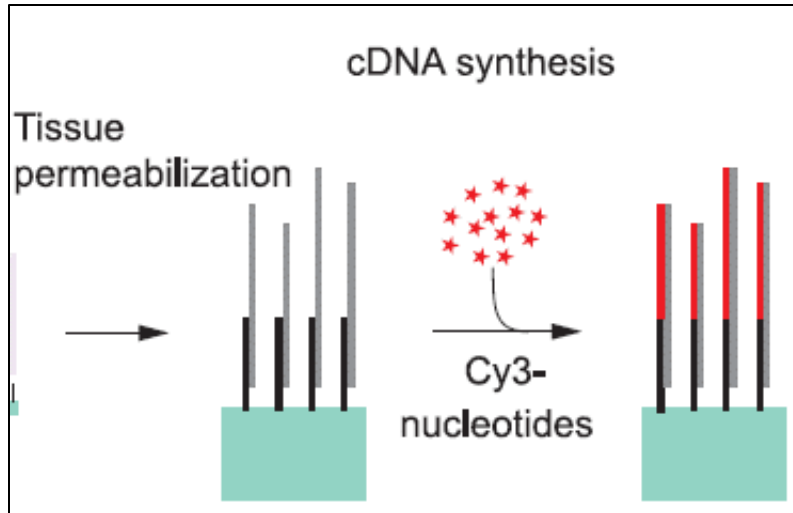
“under conditions that release the plurality of oligonucleotides from the first location [and second location] of the tissue sample”

105. In my opinion, Stahl 2016 discloses collecting the mRNAs under conditions that release the mRNAs from their locations in the tissue sample. This is because Stahl 2016 discloses a process of tissue “permeabilization,” in which the tissue sample was treated with “0.01% pepsin ... dissolved in 0.1M HCl,” to release the mRNAs from the tissue so they diffuse onto the array. EX1016, 78 (middle col.), Suppl. Materials, 4-5, FIG. 1A.

106. Thus, Stahl 2016 teaches collecting a plurality of oligonucleotides (mRNAs) at multiple locations in a tissue sample—*i.e.*, a first plurality and second plurality of oligonucleotides. Accordingly, it is my opinion that Stahl 2016 discloses claim 1’s collecting steps.

ii. “c) [and (d)] synthesizing a first plurality [and second plurality] of DNA products by performing a synthesis reaction ...”

107. It is my opinion that Stahl 2016 discloses the synthesis steps (c) and (d) because its method includes a reverse transcription reaction to “synthesize” cDNA products using the captured mRNAs as templates. Stahl 2016 teaches “[s]patially localized *cDNA synthesis*” and “*cDNA synthesis* with Cy3-labeled nucleotides.” EX1016, 79 (emphasis added), FIG. 1, 78 (middle col. and right col.) (“[w]e used fluorescently labeled nucleotides to visualize the *synthesized cDNA*.”) (emphasis added); Suppl. Materials, 4-5. Stahl 2016’s Figure 1A depicts cDNA synthesis as red bars, wherein the nucleotides are incorporated into the immobilized capture probes to generate cDNA products:



EX1016, FIG. 1A.

108. Accordingly, for these reasons, Stahl 2016’s method includes synthesizing a first and second plurality of DNA products by performing a synthesis reaction. Thus, in my opinion, Stahl 2016’s method includes claim 1’s synthesizing step.

“that uses the plurality of oligonucleotides collected in step (a) [and step (b)] as templates ...”

109. In my opinion, Stahl 2016’s synthesis reactions use the first and second plurality of oligonucleotides (mRNAs) collected in steps (a) and (b) (respectively) as templates. Specifically, Stahl 2016’s method uses reverse transcription to synthesize a “complementary” DNA, *i.e.*, cDNA, strand using the captured (collected) mRNA as a template. EX1016, 78-79, Suppl. Materials, 4-5. For example, Stahl 2016 discloses: “We asked whether it would be possible to introduce positional molecular barcodes in the *complementary DNA (cDNA)*

synthesis reaction within the context of an intact tissue section.” EX1016, 78

(middle col.) (emphasis added). Similarly, Stahl 2016’s Figure 1A (reproduced above) depicts captured mRNAs (collected first and second plurality of oligonucleotides) as grey bars serving as templates for the cDNA synthesis, which are shown as red bars. EX1016, FIG. 1A.

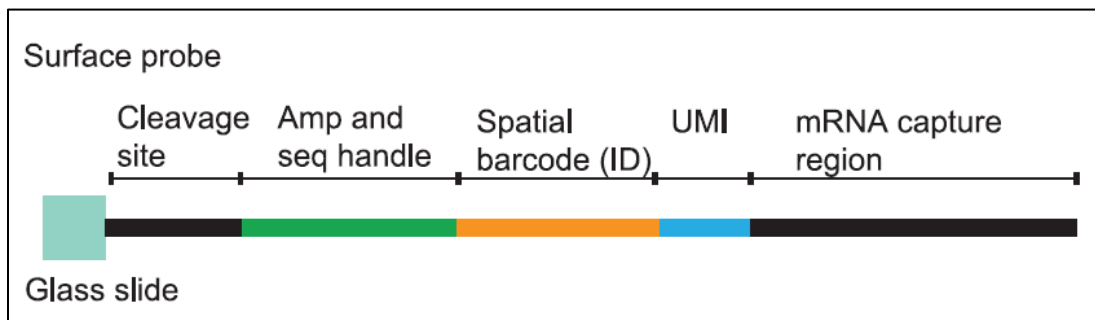
110. Further, Stahl 2016’s synthesis reactions take place simultaneously in different spots on the array. *See, e.g.*, EX1016, FIGs. 2A, S2. Thus, it is my opinion that Stahl 2016’s method synthesizes a first and second plurality of DNA products, as recited in claim 1.

“and incorporates at least one nucleic acid sequence that identifies the first location [and second location] of the tissue sample into each of the first plurality [and second plurality] of DNA products”

111. In my opinion, Stahl 2016’s synthesis reactions also incorporate at least one nucleic acid sequence that identifies the first (and second) location of the tissue sample into the first (and second) plurality of DNA products.

112. Stahl 2016 discloses that each capture probe on its array includes a “spatial barcode,” which is used to “sort[] the RNA-seq data to its corresponding array features.” EX1016, 78 (right col.), 79. Further, Stahl 2016 explains that “[e]ach array feature contains unique *DNA-barcoded probes* containing a cleavage site, a T7 amplification and sequencing handle, *a spatial barcode*, a unique molecular identifier (UMI), and an oligo(dT) VN-capture region.” EX1016, 80,

FIG. 2A (emphasis added). Stahl 2016's spatially barcoded capture probe is depicted in Figure 2A, having the spatial barcode located between the "Amp and seq handle" and the "UMI" regions:



EX1016, FIG. 2A.

113. Stahl 2016's method further describes the structure of the capture probes as: "surface *spatially barcoded* oligonucleotides...18MER_*SPATIALBARCODE*".

EX1016, Suppl. Materials, 3 (emphasis added). Thus, Stahl 2016's spatially barcoded capture probes include an 18-nucleotide long spatial barcode that is incorporated into each synthesized cDNA molecule.

114. Accordingly, in my opinion, Stahl 2016's synthesis reaction synthesizes a first plurality and second plurality of DNA products (cDNA products) using the collected oligonucleotides (mRNAs) as templates, incorporating a nucleic acid sequence that identifies a location on the array (spatial barcode) into the DNA products. EX1016, FIGs. 1-2, Suppl. Materials, 8 (referring to "*barcoded cDNA*" (emphasis added)).

115. Thus, in my opinion, Stahl 2016's method includes claim 1's synthesizing steps.

- iii. **“e) identifying the first plurality of DNA products and the second plurality of DNA products synthesized in step (c) and step (d) by sequencing the first plurality of DNA products and the second plurality of DNA products, thereby spatially detecting the plurality of oligonucleotides collected from the first location of the tissue sample and the plurality of oligonucleotides collected from the second location of the tissue sample.”**

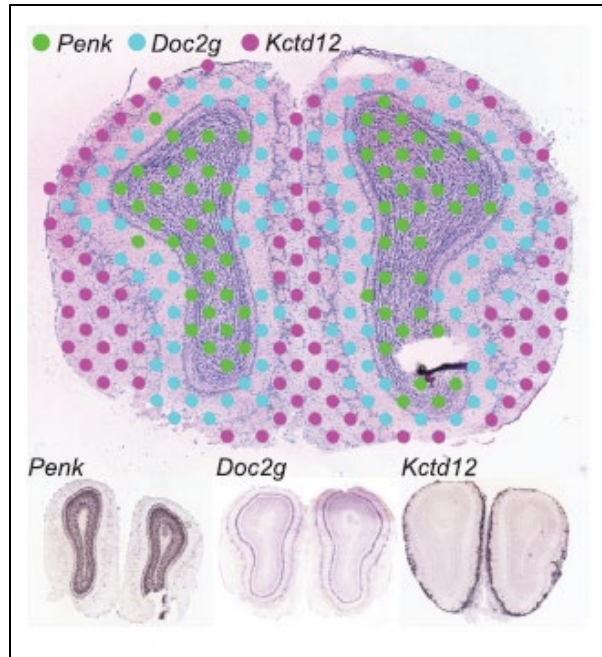
116. In my opinion, Stahl 2016's method includes the sequencing step (e) under NanoString's interpretation. I understand that NanoString agrees that “spatially detecting” means “identifying the presence of a specific target analyte within a specific region of interest in a sample.” EX1024, 31.

117. Stahl 2016 discloses generating “sequencing libraries” by collecting the spatially barcoded cDNA products synthesized from the target mRNAs on the array (the first and second plurality of DNA products) and amplifying the cDNA products. EX1016, 78 (right col.), Suppl. Materials, 6-7. Stahl 2016 discloses sequencing the amplified library “on the Illumina NextSeq platform using paired-end sequencing according to the manufacturer's protocol.” EX1016, Suppl. Materials, 7-8. Stahl 2016's method further discloses that the sequencing (“RNA-seq”) data was “sorted ... to its corresponding array features by *using the spatial barcodes*,” which “enabled visualization and analyses.” EX1016, 78 (right col.)

(emphasis added). Thus, Stahl 2016 discloses “identifying” the cDNA synthesis products by sequencing them.

118. The components and methods for sequencing by Illumina’s method and for generating the corresponding sequencing libraries were well known prior to February 2018. Illumina’s SBS method utilizes a pair of SBS primers that initiate the incorporation of fluorescently labeled reversible terminator nucleotides into the molecules generated for sequencing. EX1030; EX1005, ¶¶[00141], [00199], [00201], [00211], [00229], [00496]-[00497].

119. Stahl 2016’s method “spatially detected” the first and second plurality of oligonucleotides (mRNAs) collected from the tissue sample. This is because Stahl 2016’s method uses the spatial barcodes to identify the spatial origin of the corresponding mRNA sequence in the tissue. For example, Stahl 2016 presents results of its spatial detection methods in Figures 2 and S4. Figure 2B is shown below, depicting visualization and expression of three genes in mouse olfactory bulb tissue (upper), which Stahl 2016 confirmed with in situ hybridization (bottom):



EX1016, FIG. 2B, 78 (right col.); *see also* EX1016, 81 (left col. and middle col.), FIGs. 3A-3F, S4, S10A-10C.

120. As such, Stahl 2016's method identifies the first and second plurality of DNA products (cDNA products) by sequencing them, thereby spatially detecting the plurality of oligonucleotides (mRNAs) collected from the first and second locations of the tissue sample. Thus, in my opinion, Stahl 2016's method includes claim 1's sequencing step under NanoString's interpretation.

121. For the reasons I discuss above, Stahl 2016 teaches every element of claim 1 under NanoString's interpretation, arranged as claimed. Thus, in my opinion, Stahl 2016 anticipates claim 1 under NanoString's interpretation.

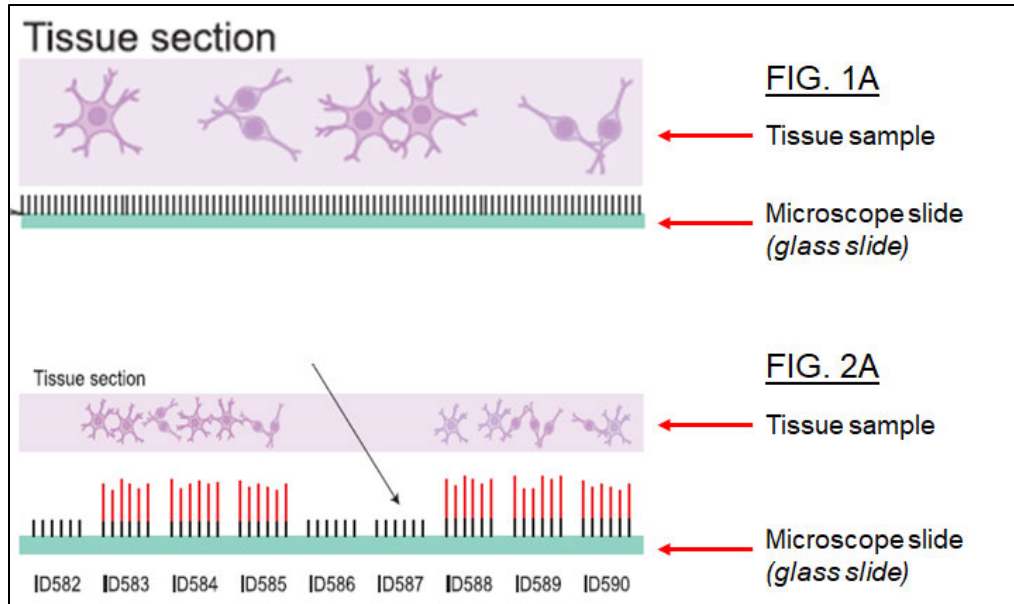
b. Claims 2-4 and 6-13

122. Claims 2-4 and 6-13 depend directly or indirectly from claim 1, and further specify the elements of claim 1. I understand that the dependent claims incorporate all the elements of the claims from which they depend.

i. Claim 2

123. Claim 2 depends from claim 1, and further requires that “the tissue sample is immobilized onto a microscope slide.” EX1043, 147:62-63. Stahl 2016 discloses the elements of claim 2.

124. Stahl 2016’s method “immobilized reverse-transcription oligo(dT) primers *on glass slides* and *placed on the slides* sections of adult *mouse olfactory bulb*.” EX1016, 78 (middle col.) (emphasis added). Specifically, Stahl 2016 discloses using “Codelink Activated *microscope glass slides*,” and that, “for Spatial Transcriptomics experiments, dried [tissue] *sections were mounted* ... and covered with a coverslip.” EX1016, Suppl. Materials, 2, 4 (emphasis added). As shown in the annotated Figures 1A and 2A below, Stahl 2016 depicts that the tissue sample is immobilized on a glass slide:



EX1016, FIGs. 1A, 2A (annotated).

125. It is my opinion that Stahl 2016 discloses that its glass slides are “microscope slides” as claimed. Specifically, Stahl 2016 discloses that, after immobilizing the tissue on the glass slide and covering it “with a coverslip,” “[b]right field imaging was carried out using a Metafer Slide Scanning Platform” (*i.e.*, a microscope). EX1016, Suppl. Materials, 4; EX1016, 79 (the “tissue is sectioned, placed onto oligo(dT) primers, stained, and imaged.”).

126. Accordingly, in my opinion, Stahl 2016 discloses the elements of claim 2 and anticipates it.

ii. Claim 3

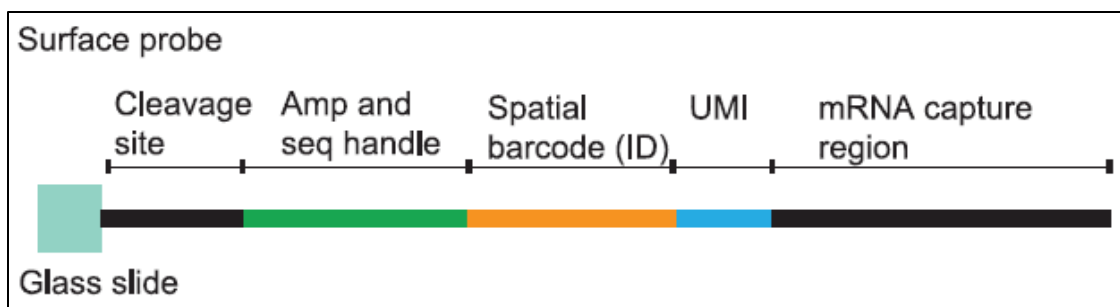
127. Claim 3 depends from claim 2, and requires that the “microscope slide comprises a plurality of primers immobilized on the microscope slide.” EX1043, 148:32-34. It is my opinion that Stahl 2016 discloses the elements of claim 3.

128. Stahl 2016 discloses that its workflow uses “*immobilized reverse-transcription oligo(dT) primers on glass slides.*” EX1016, 78 (middle col.) (emphasis added). Specifically, Stahl 2016 discloses that “poly-20TVN capture oligonucleotides (IDT)” were “*immobilized*” “on the surface of Codelink Activated *microscope glass slides.*” EX1016, Suppl. Materials, 2 (emphasis added). Stahl 2016 also discloses that its array of “1007 capture oligonucleotides with 18-mer unique barcodes, a 9-mer semi-randomized UMI and a poly-20TVN capture region” were immobilized on the same “*Codelink Activated Slides.*” EX1016, Suppl. Materials, 2 (emphasis added). Thus, it is my opinion that Stahl 2016 discloses the elements of claim 3 and anticipates it.

iii. Claim 4

129. Claim 4 depends from claim 3, and requires that the “plurality of primers is immobilized on the microscope slide at their 5’ ends.” EX1043, 148:35-36. It is my opinion that Stahl 2016 discloses the elements of claim 4.

130. Stahl 2016 discloses that its primers are immobilized on their 5’-ends, as claimed. For example, Stahl 2016’s schematic of its immobilized capture probe shows the probe immobilized on a glass slide with the “Cleavage site” at the base (5’-end), followed by the “Amp and seq handle,” spatial barcode, UMI, and mRNA capture region:



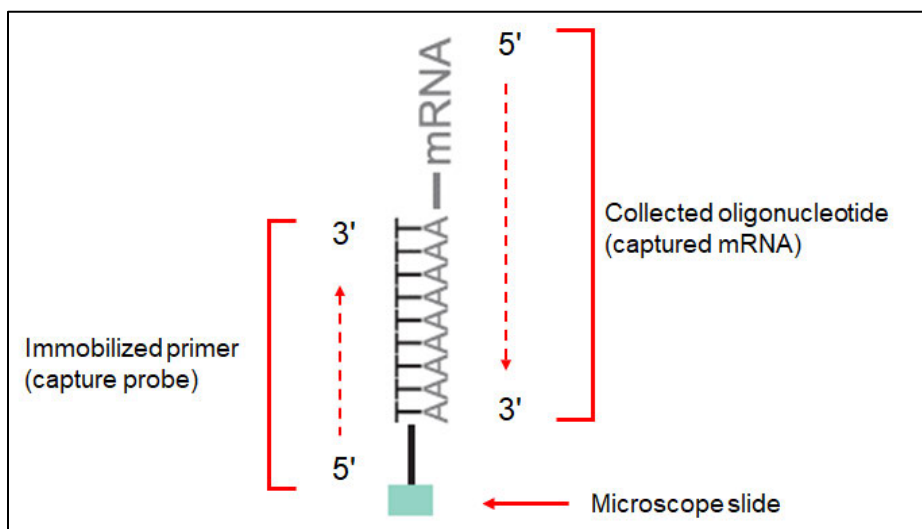
EX1016, FIG. 2A.

131. As discussed above for claim 1, Stahl 2016 discloses synthesizing cDNA products using the captured mRNAs as templates and capture probes are primer that are extended. *See* Sections VI.a, VIII.a. It was well known in the art that nucleic acid molecules have one 5'- and one 3'-end and that the nucleic acid molecules are normally enzymatically extended at their 3'-end. *See, e.g.*, EX1045, 61-62. During DNA (including cDNA) synthesis, a DNA polymerase adds deoxynucleotides at the 3'-end of a DNA molecule. *See, e.g.*, EX1045, 61-62. If a DNA molecule was immobilized to a solid support at its 3'-end, it would be impossible to incorporate a dNTP into the 3'-end. Thus, to permit extension of the capture probes in Stahl 2016's method for cDNA synthesis, the probes must be immobilized at their other end, *i.e.*, 5'-ends.

132. Furthermore, as shown in Figure 2A above, the mRNA capture region of Stahl 2016's capture probes (polyT), which hybridizes to its complementary polyA region of the mRNA, is at the free-end of the probe. EX1016, FIG. 2A. It was well known in the art that during synthesis of eukaryotic mRNAs (the process of

copying DNA into RNA) of a mRNA molecule, a poly(A) tail is added to the immature transcript. *See, e.g.*, EX1046, 201-202. During transcription, mRNA synthesis occurs in a 5' to 3' orientation, thus, the polyA region is located at the 3'-end of an mRNA molecule. *See, e.g.*, EX1046, 201-202.

133. In order for polyT mRNA capture region of the capture probe to hybridize (base pair) with the 3'-polyA tail of captured mRNA, the polyT region of the capture probe must be on the 3'-end of the immobilized capture probe—further confirming that Stahl 2016's primers are immobilized at the other end, *i.e.*, the 5'-end.



EX1016, FIG. 1A (annotated).

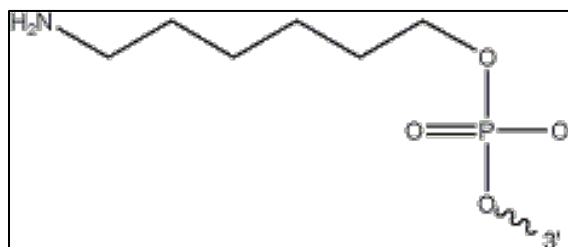
134. Stahl 2016 further confirms the 5' immobilization of its capture probes by including an “AmC6” (amino modifier C6) conjugate at the 5'-end of the probe:

Surface spatially barcoded oligonucleotides:

[AmC6]UUUUUGACTCGTAATACGACTCACTATAGGGACACGACGCTCTTCCGATCT[18
MER_SPATIALBARCODE]WSNNWSNNVTTTTTTTTTTTTTTTTTTTTTN

EX1016, Suppl. Materials, 3.

135. Stahl 2016 discloses that the sequence of its capture probe begins with the AmC6 conjugate, indicating that the AmC6 conjugate is at 5'-end of the probe. It was well known in the art that an AmC6 conjugate is used for immobilizing nucleotides onto glass substrates. *See, e.g.*, EX1040, 5. As shown below, the chemical structure of a 5' amino modifier C6 is such that an oligonucleotide that incorporates the AmC6 conjugate would have a free amine at the 5'-end.



EX1039, 2.

136. It was known prior to 2018 that the CodeLink® Activated slides used by Stahl 2016's method "covalently immobilize amine-modified DNA for use in microarray analysis." EX1047, 3. Thus, Stahl 2016's capture probes are immobilized using AmC6 conjugate at their 5'-end.

137. Accordingly, it is my opinion that Stahl 2016 discloses that its microscope slide comprises a plurality of primers immobilized at their 5'-ends and

thus discloses the elements of claim 4. As such, it is my opinion that Stahl 2016 anticipates claim 4.

iv. Claim 6

138. Claim 6 depends from claim 1, and requires that the “sequencing step is performed using a next generation sequencing reaction.” EX1043, 148:42-43. It is my opinion that Stahl 2016 discloses the elements of claim 6.

139. Stahl 2016 discloses generating “*sequencing libraries*” and provides a detailed description of methods for “cDNA library *preparation for sequencing*.” EX1016, 78 (right col.), Suppl. Materials, 6-8 (emphasis added). Specifically, Stahl 2016 discloses that the libraries are sequenced on “the *Illumina NextSeq platform* using paired-end sequencing according to the manufacturer’s protocol.” EX1016, Suppl. Materials, 7 (emphasis added). The Illumina NextSeq platform performs “next generation sequencing reaction[s],” as required by claim 6. EX1007, 1-2; EX1041, Table 1 (listing “Illumina NextSeq” as one of several “different NGS technologies”).

140. Accordingly, it is my opinion that Stahl 2016 discloses the elements of claim 6 and anticipates it.

v. Claim 7

141. Claim 7 depends from claim 1, and “further comprises amplifying a library using the first plurality of DNA products and the second plurality of DNA

products as templates.” EX1043, 148:44-46. It is my opinion that Stahl 2016 discloses the elements of claim 7.

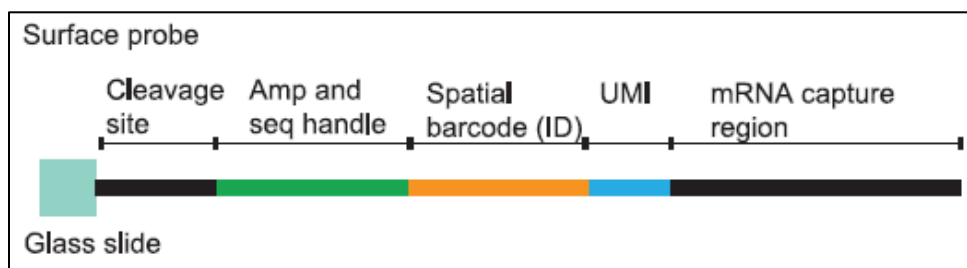
142. Stahl 2016 discloses amplifying “a library” when discussing the workflow for “cDNA library preparation for sequencing.” EX1016, Suppl. Materials, 6-7. Specifically, Stahl 2016’s method performs an initial *in vitro* transcription/amplification to produce amplified RNA molecules, followed by adding DNA adapters to the ends of the amplified RNA, reverse-transcribing the amplified RNA into cDNA, and then performing PCR to *amplify the cDNA* to add Illumina paired-end (PE) sequencing flow-cell binding sites and index sequences before sequencing. EX1016, Suppl. Materials, 7 (“2μl purified cDNA were *amplified by qPCR* ... the samples *were amplified* in a total reaction volume of 25μl.” (emphasis added)). Accordingly, it is my opinion that Stahl 2016’s PCR amplification reactions disclose amplifying “a library” using the cDNA synthesis products as templates. Thus, in my opinion, Stahl 2016 discloses the elements of claim 7 and anticipates it.

vi. Claims 8 and 9

143. Claims 8 and 9 each depends from claim 1 and require that the “at least one nucleic acid sequence that identifies the [first (claim 8) and second (claim 9)] location of the tissue sample comprise at least one unique molecular identifier.”

EX1043, 148:47-52. It is my opinion that Stahl 2016 discloses the elements of claims 8 and 9.

144. As I discuss above for claim 1, the spatial barcode in each of Stahl 2016's capture probes identifies the "first" and "second" locations of the tissue sample. EX1016, 78-80, Suppl. Materials, 8, FIGs. 1-2. Furthermore, Stahl 2016's barcoded capture probes also comprise a UMI: "[e]ach array feature contains unique *DNA-barcoded probes* containing a cleavage site, a T7 amplification and sequencing handle, a *spatial barcode*, a *unique molecular identifier (UMI)*, and an oligo(dT) VN-capture region." EX1016, 80 (emphasis added). Stahl 2016's Figure 2A provides a diagram of its spatially barcoded and UMI-containing capture probes (reproduced below), wherein the UMI is located between the spatial barcode and the mRNA capture region.



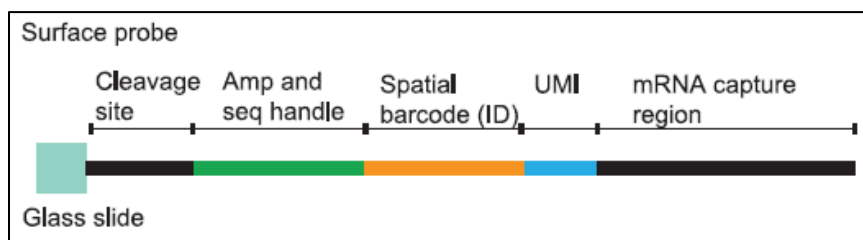
EX1016, FIG. 2A, *see also* EX1016, FIG. S5 ("UMI-filtered").

145. Accordingly, it is my opinion that Stahl 2016 discloses a nucleic acid sequence that identifies a location of the tissue sample (*i.e.*, capture probe comprising a spatial barcode) comprising a UMI. Thus, in my opinion, Stahl 2016 discloses the elements of claims 8 and 9 and anticipates them.

vii. Claims 10 and 11

146. Claims 10 and 11 each depends from claim 1, and requires that the “first plurality” (claim 10) and “second plurality” (claim 11) “of DNA products further comprises at least one amplification primer binding site.” EX1043, 148:53-58. It is my opinion that Stahl 2016 discloses the elements of claims 10 and 11.

147. The 142 patent teaches that an “amplification primer binding site” is “a nucleic acid sequence that is complementary to, or at least partially complementary to at least one amplification primer.” EX1043, 49:1-7. Stahl 2016 discloses that each of the “unique DNA-barcoded probes” on the array (located in a first and second location, as claimed) contains “a T7 amplification and sequencing handle.” EX1016, 80, FIG. 2A. Additionally, Stahl 2016’s Figure 2A depicts its capture probes comprising the “*Amp* and seq handle” (reproduced below), wherein the “*Amp* and seq handle” is located between the cleavage site and the spatial barcode.



EX1016, FIG. 2A.

148. Stahl 2016’s “*Amp* and seq handle” of the capture probe includes the “InPE1.0” PCR primer-binding site (highlighted in yellow below):

Surface spatially barcoded oligonucleotides:

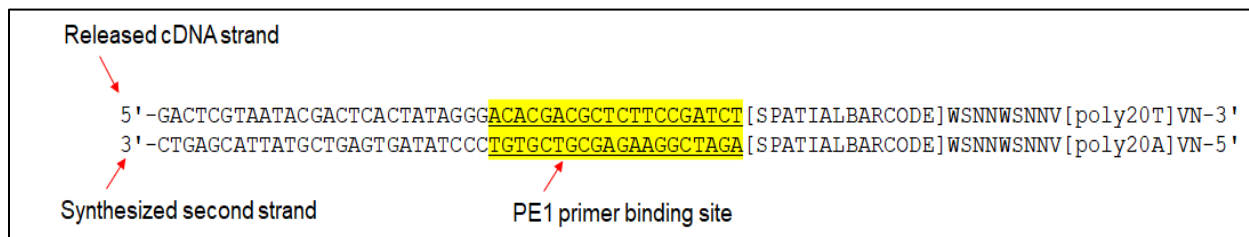
[AmC6]UUUUUGACTCGTAATACGACTCACTATAGGGACACGACGCTCTTCCGATCT[18
MER_SPATIALBARCODE]WSNNWSNNVTTTTTTTTTTTTTTTTTTTTTVN

EX1016, Suppl. Materials, 3 (annotated).

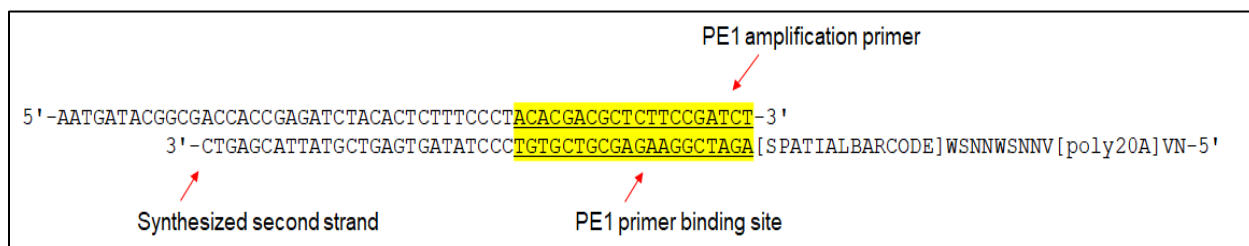
149. As I discuss above for claim 1, Stahl 2016's method synthesizes cDNA products using the captured (collected) mRNAs as templates and the capture probes as primers, thereby incorporating the sequence of the capture probes into the cDNA products. Accordingly, cDNA products synthesized from the captured (collected) mRNAs (the first and second plurality of DNA products) comprise the "Amp and seq handle" of the capture probe containing the "PE1" primer-binding site.

150. The PE1 primer-binding site is an amplification primer binding site because it is complementary to the amplification primers that are used for preparing the sequencing library. EX1016, Suppl. Materials, 6-8. Stahl 2016 discloses releasing the cDNA products (by cleaving the capture probe cleavage site "UUUUU" with USER enzyme). Subsequently, Stahl 2016's method synthesizes second-strand DNA products complementary to the released cDNA products. EX1016, Suppl. Materials, 6. These second-strand DNA products therefore include the sequence complementary to capture probe sequence.

151. The annotated figure below shows the sequence of the released cDNA product (top) and its complementary second strand (bottom), including the PE1 primer-binding site, highlighted in yellow:



152. Stahl 2016's second-strand DNA products are then PCR-amplified using PE1 amplification primers (PCR primers InPE1.0 and InPE2.0). EX1016, Suppl. Materials, 8. During amplification, the PE1 primer-binding site on the second-strand DNA products binds to its complementary sequence on the PE1 amplification primers (the InPE1.0 amplification primer). The annotated figure below shows a sequence alignment of the InPE1.0 amplification primer to Stahl 2016's second-strand DNA product:



EX1016, Suppl. Materials, 3, 8.

153. Accordingly, it is my opinion that Stahl 2016's first and second plurality of DNA products (cDNA products synthesized from the captured mRNAs)

comprise an amplification primer-binding site (PE1 primer-binding site contained in the “Amp and seq handle” of the capture probe).

154. Thus, it is my opinion that Stahl 2016 discloses the elements of claims 10 and 11 and anticipates them.

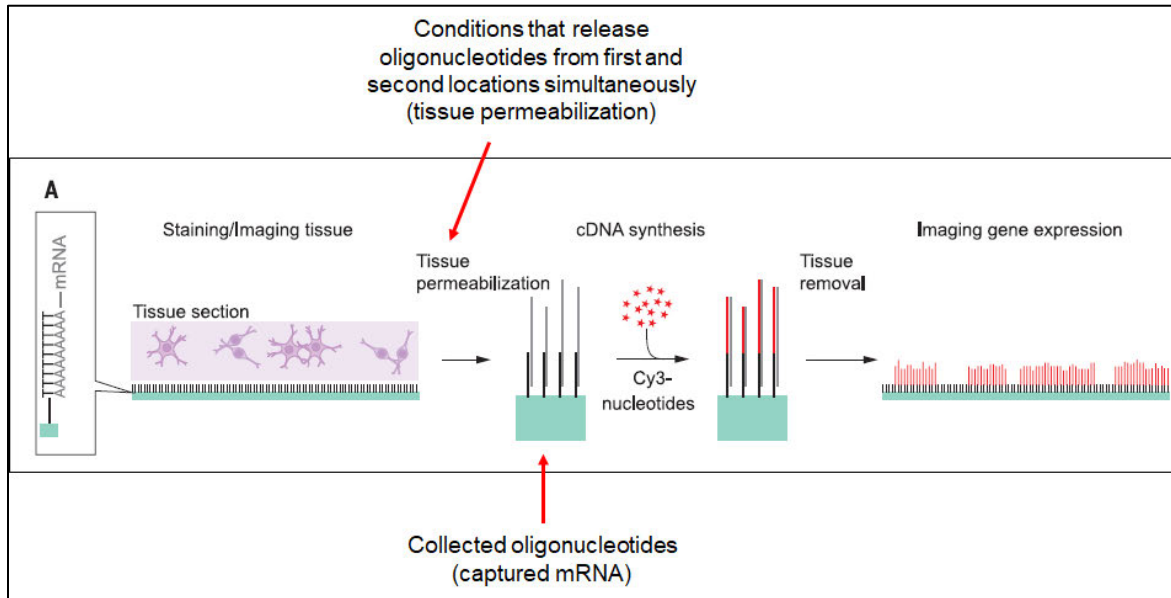
viii. Claims 12 and 13

155. Claims 12 and 13 each depends from claim 1, and requires that collecting “steps (a) and (b)” (claim 12) and synthesizing “steps (c) and (d)” (claim 13) “are performed simultaneously.” EX1043, 148:59-62. It is my opinion that Stahl 2016 discloses these elements under NanoString’s interpretation. EX1022, 11-14, 18-20; EX1015, 16-17.

156. Stahl 2016 discloses collecting the oligonucleotides (capturing the mRNAs) from the whole tissue section, all at the same time, *i.e.*, from a first and second location “simultaneously” as claimed in claim 12. EX1016, FIGs. 1-2, Suppl. Materials, 4-5. Specifically, Stahl 2016 discloses performing “[p]ermeabilization” “[f]or *each well* corresponding to *each sub-array* with a section,” and that “0.1% pepsin ... dissolved in 0.1M HCl ... was added to *each well*” to permeabilize the tissue sample. EX1016, Suppl. Materials, 4-5 (emphasis added). Thus, Stahl 2016’s method uniformly performs the permeabilization in each well (*i.e.*, across the whole array) and consequently collects the mRNAs from a first and second location of the tissue onto the array simultaneously. Stahl 2016

depicts simultaneous collection of its mRNAs from a first and second location in

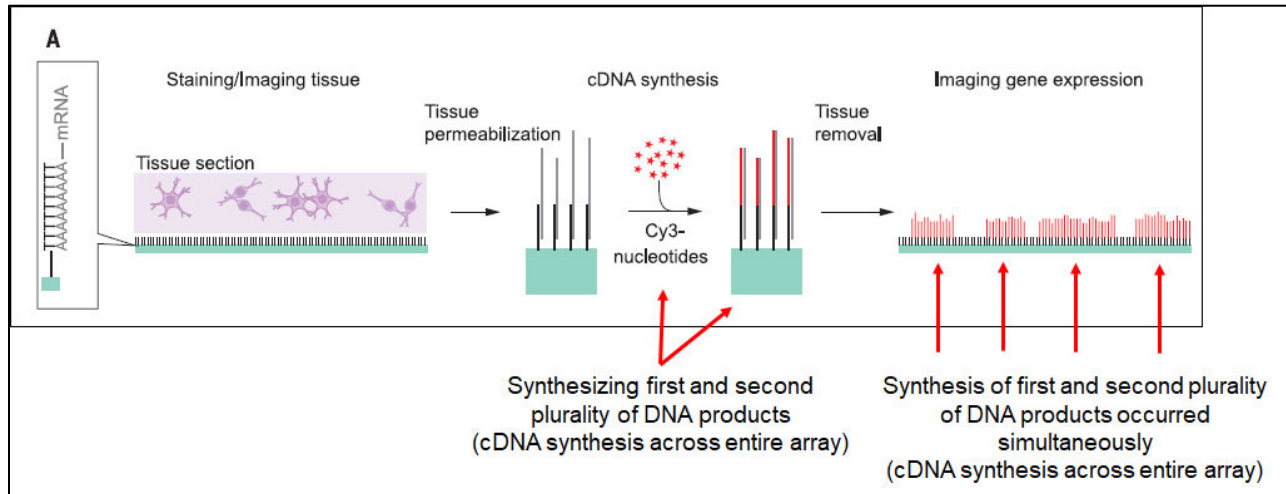
Figure 1A, as shown in the annotated figure below:



EX1016, FIG. 1A (annotated).

157. It is my opinion that Stahl 2016’s first and second plurality of DNA products synthesized from the collected oligonucleotides (cDNA products synthesized from the captured mRNAs) are synthesized across the entire array, all at the same time, *i.e.*, the first and second plurality of DNA products “simultaneously” as claimed in claim 13. EX1016, FIGs. 1-2, Suppl. Materials, 5. After permeabilizing the entire tissue section, Stahl 2016 discloses performing “cDNA synthesis” (reverse transcription) across the entire array (*i.e.*, simultaneously), such that “[e]ach well was washed ... and 70 μ l of reverse transcription mix was *added to each well* and incubated overnight ...” EX1016,

Suppl. Materials, 5 (emphasis added). Stahl 2016 illustrates these simultaneous cDNA synthesis reactions in Figure 1A, as shown in the annotated figure below:



EX1016, FIG. 1A (annotated).

158. Accordingly, it is my opinion that Stahl 2016 discloses the elements of claims 12 and 13 and anticipates them.

159. In sum, it is my opinion that Stahl 2016 teaches every element of claims 1-4 and 6-13 under NanoString's interpretation, arranged as claimed. Accordingly, it is my opinion that Stahl 2016 anticipates claims 1-4 and 6-13.

IX. GROUND 2: CLAIMS 1-13 WOULD HAVE BEEN OBVIOUS OVER STAHL 2016

160. As I explain above, Stahl 2016 teaches every element of claims 1-4 and 6-13 under NanoString's interpretation, and thus Stahl 2016 anticipates the claims. However, even assuming for argument's sake that Stahl 2016 does not anticipate

claims 1-4 and 6-13, it would have rendered claims 1-13 obvious over Stahl 2016 under NanoString's interpretation for the reasons I explain below.

a. Claims 1-4 and 6-13

161. It is my opinion that Stahl 2016 suggests practicing the claimed methods according to NanoString's interpretation, and it provides a motivation with a reasonable expectation of success for practicing the claimed methods.

162. A POSA reading Stahl 2016 would have been motivated to practice the claimed methods because, *e.g.*, Stahl 2016 discloses a method for "spatial transcriptomics" that "provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections" and "enables novel types of bioinformatics analyses." EX1016, Abstract, 82.

163. Further, it is my opinion that a POSA following the teachings of Stahl 2016 would have reasonably expected to successfully practice the claimed methods. Stahl 2016 discloses detailed descriptions and protocols with demonstrated success in the form of extensive data and analyses. *See, e.g.*, EX1016, FIGs. 1-4, Suppl. Materials 1-36. Further, a POSA would have known that Stahl's methods used commercially available materials and reagents, and that the protocol was straightforward, requiring "only a few extra steps compared with RNA-seq analysis of homogenized tissue." EX1016, 82, Suppl. Materials, 1-19.

164. Thus, a POSA reading Stahl 2016 would have been motivated to perform the claimed methods of claims 1-4 and 6-13 with a reasonable expectation of success.

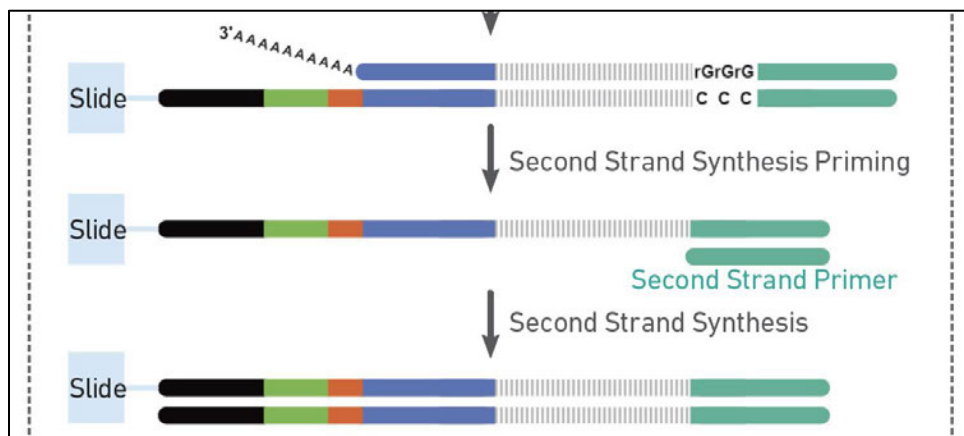
b. Claim 5

165. Claim 5 depends from claim 4 and further requires that each synthesis step (c) and (d) “comprises performing a solid-phase amplification reaction, wherein the solid-phase amplification reaction is carried out on the microscope slide using the plurality of primers immobilized on the microscope slide.” EX1043, 148:37-41.

166. I understand that because claim 5 uses “comprises,” it only requires that the solid-phase amplification take place as part of claim 1’s steps (c) and (d), before the sequencing step (e). According to the 142 patent, “‘solid-phase amplification’ ... refers to *any nucleic acid amplification* reaction carried out on or in association with a solid support such that all or *a portion of the amplified products are immobilized* on the solid support as they are formed.” EX1043, 42:41-45 (emphasis added).

167. Further, I understand that NanoString alleged in related litigation that claim 5’s solid-phase amplification reaction encompasses second-strand cDNA synthesis from the DNA products of steps (c) and (d) before the DNA products are released from the array slide. EX1022, 32-33. I have reviewed the 10x user guide

for Visium FF that NanoString identifies in its contentions. EX1015. This step of Visium FF is depicted in the figure below.



EX1015, 17.

168. Thus, under NanoString's interpretation, claim 5's solid-phase amplification reaction encompasses second-strand cDNA synthesis from the DNA products of steps (c) and (d) while the DNA products remain bound to the primers immobilized on the array slide. EX1015, 16-17.

169. As I discuss above for claim 1, because Stahl 2016's method synthesizes the cDNA products (first-strand cDNAs) on the array, it includes the synthesis steps (c) and (d). EX1016, 78-79, FIGs. 1-2, Suppl. Materials, 4-5. Further, Stahl 2016 discloses preparing in solution a "cDNA library preparation for sequencing" from the cDNA products, including an amplification step before sequencing. EX1016, Suppl. Materials, 6-7.

170. Specifically, following the release of the cDNA products from the array (microscope slide), Stahl 2016's library preparation involves performing the

following steps in solution: synthesizing second-strand cDNAs using the cDNA products as template, performing in vitro transcription to synthesize amplified RNAs (aRNAs) from cDNAs, re-synthesizing cDNAs from aRNAs by reverse transcription, and amplifying the cDNAs to generate a sequencing library.

EX1016, Suppl. Materials, 6-7. Thus, Stahl 2016's method includes processing *in-solution* the cDNA products synthesized from mRNAs from the tissue sample by second-strand cDNA synthesis and subsequent amplification.

171. A POSA would have known that the second-strand cDNA synthesis and library amplification can be performed on Stahl 2016's array, *i.e.*, microscope slide. *See, e.g.*, EX1017; EX1042; EX1049. For example, Stahl 2016's authors in their own PCT publication, Frisen, suggested that their spatial detection method using a barcoded array can include second-strand cDNA synthesis and library amplification on the array onto which the tissue sample was immobilized—which are “solid-phase amplification” under the 142 patent's definition and NanoString's interpretation. EX1017, 12:9-11, 38:20-23, 39:28-43:18.

172. It is my opinion that a POSA would have been motivated to modify Stahl 2016's method to perform the second-strand cDNA synthesis and/or library amplification on Stahl 2016's array. It was known in the art that performing these steps on a solid surface would improve “sensitivity and selectivity for nucleic acid identification.” EX1048, 1, 2, 11; *see also* EX1050, Abstract, 2075; EX1042,

17:37-18:59, 24:21-25:32, 26:19-47; EX1049, Abstract, ¶¶[0013], [0015], [0084]-[0086], [0089]-[0093], [0101], [0104], FIG. 1. For example, it was known in the art that “performing different enzymatic reactions with immobilized biomolecules on a solid support” provided “additional sensitivity, specificity and scalability of analysis”—all of which are desirable in a “high throughput” molecular bioanalytical method, such as Stahl 2016’s barcoded array-based spatial detection method using next generation sequencing. EX1048, 1; EX1016, Abstract, 78, Suppl. Materials, 1-2, 4-8; *see also* EX1050, Abstract (“solid phase amplification (SPA)” “allows the easy parallelization of DNA amplification in a single system”).

173. Additionally, it was known in the art that spatial separation of the primers on an array “significantly minimizes undesirable primer interactions hence reducing the formation of primer dimers, increasing specificity and sensitivity of amplification, and allowing for highly multiplexed analyses.” EX1048, 2; *see also* EX1050, 2075 (“Since [solid-phase amplification] results in a spatially located amplification, it is possible to amplify a large number of different DNA strands in the same experiment (i.e., onto the same solid surface) without mixing them.”).

174. Further, solid-phase amplification was desirable for high-throughput methods because it “allows the parallelization of the DNA amplification process without any direct human intervention.” EX1050, 2079. Thus, a POSA would have been motivated to modify Stahl 2016’s method to perform the second-strand

cDNA synthesis and/or library amplification on Stahl 2016's array—*i.e.*, on the microscope slide onto which tissue sample was overlaid, using the plurality of primers immobilized on the microscope slide.

175. In my opinion, a POSA would have had a reasonable expectation of successfully performing second-strand cDNA synthesis and/or library amplification on Stahl 2016's array. This is because such solid-phase steps were routinely performed in the art, particularly in high-throughput molecular bioanalytical methods such as Stahl 2016's method. *See, e.g.*, EX1005, ¶¶[0034]-[0035], [00109], [00141]-[00143], [00151]-[00158], [00307]-[00320], FIGs. 1, 2, 24-25; EX1017, 8:18-10:5, 7:29-8:3, 12:9-11, 38:20-23, 38:27-39:16, 40:13-41:21, 42:20-43:7, 43:19-43:33, 103:12-19; EX1042, 1:61-2:9, 3:11-34, 17:37-18:59, 24:21-25:32, 25:48-62, 26:19-47; EX1049, Abstract, ¶¶[0013], [0015], [0084]-[0086], [0089]-[0093], [0101], [0104], FIG. 1. For example, Frisen details "suitable means" for solid-phase second-strand synthesis and provides an exemplary method in Example 11. EX1017, 12:9-11, 38:20-23, 38:27-32, 39:5-6, 40:13-41:21, 42:28-43:7, 42:28-43:7, 103:12-19. Further, Stahl 2016's method already synthesized the first-strand cDNA synthesis on the array, *i.e.*, "solid-phase," and performing second-strand cDNA synthesis on the array was extending known and well applied DNA synthesis technologies on a solid substrate.

176. A POSA would have also known of other successful examples in the art teaching solid-phase second-strand synthesis and subsequent solid-phase library amplification—both of which are solid-phase amplification according to the 142 patent and NanoString’s interpretation. *See, e.g.*, EX1005, ¶¶[00109], [00156], [00307]-[00318], FIGs. 24-25; EX1042, 25:6-18, 25:48-62, 26:19-47; EX1049, Abstract, ¶¶[0013], [0015], [0084]-[0086], [0089]-[0093], [0101], [0104], FIG. 1. Thus, it is my opinion that Frisen discloses performing a solid-phase amplification on an array, specifically an array for use in a high-throughput genomic method.

177. Thus, in my opinion, a POSA would have been motivated to include solid-phase amplification of the synthesized cDNA products in Stahl 2016’s method with a reasonable expectation of success, wherein the reaction is carried out on Stahl 2016’s microscope slide using the plurality of primers immobilized on the microscope slide under the patent’s definition and NanoString’s interpretation.

178. I understand that NanoString did not assert any objective indicia of non-obviousness. I also understand that 10x is not aware of any objective indicia of non-obviousness. Accordingly, it is my opinion that under NanoString’s interpretation, claims 1-13 would have been obvious over Stahl 2016.

X. GROUND 3: CLAIM 5 WOULD HAVE BEEN OBVIOUS OVER STAHL 2016 AND WO 2012/140224 (FRISEN)

179. As I discuss above for Grounds 1 and 2, it is my opinion that Stahl 2016 either anticipates claims 1-4 and 6-13 or renders claims 1-13 obvious. Even

assuming Stahl 2016 alone does not render claim 5 obvious, it is my opinion that it would have rendered the claim obvious in combination with Frisen.

180. Stahl 2016 and Frisen combined teach or suggest including in Stahl 2016's method a solid-phase amplification reaction, wherein the amplification reaction is performed on the microscope slide using the primers immobilized on the microscope slide, as recited in claim 5 of the 142 patent. As the 142 patent recites, "'solid-phase amplification' ... refers to *any nucleic acid amplification reaction carried out on or in association with a solid support such that all or a portion of the amplified products are immobilized on the solid support as they are formed.*" EX1043, 42:41-45 (emphasis added). Further I understand that NanoString alleged in related litigation that claim 5's solid-phase amplification reaction encompasses second-strand cDNA synthesis from the DNA products of steps (c) and (d) while the DNA products remain bound to the primers immobilized on the array slide. EX1022, 32-33.

181. As I discussed above for claim 1, Stahl 2016's method synthesizes the cDNA products from mRNAs (first-strand cDNAs) on the array—performing claim 1's synthesis steps. EX1016, 78-79, FIGs. 1-2, Suppl. Materials, 4-5. Stahl 2016's method further includes "cDNA library preparation for sequencing." EX1016, Suppl. Materials, 6-8.

182. Stahl 2016's library preparation method includes in-solution second-strand cDNA synthesis and amplification, and it begins by first releasing the cDNA products from the array (microscope slide), followed by performing (in solution) each of: (i) *synthesizing second-strand cDNAs* using the released cDNA products as templates, (ii) synthesizing amplified RNAs (aRNAs) by reverse transcribing the cDNA duplexes, (iii) reverse transcribing the aRNAs to resynthesize the cDNA, and (iv) generating a sequencing library by *amplifying* the cDNAs. EX1016, Suppl. Materials, 6-7. Thus, it is my opinion that prior to performing library amplification steps in solution, Stahl 2016 discloses performing a second-strand cDNA synthesis step in solution.

183. Frisen, a PCT publication by Stahl 2016's authors, teaches that their array-based spatial detection method using a barcoded array can include performing second-strand cDNA synthesis and library amplification on the array onto which the tissue sample was immobilized. EX1017, 12:9-10, 38:20-23, 42:28-43:7. Frisen discloses a method for "determining and/or analyzing a transcriptome of a tissue sample." EX1017, 8:18-20. The method comprises "generating cDNA molecules from the captured RNA[s]" that are hybridized to the spatially barcoded probes immobilized on an array to which a tissue sample was contacted and "optionally amplifying said cDNA molecules" on the array. EX1017, 8:21-35. The method then continues by releasing "cDNA molecules and/or optionally their

amplicons from the surface of the array,” for further processing for sequencing.

EX1017, 9:1-5.

184. Frisen clarifies that in some instances, it “will be necessary to enhance, i.e.,] amplify, the amount of secured nucleic acid, e.g. synthesized cDNA[,] to yield quantities that are sufficient for DNA sequencing.” EX1017, 39:9-16; *see also* EX1017, 42:32-33 (“Thus in a preferred embodiment, the method of the invention may comprise a step of amplifying the DNA, e.g. cDNA molecules.”). Frisen also discloses that its methods include “where amplification is performed in situ on the array.” EX1017, 55:24-26, 23:18-20.

185. Additionally, Frisen discloses that its methods include synthesizing double stranded DNA from the cDNA products in a “single reaction to generate only a second DNA, e.g. cDNA, strand” or in an “amplification reaction to generate multiple copies of the second strand.” EX1017, 38:9-19. Further, Frisen specifies that the “step of second strand DNA, e.g. cDNA, synthesis may take place *in situ on the array*, either as a discrete step of second strand synthesis ... or in the initial step of an amplification reaction.” EX1017, 38:20-23 (emphasis added). For instance, Frisen discloses in Example 11 a method that includes generating a second-strand cDNA together with the first-strand cDNA on a chip, *i.e.*, solid surface. EX1017, 103:12-19.

186. Thus, it is my opinion that Frisen discloses methods comprising a solid-phase amplification reaction that is carried out on the array onto which the tissue sample was overlaid, using the primers immobilized on the array under the patent's definition and NanoString's interpretation.

187. **Motivation:** In my opinion a POSA would have been motivated to modify Stahl 2016's method to include second-strand cDNA synthesis and/or library amplification on its array (*i.e.*, microscope slide) as disclosed in Frisen for several reasons. *First*, in my opinion, a POSA would have appreciated that Frisen discloses array-based spatial detection methods using solid-phase second-strand cDNA synthesis and solid-phase library amplification steps. For example, both Stahl 2016 and Frisen provide methods coined as "spatial transcriptomics," and provide overlapping spatial transcriptomics workflows. Further, both Stahl 2016 and Frisen include overlapping authors and inventors: Frisen lists inventors as Jonas Frisen, Patrik Stahl, and Joakim Lundeberg, each of whom is a co-author on Stahl 2016. EX1016, 78; EX1017, Face, 1:18.

188. *Second*, a POSA would have known that producing the cDNA products and amplicons on the array in allocated spatial spots prior to collection by performing Frisen's solid-phase second-strand cDNA synthesis and/or solid-phase library amplification offers certain advantages. It was known in the art that performing these steps on a solid surface would improve "sensitivity and

selectivity for nucleic acid identification.” EX1048, 1, 2, 11; *see also* EX1050, Abstract, 2075; EX1042, 17:37-18:59, 24:21-25:32, 26:19-47; EX1049, Abstract, ¶¶[0013], [0015], [0101], [0104], FIG. 1.

189. For example, it was known in the art that “performing different enzymatic reactions with immobilized biomolecules on a solid support” provided “additional sensitivity, specificity and scalability of analysis”—all of which are desirable in a “high throughput” molecular bioanalytical method, such as Stahl 2016’s barcoded array-based spatial detection method using next generation sequencing. EX1048, 1; EX1016, Abstract, 78, Suppl. Materials, 1-2, 4-8; *see also* EX1050, Abstract (“solid phase amplification (SPA)” “allows the easy parallelization of DNA amplification in a single system”).

190. Additionally, it was known in the art that spatial separation of primers on an array “significantly minimizes undesirable primer interactions hence reducing the formation of primer dimers, increasing specificity and sensitivity of amplification, and allowing for highly multiplexed analyses.” EX1048, 2; *see also* EX1050, 2075 (“Since [solid-phase amplification] results in a spatially located amplification, it is possible to amplify a large number of different DNA strands in the same experiment (i.e., onto the same solid surface) without mixing them.”). Further, solid-phase amplification is desirable for high-throughput methods

because it “allows the parallelization of the DNA amplification process without any direct human intervention.” EX1050, 2079.

191. *Finally*, Frisen explicitly suggests implementing solid-phase second-strand synthesis and library amplification into a workflow like Stahl 2016’s workflow. EX1017, 12:9-11, 38:20-23, 38:27-32, 39:5-6, 42:28-43:7, 103:12-19.

192. Thus, a POSA would have been motivated to modify Stahl 2016’s method to perform the second-strand cDNA synthesis and/or library amplification on Stahl 2016’s array—*i.e.*, on the microscope slide onto which tissue sample was overlaid, using the plurality of primers immobilized on the microscope slide.

193. **Reasonable expectation of success:** In my opinion, a POSA would have had a reasonable expectation to successfully modify Stahl 2016’s workflow to implement Frisen’s solid-phase second-strand cDNA synthesis and/or solid-phase library amplification steps. This is because doing so required art-recognized techniques and ordinary skill and was routinely performed, particularly in high-throughput molecular bioanalytical methods such as Stahl 2016’s method. *See, e.g.*, EX1005, ¶¶[0034]-[0035], [00109], [00141]-[00143], [00151]-[00158], [00307]-[00320], FIGs. 1, 2, 24-25; EX1017, 8:18-10:5, 7:29-8:3, 12:9-11, 38:20-23, 38:27-39:16, 40:13-41:21, 42:20-43:7, 43:19-43:33, 103:12-19; EX1042, 1:61-2:9, 3:11-34, 17:37-18:59, 24:21-25:32, ; EX1049, Abstract, ¶¶[0013], [0015], [0084]-[0086], [0089]-[0093], [0101], [0104], FIG. 1. Further, Stahl 2016’s method

already synthesized the first-strand cDNA synthesis on the array, *i.e.*, “solid-phase,” and performing second-strand cDNA synthesis on the array was a straightforward modification. EX1016, 78, middle col. and right col., 79-80, FIGs. 1A, 2A, Suppl. Materials, 4-5.

194. Further, methods of solid-phase cDNA synthesis and amplification were commonplace in molecular biology laboratories and reagents such as primers, buffers, nucleotides, polymerase enzymes, and materials were all standard commercial products. *See, e.g.*, EX1017, 8:18-10:5, 7:29-8:3, 12:9-11, 38:20-23, 38:27-39:16, 40:13-41:21, 42:20-43:7, 43:19-43:33, 103:12-19; EX1005, ¶¶[0034]-[0035], [00109], [00141]-[00143], [00151]-[00158], [00307]-[00320], FIGs. 1, 2, 24-25; EX1042, 1:61-2:9, 3:11-34, 17:37-18:59, 24:21-25:32, 25:48-62, 26:19-47; EX1049, Abstract, ¶¶[0013], [0015], [0084]-[0086], [0089]-[0093], [0101], [0104], FIG. 1. Indeed, Frisen provides “suitable means” for second-strand synthesis and includes an exemplary method in Example 11. EX1017, 12:9-11, 38:20-23, 38:27-32, 39:5-6, 40:13-41:21, 42:28-43:7, 42:28-43:7, 103:12-19.

195. Thus, in my opinion, a POSA would have been motivated to include Frisen’s solid-phase amplification of the synthesized cDNA products in Stahl 2016’s method with a reasonable expectation of success, wherein the reaction is carried out on Stahl 2016’s microscope slide (array) using the plurality of primers immobilized on the microscope slide.

196. I understand that NanoString did not assert any objective indicia of non-obviousness during prosecution. I also understand that 10x is not aware of any objective indicia of non-obviousness. Accordingly, it is my opinion that under NanoString's interpretation, claim 5 would have been obvious over Stahl 2016 and Frisen.

XI. GROUND 4: CLAIMS 1-13 ARE ANTICIPATED BY WO 2017/019456 (SO-456)

197. In my opinion, claims 1-13 of the 142 patent are anticipated by So-456 under NanoString's interpretation of the claims. Specifically, So-456 discloses a method meeting all the elements of the claimed methods. I understand that So-456 is presumed enabled. I understand that So-456 was published before February 12, 2018, and thus it qualifies as prior art to the 142 patent.

a. Claim 1

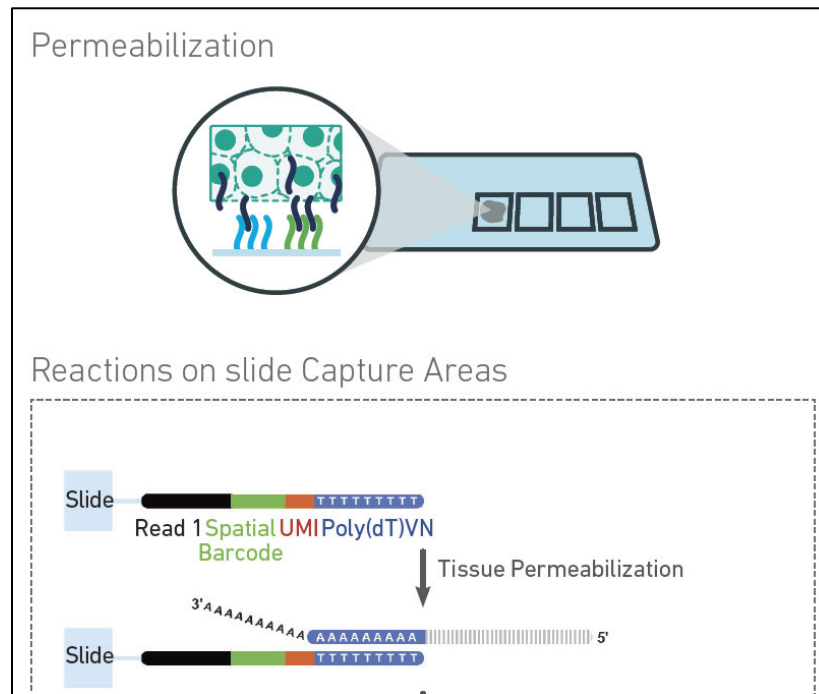
198. For the reasons I explain below, So-456 anticipates claim 1. *See also* Appendix B.

i. "A method comprising: a) [and (b)] collecting a plurality of oligonucleotides from a first location [and second location] of a tissue sample ..."

199. In my opinion, So-456's method includes the collecting steps (a) and (b) under NanoString's interpretation. I understand that NanoString alleged in related litigation that 10x's Visium FF infringes the 142 patent claims. EX1022. Under NanoString's interpretation, the collecting steps of claim 1 encompass certain of

the steps of 10x's Visium FF workflow. EX1022, 11-14, 18. I have reviewed the 10x user guide for Visium FF that NanoString identifies in its contentions.

EX1015. In Visium FF, mRNAs are diffused from and captured across the *whole* tissue sample, all at the same time. EX1015, 16-17. These steps of Visium FF are depicted in the figure below.



EX1015, 17.

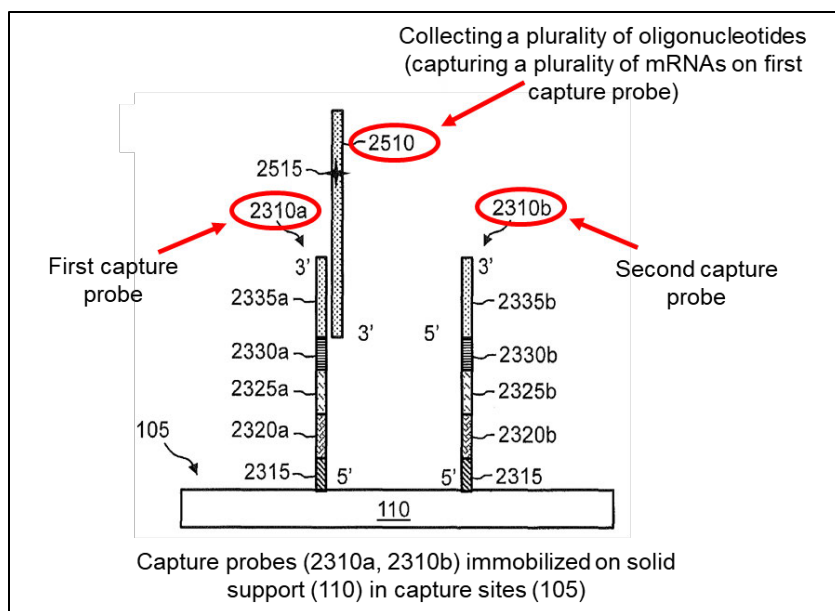
200. Thus, under NanoString's interpretation, the collecting steps of claim 1 encompass diffusion and capture of mRNAs across the *whole* tissue sample, all at the same time. EX1022, 11-14, 18; EX1015, 16-17.

201. It is my opinion that So-456's method of Figures 24-25 includes the steps of collecting a plurality of oligonucleotides from a "first" and "second" location under NanoString's interpretation. So-456 discloses contacting "a tissue sample ...

compris[ing] a plurality of mRNA molecules” with a “capture array.” EX1005,

¶¶[00308], [00314], FIGs. 24, 25. So-456’s capture array comprises for each mRNA molecule (2510) two “capture probes” (2310) that are immobilized in the “5’ to 3’ direction (i.e., the 5’ end of capture probes ... are attached to the surface of capture site).” EX1005, ¶¶[00308], [00314]. So-456 explains that the capture probes used in Figures 24-25’s method are the same as the capture probes used in Figure 23’s method. EX1005, ¶[00314].

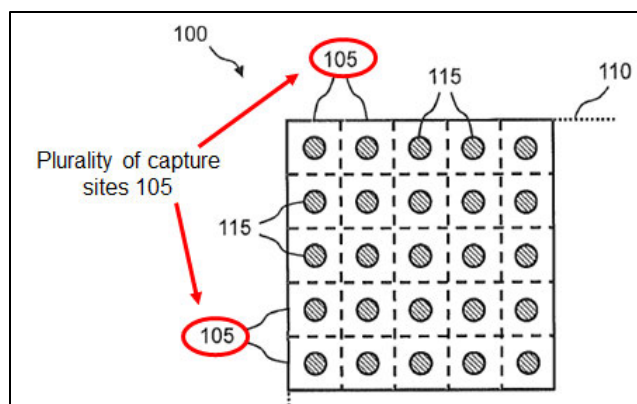
202. So-456’s method captures (collects) the mRNAs (oligonucleotides) from the tissue sample on the first capture probes immobilized on the array. EX1005, ¶¶[00308], [00314]-[00315]. The first capture probe (2310a) comprises “sequences specific for the 3’ end of the mRNA molecule” (2335a) and that the “*mRNA molecules are captured* onto the array by hybridization of the 3’ end of the mRNA to gene-specific regions [(2335a)] on the first capture probe.” EX1005, ¶¶[00308] (emphasis added), [00314]-[00315]. This is illustrated in the annotated version of So-456’s Figure 25A shown below.



EX1005, FIG. 25A (annotated).

203. Thus, in my opinion, So-456 discloses collecting (capturing) a plurality of oligonucleotides (mRNAs).

204. Furthermore, So-456's method collects oligonucleotides (mRNAs) from a first and second location of the tissue sample under NanoString's interpretation. EX1022, 11-14, 18; EX1015, 16-17. So-456's method captures mRNAs released from the tissue sample onto capture array (100) comprising "an arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110." EX1005, ¶¶[00141], [00307], [00314]. As illustrated below in an annotated version of So-456's Figure 1A, So-456 discloses capture probes that are immobilized at each of the capture sites of the array. EX1005, ¶¶[00307], [00314].



EX1005, FIG. 1A (annotated).

205. So-456's method specifies that the "target nucleic acid in the tissue section is transferred onto an array such that the position of a nucleic acid on the array can be correlated with a position in the tissue section." EX1005, ¶¶[00154]; *see also* EX1005, ¶[0093] ("facilitating the transfer of nucleic acids from a tissue sample onto a capture array"). Additionally, So-456's methods "substantially maintain information encoding the spatial orientation of the targets in the sample." EX1005, ¶[00155]. Thus, in my opinion, So-456 teaches collecting mRNAs from a first and a second location (different positions) of the tissue sample. EX1005, ¶[00155].

206. So-456's method captures (collects) mRNAs simultaneously at multiple locations of the tissue sample and consequently at multiple capture sites on the array. EX1005, ¶¶[00141], [00307]-[00308], [00314]-[00315], FIGs. 1A, 24-25. Accordingly, in my opinion So-456's method collects mRNAs from the *whole*

tissue sample, all at the same time, according to NanoString's interpretation.

EX1015, 16-17; EX1022, 11-14, 18.

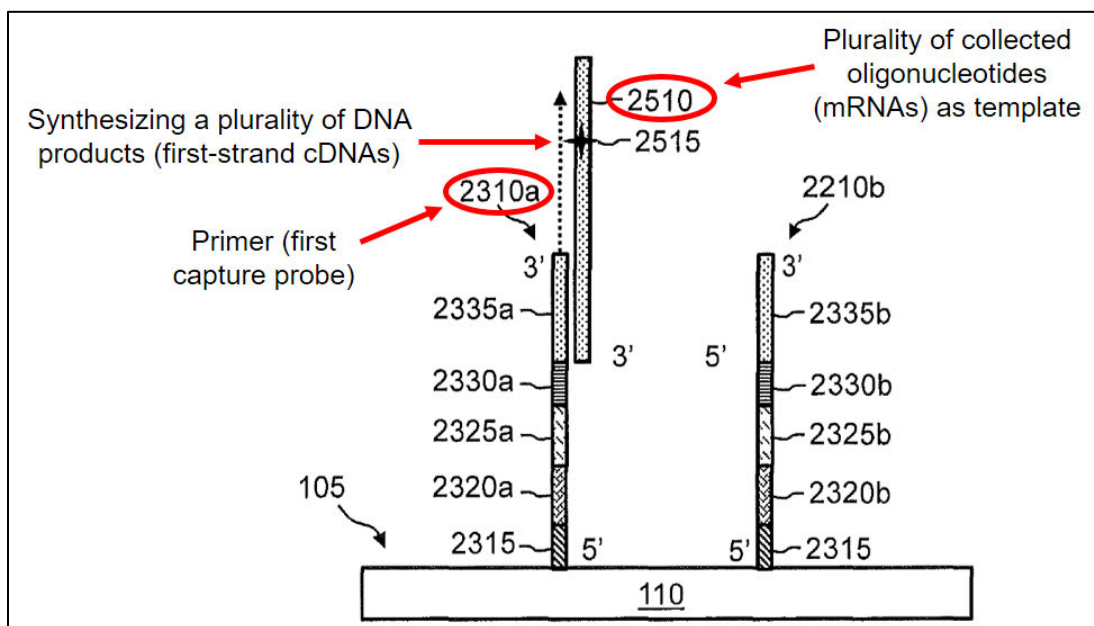
**“under conditions that release the plurality of
oligonucleotides from the first location [and second
location] of the tissue sample”**

207. In my opinion, So-456 discloses collecting the oligonucleotides (mRNAs) under conditions that release the oligonucleotides from their locations in the tissue sample. This is because So-456 discloses that the “*tissue sample is prepared for analysis*” and the mRNAs from the tissue sample are “captured onto the array,” *e.g.*, the “capture array 100 of Figure 1 A.” EX1005, ¶¶[00152] (emphasis added), [00308], [00315]; *see also* EX1005, ¶[00154] (“the target nucleic acid [(*e.g.*, mRNA)] in the tissue section is transferred onto an array such that the position of a nucleic acid on the array can be correlated with a position in the tissue section”). Additionally, So-456 discloses that the “target nucleic acid” (mRNA) “diffuse[s] from the tissue” onto the array. In my opinion, this further confirms that the tissue sample was treated to create a condition to “release” the mRNA from the tissue onto the array. EX1005, ¶[00155].

208. Thus, So-456 discloses collecting the oligonucleotides (mRNAs) under conditions that release the oligonucleotides from their locations of the tissue sample. Accordingly, it is my opinion that So-456 discloses claim 1's collecting steps.

ii. “c) [and (d)] synthesizing a first plurality [and second plurality] of DNA products by performing a synthesis reaction ...”

209. It is my opinion that So-456 discloses the synthesis steps (c) and (d). So-456's method includes "gene-specific capture regions on the first capture probe" (2310a) as "reverse transcriptase primers" for "synthesis of first strand cDNA from captured mRNA molecules." EX1005, ¶¶[00309], [00316]. This cDNA synthesis is depicted as a dotted arrow in the annotated version of So-456's Figure 25A shown below:



EX1005, FIG. 25A (annotated).

210. Accordingly, for these reasons, So-456's method includes synthesizing a first and second plurality of DNA products (cDNAs) because it produces the

cDNAs from the “captured mRNA *molecules*.” EX1005, ¶¶[00309] (emphasis added), [00316], FIGs. 24, 25A. So-456’s synthesis reactions take place simultaneously in different spots on the array. *See, e.g.*, EX1005, ¶¶[00141], [00307]-[00308], [00314]-[00315], FIGs. 1A, 24-25. Thus, it is my opinion that So-456’s method includes synthesizing a first and second plurality of DNA products, as claimed.

“that uses the plurality of oligonucleotides collected in step (a) [and step (b)] as templates ...”

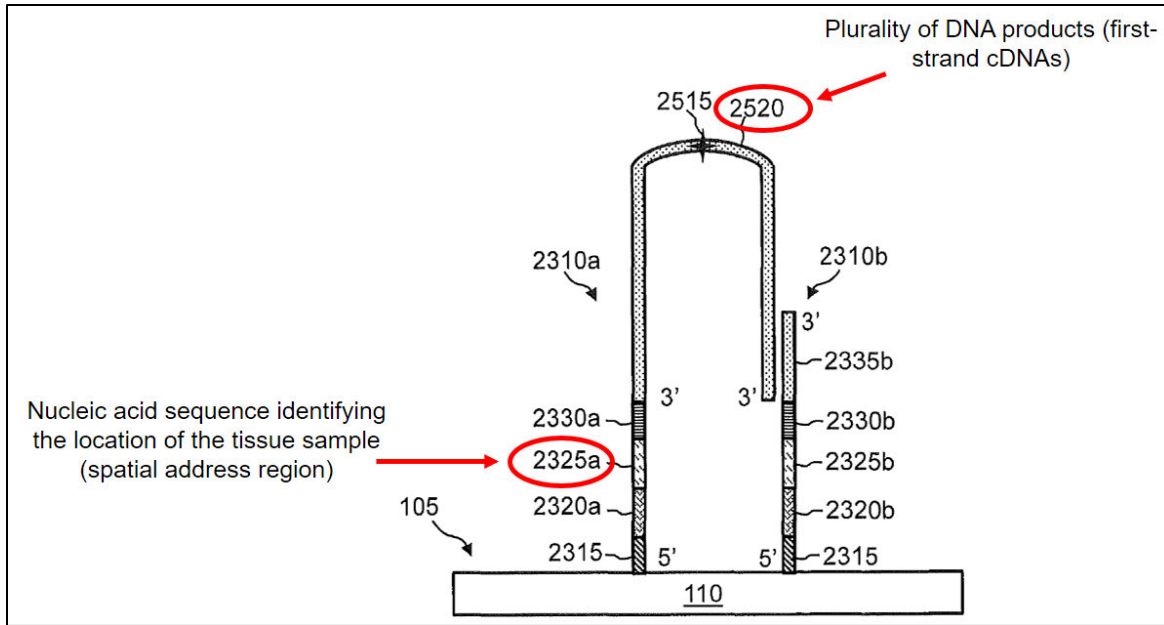
211. In my opinion, So-456’s synthesis reactions use the first and second plurality of oligonucleotides (mRNAs) collected in steps (a) and (b) (respectively) as templates. Specifically, So-456’s method includes a reverse transcription reaction to synthesize a “complementary” DNAs, *i.e.*, cDNAs, strand using the captured (collected) mRNAs as templates. EX1005, ¶¶[00309], [00316], FIG. 25A. So-456’s Figure 25A (reproduced above) shows the captured mRNAs (2510) (collected first and second plurality of oligonucleotides) serving as templates for the cDNA synthesis (depicted as a dotted arrow). EX1005, FIG. 25A. Thus, it is my opinion that So-456’s method uses the plurality of oligonucleotides (mRNAs) collected in steps (a) and (b) as templates, as claimed.

“and incorporates at least one nucleic acid sequence that identifies the first location [and second location] of the tissue sample into each of the first plurality [and second plurality] of DNA products”

212. In my opinion, So-456’s synthesis reactions also incorporate at least one nucleic acid sequence that identifies the first (and second) location of the tissue sample into the first (and second) plurality of DNA products, as claimed.

213. So-456’s method of Figures 24-25 uses capture probes (2310) that are “essentially the same” as the capture probes in So-456’s method of Figure 23A. EX1005, ¶[00314]. So-456 teaches that these capture probes comprise a “spatial address region” that includes a “unique spatial address sequence.” EX1005, ¶¶[00302], [00314]. So-456 explains that the spatial address region “corresponds to the position of capture probe 115 in capture array 100.” EX1005, ¶[00141].

214. This is illustrated in the annotated version of Figure 25B below, wherein the first strand cDNA (2520) (synthesized using the first capture probe (2310a) as primer and mRNA as template) includes the spatial address region (2325a):



EX1005, FIG. 25B (annotated).

215. For these reasons, it is my opinion that So-456's synthesis reaction synthesizes a first plurality and second plurality of DNA products (cDNA products) using the collected oligonucleotides (mRNAs) as templates, incorporating a sequence that identifies a location on the array (spatial barcode) into the DNA products. EX1005, FIGs. 25A-B. Thus, in my opinion, So-456 discloses claim 1's synthesizing steps.

- iii. **“e) identifying the first plurality of DNA products and the second plurality of DNA products synthesized in step (c) and step (d) by sequencing the first plurality of DNA products and the second plurality of DNA products, thereby spatially detecting the plurality of oligonucleotides collected from the first location of the tissue sample and the plurality of oligonucleotides collected from the second location of the tissue sample.”**

216. In my opinion, So-456 discloses claim 1’s sequencing step (e) under NanoString’s interpretation. I understand that NanoString agrees that “spatially detecting” means “identifying the presence of a specific target analyte within a specific region of interest in a sample.” EX1024, 31.

217. After synthesizing the first-strand cDNAs (first and second plurality of DNA products), So-456’s method generates a sequencing library and sequences the library. EX1005, ¶[00310]-[00313], [00317]-[00320]. Specifically, So-456’s method synthesizes the second-strand cDNAs using the first-strand cDNAs as templates and the second capture probes on the arrays as primers, and then it releases the second-strand cDNAs from the array by a cleavage reaction. EX1005, ¶¶[00310]-[00312], [00317]-[00319]. So-456’s released second-strand cDNAs comprise the sequence complementary to the first-strand cDNAs (which includes spatial address region 2325a) and the sequence of the second capture probe. Thus, the released cDNAs of So-456’s method include the spatial address region (2325a) of the first capture probe. EX1005, ¶[00319].

218. So-456 method then amplifies the released cDNA molecules (containing the spatial address regions) to “generate a sequencing library.” EX1005, ¶¶[00313], [00320]. Next, “the library is sequenced” and “the sequence data is *analyzed* ... and the *spatial information is decoded*.” EX1005, ¶¶[0089], [00157]-[00158] (emphasis added). The decoded spatial information is “used to provide *information as to the location* of the nucleic acid in the tissue section” (*i.e.*, spatially detect the plurality of oligonucleotides collected from the first and second locations of the tissue sample, as claimed). EX1005, ¶¶[0089], [00158] (emphasis added).

219. During decoding, the “sequence-identical nucleic acid molecules originating from *different regions* in a tissue sample can be distinguished based on their spatial address and can be *mapped onto their regions of origin* in the tissue sample.” EX1005, ¶[0089] (emphasis added). Accordingly, So-456’s method “enable[s] the identification of *the location of a cell or a cell cluster* in a tissue” sample. EX1005, ¶¶[0002], [0088] (emphasis added). Thus, So-456 includes claim 1’s sequencing step under NanoString’s interpretation.

220. For the reasons I discuss above, So-456 teaches every limitation of claim 1 under NanoString’s interpretation, arranged as claimed. Thus, in my opinion, So-456 anticipates claim 1 under Nanostring’s interpretation.

b. Claims 2-13

221. Claims 2-13 depend directly or indirectly from claim 1, and further specify the elements of claim 1. I understand that the dependent claims incorporate all the elements of the claims from which they depend.

i. Claim 2

222. Claim 2 depends from claim 1 and further requires that “the tissue sample is immobilized onto a microscope slide.” EX1043, 147:62-63. It is my opinion that So-456 discloses the elements of claim 2.

223. So-456 discloses fixing, sectioning, and mounting a “tissue sample” on “a *microscope slide*.” EX1005, ¶[00125] (emphasis added). Moreover, So-456’s method in Figure 2 discloses that the tissue sample, *e.g.*, fresh tissue or frozen tissue, is “sectioned onto a *slide*.” EX1005, ¶[00152] (emphasis added), FIG. 2.

224. Thus, in my opinion, So-456 discloses the elements of claim 2 and anticipates it.

ii. Claim 3

225. Claim 3 depends from claim 2 and requires that the “microscope slide comprises a plurality of primers immobilized on the microscope slide.” EX1043, 148:32-34. It is my opinion that So-456 discloses the elements of claim 3.

226. So-456 disclose that in its method “a tissue sample that comprises a plurality of mRNA molecules is contacted with a capture array,” *e.g.*, the “capture array 100 of Figure 1A.” EX1005, ¶¶[00308], [00314]-[00315]. Further, So-456

discloses that “*any number* of capture probes 115 can be *immobilized on solid support* 110 at each capture site 105” of the array of Figure 1A and that the solid support has “an *array of capture sites*” that include “*a plurality of capture probes.*” EX1005, ¶¶[00141] (emphasis added), [00154], FIGs. 1A-1B.

227. For these reasons, it is my opinion that So-456 discloses that its microscope slide comprises a plurality of primers immobilized on the slide. Thus, in my opinion, So-456 discloses the elements of claim 3 and anticipates it.

iii. Claim 4

228. Claim 4 depends from claim 3 and requires that the “plurality of primers is immobilized on the microscope slide at their 5’ ends.” EX1043, 148:35-36. It is my opinion that So-456 discloses the elements of claim 4.

229. Specifically, So-456 discloses that “the 5’ end of capture probes 2310a and 2310b are attached to the surface of capture site 105.” EX1005, ¶[00314]; *see also* EX1005, ¶[00109] (“[o]ligonucleotides to be used as capture primers ... can be immobilized such that a 3’-end is available for enzymatic extension” (emphasis added)). For these reasons, it is my opinion that So-456 discloses that its microscope slide comprises a plurality of primers immobilized at their 5’-ends. Thus, in my opinion, So-456 discloses the elements of claim 4 and anticipates it.

iv. Claim 5

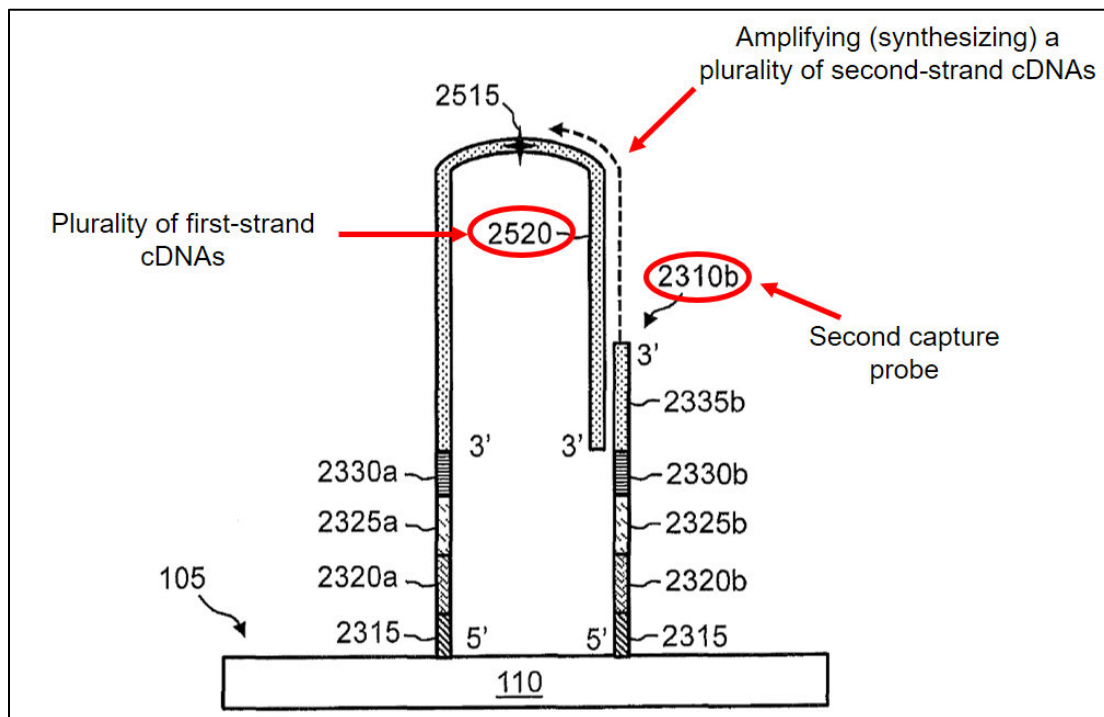
230. Claim 5 depends from claim 4, and requires that each synthesis step (c) and (d) “comprises performing a solid-phase amplification reaction, wherein the solid-phase amplification reaction is carried out on the microscope slide using the plurality of primers immobilized on the microscope slide.” EX1043, 148:37-41. I understand that because claim 5 uses “comprises,” it only requires that solid-phase amplification take place as part of claim 1’s steps (c) and (d), before step (e). In my opinion, So-456 discloses the elements of claim 5.

231. According to the 142 patent, “‘solid-phase amplification’ ... refers to *any nucleic acid amplification* reaction carried out on or in association with a solid support such that all or *a portion of the amplified products are immobilized* on the solid support as they are formed.” EX1043, 42:41-45 (emphasis added). I understand that, in related litigation, NanoString alleged that claim 5’s solid-phase amplification reaction encompasses second-strand cDNA synthesis from the DNA products of steps (c) and (d) while the DNA products remain bound to the primers immobilized on the array slide. EX1022, 32-33.

232. So-456 discloses that its “capture probes” immobilized on the microscope slide can be “*amplification primers ... immobilized* such that a 3’-end is available for enzymatic extension.” EX1005, ¶¶[00109] (emphasis added), [00173]. So-456 further specifies that the “[l]ibrary preparation may be

accomplished *on the capture array substrate.*” EX1005, ¶[00156] (emphasis added). Thus, it is my opinion that So-456’s method includes solid-phase amplification as required by claim 5 under the 142 patent’s definition and NanoString’s interpretation.

233. Additionally, it is my opinion that the second-strand cDNA synthesis step So-456’s method constitutes a solid-phase amplification reaction under the 142 patent’s definition and NanoString’s interpretation. This is illustrated in the annotated version of So-456’s Figure 25B below.



EX1005, FIG. 25B (annotated).

234. As shown in Figure 25B above, the first-strand cDNAs are captured on the second capture probes (2310b) (which serve as primers) and copied (amplified) by synthesis reaction into the second-strand cDNAs (depicted as a dotted arrow). EX1005, ¶¶[00310], [00317]-[00318], FIGs. 24, 25B.

235. Thus, in my opinion, So-456's cDNA synthesis reaction is a solid-phase amplification according to the 142 patent's definition and NanoString's interpretation. Furthermore, it is my opinion that So-456's cDNA synthesis reaction is carried out on the microscope slide (110) using the plurality of primers (capture probes) that are immobilized on the microscope slide, as claimed. EX1005, FIG. 1. Accordingly, it is my opinion that So-456 discloses the elements of claim 5 and anticipates it.

v. Claim 6

236. Claim 6 depends from claim 1, and requires that the "sequencing step is performed using a next generation sequencing reaction." EX1043, 148:42-43. It is my opinion that So-456 discloses the elements of claim 6.

237. So-456 discloses that the "spatially-tagged DNA molecules are released from the array and analyzed, for example, by high throughput *next generation sequencing* (NGS), such as sequencing-by-synthesis (SBS)." EX1005, ¶[00127] (emphasis added); *see also* EX1005, ¶¶[00156], [00595]. Accordingly, it is my opinion that So-456 discloses the elements of claim 6 and anticipates it.

vi. Claim 7

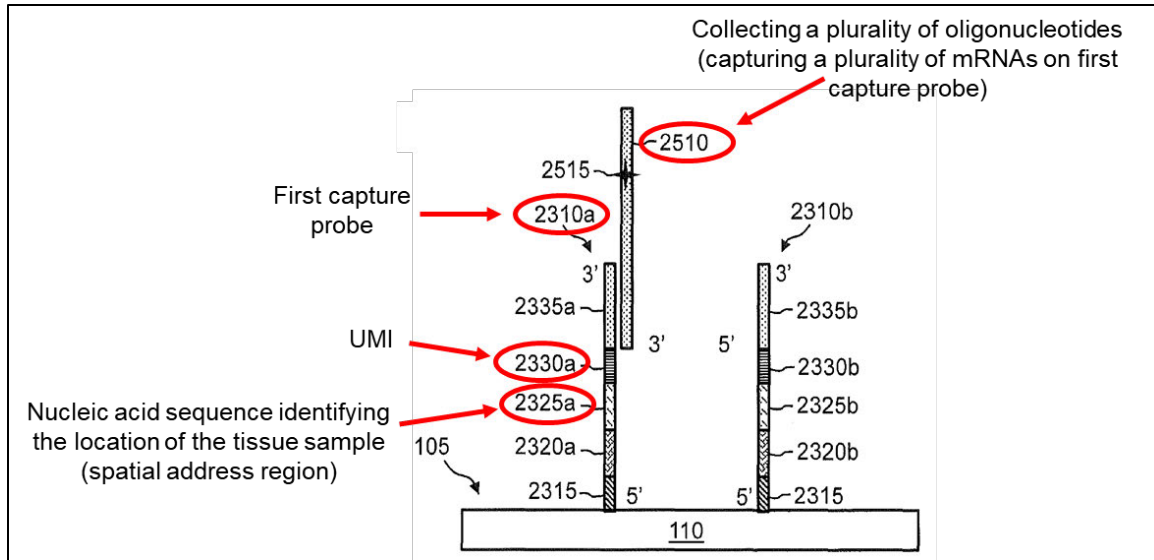
238. Claim 7 depends from claim 1, and “further comprises amplifying a library using the first plurality of DNA products and the second plurality of DNA products as templates.” EX1043, 148:44-46. It is my opinion that So-456 discloses the elements of claim 7.

239. As I discussed above under claim 5, So-456’s method copies (amplifies) the first-strand cDNAs (DNA products) synthesized from the captured mRNAs into the second-strand cDNAs by using the first-strand cDNAs as templates and second capture probes as primers. EX1005, ¶¶[00310], [00317]-[00318], FIGs. 24, 25B. Subsequently, the second-strand cDNAs (2525) are released from the array and “*amplified* to generate a sequencing library.” EX1005, ¶¶[00312]-[00313] (emphasis added), [00319]-[00320]. So-456 elaborates that “cDNA molecule 2525 is *amplified* using an SBS primer 2530 and a second SBS primer 2535” to generate a “library *amplicon* 2550.” EX1005, ¶[00320] (emphasis added); *see also* EX1005, ¶¶[00141], [00142], [00182]. For these reasons, it is my opinion that So-456 discloses amplifying a library using the cDNAs (*i.e.*, first and second plurality of DNA products) as templates and thus discloses the elements of claim 7 and anticipates it.

vii. Claims 8 and 9

240. Claims 8 and 9 each depend from claim 1 and require that the “at least one nucleic acid sequence that identifies the [first (claim 8) and second (claim 9)] location of the tissue sample comprise at least one unique molecular identifier.” EX1043, 148:47-52. It is my opinion that So-456 discloses the elements of claims 8 and 9.

241. As I discuss above for claim 1, the “spatial address region” in each of So-456’s capture probes identifies the first and second locations of the tissue sample. EX1005, ¶¶[00302], [00314]. Additionally, So-456’s capture probes also comprise a UMI: “Capture probes 2310 include ... a *spatial address region 2325*, a *unique molecular identifier (UMI) 2330*, and a capture region 2335.” EX1005, ¶[00302] (emphasis added). For example, capture probe 2310a comprises “*spatial address region 2325a*, which comprises a unique spatial address sequence ...; *UMI region 2330a*, which comprises a unique sequence for capture probe 2310a.” EX1005, ¶[00302] (emphasis added). I have illustrated this in an annotated version of Figure 25B below:



EX1005, FIG. 25B (annotated).

242. For these reasons, it is my opinion that So-456 teaches a nucleic acid sequence that identifies a location of the tissue sample (*i.e.*, capture probe comprising a spatial address region) comprising a UMI. Thus, in my opinion, So-456 discloses the elements of claims 8 and 9 and anticipates them.

viii. Claims 10 and 11

243. Claims 10 and 11 each depends from claim 1, and requires that the “first plurality” (claim 10) and “second plurality” (claim 11) “of DNA products further comprises at least one amplification primer binding site.” EX1043, 148:53-58. It is my opinion that So-456 discloses the elements of claims 10 and 11.

244. The 142 patent teaches that an “amplification primer binding site” is “a nucleic acid sequence that is complementary to, or at least partially complementary to at least one amplification primer.” EX1043, 49:1-7. So-456’s

The diagram illustrates a microarray structure. A base layer 110 supports a probe array 105. The probe array 105 consists of a first capture probe array 2310a and a plurality of DNA products (first-strand cDNAs) 2520. The first capture probe array 2310a is composed of a first capture probe 2315 and a first capture probe binding site (SBS primer region) 2320a. The first capture probe 2315 is a short DNA sequence, and the SBS primer region 2320a is a longer DNA sequence. The first capture probe binding site (SBS primer region) 2320a is further divided into segments 2320b, 2325b, 2330b, and 2335b. The first capture probe 2315 is also divided into segments 2315a, 2325a, 2330a, and 2335a. The first capture probe binding site (SBS primer region) 2320a is also divided into segments 2320b, 2325b, 2330b, and 2335b. The first capture probe binding site (SBS primer region) 2320a is also divided into segments 2320b, 2325b, 2330b, and 2335b. The first capture probe binding site (SBS primer region) 2320a is also divided into segments 2320b, 2325b, 2330b, and 2335b.

EX1005, FIG. 25B (annotated).

246. So-456 further discloses that its method synthesizes the second-strand cDNAs using the first-strand cDNAs as templates and the second capture probes on the arrays as primers, and then it releases the second-strand cDNAs from the array by a cleavage reaction. EX1005, ¶¶[00310]-[00312], [00317]-[00319]. So-456's released second-strand cDNAs (2525) comprise the sequence complementary to the first-strand cDNAs (which includes SBS primer region 2320a). Thus, the released cDNAs (2525) include SBS primer region (2320a). EX1005, ¶[00319].

247. So-456 further explains that the released "cDNA molecule 2525 is amplified using an SBS primer 2530" that comprises "SBS region 2320a' that is complementary to *SBS primer region 2320a*." EX1005, ¶[00320] (emphasis added); *see also* EX1005, ¶[00141]. Thus, So-456's first and second plurality of DNA products (first-strand cDNAs (2520) synthesized from the captured mRNAs) comprise at least one amplification primer binding site (SBS primer region 2320a). Thus, So-456 discloses the elements of claims 10 and 11 and anticipates them.

ix. Claim 12

248. Claim 12 depends from claim 1 and requires that collecting "steps (a) and (b)" "are performed simultaneously." EX1043, 148:59-60. It is my opinion that So-

456 discloses these elements under NanoString's interpretation. EX1022, 11-14, 18; EX1015, 16-17.

249. So-456's method discloses collecting the oligonucleotides (capturing the mRNAs) from the whole tissue section, all at the same time, *i.e.*, from a first and second location "simultaneously," as claimed in claim 12. EX1005, ¶[00144]. So-456's method uses a "capture *array*" comprising capture sites arranged in "rows and columns" (*i.e.*, first and second locations). EX1005, ¶[00141] (emphasis added). So-456 discloses simultaneously capturing (*i.e.*, collecting) at different locations the mRNAs because it discloses that the "mRNA molecules are *captured onto the array* by hybridization of the 3' end of the mRNA to gene-specific regions on the first capture probe." EX1005, ¶[00308] (emphasis added); *see also* EX1005, ¶[00315] ("mRNA molecule 2510 is captured at capture site 105 by hybridization of the 3' end of mRNA molecule 2510 to capture region 2335a."). For these reasons, it is my opinion that So-456 discloses the elements of claim 12 and anticipates it.

x. Claim 13

250. Claim 13 depends from claim 1 and requires that synthesizing "steps (c) and (d)" "are performed simultaneously." EX1043, 148:61-62. It is my opinion that So-456 discloses the elements of claim 13.

251. So-456's method performs the synthesizing steps simultaneously because it uses capture probes as primers for "synthesis of first strand cDNA *from captured mRNA molecules*" that are "captured onto the array" as a whole. EX1005, ¶¶[00308]-[00309] (emphasis added); *see also* EX1005, ¶[00109]. So-456's method further discloses that the spatial address region "can be *encoded in the nucleic acid* in the process of preparing the nucleic acid for sequencing." EX1005, ¶[0089] (emphasis added). Thus, So-456's method performs the synthesizing steps at multiple capture sites on the array. Accordingly, it is my opinion that So-456 discloses the elements of claim 13 and anticipates it.

252. In sum, it is my opinion that So-456 teaches every element of claims 1-13 under NanoString's interpretation, arranged as claimed. Accordingly, it is my opinion that So-456 anticipates claims 1-13.

XII. GROUND 5: CLAIMS 1-13 WOULD HAVE BEEN OBVIOUS OVER WO 2017/019456 (SO-456)

253. As I explain above, So-456 teaches every element of claims 1-13 under NanoString's interpretation, and thus So-456 anticipates the claims. However, even assuming for argument's sake that So-456 does not anticipate all of the claims, it would have rendered the claims obvious over So-456 under NanoString's interpretation for the reasons I explain below.

254. In my opinion, even assuming that So-456 does not expressly teach all the elements of claims 1-13, it suggests practicing the claimed methods according

to NanoString's interpretation. *See* Section XI. Further, So-456 provides a motivation with a reasonable expectation of success for practicing the claimed methods. A POSA reading So-456 would have been motivated to practice the methods of claims 1-13 because So-456 teaches a “*method ... of spatial detection and analysis of a nucleic acid [i.e., at least one target analyte] in a tissue sample*” using spatially barcoded arrays. EX1005, ¶¶[00151] (emphasis added), [00150], [00307]-[00320], FIGs. 2, 24, 25. Indeed, So-456 discloses that its methods “enable the characterization of transcriptomes and/or genomic variations in tissues while preserving spatial information about the tissue.” EX1005, Abstract; *see also* EX1005, ¶¶[0002], [0085], [0088].

255. Further, it is my opinion that a POSA following So-456's teaching would have had a reasonable expectation of successfully practicing the claimed methods because So-456 provides detailed descriptions and protocols. *See, e.g.*, EX1005, ¶¶[0034]-[0035], [00109], [00141]-[00143], [00151]-[00158], [00307]-[00320], FIGs. 1, 2, 24-25. Additionally, a POSA would have known that she could practice So-456's methods using standard laboratory materials and reagents with only ordinary skill, which in my opinion, would have further bolstered the reasonable expectation of success. *See, e.g.*, EX1005, ¶¶[0034]-[0035], [00109], [00141]-[00143], [00151]-[00158], [00307]-[00320], FIGs. 1, 2, 24-25; EX1042, 1:61-2:9,

3:11-34, 17:37-18:59, 24:21-25:32, 25:48-62, 26:19-47; EX1049, Abstract,

¶¶[0013], [0015], [0084]-[0086], [0089]-[0093], [0101], [0104], FIG. 1.

256. I understand that NanoString did not assert any objective indicia of non-obviousness during prosecution. I also understand that 10x is not aware of any objective indicia of non-obviousness. Accordingly, it is my opinion that under NanoString's interpretation, claims 1-13 would have been obvious over So-456.

XIII. CONCLUSION

257. I conclude that in view of the state of the art before February 12, 2018, Stahl 2016 anticipated claims 1-4 and 6-13 (Ground 1). Stahl 2016 teaches each and every element of claims 1-4 and 6-13, arranged as claimed. I understand that Stahl 2016 is presumed enabled. Thus, for the reasons detailed under Ground 1, Stahl 2016 anticipated claims 1-4 and 6-13 of the 142 patent under NanoString's interpretation.

258. I conclude that in view of the state of the art before February 12, 2018, Stahl 2016 also would have rendered obvious claims 1-13 (Ground 2). Stahl 2016 teaches or suggests all elements of the methods of claims 1-13 and a POSA would have been motivated to perform the methods of these claims with a reasonable expectation of success. Thus, for the reasons detailed under Ground 2, Stahl 2016 would have rendered claims 1-13 of the 142 patent obvious under NanoString's interpretation.

259. I conclude that in view of the state of the art before February 12, 2018, Stahl 2016 and Frisen would have further rendered claim 5 obvious (Ground 3). Stahl 2016 and Frisen in combination teach or suggest all elements of the method of claim 5. A POSA would have been motivated to perform the method of this claim with a reasonable expectation of success. Thus, for the reasons detailed under Ground 3, Stahl 2016 and Frisen would have rendered claim 5 of the 142 patent obvious under NanoString's interpretation.

260. I conclude that in view of the state of the art before February 12, 2018, So-456 anticipated claims 1-13 (Ground 4). So-456 teaches each and every element of claims 1-13, arranged as claimed. I understand that So-456 is presumed enabled. Thus, for the reasons detailed under Ground 4, So-456 anticipated claims 1-13 of the 142 patent under NanoString's interpretation.

261. I conclude that in view of the state of the art before February 12, 2018, So-456 also would have rendered claims 1-13 obvious (Ground 5). So-456 teaches or suggests all elements of the method of claims 1-13. A POSA would have been motivated to perform the method of this claim with a reasonable expectation of success. Thus, for the reasons detailed under Ground 5, So-456 would have also rendered claims 1-13 of the 142 patent obvious under NanoString's interpretation.

Inter Partes Review of U.S. Patent 11,473,142
Declaration of Paul T. Spellman, Ph.D.

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 dark ink, appearing to read "Paul T. Spellman", followed by a long horizontal flourish line extending to the right.

Paul T. Spellman, Ph.D.

Date: September 25, 2023

APPENDIX A¹

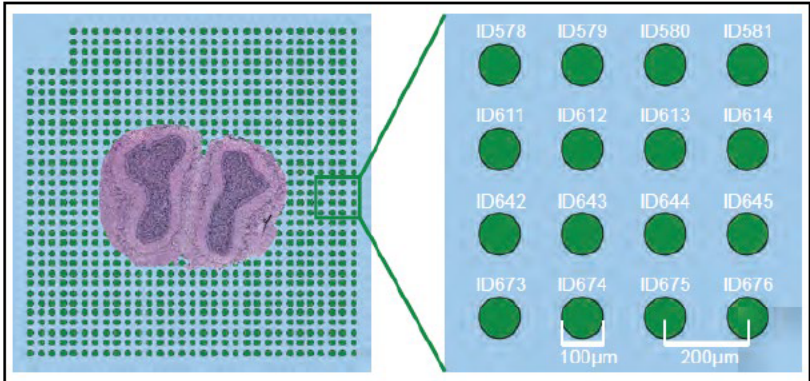
Claim 1	Stahl 2016 (EX1016)
1. A method comprising: a) collecting a plurality of oligonucleotides from a first location of a tissue sample under conditions that release the plurality of oligonucleotides from the first location of the tissue sample; b) collecting a plurality of oligonucleotides from a second location of the tissue sample under conditions that release the plurality of oligonucleotides from the second location	“Analysis of the pattern of proteins or messenger RNAs (mRNAs) in histological tissue sections is a cornerstone in biomedical research and diagnostics. This typically involves the visualization of a few proteins or expressed genes at a time. We have devised a strategy, which we call “spatial transcriptomics,” that allows visualization and quantitative analysis of the transcriptome with spatial resolution in individual tissue sections. By positioning histological sections on arrayed reverse transcription primers with unique positional barcodes, we demonstrate high-quality RNA-sequencing data with maintained two-dimensional positional information from the mouse brain and human breast cancer. Spatial transcriptomics provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections and enables novel types of bioinformatics analyses, valuable in research and diagnostics.” (Abstract) “The Spatial Transcriptomics method follows the following workflow: array printing, tissue sectioning, tissue fixation, tissue staining, imaging, pre-permeabilization, permeabilization, cDNA synthesis, tissue removal, probe release, library preparation, sequencing, data processing, data visualization and analysis. When the method refers to separate wells, these wells are created on the glass slide by using a rubber mask (ArrayIT hybridization cassette) to separate the surface into reaction compartments, suitable for the size of the printed arrays, where on-chip reactions can take place. Typically, from tissue types that the protocol has been optimized for, 75% of tissue sections put onto the array will produce high quality data. This is an approximate estimate based on reproducibility between samples of the same type,

¹ Emphasis added in bold.

Claim 1	Stahl 2016 (EX1016)
of the tissue sample;	given the RNA (RIN > 8) and tissue morphology is of good quality. We specify "high quality data" as sample data with an average number of genes per feature above a certain threshold value, as well as correct tissue placement on the spatial array (tissue inside the area, few folded areas and missing pieces etc.). The approximate values differ between different types of samples. For mouse olfactory bulb we require at least 3000 genes per feature at average and at least 90% of the bulb to be intact and placed within the spatial array. For breast cancer samples we require at least 3000 genes per feature at average in the tumor regions and that all of the tumor regions are placed within the spatial array." (Suppl. Materials, 1-2) "The realization that it is possible to capture mRNA in tissue sections with minimal diffusion and maintained positional representation motivated us to array oligonucleotides with positional barcodes (Fig. 2A), and we denoted this strategy "spatial transcriptomics." We deposited ~200 million oligonucleotides in each of 1007 features, with a diameter of 100 nm and a center-to-center distance of 200 nm, over an area of 6.2 mm by 6.6 mm (fig. S2)." (78, right col.) "After capturing and reverse-transcribing mRNA, we generated sequencing libraries based on amplification by in vitro transcription (fig. S3, A and B) (7, 8). Comparison with data from RNA extracted and fragmented in solution revealed that ~95% of the genes found with one of the methods was also found with the other (fig. S3C). The correlation between the surface and in-solution libraries was $r = 0.94$, with even representation of genes having high or low expression (fig. S3D). Replicates of surface-based experiments of adjacent tissue sections showed a correlation of $r = 0.97$ (fig. S3E). Thus, cDNA synthesis from tissue with arrayed oligonucleotides on a surface is efficient and does not introduce bias compared with in-solution protocols (fig. S3F and table S1)." (78, right col.)

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	“Fig. 2. Spatially resolved gene expression. (A) Each array feature contains unique DNA-barcoded probes containing a cleavage site, a T7 amplification and sequencing handle, a spatial barcode, a unique molecular identifier (UMI), and an oligo(dT) VN-capture region, where V is anything but T and where N is any nucleotide. cDNA (red) is generated from captured mRNA by reverse transcription.” (80) The diagram, labeled 'A', illustrates a workflow for spatially resolved gene expression. On the left, a vertical array of probes is shown, each containing a unique DNA barcode and an oligo(dT) VN-capture region. mRNA (red) is captured by these probes. A tissue section is placed on top of the array, and the tissue is stained/imaged. The tissue is then permeabilized, and cDNA (red) is released from the array features. The diagram is divided into three main sections: 'Staining/Imaging tissue', 'Tissue section', and 'Tissue permeabilization'. The final result is a slide with cDNA coupled to the arrayed oligonucleotides. (FIG. 1A) “After permeabilization, we added reverse-transcription reagents on top of the tissue. We used fluorescently labeled nucleotides to visualize the synthesized cDNA (Fig. 1A and fig. S1). The tissue was then enzymatically removed, which left cDNA coupled to the arrayed oligonucleotides on the slide (6). The fluorescent cDNA showed a pattern in detail corresponding to the tissue structure revealed by the general histology (Fig. 1, B and C), and the cDNA was strictly localized directly under individual cells (Fig. 1, D to G’). By comparing the hematoxylin-and-eosin and fluorescent signals, we could measure the average distance of diffusion outside the border of a cell to 1.7 ± 2 mm (mean $\pm$ SD) (fig. S1, E to H).” (78, middle col. and right col.) “For each well, corresponding to each sub-array with a section 70μl of 1x Exonuclease I Reaction Buffer ... with 0.19μg/μl BSA ... was added and incubated at 37°C for 30 minutes.

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	Each well was washed with 100µl 0.1x SSC, diluted in deionized water from stock solution Next, 70µl of 0.1% pepsin ... dissolved in 0.1M HCl ... was added to each well and incubated at 37°C for 10 minutes. Each well was washed as previously described and 70µl of reverse transcription mix was added to each well and incubated overnight (ON). For spatially barcoded arrays belonging to samples intended for sequencing, the reverse transcription mix contained 1x First Strand Buffer ..., 5mM DTT ..., 500µM of each dNTP ..., 0.19µg/µl BSA, 50ng/µl Actinomycin D ..., 1% DMSO ..., 20U/µl Superscript III ... and 2U/µl RNaseOUT For spatially barcoded arrays and surfaces with poly-T reverse transcription primer intended for generation of fluorescent cDNA footprints, the reverse transcription mix contained the same reagents as above except for 500µM of each dATP/dGTP/dTTP, 12.5µM of dCTP, 25µM of Cyanine 3-dCTP For experiments optimizing the conditions for permeabilization the permeabilization was carried out for 0, 2, 10 and 30 minutes. For experiments optimizing the conditions for cDNA synthesis the cDNA synthesis was carried out for 1 hour, 3 hours, 6 hours and overnight.” (Suppl. Materials, 4-5) Tissue section ID582 ID583 ID584 ID585 ID586 ID587 ID588 ID589 ID590 (FIG. 2A)

Claim 1	Stahl 2016 (EX1016)
	 (FIG. S2) See also FIGs. S1, S3.
c) synthesizing a first plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (a) as templates and incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the first	“We asked whether it would be possible to introduce positional molecular barcodes in the complementary DNA (cDNA) synthesis reaction within the context of an intact tissue section before RNA-seq. We first assessed whether it was feasible to generate cDNA from messenger RNA (mRNA) in tissue sections on a surface. We immobilized reverse-transcription oligo(dT) primers on glass slides and placed on the slides sections of adult mouse olfactory bulb, a brain region with clear histological landmarks and ample gene- expression reference data. The tissue was fixed, stained, and imaged (Fig. 1A) (6).” (78, middle col.) “After permeabilization, we added reverse-transcription reagents on top of the tissue. We used fluorescently labeled nucleotides to visualize the synthesized cDNA (Fig. 1A and fig. S1). The tissue was then enzymatically removed, which left cDNA coupled to the arrayed oligonucleotides on the slide (6). The fluorescent cDNA showed a pattern in detail corresponding to the tissue structure revealed by the general histology (Fig. 1, B and C), and the cDNA was strictly localized directly under individual cells (Fig. 1, D to G’). By comparing the hematoxylin-and-eosin and fluorescent signals, we could measure the average distance of diffusion outside the

Claim 1	Stahl 2016 (EX1016)
plurality of DNA products; d) synthesizing a second plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotide s collected in step (b) as templates and incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the second plurality of DNA products; and	border of a cell to 1.7 ± 2 mm (mean $\pm$ SD) (fig. S1, E to H).” (78, middle col. and right col.) <i>“Permeabilization and reverse transcription</i> For each well, corresponding to each sub-array with a section 70μl of 1x Exonuclease I Reaction Buffer ... with 0.19μg/μl BSA ... was added and incubated at 37°C for 30 minutes. Each well was washed with 100μl 0.1x SSC, diluted in deionized water from stock solution Next, 70μl of 0.1% pepsin ... dissolved in 0.1M HCl ... was added to each well and incubated at 37°C for 10 minutes. Each well was washed as previously described and 70μl of reverse transcription mix was added to each well and incubated overnight (ON). For spatially barcoded arrays belonging to samples intended for sequencing, the reverse transcription mix contained 1x First Strand Buffer ..., 5mM DTT ..., 500μM of each dNTP ..., 0.19μg/μl BSA, 50ng/μl Actinomycin D ..., 1% DMSO ..., 20U/μl Superscript III ... and 2U/μl RNaseOUT For spatially barcoded arrays and surfaces with poly-T reverse transcription primer intended for generation of fluorescent cDNA footprints, the reverse transcription mix contained the same reagents as above except for 500μM of each dATP/dGTP/dTTP, 12.5μM of dCTP , 25μM of Cyanine 3-dCTP For experiments optimizing the conditions for permeabilization the permeabilization was carried out for 0, 2, 10 and 30 minutes. For experiments optimizing the conditions for cDNA synthesis the cDNA synthesis was carried out for 1 hour, 3 hours, 6 hours and overnight.” (Suppl. Materials, 4-5) “After capturing and reverse-transcribing mRNA, we generated sequencing libraries based on amplification by in vitro transcription (fig. S3, A and B) (7, 8). Comparison with data from RNA extracted and fragmented in solution revealed that ~95% of the genes found with one of the methods was also found with the other (fig. S3C). The correlation between the surface and in-solution libraries was $r = 0.94$, with even

Claim 1	Stahl 2016 (EX1016)
	representation of genes having high or low expression (fig. S3D). Replicates of surface-based experiments of adjacent tissue sections showed a correlation of $r = 0.97$ (fig. S3E). Thus, cDNA synthesis from tissue with arrayed oligonucleotides on a surface is efficient and does not introduce bias compared with in-solution protocols (fig. S3F and table S1).” (78, right col.) “Fig. 1. Spatially localized cDNA synthesis. (A) The tissue is sectioned, placed onto oligo(dT) primers, stained, and imaged. cDNA synthesis with Cy3-labeled nucleotides reveals fluorescent cDNA after tissue removal.” (79) The diagram illustrates the process of cDNA synthesis. On the left, a tissue section is shown being permeabilized. An arrow points to a central stage where the tissue is placed on an array of oligo(dT) primers. To the right, Cy3-labeled nucleotides (represented by red stars) are added, leading to cDNA synthesis. The final stage shows the tissue removed, leaving behind fluorescent cDNA (represented by red bars) on the array. (FIG. 1A) The diagram shows a tissue section (represented by a purple band with cell-like structures) positioned above an array of oligo(dT) primers (represented by a green bar with vertical lines). An arrow points from the tissue section to the array. Below the array, the IDs of the primers are listed: ID582, ID583, ID584, ID585, ID586, ID587, ID588, ID589, ID590. (FIG. 2A)

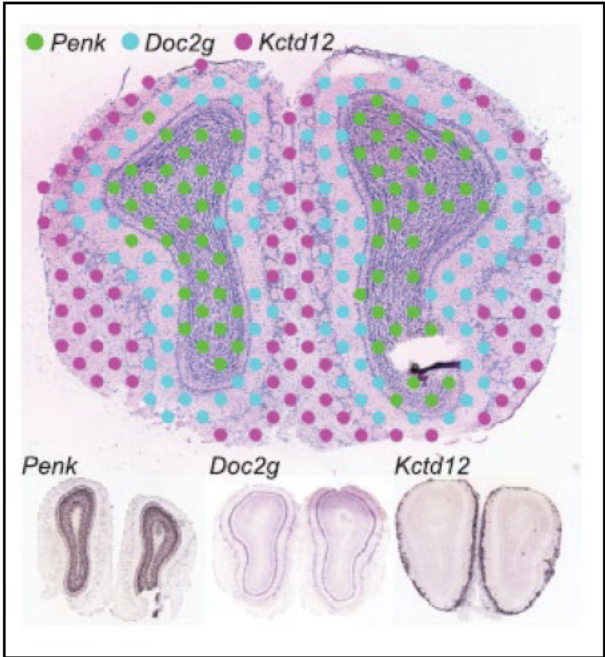
Claim 1	Stahl 2016 (EX1016)
	(FIG. S2) “We sorted the RNA-seq data to its corresponding array features by using the spatial barcodes and aligned the tissue image with the features of the array, which enabled visualization and analyses. Examples of gene-expression patterns revealed by spatial transcriptomics and validation by in situ hybridization are shown in Fig. 2B and fig. S4, A to C. Transcripts expressed at very low levels, such as olfactory receptor mRNAs (9), were also detected with spatial transcriptomics (fig. S4D).” (78, right col.) “The number of genes (10) (Fig. 2C) and unique transcripts (fig. S5A) per individual feature varied between cell layers with different cell density (Fig. 2D and table S2). For the vast majority of genes, the coefficient of variation decreased as the average expression increased (fig. S5B). The number of genes and transcripts captured was at least twice as high as when using laser capture microdissection (11), and spatial transcriptomics detected almost twice as many genes as examination by in situ hybridization in the Allen Brain Atlas (fig. S5, C and D). Furthermore, we compared spatial transcriptomics with the near-100% sensitivity of single-molecule fluorescent in situ hybridization in adjacent tissue sections. The sensitivity of spatial transcriptomics was $6.9 \pm 1.5\%$ of single- molecule fluorescent in situ hybridization (fig. S6). By comparison, single-cell RNA sequencing has been

Claim 1	Stahl 2016 (EX1016)
	reported to have about 5 to 40% sensitivity (12).” (78, right col., 79) “Fig. 2. Spatially resolved gene expression. (A) Each array feature contains unique DNA-barcoded probes containing a cleavage site, a T7 amplification and sequencing handle, a spatial barcode, a unique molecular identifier (UMI), and an oligo(dT) VN-capture region, where V is anything but T and where N is any nucleotide. cDNA (red) is generated from captured mRNA by reverse transcription.” (80) The diagram shows a horizontal bar representing a surface probe. Above the bar, five segments are labeled from left to right: 'Cleavage site', 'Amp and seq handle', 'Spatial barcode (ID)', 'UMI', and 'mRNA capture region'. The bar itself is color-coded: a light blue segment for the cleavage site, a green segment for the amp and seq handle, an orange segment for the spatial barcode, a light blue segment for the UMI, and a black segment for the mRNA capture region. The entire bar is labeled 'Surface probe' above it and 'Glass slide' below it. (FIG. 2A) “For comparing the efficiency of cDNA generation on an array with cDNA generation in solution, and for generation of Spatial Transcriptomics data, we arrayed 1007 capture oligonucleotides with 18-mer unique barcodes, a 9-mer semi-randomized UMI and a poly-20TVN capture region (IDT) onto the surface of Codelink Activated Slides according to the manufacturer's (Surmodics) instructions. The oligonucleotides were immobilized in 100µm features with 200µm center-to-center distance between the spots. According to the slide manufacturer approximately 200 million oligonucleotides were immobilized in each spot. An area in the top left corner of the array was left blank for the purpose of orientation. Six 6200x6600µm arrays were prepared per glass slide. The oligonucleotides immobilized on the spatially barcoded arrays were: Surface spatially barcoded oligonucleotides: [AmC6]UUUUUGACTCGTAATACGACTCACTATAGGG ACACGACGCTCTTCCGATCT[18MER SPATIALBARC

Claim 1	Stahl 2016 (EX1016)
	ODE]WSNNWSNNVTTTTTTTTTTTTTTTTTTTTTVN" (Suppl. Materials, 2-3) "For spatially barcoded arrays belonging to samples intended for sequencing where barcoded cDNA had been released 70µl of hybridization mix containing 0.96x PBS, 0.2µM Cy3_anti_A_probe (Eurofins) and 0.2µM Cy3_anti_Frame_probe (Eurofins) was added and incubated at RT for 10 minutes. Each well was washed with 100µl 0.1x SSC, then 2x SSC with 0.1% SDS at 50°C for 10 minutes, 0.2x SSC at RT for 1 minute, 0.1x SSC at RT for 1 minute and finally spin-dried. Both spatially barcoded arrays belonging to samples intended for sequencing, and spatially barcoded arrays and surfaces with poly-T reverse transcription primer intended to show fluorescent cDNA footprints were mounted with SlowFade Gold Antifade Reagent ... and covered with a coverslip. (Suppl. Materials, 8-9) <i>See also</i> FIGs. 1, 2.
e) identifying the first plurality of DNA products and the second plurality of DNA products synthesized in step (c) and step (d) by sequencing the first plurality of DNA products and the second plurality of DNA products, thereby	"After capturing and reverse-transcribing mRNA, we generated sequencing libraries based on amplification by in vitro transcription (fig. S3, A and B) (7, 8). Comparison with data from RNA extracted and fragmented in solution revealed that ~95% of the genes found with one of the methods was also found with the other (fig. S3C). The correlation between the surface and in-solution libraries was $r = 0.94$, with even representation of genes having high or low expression (fig. S3D). Replicates of surface-based experiments of adjacent tissue sections showed a correlation of $r = 0.97$ (fig. S3E). Thus, cDNA synthesis from tissue with arrayed oligonucleotides on a surface is efficient and does not introduce bias compared with in-solution protocols (fig. S3F and table S1)." (78, right col.) "cDNA library preparation for sequencing For each well, 70µl of release mix containing 1.1x Second Strand Buffer ... 8.75µM of each dNTP, 0.20µg/µl BSA and

Claim 1	Stahl 2016 (EX1016)
spatially detecting the plurality of oligonucleotides collected from the first location of the tissue sample and the plurality of oligonucleotides collected from the second location of the tissue sample.	0.1U/μl USER Enzyme ... was added and incubated at 37°C for 1-2 hour and 65μl from each well were collected. 5μl of second strand mix containing 2.7x First Strand Buffer, 3.7U/μl DNA polymerase I ... and 0.18U/μl RNaseH ... was added and samples were incubated at 16°C for 2 hours, 5μl T4 DNA polymerase ... was added and samples were incubated at 16°C for another 20 minutes... 5.6μl of the samples were mixed with 10.4μl In Vitro Transcription mix with a final content of 1x T7 Reaction Buffer ... 7.5mM of each NTP ... 1x T7 Enzyme Mix ... and 1U/μl SUPERaseIN ... Samples were incubated at 37°C for 14 hours. The samples were purified using Agencourt RNAClean XP beads according to the manufacturer's protocol and eluted into 10μl deionized water. The amount and average fragment length of amplified RNA (aRNA) was determined by using the RNA 6000 Pico Kit ... with a 2100 Bioanalyzer (Agilent) according to the manufacturer's protocol. The remaining sample and 2.5μl Ligation adapter (IDT) was added to a final concentration of 0.71μM. Samples were heated at 70°C for 2 min. then placed on ice before 4.5μl ligation mix was added to a final content of 1x T4 RNA Ligase Reaction Buffer ... 20U/μl T4 RNA Ligase2, truncated ... 4U/μl RNase Inhibitor, Murine ... and 0.5μM Ligation Adapter. Samples were incubated at 25°C for 1 hour. The samples were purified using Agencourt RNAClean XP beads according to the protocol previously described and mixed with 1μl RT-primer (IDT) to a final concentration of 1.7μM and 1μl dNTPs and a final concentration 0.83mM of each dNTP. Samples were heated at 65°C for 5 min and then placed on ice, 8μl reverse transcription mix was added to a final content of 1x First Strand Buffer, 0.05M DTT, 500μM of each dNTP, 1mM RT-primer, 10U/μl Superscript III and 2U/μl RNaseOUT. Samples were incubated at 50°C for 1 hour before placed on ice and the samples were subsequently purified using Agencourt RNAClean XP beads according to the protocol previously described. A total reaction volume of 10μl

Claim 1	Stahl 2016 (EX1016)
	containing 1xKAPA HiFi HotStart ReadyMix ... 1x EVA green ... 0.5µM PCR InPE1.0 ... 0.01µM PCR InPE2.0 ... 0.5µM PCR Index ... and 2µl purified cDNA were amplified by qPCR with the following protocol: 98°C for 3 minutes, followed by cycling at 98°C for 20 seconds, 60°C for 30 seconds and 72°C for 30 seconds. After determining the amount of cycles needed based on the qPCR, the samples were amplified in a total reaction volume of 25µl. The libraries were purified ... and the average fragment length of finished libraries was determined by using the DNA 1000 Kit ... with a 2100 Bioanalyzer according to the manufacturer's protocol. The concentration of the finished libraries was determined with Qubit dsDNA HS Assay Kit ... according to the manufacturer's protocol. Finished libraries were diluted to 4nM and sequenced on the Illumina NextSeq platform using paired-end sequencing according to the manufacturer's protocol. Typically, 31 or 51 bases were sequenced on read one, and 121 or 101 bases were sequenced on read two.” (Suppl. Materials, 6-8) “We sorted the RNA-seq data to its corresponding array features by using the spatial barcodes and aligned the tissue image with the features of the array, which enabled visualization and analyses. Examples of gene-expression patterns revealed by spatial transcriptomics and validation by in situ hybridization are shown in Fig. 2B and fig. S4, A to C. Transcripts expressed at very low levels, such as olfactory receptor mRNAs (9), were also detected with spatial transcriptomics (fig. S4D).” (78, right col.)

Claim 1	Stahl 2016 (EX1016)
	 (FIG. 2B) <i>See also</i> 81 (left col. and middle col.), FIGs. 2, 3, S3, S4, S10A-S10C.

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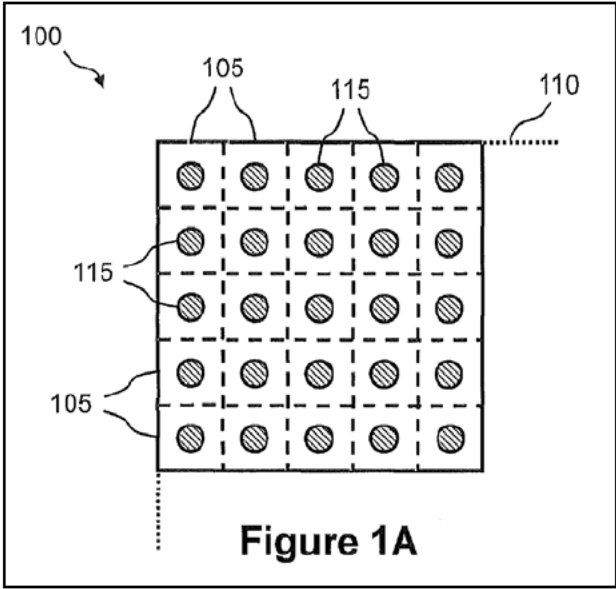
APPENDIX B¹

Claim 1	So-456 (EX1005)
1. A method comprising: a) collecting a plurality of oligonucleotides from a first location of a tissue sample under conditions that release the plurality of oligonucleotides from the first location of the tissue sample; b) collecting a plurality of oligonucleotides from a second location of the tissue sample under conditions that release the plurality of oligonucleotides from the second location of the tissue sample;	“At a step 2410, a tissue sample that comprises a plurality of mRNA molecules is contacted with a capture array. The capture array can be, for example, capture array 100 of Figure 1A For each gene-specific mRNA molecule two capture probes are used, i.e., a first capture probe that comprises sequences specific for the 3' end of the mRNA molecule and a second capture probe that comprises sequences specific for the 3' end of a corresponding first strand cDNA molecule. mRNA molecules are captured onto the array by hybridization of the 3' end of the mRNA to gene-specific regions on the first capture probe. An example of the capture probes on capture array 100 are described in more detail with reference to Figure 25A.” (§[00308]) “Figures 25A, 25B, and 25C illustrate the steps of method 2400 of Figure 24. In this example, a single capture site 105 of capture array 100 of Figure 1A is shown. Capture site 105 is essentially the same as described above with reference to Figure 23A except that capture probes 2310 are oriented in the same direction at capture site 105, i.e., both capture probe 2310a and capture probe 2310b are immobilized at capture site 105 in the 5' to 3' direction (i.e., the 5' end of capture probes 2310a and 2310b are attached to the surface of capture site 105). Capture region 2335a is specific for the 3' end of a mRNA molecule and capture region 2335b is specific for the 3' end of the

¹ Emphasis added in bold.

Claim 1	So-456 (EX1005)
	corresponding first strand cDNA molecule.” ([00314]) “At step 2410, a mRNA molecule 2510 in a tissue sample is captured on capture probe 2310a. For example, the tissue sample containing mRNA molecule 2510 or a substrate comprising mRNA molecule 2510 derived from the tissue sample (not shown) is contacted with capture probes 2310. mRNA molecule 2510 can include a mutation 2515. mRNA molecule 2510 is captured at capture site 105 by hybridization of the 3' end of mRNA molecule 2510 to capture region 2335a.” ([00315]) (FIG. 25A) “Figure 1A illustrates a plan view of an exemplary embodiment of a capture array 100 for capture of nucleic acid in a tissue sample. Capture array 100 comprises an arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110. In some embodiments, solid support 110 is a planar glass substrate. In some embodiments, solid support 110 is a bead (e.g., see Figure 1C). At least one capture

Claim 1	So-456 (EX1005)
	probe 115 is immobilized at each of the capture sites 105 of capture array 100, as shown in Figure 1B. Namely, Figure 1B illustrates a side view of one capture site 105 of capture array 100, wherein the one capture site 105 comprises at least one capture probe 115 for capture of nucleic acid in a tissue sample. Figure 1B shows a capture probe 115 immobilized on the surface of solid support 110. In this embodiment, a single capture probe 115 is shown, but any number of capture probes 115 can be immobilized on solid support 110 at each capture site 105. Capture probe 115 may optionally comprise a cleavable sequence in a cleavable region 120, an SBS primer sequence in a SBS primer binding site 125, a spatial address sequence in a spatial address region 130, and a capture sequence in a capture region 135. Cleavable region 120 can be used to release captured nucleic acid from capture array 100 such that spatial address region 130 is included in the released nucleic acid and the nucleic acid is "tagged." SBS primer region 125 can comprise an SBS primer sequence (e.g., SBS12 or SBS3) that can be used in a sequencing-by-synthesis (SBS) process. Alternatively, SBS primer sequence 125 or some portion thereof may be added subsequently, e.g., by ligation or by PCR synthesis. SBS primer region 125 can also be used in an amplification reaction to generate a sequencing library as described in more detail with reference to Figure 12 and Figure 13. Spatial address region 130 corresponds to the position of capture probe 115 in capture array 100. Each capture probe 115 at a capture site 105 comprises a unique spatial address region 130. The position of capture probe 115 in capture array 100 can be correlated with a position in the tissue sample.” (§[00141])

Claim 1	So-456 (EX1005)
	“Figure 24 illustrates a flow diagram of an example of an alternative method 2400 of using a combinatorial indexing system for generation of a spatially addressed cDNA sequencing library. Method 2400 comprises, but is not limited to, some or all of the following steps.” (§[00307])  Figure 1A (FIG. 1A) “At a step 220, the target nucleic acid in the tissue section is transferred onto an array such that the position of a nucleic acid on the array can be correlated with a position in the tissue section. In some embodiments, the target nucleic acid comprises an mRNA. In some embodiments, the target nucleic acid comprises a cDNA synthesized in situ. In some embodiments, the nucleic acid comprises genomic DNA amplicons generated by in situ amplification. In some embodiments, the array is an array of capture sites, such as capture sites 105 of capture array 100 shown in Figure 1A and Figure 1B. In some embodiments, the array is an array of beads

Claim 1	So-456 (EX1005)
	(e.g., universal capture beads 150 of Figure 1C) that include a plurality of capture probes. Other examples of an array include an array of wells or pores or projections or a sequencing flow cell that includes a plurality of capture probes. The nucleic acid can be captured onto the array, for example, by hybridizing the nucleic acid to the capture probes on the array. In some embodiments, the nucleic acid can be captured onto the array by single-strand ligation of the nucleic acid onto universal adaptor oligonucleotides.” (¶[00154]) “The present disclosure is further based, in part, on the realization that the robustness and data quality of spatial transcriptomics experiments can be enhanced by facilitating the transfer of nucleic acids from a tissue sample onto a capture array, e.g., a capture array of spatially addressed capture probes. For example, electrophoretic transfer of nucleic acids can be used to improve transfer yields and transfer kinetics of nucleic acids. High-yield nucleic acid transfer from tissue samples onto capture arrays can facilitate the detection of rare SNVs. Fast transfer kinetics can be used limit nucleic acid diffusion during the transfer process and help increase the resolution of spatial addressing. Other methods described herein involve the use of intermediate nucleic acid substrates, such as particles (e.g., electromagnetic nanoparticles), membranes (e.g., nylon membranes) or microwell plates to facilitate nucleic acid capture in the tissue sample, to facilitate nucleic acid transfer onto capture arrays, and to limit diffusion and improve spatial resolution. Additional methods, involving, e.g., tagmentation of genomic DNA are described that can be used to efficiently add spatial addresses to nucleic acids, e.g., on the surface of a capture array.” (¶[00093])

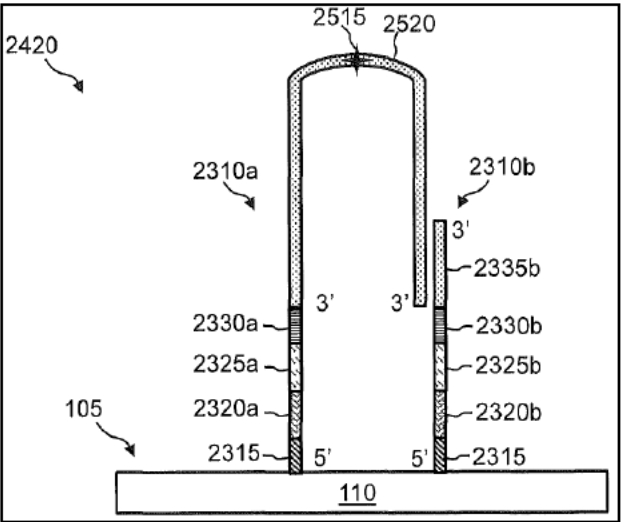
Claim 1	So-456 (EX1005)
	“In this and other embodiments described herein, the probes may be contacted with target nucleic acid by placing the tissue directly on the surface comprising the probes; placing the tissue on a substance such as a filter or a gel or a thin buffer layer separating the tissue from the probes such that the target nucleic acid may diffuse from the tissue, through the substance to the probes; placing the tissue on a substance, such as a filter or a gel or a thin buffer layer separating the tissue from the probes such that the probes may diffuse from the surface comprising the probes, through the substance to the target nucleic acid; extracting the target nucleic acid from the tissue onto an intermediate substrate (e.g., a gel, filter, solid substrate, or combinations of the foregoing), which is then placed on the surface supporting the probes; and combinations of the foregoing. In each case, the technique is selected to substantially maintain information encoding the spatial orientation of the targets in the sample.” (¶[00155]) “At a step 210, a tissue sample is prepared for analysis. In some embodiments, the tissue sample is a FFPE tissue sample that is sectioned onto a slide. Other examples include fresh tissue, frozen tissue, etc.” (¶[00152]) <i>See also</i> FIGs. 1, 2, 24, 25.
c) synthesizing a first plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (a) as	“At a step 2415, first strand cDNA is synthesized. For example, gene-specific capture regions on the first capture probe are used as reverse transcriptase primers for synthesis of first strand cDNA from captured mRNA molecules.” (¶[00309])

Claim 1	So-456 (EX1005)
templates and incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the first plurality of DNA products; d) synthesizing a second plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (b) as templates and incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the second plurality of DNA products; and	“At step 2415, first strand cDNA is synthesized using capture region 2335a as a primer in a reverse transcription reaction.” (§[00316]) (FIG. 25A) “Figure 1A illustrates a plan view of an exemplary embodiment of a capture array 100 for capture of nucleic acid in a tissue sample. Capture array 100 comprises an arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110. In some embodiments, solid support 110 is a planar glass substrate. In some embodiments, solid support 110 is a bead (e.g., see Figure 1C). At least one capture probe 115 is immobilized at each of the capture sites 105 of capture array 100, as shown in Figure 1B. Namely, Figure 1B illustrates a side view of one capture site 105 of capture array 100, wherein the one capture site 105 comprises at least one capture probe 115 for capture of nucleic acid in a tissue sample. Figure 1B shows a capture probe 115 immobilized on the surface of solid support 110. In this embodiment, a single capture probe 115 is shown, but any number of capture probes 115 can be immobilized on solid

Claim 1	So-456 (EX1005)
	support 110 at each capture site 105. Capture probe 115 may optionally comprise a cleavable sequence in a cleavable region 120, an SBS primer sequence in a SBS primer binding site 125, a spatial address sequence in a spatial address region 130, and a capture sequence in a capture region 135. Cleavable region 120 can be used to release captured nucleic acid from capture array 100 such that spatial address region 130 is included in the released nucleic acid and the nucleic acid is "tagged." SBS primer region 125 can comprise an SBS primer sequence (e.g., SBS12 or SBS3) that can be used in a sequencing-by-synthesis (SBS) process. Alternatively, SBS primer sequence 125 or some portion thereof may be added subsequently, e.g., by ligation or by PCR synthesis. SBS primer region 125 can also be used in an amplification reaction to generate a sequencing library as described in more detail with reference to Figure 12 and Figure 13. Spatial address region 130 corresponds to the position of capture probe 115 in capture array 100. Each capture probe 115 at a capture site 105 comprises a unique spatial address region 130. The position of capture probe 115 in capture array 100 can be correlated with a position in the tissue sample.” (§[00141]) “Figure 24 illustrates a flow diagram of an example of an alternative method 2400 of using a combinatorial indexing system for generation of a spatially addressed cDNA sequencing library. Method 2400 comprises, but is not limited to, some or all of the following steps.” (§[00307]) “At a step 2410, a tissue sample that comprises a plurality of mRNA molecules is contacted with a capture array. The capture array can be, for example, capture array 100 of Figure 1A For each

Claim 1	So-456 (EX1005)
	gene-specific mRNA molecule two capture probes are used, i.e., a first capture probe that comprises sequences specific for the 3' end of the mRNA molecule and a second capture probe that comprises sequences specific for the 3' end of a corresponding first strand cDNA molecule. mRNA molecules are captured onto the array by hybridization of the 3' end of the mRNA to gene-specific regions on the first capture probe. An example of the capture probes on capture array 100 are described in more detail with reference to Figure 25A.” (§[00308]) “Figures 25A, 25B, and 25C illustrate the steps of method 2400 of Figure 24. In this example, a single capture site 105 of capture array 100 of Figure 1A is shown. Capture site 105 is essentially the same as described above with reference to Figure 23A except that capture probes 2310 are oriented in the same direction at capture site 105, i.e., both capture probe 2310a and capture probe 2310b are immobilized at capture site 105 in the 5' to 3' direction (i.e., the 5' end of capture probes 2310a and 2310b are attached to the surface of capture site 105). Capture region 2335a is specific for the 3' end of a mRNA molecule and capture region 2335b is specific for the 3' end of the corresponding first strand cDNA molecule.” (§[00314]) “At step 2410, a mRNA molecule 2510 in a tissue sample is captured on capture probe 2310a. For example, the tissue sample containing mRNA molecule 2510 or a substrate comprising mRNA molecule 2510 derived from the tissue sample (not shown) is contacted with capture probes 2310. mRNA molecule 2510 can include a mutation 2515. mRNA molecule 2510 is captured at capture site 105

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	by hybridization of the 3' end of mRNA molecule 2510 to capture region 2335a.” (¶[00315]) “Figures 23A and 23B illustrate the steps of method 2200 of Figure 22. In this example, a single capture site 105 of capture array 100 of Figure 1A is shown. Capture site 105 comprises a first capture probe 2310a and a second capture probe 2310b. Capture probes 2310 include a cleavable domain 2315, an SBS primer region 2320, a spatial address region 2325, a unique molecular identifier (UMI) 2330, and a capture region 2335. For example, capture probe 2310a comprises cleavable region 2315; SBS primer region 2320a, which comprises, e.g., a SBS3 sequence; spatial address region 2325a, which comprises a unique spatial address sequence for the X dimension; UMI region 2330a, which comprises a unique sequence for capture probe 2310a; and a gene-specific capture region 2335a, which is specific for the 3' end of a mRNA molecule. Capture probe 2320a is immobilized at capture site 105 in the 5' to 3' orientation (i.e., the 5' end of capture probe 2320a is attached to the surface of capture site 105). Similarly, capture probe 2310b comprises cleavable sequence 2315; SBS primer region 2320b, which comprises a SBS12 sequence; spatial address region 2325b, which comprises a unique spatial address region for the Y dimension; UMI region 2330b, which comprises a unique sequence for capture probe 2310b; and a gene-specific capture region 2335b, which is specific for the 5' end of the mRNA molecule. Capture probe 2320b is immobilized at capture site 105 in the 3' to 5' orientation (i.e., the 3' end of capture probe 2320a is attached to the surface of capture site 105).” (¶[00302])

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	 (FIG. 25B) See also FIGs. 1, 2, 24, 25.
e) identifying the first plurality of DNA products and the second plurality of DNA products synthesized in step (c) and step (d) by sequencing the first plurality of DNA products and the second plurality of DNA products, thereby spatially detecting the plurality of oligonucleotides collected from the first location of the tissue sample and the plurality of oligonucleotides collected from the	“At a step 2420, first strand cDNA is captured on the second capture probe. First strand cDNA is captured on the second capture probe by hybridization of the 3' end of the cDNA to a gene-specific region on the second capture probe.” (¶00310)] “At a step 2425, second strand cDNA is synthesized. For example, gene-specific regions on the second capture probes are used as primers for synthesis of second strand DNA molecules.” (¶00311)] “At a step 2430, second strand cDNA molecules are released from the capture array. For example, cDNA molecules are released from the capture array using a cleavage reaction.” (¶00312)] “At a step 2435, cDNA molecules are amplified to generate a sequencing library.” (¶00313)]

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second location of the tissue sample.	“At step 2420, a first strand cDNA molecule 2520 is captured on capture probe 2310b by hybridization of the 3' end of cDNA molecule 2520 to capture region 2335b.” (§00317]) “At step 2425, second strand cDNA is synthesized in an extension reaction using capture region 2335b as a primer.” (§00318]) “At step 2430, a cDNA molecule 2525 is released from capture site 105 by cleavage of cleavable region 2315. cDNA molecule 2525 comprises SBS primer region 2320a (i.e., SBS3), spatial address region 2325a (i.e., unique spatial address for the X dimension), UMI region 2330a, UMI region 2330b, spatial address region 2325b (i.e., unique spatial address for the Y dimension), and SBS primer region 2320b (i.e., SBS 12).” (§00319]) “At step 2435, cDNA molecule 2525 is amplified using an SBS primer 2530 and a second SBS primer 2535. SBS primer 2530 comprises an SBS region 2320a' that is complementary to SBS primer region 2320a and a PS region 2540. SBS primer 2535 comprises an SBS region 2320b' that is complementary to SBS primer region 2320b and a P7 region 2545. A library amplicon 2550 synthesized using SBS primers 2530 and 2535 is flanked on the 5' end by PS region 2540, SBS primer region 2320a (i.e., SBS3), spatial address region 2325a (i.e., unique spatial address for the X dimension), UMI region 2330a and on the 3' end by UMI region 2330b, spatial address region 2325b (i.e., unique spatial address for the Y dimension), SBS primer region 2320b (i.e., SBS12), and P7 region 2545.” (§00320])

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	“The present disclosure is based, in part, on the realization that information related to the spatial origin of a nucleic acid in a tissue sample can be encoded in the nucleic acid in the process of preparing the nucleic acid for sequencing. For example, nucleic acids from a tissue sample can be tagged by probes including location-specific sequence information (a "spatial address"). Spatially addressed nucleic acid molecules from a tissue sample can then be sequenced in bulk. The sequence-identical nucleic acid molecules originating from different regions in a tissue sample can be distinguished based on their spatial address and can be mapped onto their regions of origin in the tissue sample.” (¶00089)] “At a step 230, the library is sequenced. Sequencing may be accomplished using any sequencing technique. Examples of suitable sequencers include those available from, or being developed by, Illumina, Inc., F. Hoffmann-La Roche AG, Life Technologies, Inc., Beckman Coulter, Inc., Pacific Biosciences, Inc., Oxford Nanopore, Inc., and/or their affiliates.” (¶00157)] “At a step 235, the sequence data is analyzed (e.g., mutations and/or variant calling) and the spatial information is decoded. The spatial information can be used to provide information as to the location of the nucleic acid in the tissue section.” (¶00158)] “The present disclosure provides methods and compositions that facilitate the characterization of transcriptomes and/or genomic variation in tissues while preserving spatial information related to the origin of target nucleic acids in the tissue. For example, the methods disclosed herein can enable the

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	identification of the location of a cell or a cell cluster in a tissue biopsy that carries an aberrant mutation. The methods provided herein can therefore be useful for diagnostic purposes, e.g., for the diagnosis of cancer, and possibly aid in the selection of targeted therapies.” (§0002]) “Described herein are a variety of methods and compositions that allow for the characterization of transcriptomes and/or genomic variation in tissues while preserving spatial information related to the origin of target nucleic acids in the tissue. For example, the methods disclosed herein can enable the identification of the location of a cell or a cell cluster in a tissue biopsy that carries an aberrant mutation. The methods provided herein can therefore be useful for diagnostic purposes, e.g., for the diagnosis of cancer, and possibly aid in the selection of targeted therapies.” (§0088]) <i>See also</i> FIGs. 1, 2, 24, 25.

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