

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10X GENOMICS, INC,
Petitioner,

v.

NANOSTRING TECHNOLOGIES, INC.,
Patent Owner.

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

**PATENT OWNER'S PRELIMINARY RESPONSE TO PETITION FOR
INTER PARTES REVIEW OF U.S. PATENT NO. 11,473,142**

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EXHIBIT LIST

Exhibit No.	Description
2001	USER Enzyme, https://www.neb.com/en-us/products/m5505-user-enzyme
2002	MEGAscript T7 Transcription Kit, https://www.thermofisher.com/order/catalog/product/AM1333

I. INTRODUCTION

Patent Owner NanoString Technologies, Inc. (“NanoString” or “Patent Owner”) submits this Preliminary Response to Petitioner 10X Genomics, Inc.’s (“10X” or “Petitioner”) Petition for *Inter Partes* Review of U.S. Patent No. 11,473,142 (“Petition”). For the reasons stated below, the Board should not institute review.

NanoString is a pioneer in the field of spatial biology. As a respected, leading innovator of translational research tools, NanoString has developed and brought to market breakthrough technologies that enable scientists across the globe to envision molecular interactions in three dimensions. NanoString’s spatial profiling patents and products are the first of their kind—they uniquely combine high-plex and high-throughput spatial profiling, allowing researchers to rapidly and quantitatively assess the biological implications of heterogeneity within tissues. Since its launch, NanoString’s products have been amply described in prestigious scientific publications, and studies performed using NanoString’s products have been widely presented at industry conferences.

U.S. Patent No. 11,473,142 (the “’142 Patent”) represents a novel approach for the spatial detection and quantification of target analyte in a tissue sample. As its Abstract explains, the ’142 Patent is directed to an improved and novel way of

spatially detecting target analytes in regions of interest. EX1043 (“’142 Patent”) at Abstract. More specifically, the method claimed in the ’142 Patent permits the simultaneous, multiplexed detection and quantification of protein and/or nucleic acid expression in a user-defined region of a tissue, cell, and/or subcellular structure within a cell, while maintaining the morphological context of the sample. The claimed invention of the ’142 Patent is an improvement over prior means of detecting target analytes in a sample. Conventional immunohistochemical methods allowed for simultaneous detection of six to ten protein targets at most. Similarly, in situ hybridization methods were limited to less than ten nucleic acid targets. *Id.* at 16:30-35. Thus, these methods do not enable the simultaneous detection of a large number of genes, proteins, or other biologically active molecules in the same sample. While laser microdissection methods are able to capture many genes, they are limited to a small number of locations and are cost prohibitive.

The claimed invention of the ’142 Patent uniquely addresses and solves these problems. The invention offers a spatial profiling method for RNA and/or protein in a tissue sample of much higher plex and sensitivity than previously available, providing researchers the ability to look at many biological targets at many locations in the same sample at the same time.

As the '142 Patent explains, the “present disclosure provides detection of large combinations of nucleic acid targets and/or protein targets from a defined region of a sample. The present disclosure provides an increase in objective measurements by digital quantification and increased reliability and consistency, thereby enabling comparison of results among multiple centers.” *Id.* at 16:37-43. Specifically, more than 1000 targets can be detected, and there is “no pre-defined upper limit to the number of regions of interest and comparisons that can be made.” *Id.* at 16:21-23; 40:61-64. Furthermore, the invention is compatible with existing, readily available sequencing technologies, making it accessible to a large number of users. *Id.* at Abstract. The invention also allows for an economical assay design, as inexpensive and widely available synthetic DNA oligonucleotides can be used instead of more expensive probes. *Id.* at 37:50-55.

Accused by NanoString of infringing the '142 Patent, 10X now asks the Board to institute review on all 13 claims of the '142 Patent based on five grounds. These grounds, however, lack merit. We ask the Board not to institute review.

Grounds 1, 2, and 3 all rely on a reference by Stahl et al (“Stahl”). *See* EX1016. These grounds—whether based on anticipation or obviousness—necessarily fail, as Stahl does not disclose or render obvious at least two claim elements required by the '142 Patent claims.

First, Stahl does not disclose identifying the DNA products by sequencing the DNA products. Second, Stahl does not disclose the required synthesis step that incorporates a nucleic acid that identifies a location of the tissue sample, required by all of the '142 Patent claims.

Due to the missing claim elements, Stahl does not anticipate or render obvious the claims of the '142 Patent (Grounds 1 and 2). Petitioner's reliance on Frisen to argue obviousness (Ground 3) also fails, as Petitioner does not rely on Frisen to provide the missing claim elements.

Grounds 4 and 5 rely on a reference by So et al. ("So-456"). *See* EX1005. These grounds likewise fail, as So-456 does not disclose or render obvious at least three claim elements required by the '142 Patent claims. As documented herein, So-456 discloses nothing more than a speculative and unproven scheme that is nowhere close to the claimed inventions of the '142 Patent.

First, So-456 does not disclose identifying the DNA products by sequencing the DNA products. Second, So-456 does not disclose a required synthesis reaction that incorporates a nucleic acid that identifies a location of the tissue sample, required by all of the '142 Patent claims. Third, So-456 does not disclose collecting oligonucleotides under conditions that release them. Petitioner makes conclusory assertions that the claim element is either anticipated or obvious.

Neither contention is persuasive, however, as Petitioner fails to meet the burden to demonstrate anticipation and provides only conclusory statements of motivation to combine relating to obviousness.

Due to the missing claim elements, So-456 does not anticipate or render obvious the claims of the '142 Patent (Grounds 4 and 5).

Petitioner fails to meet the burden of demonstrating a reasonable likelihood of prevailing with respect to at least one of the challenged claims. The Petition should be denied.

II. BACKGROUND

A. The '142 Patent

The '142 Patent is directed to a method of spatially detecting target analytes in regions of interest. This method can detect and quantify proteins and/or nucleic acids from particular locations in a tissue sample. Specifically, the '142 Patent relates to “probes, compositions, methods, and kits for simultaneous, multiplexed detection and quantification of protein and/or nucleic acid expression in a user-defined region of a tissue, user-defined cell, and/or user-defined subcellular structure within a cell that are adaptable for use with existing sequencing technologies.” '142 Patent at Abstract.

Claim 1 recites:

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;
- 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.

'142 Patent Claim 1.

The claimed methods are directed to the spatial detection of target analyte in a first and second location of a tissue sample. At a high level, the method involves collecting oligonucleotides from a first and second location of a tissue sample, performing a synthesis reaction on the collected sample oligonucleotides to

incorporate a nucleic acid sequence that identifies a location, and identifying the location of the extension products via sequencing. Claims 2-13 depend on claim 1.

The '142 Patent was issued on October 18, 2022, from U.S. Patent Application No. 17/476,712 ("’712 Application"), filed on September 16, 2021. The '712 Application is a continuation of U.S. Patent Application No. 16/272,487 ("’487 Application"), filed February 11, 2019, which claims priority to U.S. Provision Applications Nos. 62/629,180, filed February 18, 2018, and 62/771,212, filed November 26, 2018. The specification of the '712 Application and the '487 Application are identical.¹

B. The Level Of Ordinary Skill In The Art

For the limited purpose of this Preliminary Response, NanoString does not challenge the definition of a POSA set forth in the Petition. NanoString reserves the right to challenge this definition should the *inter partes* review be instituted.

¹ During prosecution of the '712 Application, the examiner did not issue a rejection based on 35 U.S.C. § 103. As such, NanoString had no opportunity to submit secondary considerations of nonobviousness and reserves the right to do so in the event the Board institutes review based on a ground involving obviousness.

C. The Prior Art Cited In The Petition

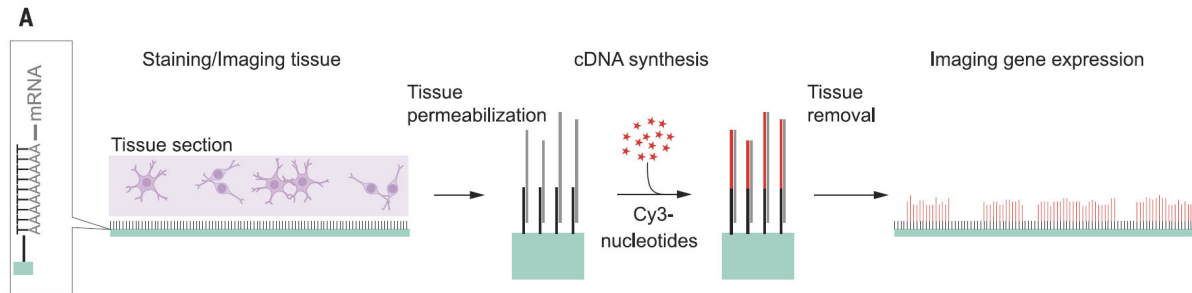
The Petition cites three references. A summary is provided below as relevant background.

1. Stahl

Stahl is a *Science* article entitled “Visualization and analysis of gene expression in tissue sections by spatial transcriptomics” that published in 2016. EX1016. The article purports to describe using “spatially barcoded arrays” that use unique barcodes and capture oligonucleotides on the surface of microscope glass slides. *Id.* at 8. By placing histological sections on the prepared arrays, “RNA-sequencing data with maintained two-dimensional positional information from [] mouse brain and human breast cancer” were collected to allow “quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections.” *Id.* at 1.

Stahl teaches a reverse transcription step to synthesize cDNA from messenger RNA (mRNA) in the tissue sections on a surface. *Id.* at 1. For this purpose, Stahl uses “immobilized reverse-transcription oligo(dT) primers on glass slides” placed

on the slides sections of the sample tissue. *Id.* The tissue is “fixed, stained and imaged.” *Id.* Figure 1A illustrates the cDNA synthesis:



Id. at Fig. 1A. As is evident in the figure, the reverse transcription reaction incorporates the cDNA sequence (shown in red) to the reverse-transcription oligo(dT) primer/surface probe (shown in black) using the sample mRNA (shown in grey) as a template. The surface probe contains “a cleavage site, a T7 amplification and sequencing handle, a spatial barcode, a unique molecular identifier (UMI), and an oligo(dT) VN-capture region.” *Id.* at 2. The reverse transcription reaction does not incorporate the spatial barcode, which is already present in the surface probe, is not synthesized, and is otherwise passive during the synthesis reaction.

After the synthesis reaction that resulted in the first cDNA products, cleavage of the surface probe releases the first cDNA products. *See* EX1016, Suppl. Materials at 6. Subsequently, a second set of cDNA products are generated, which are further cleaned up to generate amplified RNA. *Id.* Thereafter, ligation adapters are appended to the amplified RNA and single stranded cDNA are

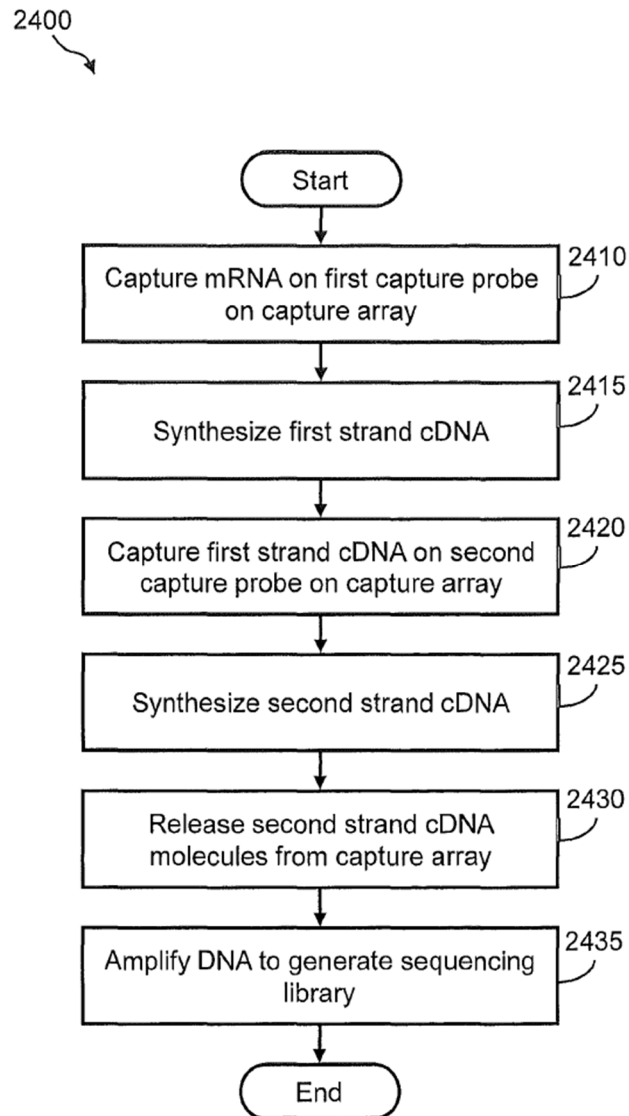
generated from the amplified RNA. *Id.* at 6-7. These single stranded cDNA products are then sequenced from the amplified RNA to analyze the visualization and quantitative analysis of the tissue transcriptome. *Id.* at 1 (“[a]fter capturing and reverse-transcribing mRNA, we generated sequencing libraries based on amplification by in vitro transcription...”).

Thus, unlike the ’142 Patent claims, Stahl does not disclose performing a synthesis reaction to incorporate a spatial barcode, or identifying the DNA products by sequencing DNA products.

2. SO-456

So-456 is a PCT application entitled “Spatial Mapping of Nucleic Acid Sequence Information” that published on February 2, 2017, as No. WO2017/019456. EX1005 (“So-456”). So-456 purports to provide “methods and compositions for spatial detection and analysis of nucleic acids in a tissue sample.” *Id.* at Abstract. So-456, however, is a speculative patent application that describes an incomplete method.

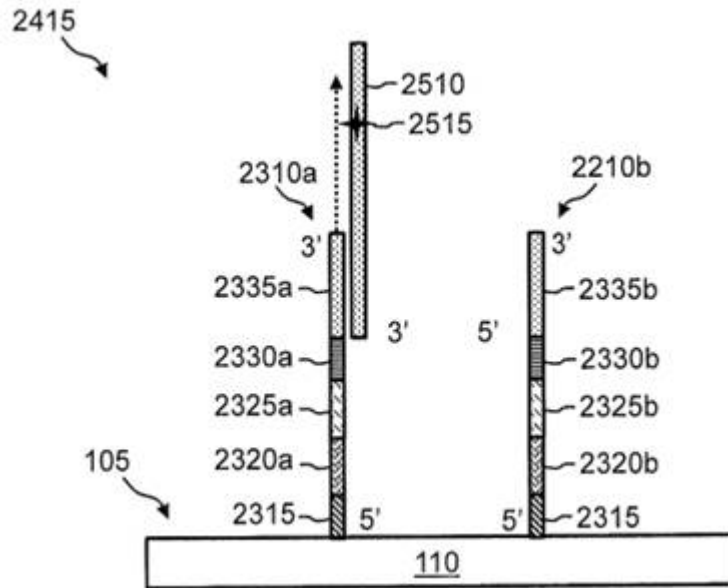
So-456 describes a method described in Figure 24:



EX1005 at Fig. 24. As an initial step, mRNA in a tissue sample is supposedly captured on a capture probe. *See id.* ¶ [00315]. So-456, however, does not provide any detail or explanation regarding how mRNA is released from a tissue sample.

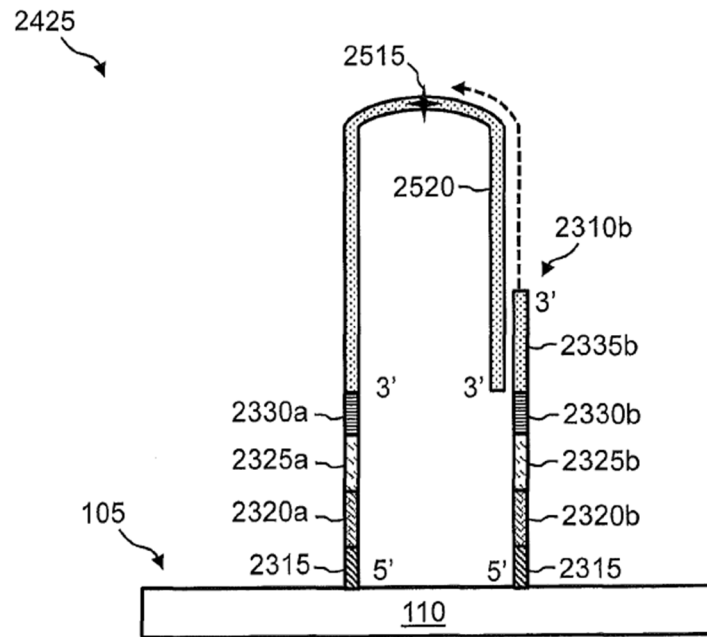
The capture probe contains a spatial barcode sequence, a sequencing primer, and an optional cleavable region. *Id.* at Fig. 25A, ¶ [00302]. So-456 describes

immobilizing the capture probes “such that the 3’-end is available for enzymatic extension.” *Id.* ¶ [00109]. Synthesis of the first strand cDNA is depicted in Figure 25A:



Id. at Fig. 25A. Thus, the synthesis reaction taught by So-456 incorporates the target analyte sequence onto a capture probe that already contains a SBS primer sequence and a spatial address region. The extension taught by So-456 does not incorporate any nucleic acid sequence that identifies the location of the tissue sample because any such sequence that might serve such a function was present in the capture probe and is completely passive with respect to the extension reaction.

Following the extension reaction, the 3’ end of the first strand cDNA is captured on an adjacent capture probe. *Id.* ¶ [00317]. A second strand is subsequently synthesized:



Id. at Fig. 25B, ¶ [00318]. This second strand cDNA has a different sequence than the first strand cDNA. The second strand cDNA is released into solution via cleavage of a cleavage region. *See id.* ¶ [00319]. Subsequent amplification of the second strand cDNA incorporates sequences necessary for sequencing. *See id.* ¶ [00320]. The final product is sequenced and the sequencing data is analyzed “to provide information as to the location of the nucleic acid in the tissue section.” *Id.* ¶¶ [00156]-[00158].

As documented further herein, unlike the '142 Patent claims, Petitioner fails to show that So-456 discloses performing a synthesis reaction to incorporate a spatial barcode, identifying the DNA products by sequencing the first strand cDNA

products, or conditions wherein mRNA are released from a tissue sample in a way that maps to the claims as drafted.

3. Frisen

Frisen is a PCT application filed by Jonas Frisen, Patrik Stahl, and Joakim Lundeberg, entitled “Method And Product For Localised Or Spatial Detection Of Nucleic Acid In A Tissue Sample” that published on October 18, 2012 as WO2012/140224. EX1017. Frisen purports to provide “methods and products for the localised or spatial detection of nucleic acid in a tissue sample.” *Id.* at Abstract.

III. CLAIM CONSTRUCTION

Patent Owner does not dispute that the “collecting” steps in claim 1 includes the capture of mRNAs throughout the tissue sample. *See* Pet. at 22. Patent Owner reserves the right to present claim construction if inter partes review is instituted.

IV. THE PROPOSED GROUNDS DO NOT RENDER THE ’142 PATENT INVALID

A. Stahl Does Not Anticipate Or Render Obvious The Challenged Claims Because Of Failure To Disclose Required Steps

Petitioner relies on Stahl as the primary reference for Grounds 1, 2, and 3, arguing that Stahl anticipates or, in the alternative, renders obvious all of the claims of the ’142 Patent. The Board should deny the Petition due to crucial technical

differences between Stahl and the '142 Patent. For example, Petitioners glosses over at least two required claim elements that Stahl does not disclose or render obvious. Specifically, Stahl fails to disclose (1) the step identifying the DNA products by sequencing; and (2) a synthesis reaction that uses the collected oligonucleotide as a template and incorporates a nucleic acid sequence that identifies a location.

1. Stahl Does Not Disclose Identifying the DNA Products by Sequencing DNA Products

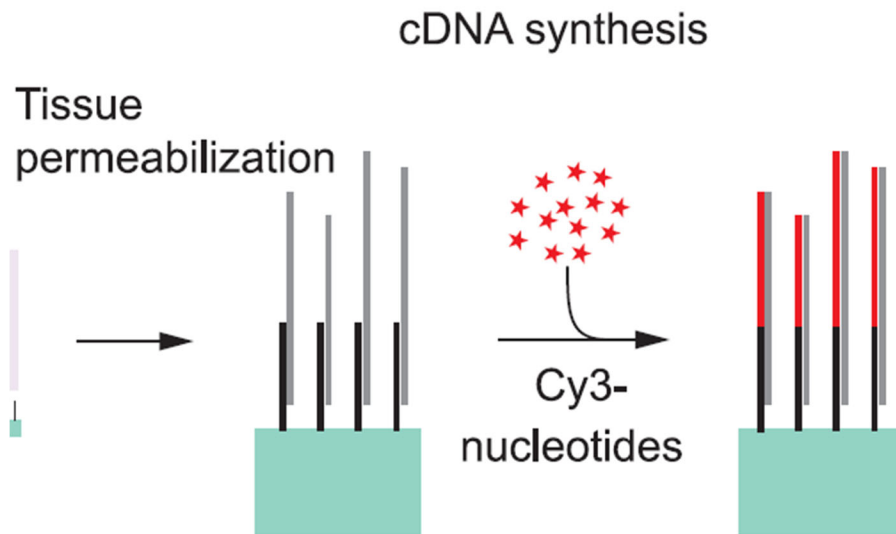
First, Stahl does not teach or render obvious the “identifying” step in independent claim 1. As required in claim 1, “the first plurality of DNA products and the second plurality of DNA products” must be identified by sequencing the DNA products:

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.

'142 Patent Claim 1(e).²

² Emphasis added throughout unless otherwise noted.

Notably, Stahl does not sequence the DNA products. In Stahl, a reverse transcription of the sample mRNA generates cDNA products, shown in Figure 1A:



EX1016 at Fig. 1A. Petitioner contends that the cDNA generated from the reverse transcription of sample mRNA satisfies the “DNA products” claim element in the ’142 Patent claims. See Pet. at 26 (“Stahl’s method uses a reverse transcription reaction to ‘synthesize’ cDNA products using the captured mRNAs as templates.”). Stahl, however, does not sequence these cDNA products.

Instead, Stahl teaches sequencing *different* products—amplified RNA molecules. After synthesis of the first set of cDNA products via reverse transcription, Stahl describes releasing the first cDNA products and using the released cDNA to generate entirely new single strands of amplified RNA with adapters. Subsequently, Stahl describes performing reverse transcription to

generate a second set of cDNA products and adding sequences for sequencing. Thus, Stahl describes sequencing amplified RNA.

Stahl describes this process in detail. First, Stahl describes releasing the first cDNA products—reverse-transcribed extension products—from the surface by cleaving the leading uracil nucleotides of the surface oligonucleotide. *See* EX1016, Suppl. Materials at 6 (“For each well, 70µl of release mix containing...0.1U/µl USER Enzyme (#M5505, NEB) was added and incubated.”); EX2001 (“USER (Uracil-Specific Excision Reagent) Enzyme (1) generates a single nucleotide gap at the location of a uracil residue.”) (<https://www.neb.com/en-us/products/m5505-user-enzyme>). Once in solution, a second DNA strand was generated. *See* EX1016, Suppl Materials at 6 (“5µl of second strand mix...was added.”). After the ends of the double-stranded DNA were blunted and a cleanup was performed, amplified RNA products were created. *See id.* (“5.6µl of the sample were mixed with 10.4µl In Vitro Transcription mix with a final content of...1xT7 Enzyme Mix (#AM1333, Ambion).”); EX2002 (“MEGAscript T7 Transcription Kit”) (“Final Product Type: Synthetic mRNA, mRNA”) (<https://www.thermofisher.com/order/catalog/product/AM1333>). The product at this stage are amplified single-stranded RNA products.

After an analysis of the amplified RNA products, ligation adapters were appended to the amplified RNA. *See* EX1016, Suppl. Materials at 6-7 (“Samples were heated...then placed on ice before 4.5μl ligation mix was added to a final content of...0.5μM Ligation Adapter.”). Single-stranded cDNA was synthesized from the amplified RNA. *See id.* at 7 (“8μl of reverse transcription mix was added.”). Amplification was then performed with PCR Index, InPe1.0, and InPE2.0 primers. *See id.* The finished products were analyzed before sequencing on the Illumina NextSeq platform. *See id.* Accordingly, the products sequenced in Stahl are ***not*** the first cDNA products purported by Petitioner to be the “DNA products” required by the ’142 Patent.

Petitioner attempts to obfuscate the crucial difference between the “DNA products” required to be sequenced by the ’142 Patent claims and the molecules actually sequenced in Stahl. *See* Pet. at 30 (“Stahl discloses generating ‘sequencing libraries’ by collecting the spatially barcoded cDNA synthesis products, amplifying them, and sequencing the amplified library ‘on the Illumina NextSeq platform using paired-end sequencing according to the manufacturer’s protocol.’”).

But as Petitioner acknowledges, Stahl teaches sequencing cDNA reverse-transcribed ***from amplified RNA***. *See* Pet. at 37 (“After performing an initial *in vitro* transcription/amplification to produce amplified RNA molecules, Stahl

discloses 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.*”). Thus, the sequenced cDNA is reverse-transcribed from amplified RNA and is not the cDNA reverse-transcribed from the sample mRNA, the alleged “DNA products.” As such, at least because Stahl does not disclose sequencing “DNA products,” Stahl does not anticipate the claims of the ’142 Patent. As claims 2-4 and 6-13 depend on independent claim 1, Stahl likewise does not anticipate those claims.

Petitioner also argues that claim 1 would have been obvious over Stahl. 10X, however, offers only conclusory statements of why it would have been obvious to a POSA. *See* Pet. at 45 (“A POSA reading Stahl would have been motivated to practice the claimed methods because Stahl teaches that its ‘spatial transcriptomics’ method ‘provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections,’ ‘enabling novel types of bioinformatics analyses.’”). Moreover, 10X states that Stahl’s “protocol was straightforward, requiring ‘only a few extra steps compared with RNA-seq analysis of homogenized tissue.’” *Id.* Thus, 10X provides no motivation or expectation for why a POSA would be successful in radically modifying Stahl’s method by

ignoring the steps in what Petitioner calls a “straightforward” protocol to generate amplified RNA from the first set of cDNA and then generate a second set of cDNA from the amplified RNA.

In addition, strand preservation via RNA amplification is a major component of Stahl’s method. For example, Stahl emphasizes that each probe contains a T7 promoter sequence. *See* EX1016 at 3 (“Each array feature contains unique DNA-barcoded probes containing...a T7 amplification and sequencing handle.”). Stahl describes using this T7 sequence only with a T7 RNA polymerase to generate amplified RNA. *See id.*, Suppl Materials at 6. Petitioner provides no reasonable argument why a POSA would modify Stahl’s method to result in a method without strand information.

Accordingly, it would not have been obvious to a POSA to modify Stahl to practice claim 1 of the ’142 Patent. As claims 2-13 depend on claim 1, those claims would also not have been obvious to a POSA in view of Stahl. Although 10X also argues that claim 5 would have been obvious over Stahl and Frisen, 10X does not argue that Frisen discloses sequencing “DNA products,” as required in independent claim 1. As such, dependent claim 5 likewise would not have been obvious over Stahl and Frisen.

2. Stahl Does Not Disclose Synthesizing DNA Products That Incorporates A Nucleic Acid Sequence that Identifies Location

Stahl does not teach or render obvious the “synthesis reaction” steps required in independent claim 1. This claim requires that “a synthesis reaction” be performed that uses the collected sample oligonucleotides as templates and incorporates a nucleic acid sequence that identifies the location of the target analyte:

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;

’142 Patent Claim 1(c), *see also id.* Claim 1(d).

Stahl does not disclose the required “synthesis reaction” step that incorporates a spatial barcode, nor does Stahl render this step obvious.

a. The Claimed Synthesis Step Is Not Anticipated By Stahl

Petitioner’s one-page gloss of Stahl fails to meet its burden to establish that Stahl anticipates the claimed synthesis step. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015); *see also* Pet. at 28-29.

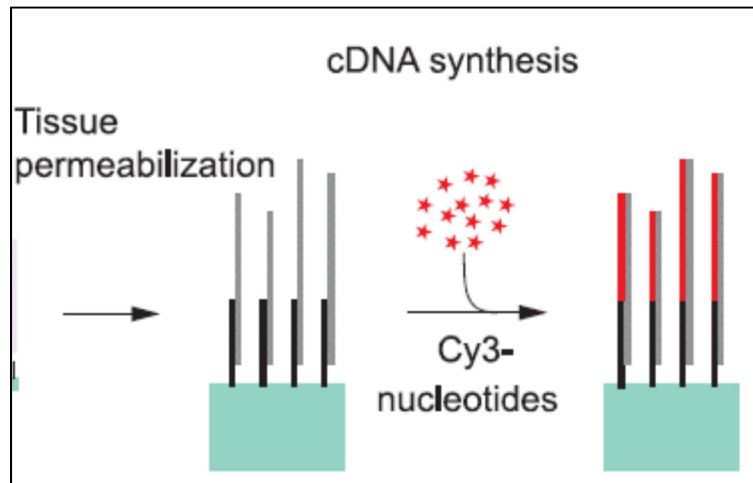
Petitioner does not—and cannot—demonstrate that the protocols disclosed in Stahl would result in incorporating the spatial address region via a synthesis reaction. Petitioner offers merely a conclusory assertion that “Stahl’s synthesis reaction

produces cDNA products comprising the spatial barcode” because the surface probe contains a spatial barcode region. Pet. at 29. Petitioner fails to explain, however, how the spatial barcode is “incorporated” by the synthesis reaction as claimed. ’142 Patent at Claim 1(c)-(d).

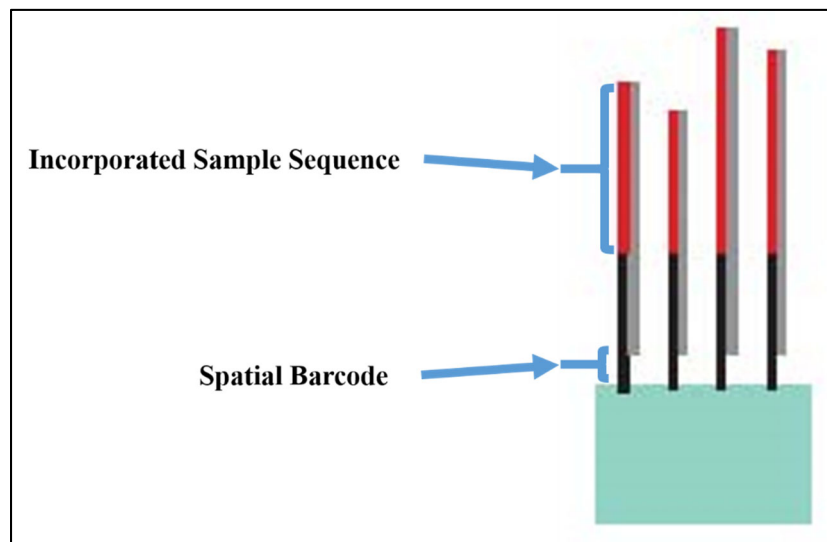
Petitioner points to Stahl’s reverse transcription reaction as the claimed “synthesis reaction.” Pet. at 26 (“Stahl discloses the synthesis steps (c) and (d) because Stahl’s method uses a reverse transcription reaction to ‘synthesize’ cDNA products using the captured mRNAs as templates.”). This reverse transcription reaction does *not* incorporate a spatial barcode and Petitioner does not contend that it does. Indeed, Stahl’s surface probe already contains a spatial barcode—as acknowledged by Petitioner. See Pet. at 28 (“Stahl discloses that each capture probe on its array comprises a ‘spatial barcode’”). Thus, there is no synthesis of cDNA that includes a spatial barcode.

Rather, Petitioner argues that because the reverse transcription reaction uses the surface probe as a primer, the “synthesis reaction produces cDNA products comprising the spatial barcode.” *Id.* at 29. While the resulting products comprise a spatial barcode, Stahl does *not* disclose the claimed method because the synthesis reaction *does not incorporate* the spatial barcode.

Petitioner’s reliance on Stahl’s Figure 1A, shown below, is misplaced:



EX1016, Fig. 1A. Fig. 1A is clear that the spatial barcode (part of the capture probe, shown in black) is *not* incorporated by the synthesis reaction, as claimed. Rather, the sample DNA sequence (shown in red) is incorporated onto the capture probe, which already contains a spatial barcode:



EX1016, Fig. 1A (annotated). As shown above and described in Stahl, the reverse transcription reaction (synthesis reaction) synthesizes the sample sequence (shown

in red) onto the capture probe (shown in black) using the sample oligonucleotide (shown in grey) as a template. The “nucleic acid sequence that identifies the first location,” i.e. Stahl’s “spatial barcode,” is contained at the base of the capture probe. The spatial barcode is **not** incorporated through the reverse transcription reaction.

Petitioner points to no other disclosure in Stahl, and indeed there is none, where “a synthesis reaction” is performed that “incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the” “DNA products.” ’142 Patent at Claim 1(c)-(d). The ’142 Patent, in contrast, teaches embodiments wherein the spatial identifier is incorporated through a synthesis reaction. Specifically, the ’142 Patent describes an extension reaction using a “nucleic acid template” that comprises “a nucleic acid sequence which identifies the specific location of the tissue sample from which the” sample oligonucleotide was released. ’142 Patent at 5:15-20. Thus, the ’142 Patent describes an extension reaction (synthesis reaction) that incorporates the spatial identifier.

Unlike the claims and specification of the ’142 Patent, Stahl does not describe a synthesis reaction that incorporates a nucleic acid sequence. In the sole embodiment relied on by Petitioner, represented by Figure 1A, a single reverse transcription reaction takes place to incorporate the sequence of the target oligonucleotide into the capture probe. This reaction does **not** incorporate a nucleic

acid sequence that identifies a location. Accordingly, Petitioner fails to meet its burden to establish that the claimed “synthesis reaction” is disclosed by Stahl.

b. Stahl Does Not Render The Claimed Synthesis Step Obvious

Petitioner’s Ground 2 argues in alternative to the Ground 1 anticipation theory, that to the extent “Stahl does not disclose all the elements of claims 1-4 and 6-13, these claims still would have been obvious in view of Stahl.” Pet. at 44. To support its obviousness argument, Petitioner relies on substantially the same argument that they rely on to argue anticipation. *Id.* at 44-45 (“Even assuming *arguendo* Stahl does not expressly teach the claimed methods, it suggests practicing them”). Petitioner, however, only supports its obviousness argument with bare-bones conclusory assertions that a “POSA would have had a reasonable expectation of successfully practicing the claimed methods, because Stahl provides detailed descriptions and protocols” and cites generally to the entirety of Stahl’s supplemental materials without any explanation. *Id.* at 45. Such conclusory statements accompanied by broad citation without explanation cannot possibly support Petitioner’s obviousness argument.

For the same reasons discussed above, Stahl does not render obvious the required synthesis reaction steps because Stahl does not disclose a synthesis reaction

that incorporates a spatial barcode and, further, Petitioner fails to establish that it would have been obvious to modify the methods of Stahl to do so.

Petitioner points to no disclosure in Stahl of any synthesis reaction that “incorporates at least one nucleic acid sequence that identifies the [] location of the tissue sample.” ’142 Patent at Claim 1(c)-(d). In addition, Petitioner provides no extra details or explanation in Ground 2 than was provided in Ground 1. Rather, Petitioner simply asserts that to the extent “Stahl does not disclose all the elements of claims 1,” these elements “would have been obvious in view of Stahl.” Pet. at 44.

Petitioner provides no compelling reason to modify the method of Stahl that would render it obvious. Petitioner only provides a conclusory assertion that a “POSA reading Stahl would have been motivated to practice the claimed methods because Stahl teaches that its ‘spatial transcriptomics’ method ‘provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections.’” *Id.* at 45. Petitioner’s purported motivation fails, however, because a synthesis reaction that incorporates the capture probe is not necessary or obvious to achieve cDNA products that include both the sample oligonucleotide sequence and the spatial barcode. As discussed above, a reverse transcription of the capture probe would incorporate the sample oligonucleotide sequence onto the capture

probe. Thus, this reverse transcription of the capture probe would purportedly create a single molecule with both a spatial barcode (under Petitioner's theory) and the sample oligonucleotide sequence. There would not be a motivation to modify the method to find an alternative where the purported motivation is already achieved with the disclosed method.

Petitioner's argument for a reasonable expectation of success provides no further details. Petitioner again makes a conclusory assertion that a "POSA would have known that Stahl's methods used commercially available materials and reagents, and that the protocol was straightforward." Pet. at 45. Petitioner fails, however, to provide any detail or support for this assertion beyond a broad citation to 19 pages of Stahl's supplementary materials without explanation.

Accordingly, Stahl does not anticipate or render obvious the claimed synthesis reaction step.

B. So-456 Does Not Anticipate Or Render Obvious The Challenged Claims Because Of Failure To Disclose Required Steps

Petitioner relies on So-456 as the primary reference for Grounds 4 and 5, arguing that So-456 anticipates or, in the alternative, renders obvious all of the claims of the '142 Patent. The Board should deny the Petition due to crucial technical differences between So-456 and the '142 Patent. For example,

Petitioners glosses over at least three required claim elements that So-456 does not disclose or render obvious. Specifically, So-456 fails to disclose (1) the step identifying the DNA products by sequencing; (2) a synthesis reaction that uses the collected oligonucleotide as a template and incorporates a nucleic acid sequence that identifies a location, and (3) collecting oligonucleotides under conditions that release them.

1. So-456 Does Not Disclose Identifying the DNA Products by Sequencing DNA Products

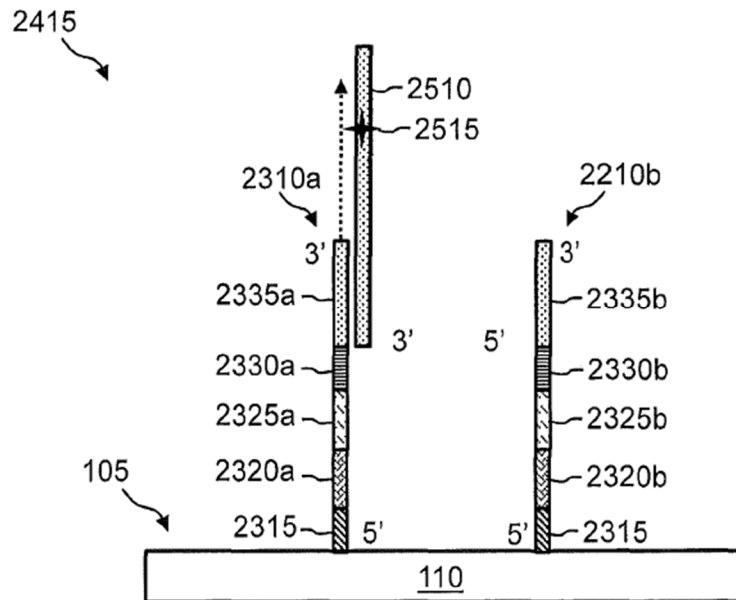
First, So-456 does not disclose the “identifying” step in independent claim 1. As required in claim 1, “the first plurality of DNA products and the second plurality of DNA products” must be identified by sequencing the DNA products:

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.

’142 Patent Claim 1(e).

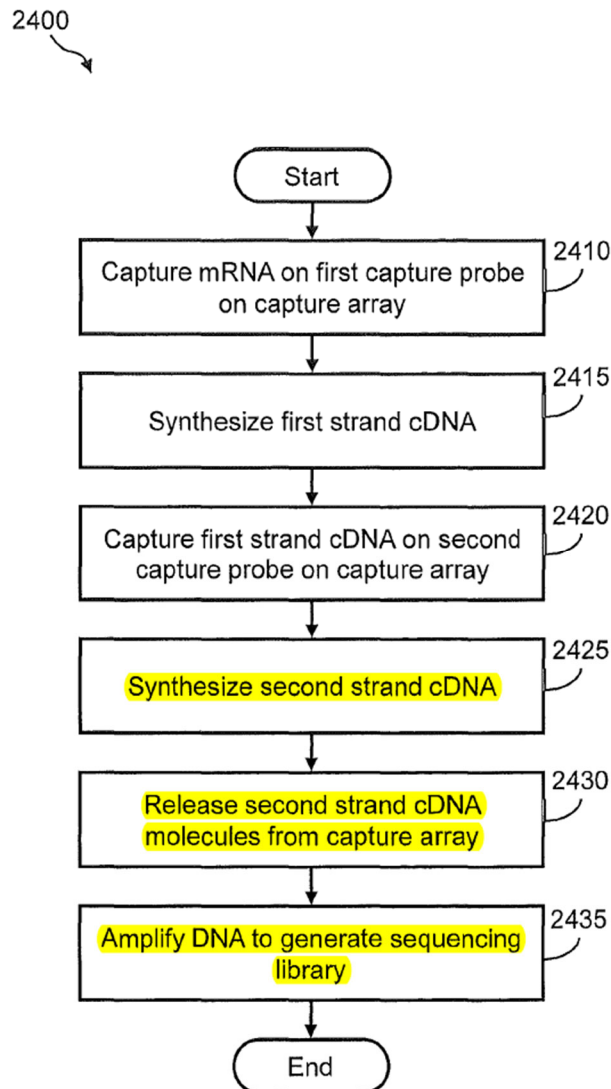
Similar to Stahl, So-456 does not sequence the DNA products required by the claims. Petitioner contends that the first-strand cDNA in So-456’s method as the “DNA product” in the ’142 Patent claims. *See* Pet. at 61 (“So-456’s method uses the ‘gene-specific capture regions on the first capture probe’ (2310a) as ‘reverse

transcriptase primers' for '*synthesis of first strand cDNA from captured mRNA molecules.*'"). Petitioner further points to the dotted lines in Figure 25A to illustrate the produced "first strand cDNA from captured mRNA molecules":



EX1005 at Fig. 25A. So-456, however, does not sequence this first strand cDNA.

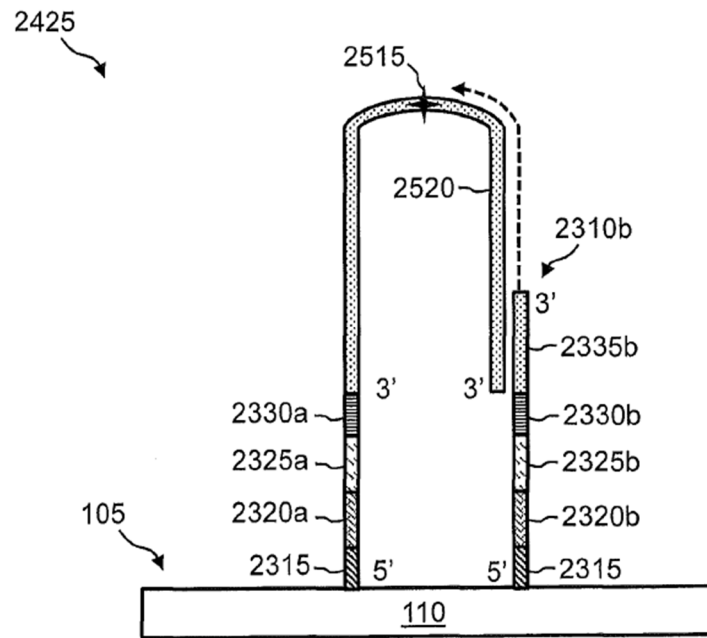
Instead, So-456 generates and sequences a second strand cDNA. After a second strand cDNA is generated, So-456 attaches sequencing adapters sequencing the resulting product. This process is described in Figure 24:



Id. at Fig. 24 (annotated).

As So-456 teaches, after the first strand cDNA is synthesized, it is captured by hybridization to a nearby capture probe, as shown in the figure below. *See id.* ¶ [00317] (“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.”). A second strand cDNA is then created. *See id.* ¶ [00318] (“At

step 2425, second strand cDNA is synthesized in an extension reaction using capture region 2335b as a primer.”). The extension reaction that generates the second strand cDNA is illustrated in Figure 25B with dotted lines:



Id. at Fig. 25B. Next, the second strand cDNA is cleaved from the solid support. *See id.* ¶ [00319] (“At step 2430, a cDNA molecule 2525 is released from capture site 105 by cleavage of cleavable region 2315.”). The second strand cDNA, once in solution, undergoes amplification wherein sequences necessary for Illumina sequencing are added. *See id.* ¶ [00320].

Accordingly, So-456 teaches sequencing the second strand cDNA and not the first strand cDNA, which Petitioner contends is the “DNA product” required in the claims of the ’142 Patent. Notably, the sequence of the first strand cDNA and the

second strand cDNA are not the same or complementary. As So-456 teaches, the second strand cDNA “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).” *Id.* ¶ [00319]. As Figure 25B above clearly shows, regions 2330b, 2325b, and 2320b are only present on the second strand cDNA and not the first strand cDNA.³ Thus, rather than sequence the first strand cDNA, or the “DNA product” in the challenged claims, So-456 teaches sequencing the second strand cDNA, a downstream product markedly different from the first strand cDNA.

For at least the reason above, So-456 does not anticipate the claims of the ’142 Patent. As Petitioner offers no motivation or expectation of success why a POSA would modify So-456’s method to sequence directly the first strand cDNA,

³ So-456 teaches that the “a” and “b” sequences for regions 2330, 2325, and 2320 are not identical and serve different functions. Region 2330 is a UMI region where 2330a “comprises a unique sequence for *capture probe 2310a*” and 2330b “comprises a unique sequence for *capture probe 2310b*.” *Id.* ¶ [00302]. Meanwhile, region 2325 is a spatial address region where 2325a “comprises a unique spatial address sequence for the *X dimension*” and region 2325b “comprises a unique spatial address region for the *Y dimension*.” *Id.* Lastly, region 2320 is a SBS primer region, where 2320a denotes a *SBS3* sequence and 2320b denotes a *SBS12* sequence. *Id.*

Petitioner has likewise failed to show that So-456 renders obvious the claims of the '142 Patent.

2. So-456 Does Not Disclose Synthesizing DNA Products That Incorporates A Nucleic Acid Sequence that Identifies Location

So-456 also does not teach or render obvious a synthesis reaction that “incorporates” a nucleic acid sequence that identifies the location of the tissue sample. Claims 1(c) and (d) of the '142 Patent require performing “a synthesis reaction” that uses the collected oligonucleotides as templates and incorporates a nucleic acid sequence that identifies the location of the target analyte:

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

'142 Patent Claims 1(c) and 1(d).

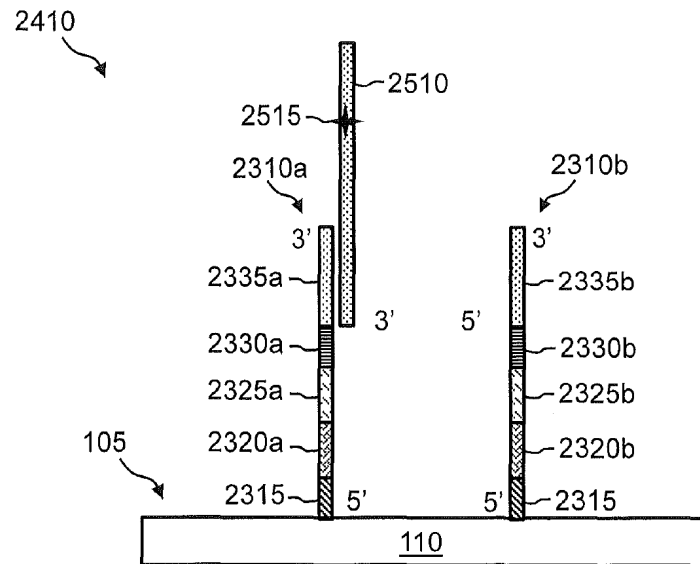
Like Stahl, So-456 does not incorporate a nucleic acid sequence that identifies the location. Petitioner identifies a “spatial address region” as the nucleic acid sequence that identifies a location. *See* Pet. at 64 (“*a sequence that identifies a*

location on the array and the corresponding location of the tissue sample (*spatial address region of the capture probe*)). So-456, however, fails to disclose how this spatial address region is “incorporated” during the synthesis reaction as claimed. ’142 Patent at Claim 1(c)-(d).

Petitioner points to So-456’s reverse transcription reaction in Figure 25A as the claimed “synthesis reaction.” *See* Pet. at 61; *see also id.* at 62 (“So-456 uses reverse transcription to synthesize a cDNA strand using the captured mRNA as a template”). Indeed, So-456 generates a cDNA product via reverse transcription.

This reverse transcription reaction that Petitioner relies upon, however, does *not* incorporate a spatial address region. The spatial address region, as acknowledged by the Petitioner, is already present in So-456’s capture probe. *Id.* at 63 (“So-456 teaches that these *capture probes comprise a ‘spatial address region’* that comprises a ‘unique spatial address sequence.’”). The Petitioner argues that because the reverse transcription reaction produces DNA products “comprising a sequence that identifies a location on the array and the corresponding location of the tissue sample (spatial address region of the capture probe),” the DNA products “incorporate” the claimed sequence. *Id.* at 64. While the resulting products may comprise a spatial address region, So-456 does *not* disclose the claimed method because the synthesis reaction *does not incorporate* the spatial address region.

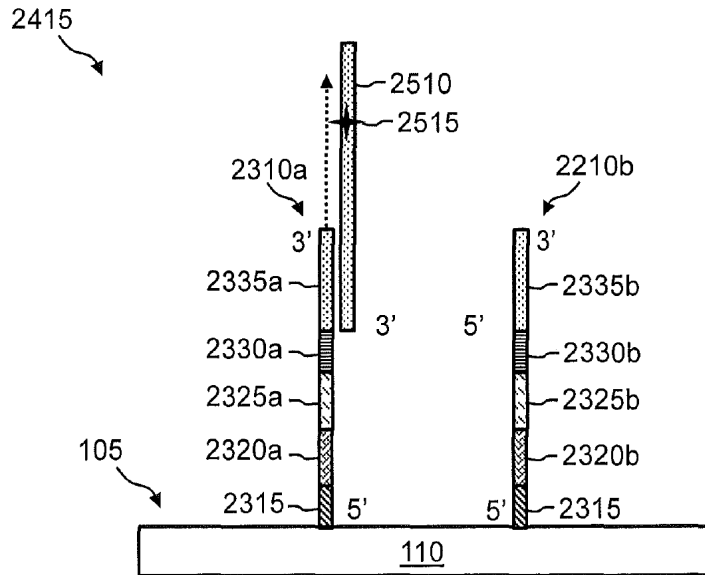
So-456's Figure 25A demonstrates the synthesis reaction step, as shown below:



Id. at Fig. 25A. The figure shows a capture probe (2310a) that includes the purported spatial address region (2325a). *See, e.g., id.* ¶ [00319]. The Petitioner acknowledges that “So-456’s method uses the ‘gene-specific capture regions on the first capture probe’ (2310a) as ‘reverse transcriptase primers’ for ‘synthesis of first strand cDNA from captured mRNA molecules.’” Pet. at 61; *see also* EX1005 ¶ [00316] (“first strand cDNA is synthesized using **capture region 2335a** as a primer in a reverse transcription reaction.”).

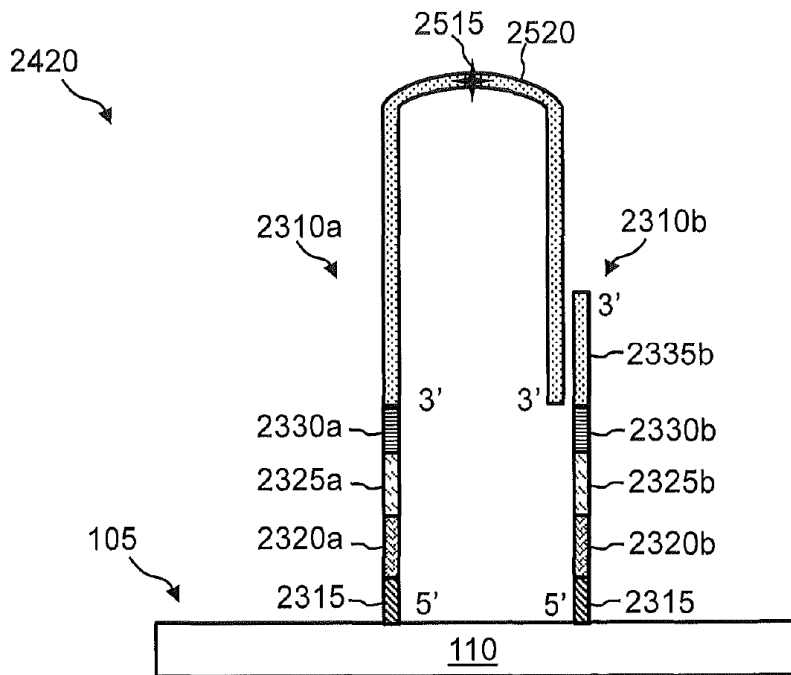
The spatial address region that Petitioner identifies as the “nucleic acid sequence that identifies location” is not being incorporated in the synthesis reaction. The purported synthesis reaction utilizes the “gene-specific capture region” (2335a)

of the capture probe (2310a) as the reverse transcription primer. As Petitioner acknowledges, “So-456’s Figure 25A depicts [the] cDNA synthesis as a dotted arrow,” shown below. Pet. at 61.



Id. at Fig. 25A. As is evident from Figure 25A, the 3' end of the sample polynucleotide (2510) is hybridized to the gene-specific capture region of the capture probe and the reverse transcription reaction moves up (along the arrow) synthesizing a complement to the sample oligonucleotide. The spatial address region is **not** synthesized in this reverse transcription reaction, and **does not need to be** because it is already present on the capture probe. Rather, only the complement to the sample oligonucleotide is synthesized.

Petitioner further relies on Figure 25B, shown below:



Id. at Fig. 25B. Petitioner makes a conclusory argument that because “So-456’s synthesis reaction produces DNA products (first-strand cDNAs) comprising a sequence that identifies a location,” So-456’s synthesis reaction incorporates “the sequence into the DNA products.” Pet. at 64. So-456 teaches no such thing. As is clear from the figure, the spatial address region (labelled as 2325a in the above figure) is already present as a part of the capture probe from the beginning of the synthesis reaction, and is **not** incorporated by the synthesis reaction.

As discussed above, the ’142 Patent, in contrast, teaches embodiments wherein the spatial identifier is incorporated through a synthesis reaction. Specifically, the ’142 Patent describes an extension reaction using a “nucleic acid template” that comprises “a nucleic acid sequence which identifies the specific

location of the tissue sample from which the” sample oligonucleotide was released. ’142 Patent at 5:15-20. Thus, the ’142 Patent describes an extension reaction (synthesis reaction) that incorporates the spatial identifier.

Accordingly, So-456 does not anticipate the challenged claims because So-456 fails to disclose a synthesis step that incorporates a nucleic acid that identifies the location. So-456 also does not render obvious the challenged claims because, similar to Stahl, Petitioner offers no reason why a POSA would modify So-456’s method.

3. So-456 Does Not Disclose Collecting Oligonucleotides Under Conditions That Release Them

As an additional independent reason, So-456 does not disclose the “collecting” steps in independent claim 1. As required in claim element 1, oligonucleotides must be collected “under conditions that release the plurality of oligonucleotides from the [] location of the tissue sample”:

- 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*;

’142 Patent Claim 1. Thus, So-456 must teach conditions that allow oligonucleotides to be released from a tissue sample.

So-456, however, provides no disclosure on any “conditions” that “release the plurality of oligonucleotides” from a tissue sample. The disclosure that is present in So-456 does not relate to conditions that release oligonucleotides from a tissue sample:

As used herein, the term “tissue sample” refers to a piece of tissue that has been obtained from a subject, fixed, sectioned, and mounted on a planar surface, e.g., a microscope slide. The tissue sample can be a formalin-fixed paraffin-embedded (FFPE) tissue sample or a fresh tissue sample or a frozen tissue sample, etc. The methods disclosed herein may be performed before or after staining the tissue sample. For example, following hematoxylin and eosin staining, a tissue sample may be spatially analyzed in accordance with the methods as provided herein. A method may include analyzing the histology of the sample (e.g., using hematoxylin and esoins staining) and then spatially analyzing the tissue.

EX1005 ¶ [00125]. Sectioning, staining, and fixing a tissue sample on a microscope slide are not processes that release oligonucleotides from a tissue sample. So-456 instead describes transferring the nucleic acids in a tissue sample onto an array without describing any specific conditions. *Id.* ¶ [00127] (“In some embodiments, nucleic acids in a tissue sample are transferred to and captured onto an array. For example, a tissue section is placed in contact with an array and nucleic acid is captured onto the array and tagged with a spatial address.”). Without any specific condition, however, it is unclear how oligonucleotides in a tissue sample that has been, for example, fixed by FFPE, are released onto the array.

Petitioner attempts to argue around this lack of disclosure by taking language from So-456 out of context. Petitioner argues that “the tissue sample was treated to create a condition to ‘release’ the mRNA from the tissue onto the array” because So-456 states that “the ‘*tissue sample is prepared for analysis*’ and the mRNAs from the tissue sample are ‘captured onto the array.’” Pet. at 60 (citing EX1005 ¶¶ [00152], [00308], [00315]) (emphasis in original).

The cited paragraphs, however, do not describe treating a tissue sample under conditions that would release mRNA:

[00152] 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.

[00308] 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 IA. 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.

[00315] 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.

EX1005 ¶¶ [00152], [00308], [00315]. The cited paragraphs only disclose sectioning a FFPE tissue sample and contacting a tissue sample with a capture array or capture probes. These processes are insufficient for mRNA to be released from a tissue sample. For example, for Stahl, Petitioner cites to “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 into the array.” Pet. at 26. Petitioner fails to cite to any similar disclosure in So-456.

Petitioner’s reliance on an alleged disclosure in So-456 that “‘target nucleic acid’ (mRNA) ‘diffuses from the tissue’ onto the array” fares no better. *Id.* at 60 (citing EX1005 ¶ [00155]). So-456 states, without more, that “a filter or a gel or a thin layer separating the tissue from the probes” may allow the target nucleic acid to “diffuse from the tissue.” *Id.* Crucially, So-456 provides no examples of these substances, no embodiments that employ such substances, and no disclosure of *how* such a scheme may even work. Simply stating that it does is far from sufficient in teaching “conditions” that allow oligonucleotides to be released from a tissue sample. Petitioner provides no argument that such a step would have been obvious to a POSA.

Accordingly, for this additional reason, So-456 does not anticipate or render obvious the challenged claims because So-456 fails to disclose conditions that release oligonucleotides from tissue sample.

V. CONCLUSION

For the reasons set forth above, 10x's Petition for *Inter Partes* Review should be denied in its entirety.

Dated: January 4, 2024

Respectfully submitted,

/Derek C. Walter/

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Reg. No. 74,656

CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24 et seq., the undersigned certifies that this document complies with the type-volume limitations. This document contains 7,679 words as calculated by the “Word Count” feature of Microsoft Word 2016, the word processing program used to create it.

Respectfully submitted,

WEIL, GOTSHAL AND MANGES LLP

Dated: January 4, 2024

By: /Derek C. Walter/
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Lead Counsel for Patent Owner
Reg. No. 74,656

CERTIFICATE OF SERVICE

I hereby certify that a true and correct copy of **PATENT OWNER'S PRELIMINARY RESPONSE TO PETITION FOR INTER PARTES REVIEW OF U.S. PATENT NO. 11,473,142, EXHIBIT 2001, and EXHIBIT 2002** were served on January 4, 2024, via email pursuant to 37 C.F.R. and § 42.107 to the Petitioner at the addresses below:

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