

**IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE**

10X GENOMICS, INC. and PRESIDENT AND
FELLOWS OF HARVARD COLLEGE,

Plaintiffs,

v.

VIZGEN, INC.,

Defendant.

C.A. No. 22-595-MFK

JURY TRIAL DEMANDED

OUTSIDE ATTORNEYS' EYES ONLY

VIZGEN, INC.,

Counterclaim-Plaintiff,

and

PRESIDENT AND FELLOWS OF HARVARD
COLLEGE,

Counterclaim-Plaintiff as to
certain claims,

v.

10X GENOMICS, INC.,

Counterclaim-Defendant as to
certain claims,

and

PRESIDENT AND FELLOWS OF HARVARD
COLLEGE,

Counterclaim-Defendant as to
certain claims.

VIZGEN'S INITIAL INFRINGEMENT CLAIM CHART
FOR U.S. PATENT NO. 11,098,303

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

Pursuant to Paragraph 4(c) of the Default Standard for Discovery of the United States Court of the District of Delaware and Paragraph 7(c) of this Court's Scheduling Order of September 19, 2022 (D.I. 45), Defendant and Counterclaim-Plaintiff Vizgen, Inc. ("Vizgen"), by and through its undersigned counsel, hereby serve on Plaintiff and Counterclaim-Defendant 10x Genomics, Inc. ("10x") the following initial infringement claim chart concerning U.S. Patent No. 11,098,303 ("the '303 patent").

Vizgen has prepared these contentions based on information and discovery currently available to it. Fact discovery remains open and expert discovery has not yet begun in this case, nor has 10x produced any source code. Vizgen's investigation into the infringement of the asserted claims of the '303 patent in this action continues, and fact depositions have not yet begun. Further, claim-construction exchanges and briefing have not yet begun, and the Court has not yet construed the claims of the '303 patent.

In addition, as explained in correspondence to 10x, upon review of 10x's alleged technical production, it is clear that 10x has withheld core technical documents that should have been produced on October 27, 2022 pursuant to Paragraph 4(b) of the Default Standard for Discovery of the United States Court of the District of Delaware and Paragraph 7(b) of this Court's Scheduling Order of September 19, 2022 (D.I. 45). *See, e.g.*, Nov. 9, 2022 Email from A. Tigchelaar to 10x counsel (stating that "[u]pon review of 10x's production, it has become clear that 10x did not produce certain categories of technical documents that should have been produced such as user manuals and protocols for the Accused In Situ Xenium Platform (*see, e.g.*, 10X595-00000677 referencing user guides for the Xenium Analyzer (CG000584) and Probe Hybridization, Ligation & Amplification protocol (CG000582), etc.)), technical notes (*see, e.g.*, 10X595-00000752), and technical documents relating to custom gene panel design, decoding, quality

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

scores, and output file exports (*see, e.g.*, Janesick (2022) at 29-30), including but not limited to protocols, user guides, codebooks, etc.”); Nov. 9 and 17, 2022 E-mail from A. Tigchelaar to 10x counsel (addressing deficiencies in 10x’s technical document production). By way of non-limiting example, 10x’s production references several user guides and protocols that were not produced by 10x and for which 10x has and/or will provide to its customers along with 10x’s Accused Products to inform and instruct others as to how to use the Accused Products. *See e.g.*, 10X595-00000654-668 (“Xenium Analyzer Site Preparation Survey”); 10X595-00000669-689 (“Xenium In Situ Gene Expression – Protocol Planner”); *Id.* at -00000671 (disclosing that 10X will provide its customers “[a] list of documents to support various steps of the in situ gene expression workflow”); *Id.* at -00000673 (disclosing that 10X will provide “On-Site Training”); *Id.* at -00000677 (disclosing customer manuals such as “Tissue Preparation Guide”, “Demonstrated Protocol CG000580”; “Prob Hybridization, Ligation & Amplification For FF & FFPE Samples on Xenium Slides”, “User Guide CG000582”; “Xenium Analyzer . . . User Guide CG000584”); *Id.* at -00000681 (disclosing customer user guides such as “Demonstrated Protocol CG000579”, “Demonstrated Protocol CG000581”, “User Guide CG000582”; “User Guide CG000584”); 10X595-00000690-753 (Xenium In Situ Overview); 10X595-00000784 at Slide 16 (disclosing materials available to customers for FFPE Protocols); *Id.* at Slide 33 (disclosing materials available to customers for FF Protocols); *Id.* at Slide 52 (disclosing user guide available to customers for Xenium In Situ Spatial Profiling). Counsel has requested that 10x supplement its production. As of the date of these contentions, 10x has refused to do so.

Accordingly, Vizgen therefore provides citations only to evidence demonstrating 10x’s infringement that is currently available to it. These disclosures are not intended to be an exhaustive citation of all such evidence. Vizgen anticipates that additional facts that it learns over the course

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

of the litigation will be highly relevant to demonstrating 10x's infringement and reserves all rights to amend or supplement these contentions to include such information. Moreover, in the event that a claim limitation is deemed to be missing under a literal infringement analysis (e.g., due to claim construction), Vizgen further reserves the right to demonstrate the presence of a substantial equivalent of such limitation and to pursue infringement under the doctrine of equivalents.

Accordingly, Vizgen reserves the right to supplement, modify, and/or enlarge the following disclosures, without prejudice, based on additional documents, information or things, further analysis, and/or in light of subsequent events in the litigation, such as rulings by the Court. For example, Vizgen reserves the right to amend these contentions where appropriate, as discovery in this case continues and/or in light of 10x's claim-construction contentions, any modification or clarification of 10x's non-infringement positions and/or invalidity contentions (if any will be provided), any additional non-infringement positions or invalidity contentions from 10x, and/or this Court's claim construction. Vizgen also reserves the right to rely on and introduce information in its infringement contentions in addition to any information provided herein during discovery, at trial, or otherwise.

These contentions are made pursuant to Federal Rule of Evidence 502 and the Protective Order in this case. To the extent that these contentions contain any information that may be protected from discovery under the attorney-client privilege, the work-product doctrine, the common-interest privilege, or any other applicable privilege or immunity, such disclosure is inadvertent and does not constitute a waiver of any such privileged or immunity.

The contentions set forth below are based solely on Vizgen's current knowledge and belief, based on the information concerning 10x's Accused Products that is currently available to Vizgen given the limited discovery that has occurred to date in this action.

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

As described herein, 10x's Accused Products literally or by the doctrine of equivalents infringes each of the asserted claim of the '303 Patent.

I. ACCUSED PRODUCTS AND ASSERTED CLAIMS

10x's manufacture, use, offer for sale, and/or sale within the United States, and/or importation into the United States, of 10x's Xenium In Situ Analysis Technology, which comprises 10x's "Xenium In Situ Analyzer," "Xenium In Situ reagents and panels," and "Xenium Software Solutions," and any prototypes, proof-of-concept, early access, or prior models thereof (collectively, "10x's Accused Products"), and methods of using the same, which are described in Vizgen's Paragraph 4(a) disclosures filed September 15, 2022 (D.I. 43), constitutes infringement under 35 U.S.C. § 271(a), (b), and/or (c), of Claims 1-12 of the '303 patent, either literally or under the doctrine of equivalents.

II. DIRECT INFRINGEMENT

For the reasons stated herein and in Vizgen's First Amended Answer, Defenses, and Counterclaims (D.I. 71), 10x's Accused Products directly infringe each asserted claim of the '303 patent. Vizgen supplies the following claim chart (*see* Exhibit A) identifying specifically where each limitation of each asserted claim is found within 10x's Accused Products. Fact discovery is ongoing, and 10x has failed to provide complete responses to Vizgen's discovery requests. As explained above, 10x has not met its burden of producing (for example) its core technical documents for 10x's Accused Products, nor has 10x produced any source code relating to 10x's Accused Products. Accordingly, the initial infringement charts set forth in Exhibit A are necessarily limited by 10x's selective and incomplete disclosure of information to date regarding 10x's Accused Products. Vizgen expressly reserves the right to supplement and/or amend these initial infringement claim charts after receiving complete responses to Vizgen's discovery

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

requests, including, without limitation, further production of documents and things relating to 10x's Accused Products, and in light of any additional fact or expert discovery.

III. INDIRECT INFRINGEMENT

For the reasons stated herein and in Vizgen's First Amended Answer, Defenses, and Counterclaims (D.I. 71), and subject to further investigation, 10x actively, knowingly, and intentionally encourages and aids its partners, collaborators, customers, and/or end users of the Xenium In Situ Analysis Technology to directly infringe the asserted claims of the '303 patent under 35 U.S.C. § 271(b). 10x has and is actively, knowingly, and intentionally inducing infringement of the '303 patent through a range of activities relating to 10x's Accused Products in the United States. 10x's instructions for the Xenium In Situ Analysis Technology have and will induce others to infringe, and 10x possesses the specific intent to induce and encourage others to infringe the asserted claims of the '303 patent. *See e.g.*, 10X595-00000654-668 ("Xenium Analyzer Site Preparation Survey"); 10X595-00000669-689 ("Xenium In Situ Gene Expression – Protocol Planner"); *Id.* at -00000671 (disclosing that 10X will provide its customers "[a] list of documents to support various steps of the in situ gene expression workflow"); *Id.* at -00000673 (disclosing that 10X will provide "On-Site Training"); *Id.* at -00000677 (disclosing customer manuals such as "Tissue Preparation Guide", "Demonstrated Protocol CG000580"; "Prob Hybridization, Ligation & Amplification For FF & FFPE Samples on Xenium Slides", "User Guide CG000582"; "Xenium Analyzer . . . User Guide CG000584"); *Id.* at -00000681 (disclosing customer user guides such as "Demonstrated Protocol CG000579", "Demonstrated Protocol CG000581", "User Guide CG000582"; "User Guide CG000584"); 10X595-00000690-753 (Xenium In Situ Overview); 10X595-00000784 at Slide 16 (disclosing materials available to customers for FFPE Protocols); *Id.* at Slide 33 (disclosing materials available to customers for FF Protocols); *Id.* at Slide 52 (disclosing user guide available to customers for Xenium In Situ Spatial

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

Profiling); *see also* 10X595-00000423 at -00000498 (disclosing that at least five purchase orders have been booked by customer for 10x's Accused Products).

Additionally, 10x knows or should know that the commercial manufacture, use, offer for sale, and/or sale within the United States, and/or importation into the United States of 10x's Accused Products induces infringement of the '303 patent. 10x also had actual knowledge of the '303 patent. *See, e.g.*, D.I. 71 ¶¶ 270-73.

For the reasons stated herein and in Vizgen's First Amended Answer, Defenses, and Counterclaims (D.I. 71), and subject to further investigation, 10x contributes to the infringement of the asserted claims of the '303 patent under 35 U.S.C. § 271(c) by supplying in the United States products designed for use in practicing the methods claimed in the '303 patent, including 10x's Accused Products, and has and will offer to sell, and sell such technology in the United States. 10x has and will provide its partners, collaborators, customers and/or end-users with 10x's Accused Products, which are specially made or adapted to infringe the '303 patent. 10x has knowledge of such infringing use and knows that 10x's Accused Products are not a staple article or commodity of commerce suitable for substantial non-infringing use, but rather are especially made and/or adapted for use in the direct infringement of the '303 patent. 10x's Accused Products do not have any substantial non-infringing uses.

IV. DOCTRINE OF EQUIVALENTS

As set forth in Exhibit A, each element of claims 1-12 of the '303 patent is literally present in 10x's Accused Products and/or method of use according to the information 10x has produced to date. 10x's documents support literal infringement of the asserted claims for all the reasons discussed in Exhibit A. As of the date of these initial infringement contentions, 10x has not disclosed any non-infringement positions. In the event that 10x contends that its Accused Products do not literally meet any element of the asserted claims, Vizgen reserves the right to demonstrate

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

the presence of a substantial equivalent of such an element and to assert infringement under the doctrine of equivalents.

Vizgen will provide expert testimony about the claimed subject matter according to the expert discovery schedule entered by the Court in this action. To the extent that 10x, in the future, identifies a specific limitation of the asserted claims of the '303 patent that it argues is not literally infringed by 10x's Accused Products, Vizgen will respond with additional details at that time regarding infringement under the doctrine of equivalents.

V. WILLFULNESS

For the reasons stated herein and in Vizgen's First Amended Answer, Defenses, and Counterclaims (D.I. 71), and subject to further investigation, 10x's infringement of the '303 patent is intentional, willful, wanton, deliberate, and egregious. At least prior to the filing of the Complaint, 10x knew or should have known that its making, using, selling, offering to sell, and/or importing 10x's Accused Products has and will constitute an unjustifiably high risk of infringement of the '303 patent. Despite having knowledge of the '303 patent, 10x nevertheless continues making, using, selling, offering to sell, and/or importing the infringing 10x's Accused Products, inducing third parties to operate those products in an infringing manner, and contributing to third parties' infringement. 10x's infringement is intentional, knowingly deliberate, and willful.

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

Dated: November 23, 2022

YOUNG CONAWAY STARGATT &
TAYLOR, LLP

OF COUNSEL:

David Bilsker
Sam Stake
QUINN EMANUEL URQUHART & SULLIVAN,
LLP
50 California Street, 22nd Floor
San Francisco, CA 94111
(415) 875-6600

Kevin Johnson
Victoria Maroulis
Andrew Bramhall
QUINN EMANUEL URQUHART & SULLIVAN,
LLP
555 Twin Dolphin Dr., 5th Floor
Redwood Shores, CA 94065
(650) 801-5000

Angus Chen
QUINN EMANUEL URQUHART & SULLIVAN,
LLP
51 Madison Avenue, 22nd Floor
New York, NY 10010
(212) 849-7000

Patrick D. Curran
Eric D. Wolkoff
QUINN EMANUEL URQUHART & SULLIVAN,
LLP
111 Huntington Avenue, Suite 520
Boston, MA 02199
(617) 712-7100

/s/ Pilar G. Kraman
James L. Higgins (No. 5021)
Pilar G. Kraman (No. 5199)
Rodney Square
1000 North King Street
Wilmington, DE 19801
(302) 571-6600
jhiggins@ycst.com
pkraman@ycst.com

Attorneys for Vizgen, Inc.

OUTSIDE COUNSEL EYES' ONLY

EXHIBIT A

EXHIBIT A – INITIAL INFRINGEMENT CLAIM CHART – US PATENT NO. 11,098,303

U.S. Patent 11,098,303	10x Accused Products
Claim 1: A method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization, comprising:	10x directly infringes and induces and contributes to the infringement of this claim by using and supplying assays and reagents with the Xenium In Situ Analysis Technology (e.g., the Accused Products), which practices all elements of this claim, or equivalents thereof. To the extent the preamble of the '303 Patent is limiting, the Accused Products are used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. The use of the Accused Products includes a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. The Accused Products can be used to identify multiple nucleic acid targets (e.g., expressed genes) in a tissue sample using <i>in situ</i> hybridization of probes that recognize targets. The Xenium Website¹ provides, e.g.: In situ profiling—just the right scale, in the right place[.] With most current spatial technologies, researchers can detect many targets at multicellular resolution, or focus on a very small number of known targets at higher resolution. In situ technology helps close this gap, offering subcellular resolution on key targets and helping generate a more comprehensive understanding of healthy biological processes and disease. Advances in in situ technologies have exponentially increased how many RNAs can be simultaneously studied within a tissue while retaining high cellular resolution. In situ profiling can create a full map of healthy and diseased tissue at subcellular resolution, and identify cell and biomarker diversity to help more accurately predict therapeutic outcomes. In situ is bounding forward the exploration of biology and disease. *** To define targets for a Xenium In Situ study, researchers can leverage 10x Genomics' powerful Chromium and Visium platforms, allowing for the identification and visualization of cell type markers, cell composition,

¹ *Xenium In Situ Analysis Technology*, 10x Genomics, <https://www.10xgenomics.com/in-situ-technology> (last visited on August 30, 2022). After the filing of Vizgen's August 30, 2022 Answer, Defenses and Counterclaims, 10x altered its website, including text and videos, such that some of the content relied upon by Vizgen throughout this claim chart no longer exists on 10x's website.

U.S. Patent 11,098,303	10x Accused Products
	or regional gene expression. Researchers can then use Xenium to dive deeper and create gene expression maps at cellular and subcellular resolution, identifying gene expression patterns in the original tissue. *** Build spatial transcriptome maps with Xenium In Situ. The Xenium workflow starts with sectioning tissues onto a microscope slide. The sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating bright, easy to image signal that has a high signal-to-noise ratio. The slide is then placed in the Xenium In Situ Analyzer where the sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section. The Xenium Website (Nov. 2022)² also evidences that the Xenium In Situ platform can be used to identify multiple nucleic acid targets (e.g., expressed genes) in a tissue sample using in situ hybridization of probes that recognize targets. The Xenium Website (Nov. 2022) provides: The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section.

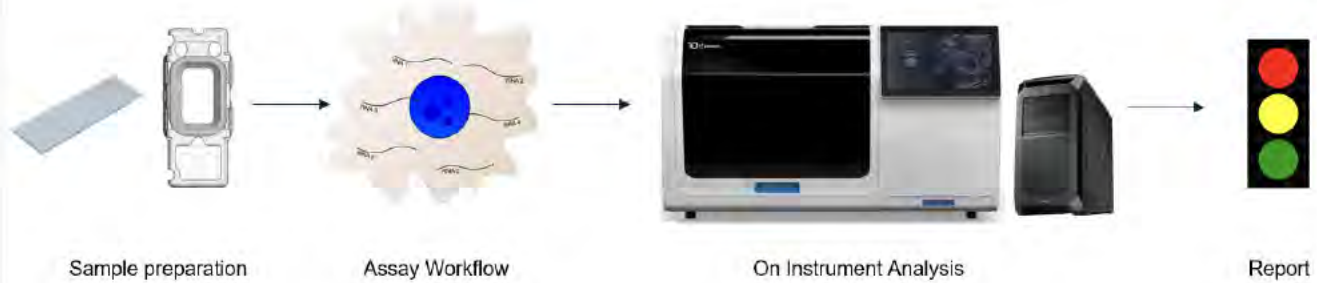
² *Xenium In Situ Analysis Technology*, 10x Genomics, <https://www.10xgenomics.com/in-situ-technology> (last visited November 17, 2022).

U.S. Patent 11,098,303	10x Accused Products
	The Xenium Website (Nov. 2022) includes a workflow evidencing that the Xenium In Situ platform can be used to identify multiple nucleic acid targets in a tissue sample using in situ hybridization of probes that recognize targets: Embedded in a section of the Xenium Website with the header “How it works[:] Build spatial transcriptome maps with Xenium In Situ,” 10x provides the Xenium Video³ to demonstrate the Accused Products. The voiceover of the Xenium Video provides (at 2:01-2:06), <i>e.g.</i>, that “[a]t the end, we can build a spatial map of the transcripts across the tissue section.”

³ The **Xenium Video** is embedded in the **Xenium Website**.

U.S. Patent 11,098,303	10x Accused Products
	Moreover, an article by 10x, Janesick A. et al., <i>High resolution mapping of the breast cancer tumor microenvironment using integrated single cell, spatial and in situ analysis of FFPE tissue</i>, BioRxiv, (“Janesick (2022)”) published on October 7, 2022 (produced as 10X595-00000001), further demonstrates the use of the Accused Products by 10x to identify multiple nucleic acid targets in a tissue sample using in situ hybridization of probes that recognize targets. The named authors of Janesick (2022) include “10x Development Teams” whose members are listed in Janesick (2022)’s Supplementary Information. The Supplementary Information identifies “10x Genomics team members who all contributed to the development of technologies used in” Janesick (2022). These members include, <i>e.g.</i>, Daniel Gyllborg, lead author of the Gyllborg article discussed herein; Xiaoyan Qian, lead author of the Qian article discussed herein; Malte Kühnemund and Toon Verheyen, named inventors of the ’732 publication discussed herein; and Preyas Shah, sole inventor of the Shah reference discussed herein. Janesick (2022) discloses that 10x used the Accused Products to analyze section of breast cancer tissue and identify 17 different cell types. It further states Xenium allowed 10x “to spatially resolve these cell types and their gene expression profiles with single cell resolution.” It further states that the Accused Products employ a method of building a spatial transcriptomic map of a tissue sample through the use of hybridizing and ligating specific DNA probes to target mRNA. <i>See, e.g.</i> in Figure 1, Janesick (2022) states: The novel Xenium In Situ platform used a microscopy based read-out. A 5 µm tissue section was sectioned onto a Xenium slide, followed by hybridization and ligation of specific DNA probes to target mRNA, followed by rolling circle amplification. The slide was placed in the Xenium Analyzer instrument for multiple cycles of fluorescent probe hybridization and imaging. Each gene has a unique optical signature, facilitating decoding of the target gene, from which a spatial transcriptomic map was constructed across the entire tissue section. 10x’s Xenium Analyzer document (10X595-000000042) further shows that 10x’s Accused Products are used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. <i>See, e.g.</i>, 10X595-000000042 at 10X595-000000044: The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. . . .

U.S. Patent 11,098,303	10x Accused Products
	An optical signature specific to each gene is generated, enabling identification of the target gene. <i>See, e.g.</i>, 10X595-00000042 at 10X595-00000046: The Xenium platform uses targeted panels to detect gene and protein expression at the subcellular level. 10x's Product Approval Committee Phase Gate Review Decision Form document (10X595-00000423) further shows that the Accused Products are used to detecting a plurality of different nucleic acid targets within a sample. <i>See, e.g.</i>, 10X595-00000423 at 10X595-00000434: Xenium In Situ High resolution, high-throughput in situ analysis • Hundreds of gene targets • Menu of pre-designed panels • Up to 100 custom targets selectable The Xenium Protocol Walkthrough (10X595-00000784) depicts the workflow for the Accused Products, showing that it is used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. <i>See, e.g.</i>, 10X595-00000784 at slides 5-6:

U.S. Patent 11,098,303	10x Accused Products
	Xenium Platform Overview  The flowchart illustrates the Xenium Platform workflow. It begins with 'Sample preparation', represented by a grey chip and a microfluidic device. An arrow points to 'Assay Workflow', depicted as a gear with a blue center and five segments labeled 'Step 1' through 'Step 5'. Another arrow leads to 'On Instrument Analysis', shown as a large white and black machine. A final arrow points to 'Report', represented by a vertical traffic light with red, yellow, and green lights.  © 10X GENOMICS, INC., 2022 CONFIDENTIAL – DO NOT DISTRIBUTE FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products are used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. <i>See, e.g.</i>, 10X595-00001004 at 10X595-00001006: Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.

U.S. Patent 11,098,303	10x Accused Products
	The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products are used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. <i>See, e.g.</i>, 10X595-00001018 at 10X595-00001020: The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section. The Xenium In Situ Gene Expression – Expertly curated, easily customizable in situ panels document (10X595-00001025) shows that the Accused Products are used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. <i>See, e.g.</i>, 10X595-00001025 at 10X595-00001025: Xenium In Situ is the next-level in situ solution for subcellular profiling of 100s of RNA targets <i>See, e.g.</i>, 10X595-00001025 at 10X595-00001026: Easily add up to 100 custom targets to any panel, letting you address your specific research objectives. <i>See, e.g.</i>, 10X595-00001025 at 10X595-00001028: ~280 genes chosen to target cell types and cell states for human breast tissue. Customize up to 100 genes for a total of 380 genes.

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products
	<i>See, e.g.</i>, 10X595-00001025 at 10X595-00001029: ~248 genes chosen to target cell types and cell states for mouse brain research. Customize up to 100 genes for total of 348 genes. The Xenium In Situ – High-performance in situ made fast and easy document (10X595-00001040) shows that the Accused Products are used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. <i>See, e.g.</i>, 10X595-00001040 at 10X595-00001040: The Xenium In Situ platform enables high-throughput subcellular mapping of 100s of RNA targets <i>See, e.g.</i>, 10X595-00001040 at 10X595-00001041: Thoughtful probe design delivers highly sensitive and specific detection of targets in fresh frozen or FFPE tissues, for data you can trust. <i>See, e.g.</i>, 10X595-00001040 at 10X595-00001044: High specificity is achieved by leveraging probe-based hybridization and ligation to the target. Individual probes are bright enough for robust detection. The Accused Products are further evidenced by patent applications assigned to 10x. By way of non-limiting example, U.S. Patent Pub. No. 2022/0084628, titled “Methods and Systems for Barcode Error Correction,” (“Shah”)⁴, assigned to 10x, also discloses a method for detecting gene target transcripts (e.g., a plurality of nucleic acid targets) in a sample using in situ hybridization. The Accused Products utilize the method for detecting

⁴ Preyas Shah is the inventor listed on U.S. Patent Pub. No. 2022/0084628. In Janesick (2022), Shah is listed as a team member who contributed to the development of 10x’s technologies.

U.S. Patent 11,098,303	10x Accused Products
	gene target transcripts (e.g., a plurality of nucleic acid targets) in a sample using in situ hybridization that is disclosed in Shah. <i>See, e.g., Shah</i> Example 1, [0277] (excerpted below) (<i>all highlighting in Shah added for emphasis</i>): Example 1: In Situ Detection of Target Gene Transcripts [0277] Target gene transcripts are assigned a codeword (e.g., a designed barcode described herein) in a sparse decoding process. In some instances, target gene transcripts are assigned a designed barcode based upon differential gene expression levels as described elsewhere herein. Probes (such as padlock probes) comprising a target binding region and a unique nucleic acid barcode sequence (chemical barcode) associated with a particular target are utilized to detect target gene transcripts. In some instances, chemical The Accused Products utilize, <i>e.g.</i>, technology developed at CartaNA and/or by CartaNA employees such as Mats Nilsson. 10x has stated that Mats Nilsson is working with 10x as a scientific advisor regarding the development and commercialization of the Accused Products. (<i>See Counterclaims at ¶¶ 32-51</i>). Publications by CartaNA or its employees, including Mats Nilsson, disclose aspects of the Accused Products. For example, Gyllborg (2020) provides (at 1-2):⁵ Multiplexed in situ hybridization offers the possibility to explore cellular diversity at subcellular resolution in a upscaled approach. As an example, our lab has developed in situ sequencing (ISS) to be used to detect RNA isoforms, transcriptomic distribution, and cell typing across tissue sections.

⁵ Gyllborg et al., Hybridization-Based In Situ Sequencing (HybISS) for Spatially Resolved Transcriptomics in Human and Mouse Brain Tissue, 48 Nucleic Acids Res. 1, (2020). Mats Nilsson is an author on the Gyllborg publication.

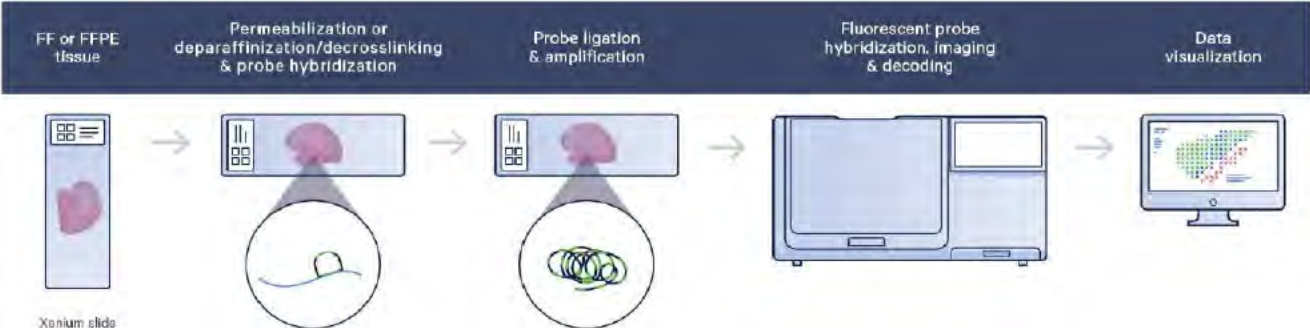
U.S. Patent 11,098,303	10x Accused Products
(a) producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes,	The use of the Accused Products include a method comprising producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes, or an equivalent thereof. <i>See, e.g.,</i>: The Xenium Website, Xenium Video, and Xenium Website (Nov. 2022) show that the use of the Accused Products include a method producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets (<i>e.g.</i>, a cell or tissue specimen). They further describe exposing the sample to a plurality of different encoding nucleic acid probes. The Xenium Website provides (<i>emphasis added</i>): The Xenium workflow starts with sectioning tissues onto a microscope slide. <i>The sections are then treated to access the RNA for labeling with circularizable DNA probes.</i> The Xenium Website (Nov. 2022) provides (<i>emphasis added</i>): Sample processing starts with sectioning tissues onto a Xenium slide. <i>Sections are then treated to access the RNA for labeling with circularizable DNA probes.</i> As further shown in the Xenium Video below, to produce said plurality of first hybridized complexes, such circularizable DNA probes—“padlock” nucleic acid probes—bind multiple different nucleic acid targets (<i>e.g.</i>, RNAs 1-6). <i>E.g.</i>, the Xenium Video (from 0:15 to 0:29, excerpt below) discloses an encoding nucleic acid probe (a padlock probe) exposed to a nucleic acid target in a sample. The Xenium Video voice-over states: “Next, the RNA is labeled with circularizable DNA probes. These probes contain two regions that hybridize to the target RNA, as well as a gene-specific sequence that we refer to as the barcode.”

U.S. Patent 11,098,303	10x Accused Products
	 A diagram showing a horizontal barcode probe with alternating black and green segments. Below it is a wavy green line representing a nucleic acid target. The word "Barcode" is written above the probe. The Xenium Video (at 0:32, excerpt below) shows the formation of a hybridized complex between portions of the encoding probe and the nucleic acid target.  A diagram showing the barcode probe hybridized to the wavy green nucleic acid target. The probe is bent into a loop, with its ends bound to the target. Small green segments on the target are highlighted with white arrows. The Xenium Video (at 1:15, excerpt below) shows multiple encoding nucleic acid probes (padlock probes) within the sample. These multiple encoding probes form hybridized complexes with multiple nucleic acid targets (<i>white arrows added for emphasis to indicate hybridization complexes</i>).

U.S. Patent 11,098,303	10x Accused Products
	The Xenium Website (Nov. 2022) also discloses an encoding nucleic acid probe (a padlock probe) exposed to a nucleic acid target in a sample, shows the formation of a hybridized complex between portions of the encoding probe and the nucleic acid target, and shows multiple encoding nucleic acid probes (padlock probes) within the sample. These multiple encoding probes form hybridized complexes with multiple nucleic acid targets. <i>See, e.g.:</i>

U.S. Patent 11,098,303	10x Accused Products
	FFPE or FF Tissue Permeabilization or deparaffinization/decrosslinking & probe hybridization Probe ligation & enzymatic amplification Load slides onto the Xenium Analyzer Successive rounds of fluorescent probe hybridization, imaging & probe removal Optical signature generation Target gene identification Similarly, Janesick (2022) provides that the Accused Products produce a plurality of hybridized complexes of specific DNA probes and target mRNA via rolling circle amplification. See, e.g., Figure 1 (<i>white arrow added for emphasis to indicate hybridization complexes</i>):

U.S. Patent 11,098,303	10x Accused Products
	Xenium Cassette Probe hybridization and ligation Rolling circle amplification Xenium Analyzer Fluorescent detection and decoding Fluorescent Output Post-Xenium IF and H&E Xenium Janesick (2022) states in the text of Figure 1 (<i>italics added for emphasis</i>): “A 5 μm tissue section was sectioned onto a Xenium slide, followed by <i>hybridization and ligation of specific DNA probes to target mRNA, followed by rolling circle amplification</i>. The slide was placed in the Xenium Analyzer instrument for multiple cycles of fluorescent probe hybridization and imaging.” 10x’s Xenium Analyzer document (10X595-00000042) further shows that the Accused Products produce a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes. <i>See, e.g., 10X595-00000042 at 10X595-00000044 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Workflow overview  The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. <i>The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence.</i> Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The Xenium workflow, which supports both FF and FFPE tissues, starts by sectioning the desired tissues onto a Xenium slide. These sections undergo treatments, such as deparaffinization and permeabilization, to make the RNA and protein analytes accessible. <i>The RNA is labeled with circularizable DNA probes containing two regions that hybridize to the target RNA and a third region that encodes a gene-specific barcode. The two ends of the probes bind the target RNA and are ligated to generate a circular DNA probe.</i> In the event that one part of the probe experiences an off-target binding event, ligation will not occur, suppressing off-target signals and ensuring high specificity. Following ligation, the circularized probe is enzymatically amplified, generating multiple copies of the gene-specific barcode for each RNA binding event.

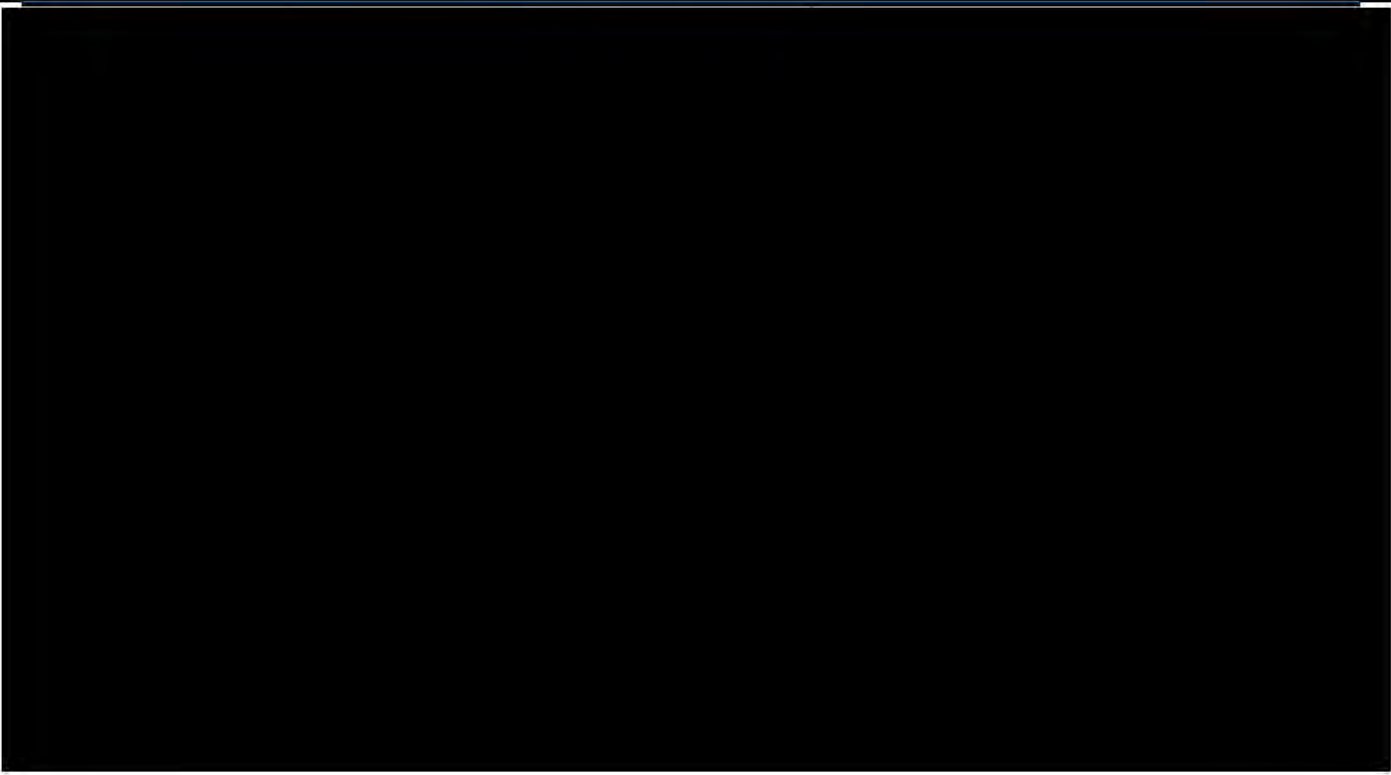
U.S. Patent 11,098,303	10x Accused Products
	10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products produce a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes. <i>See, e.g., 10X595-00000052 at 10X595-00000054 (emphasis added):</i> <i>Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization.</i> On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue. 

U.S. Patent 11,098,303	10x Accused Products

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products
	

OUTSIDE COUNSEL EYES' ONLY

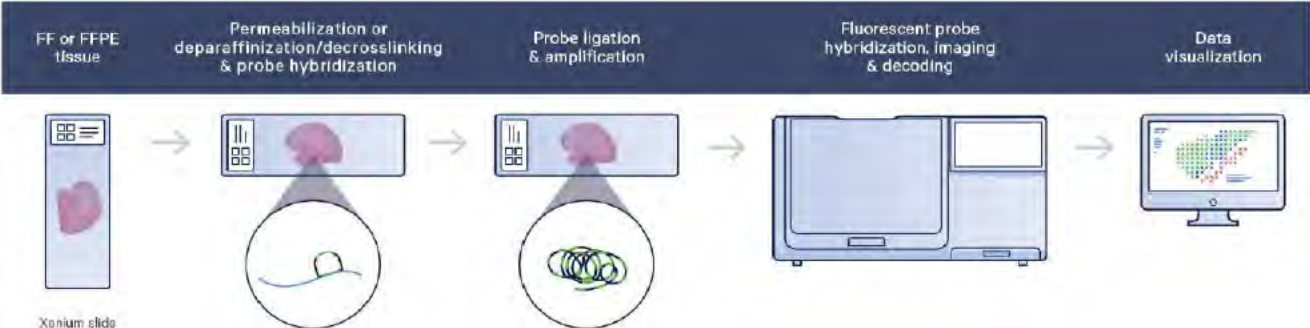
U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	Similarly, Shah [0277] provides, <i>e.g.</i>, that “[p]robes (such as padlock probes) ... are utilized to detect target gene transcripts.”

U.S. Patent 11,098,303	10x Accused Products	
		Example 1: In Situ Detection of Target Gene Transcripts [0277] Target gene transcripts are assigned a codeword (e.g., a designed barcode described herein) in a sparse decoding process. In some instances, target gene transcripts are assigned a designed barcode based upon differential gene expression levels as described elsewhere herein. Probes (such as padlock probes) comprising a target binding region and a unique nucleic acid barcode sequence (chemical barcode) associated with a particular target are utilized to detect target gene transcripts. In some instances, chemical barcodes are a designed barcode sequence as described elsewhere herein. Probes are hybridized to a biological
wherein each of the plurality of encoding nucleic acid probes comprises (i) a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid	The use of the Accused Products includes a method comprising producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes wherein each of the plurality of encoding nucleic acid probes comprises a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid target of the plurality of different nucleic acid targets, or an equivalent thereof. As shown <i>supra</i> and reproduced below, the Xenium Video discloses, <i>e.g.</i>, that each of the plurality of encoding nucleic acid probes comprises a target sequence that specifically hybridizes to a different nucleic acid target of the plurality of different nucleic acid targets. <i>E.g.</i>, the Xenium Video shows (at 0:32) the formation of a hybridized complex between each of the encoding probe's target sequence(s) and a different nucleic acid target.	

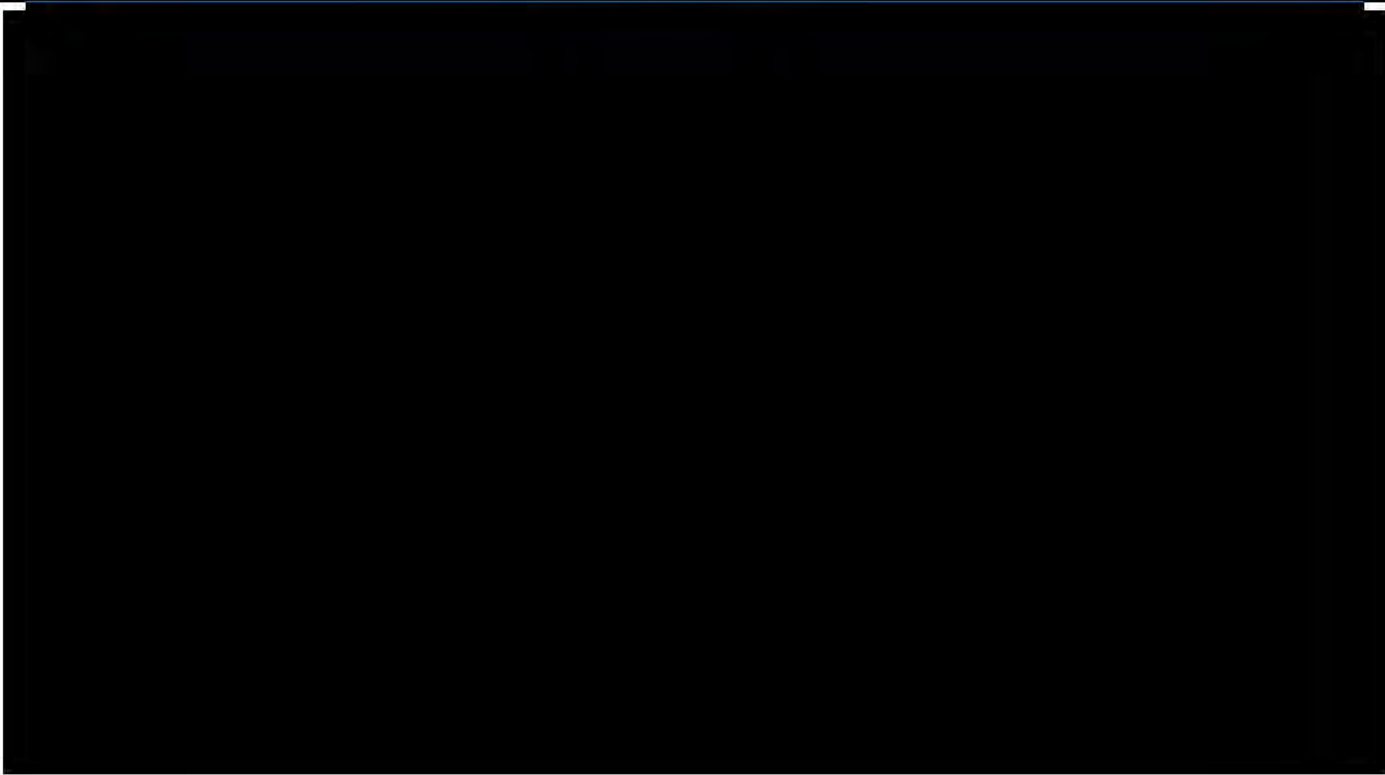
U.S. Patent 11,098,303	10x Accused Products
target of the plurality of different nucleic acid targets and	 The Xenium Website (Nov. 2022) shows the formation of a hybridized complex between each of the encoding probe's target sequence(s) and a different nucleic acid target:  Janesick (2022) also shows the formation of a hybridized complex between each of the encoding probe's target sequence(s) and a different nucleic acid target. <i>See, e.g., Figure 1 (white arrow added for emphasis to indicate hybridization complexes):</i>

U.S. Patent 11,098,303	10x Accused Products
	The diagram illustrates the Xenium workflow. It begins with a 'Xenium Cassette' used for 'Probe hybridization and ligation'. This is followed by 'Rolling circle amplification'. The process then moves to a 'Xenium Analyzer' for 'Fluorescent detection and decoding', which produces a 'Fluorescent Output' image. Finally, a 'Post-Xenium IF and H&E' image is shown. The 'Xenium' logo is visible on the right side of the diagram. Janesick (2022) states in the text of Figure 1 (<i>italics added for emphasis</i>): “A 5 µm tissue section was sectioned onto a Xenium slide, followed by <i>hybridization and ligation of specific DNA probes to target mRNA</i>, followed by rolling circle amplification. The slide was placed in the Xenium Analyzer instrument for multiple cycles of fluorescent probe hybridization and imaging.” Janesick (2022) also states that the probes used for the Accused Products “were designed to contain two complementary sequences that hybridize to the target RNA and a third region encoding a gene-specific barcode, so that the paired ends of the probe bind to the target RNA and ligate to generate a circular DNA probe.” 10x’s Xenium Analyzer document (10X595-00000042) further shows that the Accused Products produce a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes wherein each of the plurality of encoding nucleic acid probes comprises a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid target of the plurality of different nucleic acid targets. <i>See, e.g., 10X595-00000042 at 10X595-00000044 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Workflow overview  The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. <i>The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region.</i> The Xenium workflow, which supports both FF and FFPE tissues, starts by sectioning the desired tissues onto a Xenium slide. These sections undergo treatments, such as deparaffinization and permeabilization, to make the RNA and protein analytes accessible. <i>The RNA is labeled with circularizable DNA probes containing two regions that hybridize to the target RNA and a third region that encodes a gene-specific barcode. The two ends of the probes bind the target RNA and are ligated to generate a circular DNA probe.</i> In the event that one part of the probe experiences an off-target binding event, ligation will not occur, suppressing off-target signals and ensuring high specificity. Following ligation, the circularized probe is enzymatically amplified, generating multiple copies of the gene-specific barcode for each RNA binding event.

U.S. Patent 11,098,303	10x Accused Products
	10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products produce a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes wherein each of the plurality of encoding nucleic acid probes comprises a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid target of the plurality of different nucleic acid targets. <i>See, e.g.,</i> 10X595-00000052 at 10X595-00000054 (<i>emphasis added</i>): Xenium in situ analysis uses <i>circularizable probes specific to target transcripts</i> followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue. 

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products
	

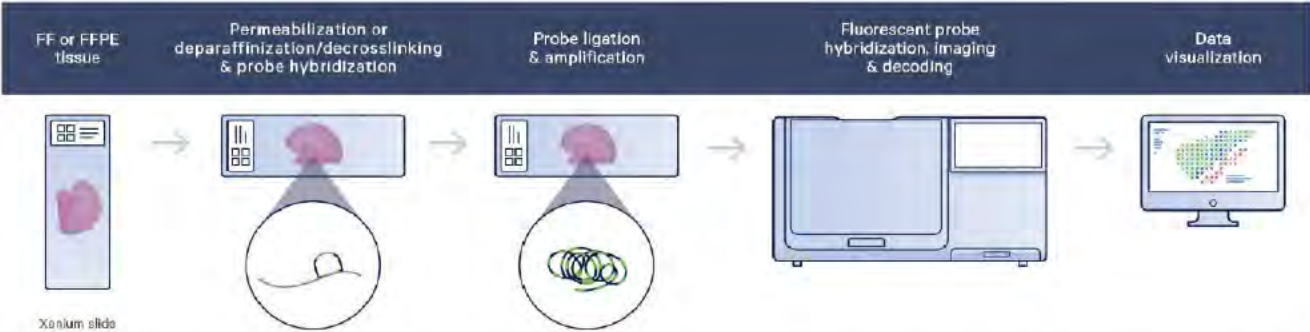
OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products	

U.S. Patent 11,098,303	10x Accused Products
	Gyllborg (2020) also discloses that each of the plurality of encoding nucleic acid probes comprises a target sequence that specifically hybridizes to a different nucleic acid target (the “gene of interest”). <i>E.g.</i>, Gyllborg (2020) at 4 and Figure 1(A) (excerpted below) provides (<i>emphasis added</i>): Similar to previous established ISS methods, PLPs [padlock probes] <i>are designed to target specific cDNA sequences</i> from reversed transcribed mRNA <i>in situ</i> (Figure 1A). An in-house padlock design pipeline produces multiple gene unique target sequences ... The customizable backbone of the PLPs contains two parts: a unique ID sequence that is paired to each gene of interest targeted and a general ‘anchor’ sequence shared by all PLPs. Here we design PLPs to target a 30 nt gene unique cDNA sequence split into two 15 nt arms, and a backbone with a 20 nt ID sequence and a 20 nt anchor sequence, resulting in a final 70 nt long PLP ...

U.S. Patent 11,098,303	10x Accused Products
	A miRNA RT cDNA PLP hybridization Gyllborg (2020) at 4 further describes hybridization of encoding nucleic acid probes at different locations of an identical nucleic acid target (<i>emphasis added</i>): The 20 nt ID sequence is a predetermined sequence unique for the transcript target (e.g. <i>five PLPs targeting five regions of one gene transcript</i> have the same ID sequence).
(ii) one or more read sequences such that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid	The use of the Accused Products include a method comprising producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes wherein each of the plurality of encoding nucleic acid probes comprises one or more read sequences such that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes of the different encoding nucleic acid probes located in its different locations, or an equivalent thereof. The Xenium Website and Xenium Video disclose that each of the plurality of encoding nucleic acid probes comprises one or more read sequences. <i>E.g.</i>, the Xenium Video voiceover states (at 0:50-1:06): “Following this ligation, the circularized probe [<i>e.g.</i>, the encoding probe] is enzymatically amplified, generating multiple copies of the gene-specific barcode [<i>e.g.</i>, one or more read sequences] for each RNA binding event. Later, fluorescent oligos will bind the amplified barcode sequence.” The “multiple copies” of the encoding probe’s barcode sequence are indicated in white arrows below, <i>added for emphasis</i> (excerpt at 0:58).

U.S. Patent 11,098,303	10x Accused Products
probes of the different encoding nucleic acid probes located in its different locations;	The Xenium Website and Xenium Video and CartaNA publications such as Gyllborg (2020) further disclose, <i>e.g.</i>, that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes located in its different locations, either literally or under the doctrine of equivalents. For example, Gyllborg (2020) provides (at 4) (<i>emphasis added</i>): Here, we design PLPs to target a 30 nt gene unique cDNA sequence ... The 20 nt ID sequence is a predetermined sequence unique for the transcript target (e.g. <i>five PLPs targeting five regions of one gene transcript</i> have the same ID sequence). ... The RCP amplicon contains multiple, repetitive ID sequence targets, allowing for the specificity of complimentary hybridization of readout oligonucleotides termed bridge-probes. 10x's Xenium Analyzer document (10X595-00000042) further shows that the Accused Products produce a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes wherein each of the plurality of encoding nucleic acid probes comprises one or more read sequences such that each of the plurality of first

U.S. Patent 11,098,303	10x Accused Products
	hybridized complexes comprises multiple encoding nucleic acid probes of the different encoding nucleic acid probes located in its different locations. <i>See, e.g., 10X595-00000042 at 10X595-00000044 (emphasis added):</i> Workflow overview  The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. <i>The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence.</i> Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The Xenium workflow, which supports both FF and FFPE tissues, starts by sectioning the desired tissues onto a Xenium slide. These sections undergo treatments, such as deparaffinization and permeabilization, to make the RNA and protein analytes accessible. <i>The RNA is labeled with circularizable DNA probes containing two regions that hybridize to the target RNA and a third region that encodes a gene-specific barcode. The two ends of the probes bind the target RNA and are ligated to generate a circular DNA probe.</i> In the event that one part of the probe experiences an off-target binding event, ligation will not occur, suppressing off-target signals and ensuring high specificity. Following ligation, the circularized

U.S. Patent 11,098,303	10x Accused Products
	probe is enzymatically amplified, generating multiple copies of the gene-specific barcode for each RNA binding event. 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products produce a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes wherein each of the plurality of encoding nucleic acid probes comprises one or more read sequences such that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes of the different encoding nucleic acid probes located in its different locations. <i>See, e.g., 10X595-00000052 at 10X595-00000054 (emphasis added):</i> Xenium in situ analysis uses <i>circularizable probes specific to target transcripts</i> followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products	

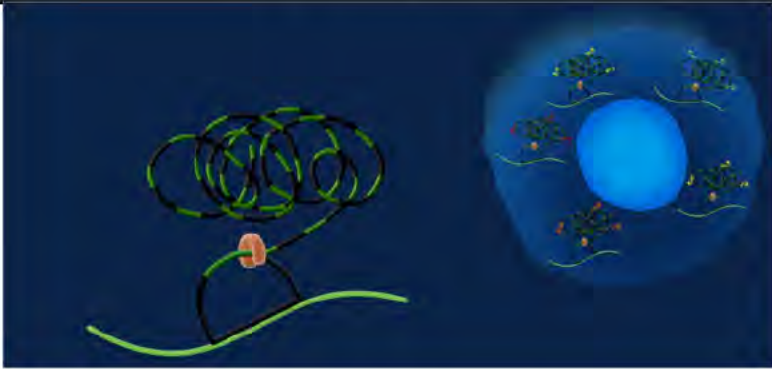
U.S. Patent 11,098,303	10x Accused Products
	Moreover, by way of non-limiting example, U.S. Patent Publication No. 2021/0198723 (“the ’723 Publication”),⁶ titled “Methods of Detecting an Analyte” and assigned to 10x Genomics, Inc., similarly discloses that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes located in its different locations, <i>e.g.</i>, at [0147]:

⁶ U.S. Patent Publication No. 2021/0198723 to Kühnemund & Verheyen (“Methods of Detecting an Analyte”). The applicant of the ’723 publication’s application is CartaNA AB. As mentioned above, the Accused Products utilize, *e.g.*, technology developed at CartaNA and/or by CartaNA employees such as Mats Nilsson.

U.S. Patent 11,098,303	10x Accused Products
	[0147] Firstly, multiple target nucleic acid molecules may be provided for each target analyte. In some embodiments, for example, multiple target nucleic acid molecules may be provided in the form of multiple probes, e.g. hybridization probes, or antibody-based or other probes comprising a nucleic acid molecule, for each target analyte. Each of the multiple probes may represent, or may comprise, or lead to the generation of, a target nucleic acid molecule. By way of representative example, multiple padlock probes, each comprising the same marker sequence, may hybridize to a single nucleic acid target analyte. Thus, for example, if 3 target Janesick (2022) also states that the probes used for the Accused Products “were designed to contain two complementary sequences that hybridize to the target RNA and a third region encoding a gene-specific barcode, so that the paired ends of the probe bind to the target RNA and ligate to generate a circular DNA probe.” Janesick (2022) further states that (<i>emphasis added</i>): <i>The mRNAs were targeted by the 313 probes described above and two negative controls: 1) probe controls to assess non-specific binding and 2) genomic DNA (gDNA) controls to ensure the signal is from RNA. Probe hybridization occurred at 50° C overnight with a probe concentration of 10 nM. After stringency washing to remove un-hybridized probes, probes were ligated at 37° C for two hours. During this step, a rolling circle amplification (RCA) primer was also annealed. The circularized probes were then enzymatically amplified (for one hour at 4° C followed by two hours at 37° C), generating multiple copies of the gene-specific barcode for each RNA binding event, resulting in a strong signal-to-noise ratio.</i>
(b) producing a plurality of readout probe-hybridized complexes by exposing	The use of the Accused Products include a method comprising producing a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal, or an equivalent thereof.

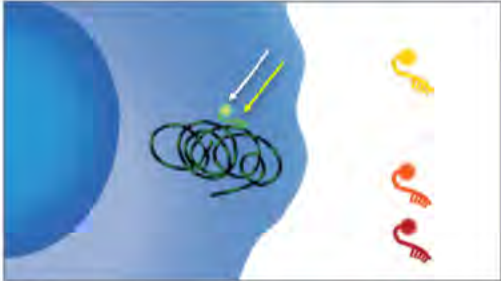
U.S. Patent 11,098,303	10x Accused Products
each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent	The Xenium Website, Xenium Video, and Xenium Website (Nov. 2022) disclose producing a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe. For example, the Xenium Website states: The slide is then placed in the Xenium In Situ Analyzer where the sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. The Xenium Website (Nov. 2022) also states: Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. Janesick (2022) also discloses: The circularized probes were then enzymatically amplified (for one hour at 4° C followed by two hours at 37° C), <i>generating multiple copies of the gene-specific barcode for each RNA binding event</i>, resulting in a strong signal-to-noise ratio. * * * On the Xenium Analyzer, image acquisition was performed in cycles. The reagents, including fluorescently labeled probes for detecting RNA, were automatically cycled in, incubated, imaged, and removed by the instrument. Following the binding of fluorescent oligos to the amplified barcode sequence, the sample underwent 15 rounds of fluorescent probe hybridization, imaging, and probe removal. The Xenium Video discloses that readout probes hybridize to hybridized complexes (<i>e.g.</i>, in excerpted and enlarged portion below). The Xenium Video voiceover script (at 1:01-1:06): “Later, fluorescent oligos will bind the amplified barcode sequence.”

OUTSIDE COUNSEL EYES' ONLY

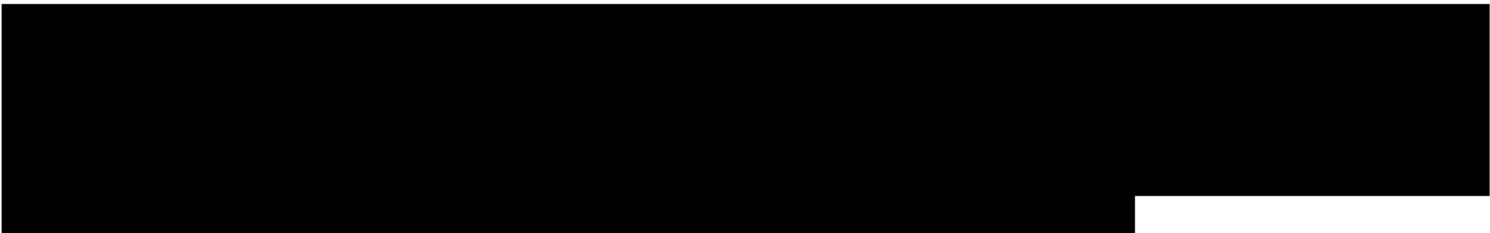
U.S. Patent 11,098,303	10x Accused Products
signaling entity capable of producing a fluorescent signal;	 Exemplary portion enlarged (<i>yellow and white arrows added for emphasis</i>):

U.S. Patent 11,098,303	10x Accused Products
	As exemplified above, the Xenium Video discloses the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. Specifically, the readout probe is comprised of a portion that hybridizes to a readout sequence (<i>indicated by added yellow arrows</i>) and a portion that comprises a fluorescent signal (<i>indicated by white arrows</i>). These two portions of the readout probe design can also be seen in the Xenium Video, e.g., at 1:40 (<i>arrows added for emphasis</i>):

U.S. Patent 11,098,303	10x Accused Products
	The Xenium Website (Nov. 2022) similarly shows that readout probes hybridize to hybridized complexes and shows the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. Specifically, the readout probe is comprised of a portion that hybridizes to a readout sequence (<i>indicated by added yellow arrow</i>) and a portion that comprises a fluorescent signal (<i>indicated by white arrow</i>). These two portions of the readout probe design can be seen below:

U.S. Patent 11,098,303	10x Accused Products
	Sucessive rounds of fluorescent probe hybridization, imaging & probe removal  10x's Xenium Analyzer document (10X595-00000042) further shows that the Accused Products produce a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00000042 at 10X595-00000044-45 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Workflow overview The diagram illustrates the Xenium workflow in five sequential steps, each with an icon and a label above it. Step 1: 'FF or FFPE tissue' with an icon of a 'Xenium slide' showing a tissue section. Step 2: 'Permeabilization or deparaffinization/decrosslinking & probe hybridization' with an icon showing a magnified view of a cell with a probe hybridizing to RNA. Step 3: 'Probe ligation & amplification' with an icon showing a magnified view of a cell with a circular DNA probe. Step 4: 'Fluorescent probe hybridization, imaging & decoding' with an icon of a laboratory instrument. Step 5: 'Data visualization' with an icon of a computer monitor displaying a data map. Arrows connect the icons from left to right. The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. <i>The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos</i>, creating a bright, easy-to-image signal with a high signal-to-noise ratio. The sample undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products produce a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically

U.S. Patent 11,098,303	10x Accused Products
	hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g.</i>, 10X595-00000052 at 10X595-00000054 (<i>emphasis added</i>): <i>Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization.</i> On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue. 

U.S. Patent 11,098,303	10x Accused Products

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

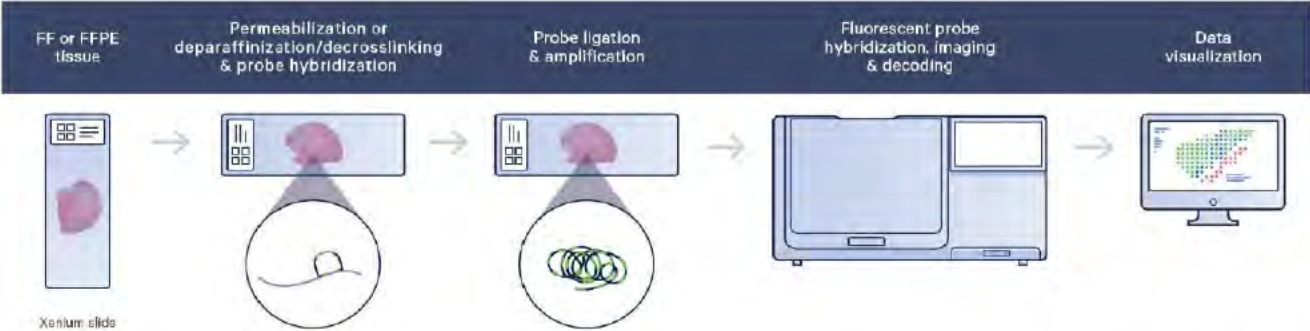
U.S. Patent 11,098,303	10x Accused Products
	The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products produce a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00001004 at 10X595-00001006 (emphasis added):</i> <i>Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization.</i> On the Xenium Analyzer, microscope

U.S. Patent 11,098,303	10x Accused Products
	images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue. The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products produce a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00001018 at 10X595-00001020 (emphasis added, including red box and arrows to diagram):</i> The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. <i>Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos</i>, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section.

U.S. Patent 11,098,303	10x Accused Products
	The flowchart illustrates the 10x Accused Products process. It begins with 'FFPE or FF Tissue' (slide with brain), followed by 'Permeabilization or deparaffinization/decrosslinking & probe hybridization' (slide with brain and probe), then 'Probe ligation & enzymatic amplification' (slide with brain and probe). The process then moves to 'Load slides onto the Xenium Analyzer' (analyzer machine). A red box highlights 'Successive rounds of fluorescent probe hybridization, imaging & probe removal' (microscope image). This is followed by 'Optical signature generation' (cell diagram) and 'Target gene identification' (cell diagram with gene labels).
(c) detecting a fluorescent	The use of 10x's Accused Products includes a method comprising detecting a fluorescent signal from each of the plurality of readout probe-hybridized complexes, or an equivalent thereof.

U.S. Patent 11,098,303	10x Accused Products
signal from each of the plurality of readout probe-hybridized complexes;	The Xenium Website, Xenium Video and Xenium Website (Nov. 2022) disclose detecting a fluorescent signal from each of the plurality of readout probe-hybridized complexes. The Xenium Website provides, <i>e.g.</i> (<i>emphasis added</i>): “[t]he slide is then placed in the Xenium In Situ Analyzer where the sample then undergoes successive rounds of <i>fluorescent probe</i> hybridization, <i>imaging</i>, and removal.” (<i>Emphasis added.</i>) The Xenium Video voiceover states (at 1:02-1:05): “[f]luorescent oligos will bind the amplified barcode sequence.” Later, the voiceover provides (at 1:34-1:41): “The sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and probe removal.” The Xenium Video shows that a fluorescent signal can be detected from multiple target complexes (<i>e.g.</i>, at 1:43): The Xenium Website (Nov. 2022) similarly provides, <i>e.g.</i> (<i>emphasis added</i>): “Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and

U.S. Patent 11,098,303	10x Accused Products
	removal.” It further shows that a fluorescent signal can be detected from multiple target complexes (excerpted below): Optical signature generation Janesick (2022) also states: “The slide was placed in the Xenium Analyzer instrument for multiple cycles of fluorescent probe hybridization and imaging. <i>Each gene has a unique optical signature</i>, facilitating decoding of the target gene, from which a spatial transcriptomic map was constructed across the entire tissue section.” (<i>Emphasis added.</i>) The following Figure also shows the detection of fluorescent signals from multiple target complexes: Probe hybridization and ligation Fluorescent detection and decoding Fluorescent Output Gyllborg (2020) Figure 1 (legend) similarly provides that “[e]very cycle consists of hybridizing bridge-probes to RCPs and reading them out with fluorophore conjugated readout detection probes.”

U.S. Patent 11,098,303	10x Accused Products
	10x's Xenium Analyzer document (10X595-00000042) further shows that the Accused Products detect a fluorescent signal from each of the plurality of readout probe-hybridized complexes. <i>See, e.g., 10X595-00000042 at 10X595-00000044-45 (emphasis added):</i> Workflow overview  The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. <i>The sample undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.</i>

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products
	10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products detect a fluorescent signal from each of the plurality of readout probe-hybridized complexes. <i>See, e.g., 10X595-00000052 at 10X595-00000054 (emphasis added):</i> Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. <i>Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i>

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products detect a fluorescent signal from each of the plurality of readout probe-hybridized complexes. <i>See, e.g., 10X595-00001004 at 10X595-00001006 (emphasis added):</i> Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. <i>On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive</i>

U.S. Patent 11,098,303	10x Accused Products
	<i>rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i> The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products detect a fluorescent signal from each of the plurality of readout probe-hybridized complexes. <i>See, e.g., 10X595-00001018 at 10X595-00001020 (emphasis added):</i> The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. <i>Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.</i> Finally, a spatial map of the transcripts is built across the entire tissue section.

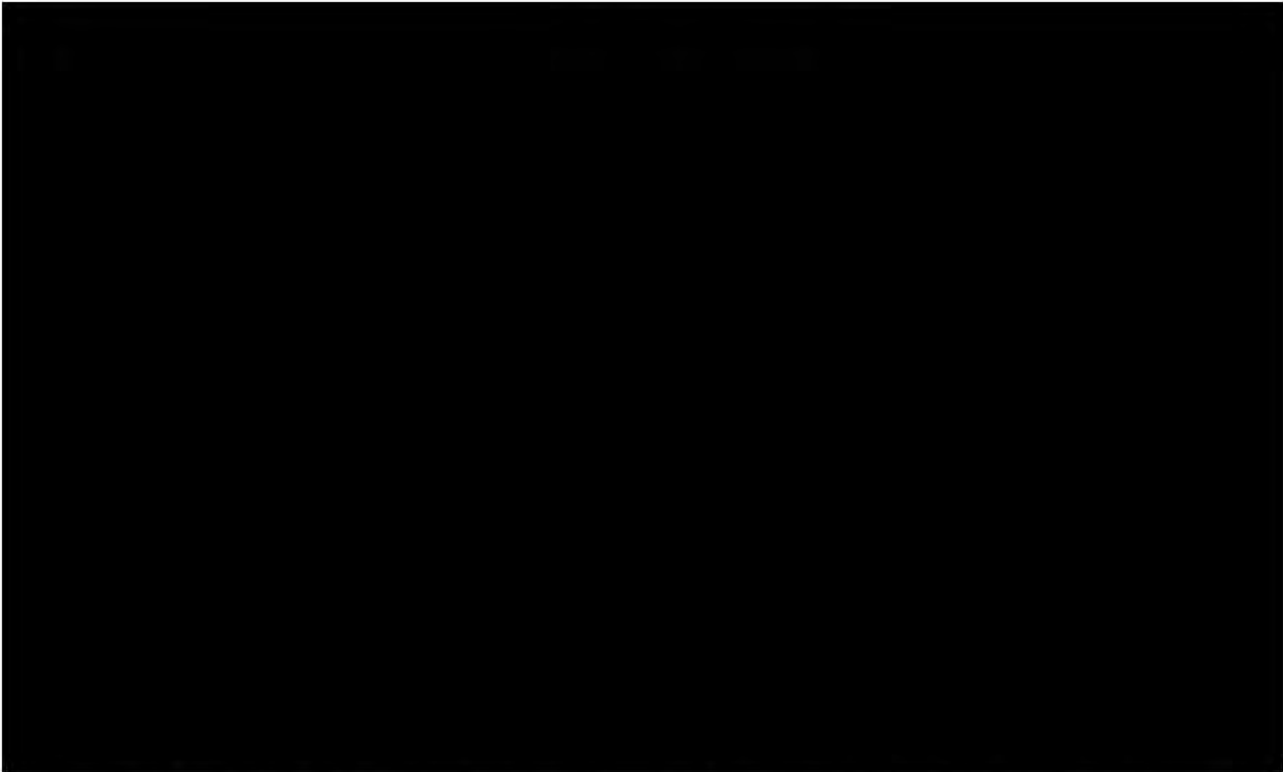
U.S. Patent 11,098,303	10x Accused Products
	FFPE or FF Tissue Permeabilization or deparaffinization/decrosslinking & probe hybridization Probe ligation & enzymatic amplification Load slides onto the Xenium Analyzer Sucessive rounds of fluorescent probe hybridization, imaging & probe removal Optical signature generation Target gene identification
(d) removing the fluorescent	The use of 10x's Accused Products includes a method comprising removing the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes, or equivalent thereof.

U.S. Patent 11,098,303	10x Accused Products
signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes;	The Xenium Website, Xenium Video and Xenium Website (Nov. 2022) disclose removing the fluorescent signal from each of the plurality of readout probe-hybridized complexes by, <i>e.g.</i>, removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes. The Xenium Website provides, <i>e.g. (emphasis added)</i>: “[t]he slide is then placed in the Xenium In Situ Analyzer where the sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and removal.” Similarly, the Xenium Video voiceover provides (at 1:34-1:41): “The sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and probe removal.” The Xenium Website (Nov. 2022) similarly provides, <i>e.g. (emphasis added)</i>: “Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal.” Janesick (2022) also discloses <i>e.g. (emphasis added)</i>: “On the Xenium Analyzer, image acquisition was performed in cycles. The reagents, including fluorescently labeled probes for detecting RNA, were automatically cycled in, incubated, imaged, and removed by the instrument. Following the binding of fluorescent oligos to the amplified barcode sequence, the sample underwent 15 rounds of fluorescent probe hybridization, imaging, and probe removal.” Gyllborg (2020) at 5 (Figure 1 legend) similarly provides, <i>e.g.</i>: “For sequential cycles, bridge-probes are then stripped off to allow for rehybridizing next round of bridge-probes.” 10x’s Xenium Analyzer document (10X595-00000042) further shows that the Accused Products remove the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes. <i>See, e.g.</i>, 10X595-00000042 at 10X595-00000044-45 (<i>emphasis added</i>):

U.S. Patent 11,098,303	10x Accused Products
	Workflow overview The diagram illustrates the Xenium workflow in five sequential steps, each with an icon and a label above it. Step 1: 'FF or FFPE tissue' with an icon of a tissue section on a 'Xenium slide'. Step 2: 'Permeabilization or deparaffinization/decrosslinking & probe hybridization' with an icon showing a tissue section with a magnified view of a cell. Step 3: 'Probe ligation & amplification' with an icon showing a tissue section with a magnified view of a cell containing circular DNA structures. Step 4: 'Fluorescent probe hybridization, imaging & decoding' with an icon of a laboratory instrument. Step 5: 'Data visualization' with an icon of a computer monitor displaying a data map. Arrows connect the icons from left to right. The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. The sample undergoes successive rounds of fluorescent probe hybridization, imaging, <i>and removal</i>. An optical signature specific to each gene is generated, enabling identification of the target gene. 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products remove the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes. <i>See, e.g., 10X595-00000052 at 10X595-00000054 (emphasis added):</i>

OUTSIDE COUNSEL EYES' ONLY

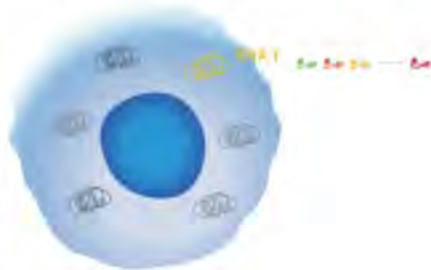
U.S. Patent 11,098,303	10x Accused Products
	Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, <i>and removal</i> creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue. 

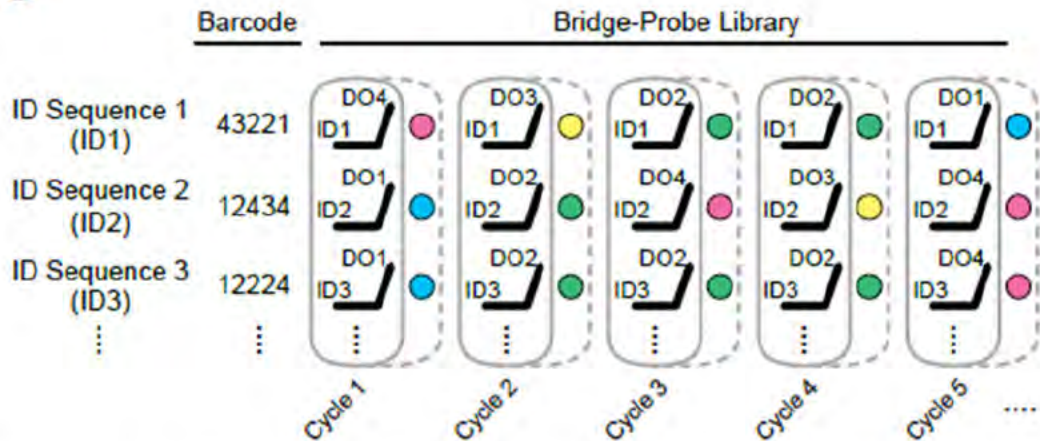
U.S. Patent 11,098,303	10x Accused Products
	 The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products remove the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes. <i>See, e.g., 10X595-00001004 at 10X595-00001006 (emphasis added):</i> Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, <i>which is then removed</i>. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.

U.S. Patent 11,098,303	10x Accused Products
	The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products remove the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes. <i>See, e.g., 10X595-00001018 at 10X595-00001020 (emphasis added):</i> The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, <i>and removal</i>. An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section.
(e) after step (d), producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout	The use of 10x's Accused Products includes a method comprising, after step (d), producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal, or equivalent thereof. The Xenium Website, Xenium Video, and Xenium Website (Nov. 2022) disclose producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe. <i>E.g., the Xenium Website provides that (emphasis added): "[t]he slide is then placed in the Xenium In Situ Analyzer where the sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and removal."</i>

U.S. Patent 11,098,303	10x Accused Products
nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal;	The Xenium Website (Nov. 2022) further provides that: “Up to two slides are then placed in the Xenium Analyzer where the samples undergo <i>successive rounds of fluorescent probe hybridization</i>, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.” Janesick (2022) also discloses e.g. (<i>emphasis added</i>): “On the Xenium Analyzer, image acquisition was performed in cycles. The reagents, <i>including fluorescently labeled probes for detecting RNA, were automatically cycled in, incubated, imaged, and removed by the instrument. Following the binding of fluorescent oligos to the amplified barcode sequence, the sample underwent 15 rounds of fluorescent probe hybridization</i>, imaging, and probe removal.” Similarly, the Xenium Video voiceover states (at 1:34-2:00): The sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and probe removal. Each barcode, and therefore each RNA label, is turned on or off in a specific color at each cycle of this process. This generates an optical signature specific to each gene. By reading out the signature, we can identify the target gene. The Xenium Video further discloses the different readout nucleic acid probes comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. As exemplified below (<i>excerpted and recombined for emphasis</i>), the Xenium Video (at 1:50-2:04) discloses sequential hybridization using different readout probes (associated with different color fluors). These readout probes have the same structure as disclosed above in step (b).

U.S. Patent 11,098,303	10x Accused Products
	The diagram illustrates four panels of RNA sequencing. Each panel shows a target RNA (RNA 1, RNA 2, RNA 4, RNA 5) being hybridized with a series of readout probes labeled 1, 2, 3, ..., N. The probes are color-coded: green, orange, yellow, and red. The RNA is shown as a tangled mass of colored lines. The probes are shown as small, colored, Y-shaped structures. The background is dark blue. Similarly, as exemplified below, the Xenium Website (Nov. 2022) discloses sequential hybridization using different readout probes (associated with different color fluors). These readout probes have the same structure as disclosed above in step (b).

U.S. Patent 11,098,303	10x Accused Products
	↓ Target gene identification  Gyllborg (2020) similarly provides, at 2-4: The unique feature of the ISS method is the probe amplification and the integrated barcoding system that can be decoded across sequential rounds of probing, imaging and stripping. *** Sequential rounds of bridge-probe hybridization, readout detection probing, imaging and stripping allows for a highly multiplexed assay without the round limitation of previous SBL-based ISS methods. Gyllborg (2020)'s Supplementary Figure 1C illustrates this serial readout of fluorescent signals as follows:

U.S. Patent 11,098,303	10x Accused Products
	C  The diagram illustrates a Bridge-Probe Library used for sequencing. It shows three ID sequences (ID1, ID2, ID3) and their corresponding barcodes (43221, 12434, 12224) across five cycles (Cycle 1 to Cycle 5). Each cycle contains a set of bridge probes, each with a specific ID sequence and a barcode. The probes are shown as a series of colored dots (pink, yellow, green, blue) representing different nucleotides. The diagram shows that the same ID sequence can be used across multiple cycles, and the barcode is used to distinguish between them. 10x's Xenium Analyzer document (10X595-00000042) further shows that the Accused Products, after step (d), produces a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00000042 at 10X595-00000044-45 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Workflow overview The diagram illustrates the Xenium workflow in five sequential steps, each with an icon and a label above it. Step 1: 'FF or FFPE tissue' with an icon of a tissue section on a 'Xenium slide'. Step 2: 'Permeabilization or deparaffinization/decrosslinking & probe hybridization' with an icon showing a tissue section being treated. Step 3: 'Probe ligation & amplification' with an icon showing a circular DNA probe being amplified. Step 4: 'Fluorescent probe hybridization, imaging & decoding' with an icon of a microscope. Step 5: 'Data visualization' with an icon of a computer monitor displaying a heatmap. Arrows connect the icons from left to right. The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. <i>The sample undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.</i> 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products, after step (d), produces a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences,

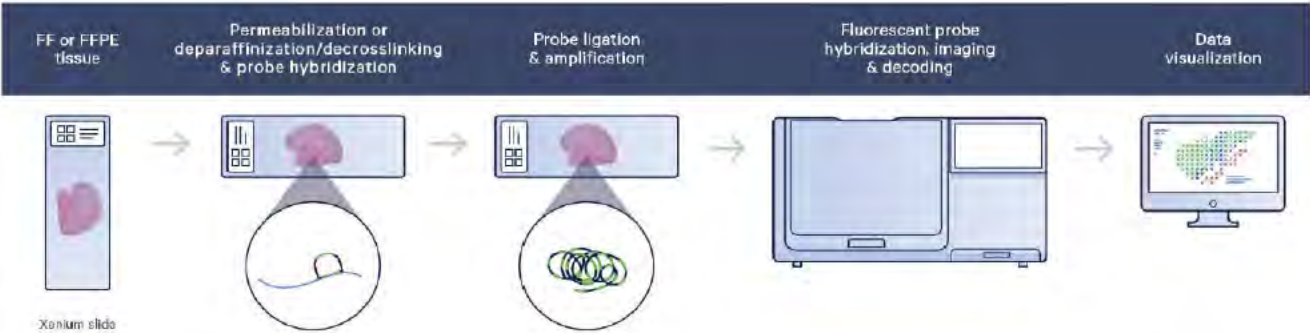
U.S. Patent 11,098,303	10x Accused Products
	and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g.,</i> 10X595-00000052 at 10X595-00000054 (<i>emphasis added</i>): Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. <i>Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i> 

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products, after step (d), it produces a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00001004 at 10X595-00001006 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. <i>On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i> The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products, after step (d), produces a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00001018 at 10X595-00001020 (emphasis added):</i> The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. Up to two slides are then placed in the Xenium Analyzer where the samples undergo <i>successive rounds of fluorescent probe hybridization, imaging, and removal</i>. An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section.
(f) detecting a fluorescent signal from each of the different plurality of	The use of 10x's Accused Products includes a method comprising detecting a fluorescent signal from each of the different plurality of readout probe hybridized complexes, or equivalent thereof. The Xenium Website, the Xenium Video, and the Xenium Website (Nov. 2022) disclose detecting a fluorescent signal from each of the different plurality of readout probe hybridized complexes. <i>See, e.g., supra</i> steps (c) and (e). <i>E.g., the Xenium Website</i> provides that “the sample then undergoes <i>successive rounds</i> of fluorescent probe

U.S. Patent 11,098,303	10x Accused Products
readout probe hybridized complexes;	hybridization, <i>imaging</i>, and removal.” (<i>emphasis added</i>); the Xenium Website (Nov. 2022) provides “samples undergo <i>successive rounds of fluorescent probe hybridization</i>, imaging, and removal” (<i>emphasis added</i>). 10x’s Xenium Analyzer document (10X595-00000042) further shows that the Accused Products detect a fluorescent signal from each of the different plurality of readout probe hybridized complexes. <i>See, e.g.</i>, 10X595-00000042 at 10X595-00000044-45 (<i>emphasis added</i>): Workflow overview  The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. <i>The sample undergoes successive rounds</i>

U.S. Patent 11,098,303	10x Accused Products
	<i>of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.</i> 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products detect a fluorescent signal from each of the different plurality of readout probe hybridized complexes. <i>See, e.g., 10X595-00000052 at 10X595-00000054 (emphasis added):</i> Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. <i>Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i>

OUTSIDE COUNSEL EYES' ONLY

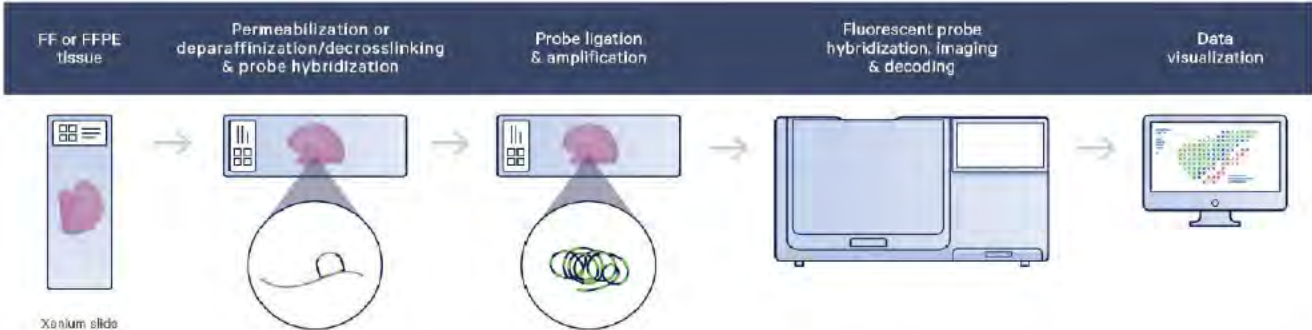
U.S. Patent 11,098,303	10x Accused Products

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products detect a fluorescent signal from each of the different plurality of readout probe hybridized complexes. <i>See, e.g., 10X595-00001004 at 10X595-00001006 (emphasis added):</i> <i>Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i>

U.S. Patent 11,098,303	10x Accused Products
	The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products detect a fluorescent signal from each of the different plurality of readout probe hybridized complexes. <i>See, e.g., 10X595-00001018 at 10X595-00001020 (emphasis added):</i> The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. <i>Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.</i> Finally, a spatial map of the transcripts is built across the entire tissue section.
(g) removing the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling	The use of 10x's Accused Products includes a method comprising removing the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes, or equivalent thereof. The Xenium Website, the Xenium Video, and the Xenium Website (Nov. 2022) disclose removing the fluorescent signal from each of the different plurality of readout probe hybridized complexes by, <i>e.g.</i>, removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes. <i>See, e.g., supra</i> step (d). <i>E.g.</i>, the Xenium Website provides that “the sample then undergoes <i>successive rounds</i> of fluorescent probe hybridization imaging, and <i>removal</i>.” (<i>emphasis added</i>) ; the Xenium Website (Nov. 2022) provides “samples undergo <i>successive rounds</i> of fluorescent probe hybridization, imaging, and <i>removal</i>.” (<i>Emphasis added</i>). 10x's Xenium Analyzer document (10X595-00000042) further shows that the Accused Products remove the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the

U.S. Patent 11,098,303	10x Accused Products
entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes;	fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes. <i>See, e.g., 10X595-00000042 at 10X595-00000044-45 (emphasis added):</i> Workflow overview  The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. The sample undergoes <i>successive rounds</i> of fluorescent probe hybridization, imaging, <i>and removal</i>. An optical signature specific to each gene is generated, enabling identification of the target gene. 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products remove the fluorescent signal from each of the different plurality of readout probe hybridized complexes by

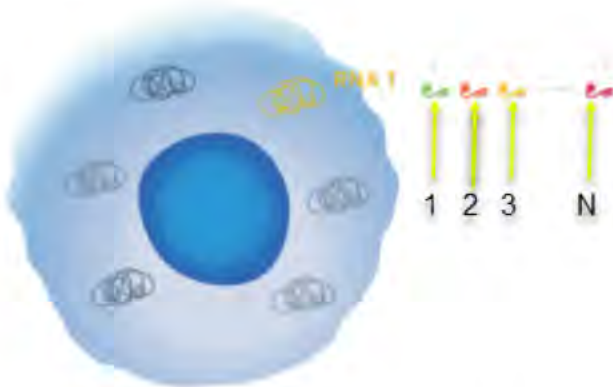
OUTSIDE COUNSEL EYES' ONLY

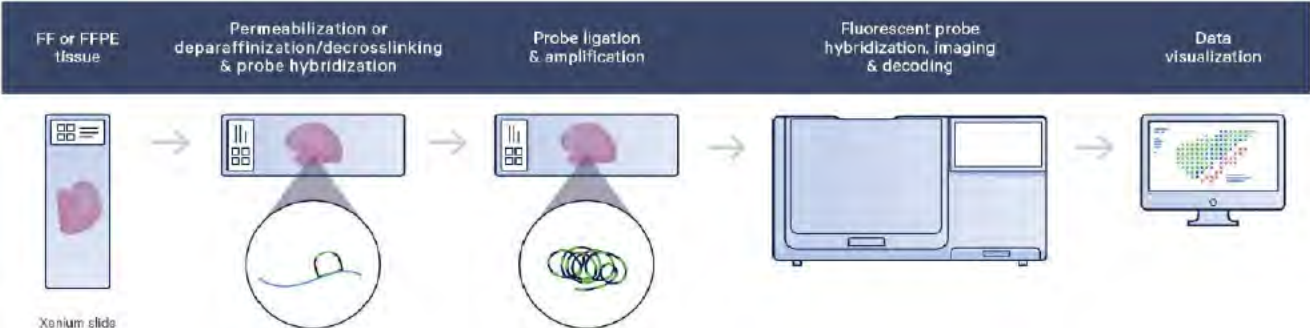
U.S. Patent 11,098,303	10x Accused Products
	inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes. <i>See, e.g.</i>, 10X595-00000052 at 10X595-00000054 (<i>emphasis added</i>): Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, <i>and removal</i> creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue. 

U.S. Patent 11,098,303	10x Accused Products
	The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products remove the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes. <i>See, e.g.,</i> 10X595-00001004 at 10X595-00001006: Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, <i>which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i>

U.S. Patent 11,098,303	10x Accused Products
	The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products remove the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes. <i>See, e.g., 10X595-00001018 at 10X595-00001020 (emphasis added):</i> The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. Up to two slides are then placed in the Xenium Analyzer where the samples undergo <i>successive rounds of fluorescent probe hybridization, imaging, and removal</i>. An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section.
(h) repeating steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout	The use of 10x's Accused Products includes a method comprising repeating steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal, or an equivalent thereof. The Xenium Website, Xenium Video, and Xenium Website (Nov. 2022) disclose repeating steps (e) to (g) for one or more times using a different readout nucleic acid probe. <i>See supra</i> steps (e)-(g); <i>see also supra</i> steps (b)-(d). For example, the Xenium Video (at 1:50-2:04, <i>excerpted and reorganized below for emphasis</i>) discloses sequential hybridization using different readout probes (associated with different color fluors) up to "N" times.

U.S. Patent 11,098,303	10x Accused Products
nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal;	As shown in the Xenium Video, the different readout nucleic acid probes comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See supra</i> step (b). Similarly, the Xenium Website (Nov. 2022) discloses sequential hybridization using different readout probes (associated with different color fluors) up to “N” times (<i>yellow arrows and numbering added for emphasis</i>).

U.S. Patent 11,098,303	10x Accused Products
	Target gene identification  10x's Xenium Analyzer document (10X595-00000042) further shows that the Accused Products repeats steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00000042 at 10X595-00000044-45 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Workflow overview  The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. <i>The sample undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.</i> 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products repeat steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00000052 at 10X595-00000054 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. <i>Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i>

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

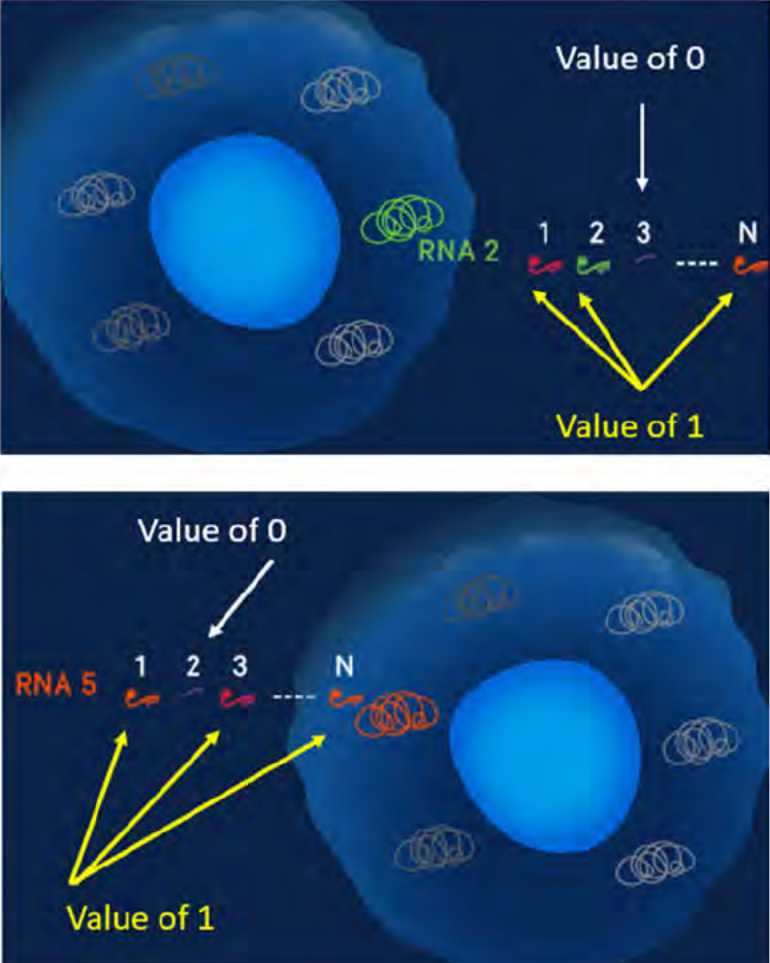
U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	 The In Situ Analysis of Cells in Their True Context document (10X595-00001004) shows that the Accused Products repeat steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00001004 at 10X595-00001006 (emphasis added):</i>

U.S. Patent 11,098,303	10x Accused Products
	Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. <i>On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i> The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products repeat steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See, e.g., 10X595-00001018 at 10X595-00001020 (emphasis added):</i> The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk. Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. <i>Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene.</i> Finally, a spatial map of the transcripts is built across the entire tissue section. Similarly, Shah [0277] discloses, <i>e.g.</i>, probes bound to the target are detected in a decoding process using fluorescently labeled probes in a plurality of detection cycles (<i>e.g.</i>, a series of imaging cycles).

U.S. Patent 11,098,303	10x Accused Products
	probes to bind with the target gene transcripts. Any number of optional processing steps can be performed either pre- or post-probe hybridization (e.g., fixation, permeabilization, washes, hydrogel embedding, probe ligation, amplification, such as rolling circle amplification, etc.). Probes that bound to the target (or an amplified or processed product thereof) are then detected in a decoding process using, e.g., fluorescently labeled probes in a plurality of detection cycles (e.g., series of imaging cycles) to detect a plurality of features and generate a decoded barcode. In some instances, the adaptive error correction methodologies described herein are utilized
(i) producing codewords representing the plurality of different nucleic acid targets at locations within the sample, wherein each of the codeword represents one of the plurality of different nucleic acid targets and comprises	The use of 10x's Accused Products includes a method comprising producing codewords representing the plurality of different nucleic acid targets at locations within the sample, wherein each of the codeword represents one of the plurality of different nucleic acid targets and comprises multiple binary values 1 and 0, wherein a value of 1 is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-hybridized complexes at a respective location within the sample while a value of 0 is obtained when the fluorescent signal is not detected from one of the plurality of readout probe-hybridized complexes or one of the different plurality of readout probe-hybridized complexes at the respective location within the sample, or an equivalent thereof. By way of non-limiting examples: The Xenium Website, Xenium Video, and Xenium Website (Nov. 2022) disclose producing codewords (e.g., an "optical signature") representing the plurality of different nucleic acid targets at locations within the sample. As exemplified below, each codeword comprises multiple binary values 1 and 0, wherein a value of 1 is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-hybridized complexes at a respective location within the sample, while a value of 0 is obtained when the fluorescent signal is not detected from one of the plurality of readout probe-hybridized complexes or one of the different plurality of readout probe-hybridized complexes at the respective location within the sample.

U.S. Patent 11,098,303	10x Accused Products
multiple binary values 1 and 0, wherein a value of 1 is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-hybridized complexes at a respective location within the sample while a value of 0 is obtained when the fluorescent signal is not	The Xenium Website provides, <i>e.g.</i>, that “[a]n optical signature specific to each gene is generated, enabling identification of the target gene.” The Xenium Website (Nov. 2022) provides, <i>e.g.</i>, that “[a]n optical signature specific to each gene is generated, enabling identification of the target gene.” It also shows the production of sequences associated with different nucleic acid targets at their respective location in the sample (excerpted below). Optical signature generation The Xenium Video discloses the production of codewords representing the plurality of different nucleic acid targets from the targets' respective locations within the sample. At 1:54 and 2:02 below (exemplary), the Xenium Video describes the production of sequences associated with different nucleic acid targets at their respective location in the sample (<i>e.g.</i>, RNA 5 and RNA 2 exemplified below).

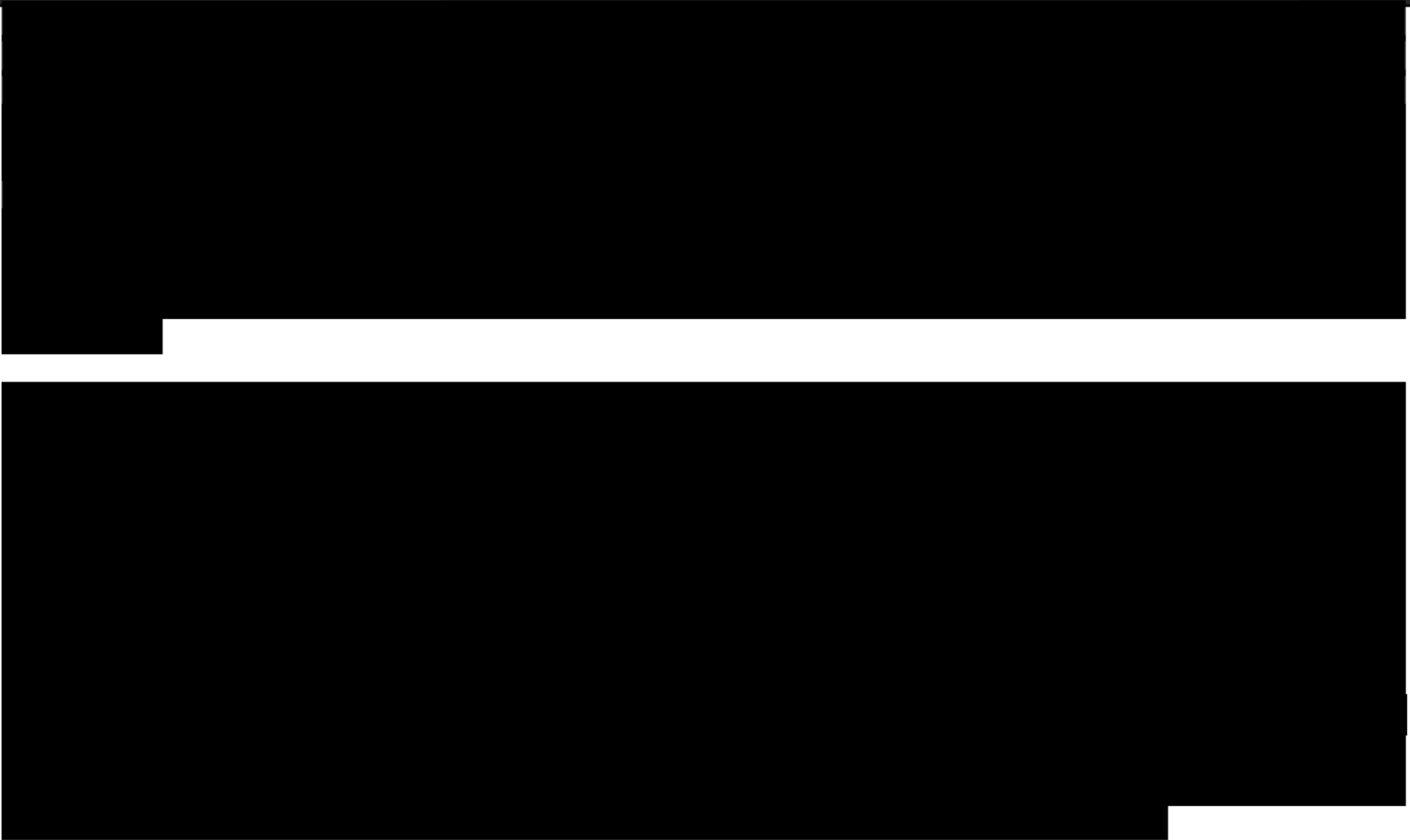
U.S. Patent 11,098,303	10x Accused Products
detected from one of the plurality of readout probe-hybridized complexes or one of the different plurality of readout probe-hybridized complexes at the respective location within the sample;	The Xenium Video states (at 1:43-1:51) (<i>emphasis added</i>): “Each barcode, and therefore each RNA label, is turned <i>on or off</i> in a specific color at each cycle of this process.” As indicated by the yellow and white arrows and text (<i>added for emphasis</i>), these sequences are comprised of either a fluorescent signal (“1”) or the failure to detect a fluorescent signal (“0”). 

U.S. Patent 11,098,303	10x Accused Products
	Janesick (2022) discloses, in part, that the decoded barcodes are determined by the detection of fluorescent intensity—i.e, detection of fluorescence (“signal brightness”) as a value of “1” and no fluorescence as a value of “0”—to create the codewords, or optical signature, that is unique for each gene and used to identify the target gene. <i>Decoding and quality scores.</i> In each cycle, fluorescently-labeled oligonucleotides are bound to amplified barcodes and <i>the fluorescent intensity in each of the four Xenium color channels is measured. Using the fluorescent intensity detected over the 15 Xenium cycles, an optical signature unique for each gene is generated and used to identify a target gene.</i> Each decoded transcript was assigned a raw Q-score, a phred-scaled Quality Value which indicates the probability that the detected object exists and was correctly identified by the decoding algorithm. <i>The Q-score is influenced by many technical factors such as signal brightness, spot localization accuracy, and signal purity.</i> Negative control codewords built into the system ensure that the reported Q-score is accurately calibrated. * * * Output file export. A variety of output files were produced by the on-instrument pipeline. The essential files used downstream were the feature-cell matrix (HDF5 and MEX formats identical to those output by Cell Ranger and Space Ranger for Chromium and Visium data, respectively), the transcripts (listing each mRNA, its 3D coordinates, and a quality score), and the cell boundaries CSV file. Shah [0100] and [0277] similarly disclose, <i>e.g.</i>, that each target gene transcript is assigned a “codeword” (<i>e.g.</i>, a barcode). The barcode comprises, <i>e.g.</i>, the detection of fluorescence signals during the decoding cycles (a value of “1”). Example 1: In Situ Detection of Target Gene Transcripts [0277] Target gene transcripts are assigned a codeword (<i>e.g.</i>, a designed barcode described herein) in a sparse decoding process. In some instances, target gene transcripts *****

U.S. Patent 11,098,303	10x Accused Products
	[0100] As used herein, a “decoding process” is a process comprising a plurality of decoding cycles in which different sets of barcode probes are contacted with target analytes (e.g., mRNA sequences) or target barcodes (e.g., barcodes associated with target analytes) present in a sample or on an array, and used to detect the target sequences or associated target barcodes, or segments thereof. In some instances, the decoding process comprises acquiring one or more images (e.g., fluorescence images) for each decoding cycle. Decoded barcode sequences are then inferred based on a set of physical signals (e.g., fluorescence signals) detected in each decoding cycle of a decoding process. In some instances, the set of physical signals (e.g., fluorescence signals) detected in a series of decoding cycles for a given target barcode (or target analyte sequence) may be considered a “signal signature” for the target barcode (or target analyte sequence). In some instances, a decoding process Paragraph [0011] of Shah also provides that, e.g., the fluorescent signal may not be detected from one of the plurality of readout probe-hybridized complexes during at least one decoding cycle (e.g., a value of “0”). guanine-cytosine (GC) content of about 50%. In some embodiments, the at least one additional characteristic comprises a specified maximum length for homopolymer sub-sequences of 7 nucleotides. In some embodiments, at least one segment of at least one target barcode sequence of the plurality encodes for an “OFF” state that is not visualized in at least one decoding cycle. In some embodiments, the at least one additional property comprises a specified decoding

U.S. Patent 11,098,303	10x Accused Products
	Gyllborg (2020) (at 4) similarly discloses the detection of a fluorescent signal (value of “1”) and not detecting a fluorescent signal (value of “0”) when decoding the identity of a target using a sequential combinatorial decoding scheme (<i>emphasis added</i>): Decoding is performed through sequential combinatorial decoding scheme similar to previous ISS methods, ... [W]ith bridge-probes, this now also allows the possibility for further error correction by <i>including ‘zeroes’ in the decoding scheme</i> where bridge-probes are simply excluded for a subset of genes in different rounds of probing. All these new features integrated into HybISS increases its flexibility and adaptability to suit the design of experiments. By way of further non-limiting examples:

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	
(j) matching the codewords with valid codewords in	The use of 10x's Accused Products includes a method comprising matching the codewords with valid codewords in a codebook by comparing the codewords to the valid codewords in the codebook, and if one of the codewords is not matched with one of the valid codewords in the codebook, applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook, or discarding the one of the codewords, wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets, or an equivalent thereof.

U.S. Patent 11,098,303	10x Accused Products
a codebook by comparing the codewords to the valid codewords in the codebook, and if one of the codewords is not matched with one of the valid codewords in the codebook, applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook,	For example, 10x “expects the Xenium Analyzer will deliver targeted gene expression information at subcellular resolution <i>at high sensitivity</i> . . .”⁷ The Xenium Website (Nov. 2022) also states that “[t]he Xenium platform achieves <i>highly specific and sensitive detection of RNA targets using a probe-based strategy</i>. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk.” By way of non-limiting example, Janesick (2022) discloses below that the decoded barcodes are determined by the detection of fluorescent intensity (i.e, detection of fluorescence as “1” and no fluorescence as “0”) to create the codewords, or optical signature, that is unique for each gene and used to identify the target gene. If a decoded transcript is not correctly matched to a valid codeword in a codebook, an error detection or correction system is applied (e.g., the decoding algorithm) to either match the decoded transcript to a valid codeword or discard the one of the codewords, wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets. <i>Decoding and quality scores.</i> In each cycle, fluorescently-labeled oligonucleotides are bound to amplified barcodes and the fluorescent intensity in each of the four Xenium color channels is measured. <i>Using the fluorescent intensity detected</i> over the 15 Xenium cycles, an optical signature unique for each gene is generated and used to identify a target gene. <i>Each decoded transcript was assigned a raw Q-score, a phred-scaled Quality Value which indicates the probability that the detected object exists and was correctly identified by the decoding algorithm.</i> The Q-score is influenced by many technical factors such as signal brightness, <i>spot localization accuracy, and signal purity. Negative control codewords built into the system ensure that the reported Q-score is accurately calibrated.</i> * * * Output file export. A variety of output files were produced by the on-instrument pipeline. The essential files used downstream were the feature-cell matrix (HDF5 and MEX formats identical to those output by Cell

⁷ 10x Genomics Showcases Robust Innovation Pipeline at Xperience 2022 Event (Feb. 22, 2022), <https://www.prnewswire.com/news-releases/10x-genomics-showcases-robust-innovation-pipeline-at-xperience-2022-event-301487919.html> (*emphasis added*).

U.S. Patent 11,098,303	10x Accused Products
or discarding the one of the codewords, wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets; and	Ranger and Space Ranger for Chromium and Visium data, respectively), the transcripts (listing each mRNA, its 3D coordinates, and <i>a quality score</i>), and the cell boundaries CSV file. Janesick (2022) states above that the “output files” are identical to those output files by Cell Ranger and Space Ranger. On information and belief, the error correction and/or detection system used by 10x as part of 10x’s Accused Product is also identical to the error correction and/or detection method utilized by, <i>e.g.</i>, Cell Ranger, which comprises a method of applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook, or discarding the one of the codewords, wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets. <i>See</i> Gene Expression Algorithms Overview - Software -Single Cell Gene Expression -Official 10x Genomics Support, https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/algorithms/overview#barcode-corr (last visited on November 23, 2022) (disclosing at least “10x barcode correction” and “UMI counting” as part of 10x’s error correction and detection algorithms); <i>see</i> How does Cell Ranger correct barcode sequencing errors? – 10X Genomics, https://kb.10xgenomics.com/hc/en-us/articles/115003822406-How-does-Cell-Ranger-correct-barcode-sequencing-errors- (last visited on November 23, 2022) (disclosing “algorithm to correct putative barcode sequences against the whitelist” utilizing Q scores). By way of non-limiting example, Shah discloses, <i>e.g.</i>, matching the codewords with valid codewords in a codebook by comparing the codewords to the valid codewords in the codebook, and if one of the codewords is not matched with one of the valid codewords in the codebook, applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook or discarding the one of the codewords, and that the codebook comprises the valid codewords of the plurality of nucleic acid targets. Shah [0277] discloses that target gene transcripts are assigned a “codeword” (<i>e.g.</i>, a barcode) in a sparse decoding process, and that these barcodes can be corrected using the error correction methodologies disclosed in Shah. Example 1: In Situ Detection of Target Gene Transcripts [0277] Target gene transcripts are assigned a codeword (<i>e.g.</i>, a designed barcode described herein) in a sparse decoding process. In some instances, target gene transcripts

U.S. Patent 11,098,303	10x Accused Products
	***** series of imaging cycles) to detect a plurality of features and generate a decoded barcode. In some instances, the adaptive error correction methodologies described herein are utilized to generate a corrected barcode. In some instances, the image registration and stitching methodologies described Shah [0158] further discloses that MERFISH is an example of a sparse decoding process. It further discloses the use of MERFISH technology to design barcodes. [0158] As a non-limiting example of a sparse decoding process, a MERFISH (multiplexed error-robust fluorescence in situ hybridization) scheme comprising 16 cycles of decoding was performed (see, e.g., Chen, et al. (2015) "Spatially Resolved, Highly Multiplexed RNA Profiling in

U.S. Patent 11,098,303	10x Accused Products
	Single Cells", Science 348(6233):aaa6090; see also, e.g., U.S. Pat. No. 11,098,303; U.S. Pat. Pub. 20190264270; and PCT/US2019/065857 (WO2020123742A1) for an exemplary description of the MERFISH probes, encoding schemes, and methodologies), where each decoding cycle comprised use of a one-color two-state-chemistry (1C2S) for detecting a binary alphabet including the OFF letter $\mathcal{A}_v = \{\omega, \eta\}$. The decoded barcodes can then be interpreted as binary strings where ω is the letter corresponding to a spatial feature visible in the single color channel. Each designed barcode sequence may be designed to have 4ω and 12η (i.e., 16 barcode segments) with a pairwise Hamming distance $H_D \geq 4$. This set of designed barcodes can be used to encode up to $D=1000$ gene transcripts. To summarize, in this merFISH scheme, designed barcodes may be drawn from starter sequences in $\{\omega, \eta\} \times \dots \times \{\omega, \eta\}$. The designed barcode sequences χ satisfy two conditions: they comprise 4ωs; and exhibit a minimum pairwise Hamming distance $H_D \geq 4$. Algorithm 1, as described above, can be used to construct the designed barcode sequences that satisfy the minimum pairwise Hamming distance $H_D \geq 4$ criterion while enforcing the 4ωs criterion using a prefilter during the iteration of sequence selection or with a post-construction filter. By way of non-limiting example, exemplary paragraphs [0101], [0132], [0252] and Figure 23 of Shah excerpted below disclose matching codewords (e.g., decoded barcodes) with valid codewords in a codebook (e.g., a "list [of] designed barcodes used to barcode . . . nucleic acids" and/or "designed barcode sequence[s]"), which comprises the valid codewords of the plurality of nucleic acid targets, by comparing the codewords to the valid codewords in the codebook.

U.S. Patent 11,098,303	10x Accused Products
	For example, Shah [0252] and Figure 23 disclose that, <i>e.g.</i>, if one of the codewords is not matched with one of the valid codewords in the codebook, applying an error detection or correction system, and, <i>e.g.</i>, matching the one of the codewords with another of the valid codewords in the codebook. <i>E.g.</i>, Shah [0252] discloses that its error correction module may detect errors following detection of barcoded sequences of barcoded nucleic acids, and that it may correct such sequences by replacement with one of the designed barcodes in the list. [0101] As used herein, a “decoded barcode” (or “decoded barcode sequence”) is a digital barcode sequence generated via a decoding process that ideally matches a designed barcode sequence, but that may include errors arising from noise in the synthesis process used to create chemical barcodes and/or noise in the decoding process itself. As ***** [0252] FIG. 23 is a flowchart of an exemplary error correction process 2300 that may be performed by the system 1600 of FIG. 16. In some instances, the error correction module 1620 retrieves a list designed barcodes used to barcode, <i>e.g.</i>, the nucleic acids 1616, in process step 2320. Thus, when the decoding module 1618 detects the barcode sequences of barcoded nucleic acids 16166, in process step 2340, the error correction module 1620 may detect errors and correct each detected and decoded barcode sequence comprising an error by replacement with one of the designed barcodes in the list that has a closest edit distances (<i>e.g.</i>, a Hamming distance) to the detected and decode barcode sequence, in process step 2360. *****

U.S. Patent 11,098,303	10x Accused Products
	<pre> graph TD 2320[Retrieve A List Of Designed Barcodes Used To Barcode Target Analytes] --> 2340[Detect & Decode The Barcode Sequences Of Barcoded Target Analytes] 2340 --> 2360[For Each Decoded Barcode, Correct The Decoded Barcode By Replacement With A Designed Barcode That Has A Closest Hamming Distance To The Decoded Barcode] </pre> FIG. 23 By way of non-limiting example, paragraph [0132] of Shah discloses correction of decoded barcodes comprising errors (<i>e.g.</i>, barcode Y). Shah's correction algorithm includes a designed barcode set χ (<i>e.g.</i>, a codebook) which contains at most one designed barcode within a specified distance (<i>e.g.</i>, barcode Y') for a decoded barcode (<i>e.g.</i>, barcode Y). Implementation of this algorithm (<i>e.g.</i>, matching error-comprising barcode Y to designed barcode set χ to identify corrected barcode Y') results in a set of corrected barcode sequences (γ').

U.S. Patent 11,098,303	10x Accused Products
	[0132] Nearest neighbor correction for decoded barcodes comprising errors may be implemented by starting with the designed barcode set χ which satisfies a condition that the minimum pairwise distances are greater than $2k$. For the query (decoded) barcode Y, there should be at most one designed barcode 10 within a distance k if the distance is a metric. Then, that designed barcode 10 is assigned as the correction for decoded barcode Y. If the error is more than k, the correction is incorrect, leading to a false positive. If there is no designed barcode 10 from the designed barcode set χ within the distance radius k, then the query (decoded) barcode Y remains uncorrected. This may be performed for every decoded barcode sequence in y to obtain a set of corrected barcode sequences y', exemplarily implemented as follows in Algorithm 2: Algorithm 2: Nearest Neighbor Barcode Correction <pre> Result: Set of corrected barcode sequences γ' Initialize empty set of final corrected sequences γ'; Initialize a BKTree storing the available design sequences χ; foreach barcode Y drawn from γ do Find $Y' = \text{neighbor of } Y \text{ within distance } k \text{ in } \chi$; if neighbor found then Insert Y' into γ' else Insert Y into γ'; end end end </pre> Exemplary claim 1 of Shah claims, <i>inter alia</i>, correcting one or more of the decoded target oligonucleotide sequences of the plurality by replacement with a known target oligonucleotide sequence (e.g., applying an error detection or correction system and matching the one of the codewords with another of the valid codewords in the codebook).

U.S. Patent 11,098,303	10x Accused Products
	1. A computer-implemented method for error correction of decoded target barcode sequences comprising: obtaining an image for each decoding cycle of a plurality of decoding cycles to obtain a series of images; detecting, in each image of the series of images, one or more locations of one or more respective barcode probe sequences of a plurality of barcode probe sequences, wherein the one or more respective barcode probe sequences are hybridized or bound to one or more target oligonucleotide sequences or segments thereof; decoding a plurality of target oligonucleotide sequences based on which decoding cycle and for which locations in one or more images of the series of images the one or more respective barcode probe sequences of the plurality are detected to obtain a plurality of decoded target oligonucleotide sequences; and correcting one or more of the decoded target oligonucleotide sequences of the plurality by replacement with a known target oligonucleotide sequence, or proxy thereof, that has a maximum likelihood as computed from a probability distribution that provides probabilities for detecting a given barcode probe sequence at a given location in a given decoding cycle.

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	Gyllborg (2020) (at 2, 4) also provides that its method(s) utilize an error correction system: The decoding scheme for then designing bridge-probes was generated via an in-house MATLAB script that specifies number of positions and minimum base difference between two individual barcodes, hamming distance set to two for five cycles of decoding (https://github.com/Moldia/iss-analysis/tree/master/lib/barcode). ***** Decoding is performed through sequential combinatorial decoding scheme similar to previous ISS methods, where each barcode has a hamming distance of at least two, allowing for correction and probabilistic assignment of barcodes with better accuracy.

U.S. Patent 11,098,303	10x Accused Products
	As described in, e.g., Qian (2020),⁸ CartaNA technology involves error correction using a “codebook” defined by probe barcodes. Qian (2020) provides (at 102, Methods Section 107-108) (<i>emphasis added</i>): In total <i>we designed 755 probes for 99 genes, but used only 161 barcodes out of 1,024 (4⁵) possible combinations to allow error correction</i> ... The barcodes were identified with an epifluorescence microscope with a X20 objective in five rounds of multi-color imaging. ***** The second processing step detected spots⁹ in all images, performed fine alignment of color channels and sequencing rounds, and computed for each spot a position in global coordinates and an intensity vector summarizing the detected fluorescence of that spot in each round and channel. ***** The last step in associating spots to genes consisted of transforming the intensity vectors to gene identities Finally, <i>we associate each spot with a gene using the codebook defined by the probe barcodes</i>. ***** For each probe <i>p</i> with barcode <i>b</i> [...], we concatenate the corresponding crosstalk vectors into a 20-dimensional vector [<i>c</i>] [...]. Each spot is called as belonging to the probe for which this vector is best matches the 20-dimensional intensity vector, ... Spots whose cosine angles fall below a threshold value are taken to represent misreads ... and discarded. In the event the use of 10x's Accused Products does not literally meet this element, then, in the alternative, the use of 10x's Accused Products is insubstantially different from a method matching the codewords with valid codewords in a codebook by comparing the codewords to the valid codewords in the codebook, and if one of the codewords is

⁸ Xiaoyan Qian et al., *Probabilistic Cell Typing Enables Fine Mapping of Closely Related Cell Types In Situ*, 17 Nature Methods 101 (2020). As mentioned above, the Accused Products utilize, e.g., technology developed at CartaNA and/or by CartaNA employees such as Xiaoyan Qian. Qian is mentioned in Janesick (2022) as a team member who contributed to the development of 10x's technologies. On information and believe, technology described in Qian is used for the Accused Products.

⁹ “Spot” refers to RNA spot; e.g., the location of the target. **Qian 2020** (at 12) provides: “We will represent the results of an in situ sequencing experiment via the location x_s and decoded gene g_s of each detected RNA spot s .”

U.S. Patent 11,098,303	10x Accused Products
	not matched with one of the valid codewords in the codebook, applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook, or discarding the one of the codewords, wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets. Therefore, 10x's Accused Products performs substantially the same function in substantially the same way to obtain the same result.
(k) determining the plurality of nucleic acid targets in the sample based on results from the matching step.	The use of 10x's Accused Products includes a method comprising determining the plurality of nucleic acid targets in the sample based on results from the matching step, or an equivalent thereof. <i>See</i> Claim 1 (i) and (j), which are incorporated herein in their entirety. The Xenium Website, the Xenium Video, and the Xenium Website (Nov. 2022) disclose determining the plurality of nucleic acid targets in the sample based on results from the matching step. <i>See, e.g., supra</i> step (e). The Xenium Video voiceover provides (at 2:01-2:06) that, “[a]t the end, we can build a spatial map of the transcripts across the tissue section.” At 2:06, for example, the Xenium Video identifies different nucleic acid targets in the sample:

U.S. Patent 11,098,303	10x Accused Products
	 The Xenium Website (Nov. 2022) also provides that “[f]inally, a spatial map of the transcripts is built across the entire tissue section,” and identifies different nucleic acid targets in the sample: Optical signature generation 

U.S. Patent 11,098,303	10x Accused Products
	Janesick (2022) further provides that “[e]ach gene has a unique optical signature, facilitating decoding of the target gene, from which a spatial transcriptomic map was constructed across the entire tissue section.” It further explains how the decoded transcripts are used to identify the target gene: <i>Decoding and quality scores.</i> In each cycle, fluorescently-labeled oligonucleotides are bound to amplified barcodes and the fluorescent intensity in each of the four Xenium color channels is measured. Using the fluorescent intensity detected over the 15 Xenium cycles, an optical signature unique for each gene is generated and used to identify a target gene. <i>Each decoded transcript was assigned a raw Q-score</i>, a phred-scaled Quality Value which indicates the probability that the detected object exists and was correctly identified by the decoding algorithm. The Q-score is influenced by many technical factors such as signal brightness, spot localization accuracy, and signal purity. Negative control codewords built into the system ensure that the reported Q-score is accurately calibrated. 10x’s Xenium Analyzer document (10X595-00000042) further shows that the Accused Products determine the plurality of nucleic acid targets in the sample based on results from the matching step. <i>See, e.g., 10X595-00000042 at 10X595-00000044-45 (emphasis added):</i> Workflow overview The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded

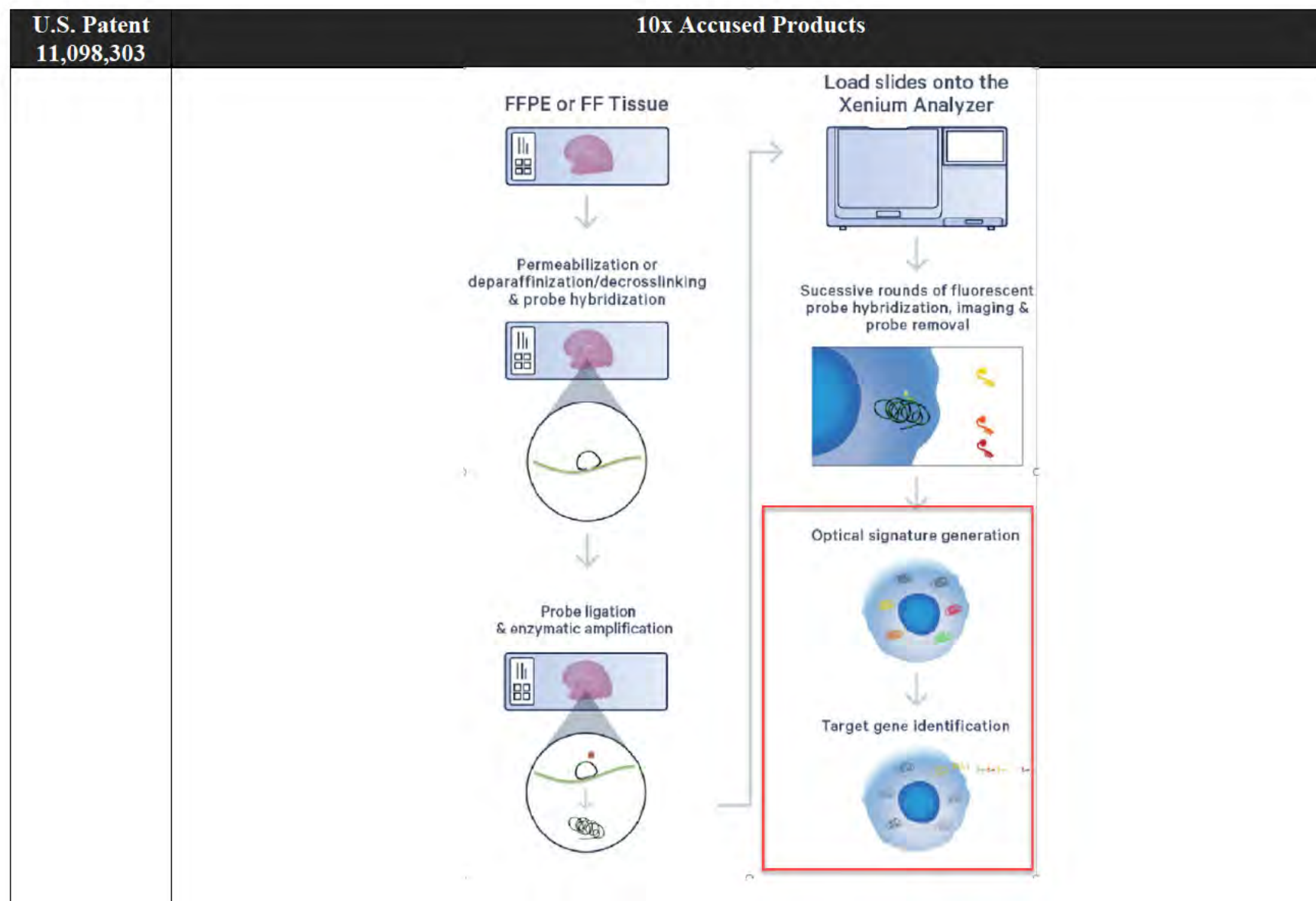
U.S. Patent 11,098,303	10x Accused Products
	antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. The sample undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. <i>An optical signature specific to each gene is generated, enabling identification of the target gene.</i> 10x's In situ analysis of cells in their true context document (10X595-00000052) further shows that the Accused Products determine the plurality of nucleic acid targets in the sample based on results from the matching step. <i>See, e.g., 10X595-00000052 at 10X595-00000054 (emphasis added):</i> Xenium in situ analysis uses circularizable probes specific to target transcripts followed by enzymatic amplification to create a target for fluorescent probe hybridization. On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe, which is then removed. Successive rounds of fluorescent probe hybridization, imaging, and removal <i>creates a unique optical signature that reveals the identity of the RNA at a location within each cell of a tissue.</i> <i>See also 10X595-00001004 at 10X595-00001006 (same);</i>

U.S. Patent 11,098,303	10x Accused Products

U.S. Patent 11,098,303	10x Accused Products
	The High-performance in situ gene expression mapping document (10X595-00001018) shows that the Accused Products determine the plurality of nucleic acid targets in the sample based on results from the matching step. <i>See, e.g.</i>, 10X595-00001018 at 10X595-00001020: The Xenium platform achieves highly specific and sensitive detection of RNA targets using a probe-based strategy. The probes are designed using a proprietary 10x Genomics algorithm that ensures no probe crosstalk.

OUTSIDE COUNSEL EYES' ONLY

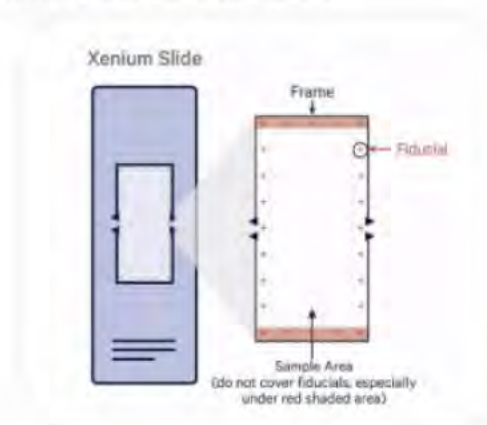
U.S. Patent 11,098,303	10x Accused Products
	Sample processing starts with sectioning tissues onto a Xenium slide. Sections are then treated to access the RNA for labeling with circularizable DNA probes. Ligation of the probes then generates a circular DNA probe which is enzymatically amplified and bound with fluorescent oligos, creating a bright, easy-to-image signal that has a high signal-to-noise ratio. Up to two slides are then placed in the Xenium Analyzer where the samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal. <i>An optical signature specific to each gene is generated, enabling identification of the target gene. Finally, a spatial map of the transcripts is built across the entire tissue section.</i>



U.S. Patent 11,098,303	10x Accused Products
	Xenium In Situ: Internal Overview Training (10X595-00000787) at 10X595-00000903 (“Decoding in line with biochemistry and imaging cycles”; “On-instrument secondary analysis performed after imaging”); <i>id.</i> at 10X595-00000904 (“Xenium analysis output overview”). Shah [0277] similarly discloses using, <i>e.g.</i>, decoded and/or corrected barcodes to identify target gene transcripts in the biological sample. features to generate a decoded barcode. In some instances, the adaptive error correction and image registration and stitching methodologies described herein are utilized to adjust the registration of one or more images of the series of images and align the locations of the features to generate the corrected barcode. Decoded and/or corrected barcodes are then utilized to identify the target gene transcripts in the biological sample.
Claim 2: The method of claim 1,	See Claim 1, which is incorporated herein by reference in its entirety.
wherein the fluorescent signal in step (c) and/or step (f) is detected at a resolution better than 300 nm.	In addition, the use of 10x’s Accused Products includes a method wherein the fluorescent signal in step (c) and/or step (f) is detected at a resolution better than 300 nm, or an equivalent thereof . See Claims 1(c) and 1(f), which are incorporated herein by reference in their entirety. See also, <i>e.g.</i>: Xenium Analyzer (10X595-00000042) at 10X595-00000045 (“The imager uses a high numerical aperture and a fast area scan camera with a low read noise sensor to achieve ~200 nm per-pixel resolution.”); 10X595-00000052 at 10X595-00000055 (“Resolution: ~0.2 microns/pixel.”). Panel Design and On-instrument Analysis (10X595-00000786) at slide 7 (“Each image pixel is roughly 200 x 200 nm”). Janesick (2022) at 10X595-00000028 (states that “[t]he imager is a fast area scan camera featuring a high numerical aperture, a low read noise sensor, and ~200 nm per-pixel resolution.”).

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products
Claim 3: The method of claim 1,	<i>See</i> Claim 1, which is incorporated herein by reference in its entirety.
wherein the sample comprises a cell.	In addition, the use of 10x's Accused Products includes a method, wherein the sample comprising a cell. <i>See, e.g.</i> , Claim 1(pre), which is incorporated herein by reference in its entirety. <i>See also e.g.</i> , Xenium Analyzer (10X595-00000042) at 10X595-00000044 ("The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1)."; "The Xenium workflow, which supports both FF and FFPE tissues, starts by sectioning the desired tissues onto a Xenium slide.").
Claim 4: The method of claim 3,	<i>See</i> Claims 1 and 3, which are incorporated herein by reference in their entirety.
wherein the cell is fixed on a coverslip.	In addition, the use of 10x's Accused Products includes a method, wherein the cell is fixed on a coverslip, or an equivalent thereof. <i>See, e.g.</i> , Claim 1(pre), which is incorporated herein by reference in its entirety. <i>See also, e.g.</i> ,: The Xenium In Situ Internal Overview Training document (10X595-00000787) showing that 10x's Accused Product includes a method, wherein the cell is fixed on a coverslip, or an equivalent thereof. . <i>See, e.g.</i> , 10X595-00000787 at 10X595-00000803, 10X595-00000806:

U.S. Patent 11,098,303	10x Accused Products
	Xenium Sample Preparation Tissue Placement Guidelines  The diagram illustrates the components for Xenium sample preparation. On the left is a blue 'Xenium Slide' with a central white rectangular area. To its right is a 'Frame' with a white interior and a red border. Inside the frame, there are several small black dots representing 'Fiducial' markers. A red circle highlights one of these markers. Below the frame, a red shaded area is labeled 'Sample Area (do not cover fiducials, especially under red shaded area)'. Arrows indicate the alignment of the slide with the frame. Fiducial Guidelines: • Image registration depends on fiducial finding • If a certain number of fiducials are covered, instrument run will fail to initiate • Sample area is outlined by a fiducial frame that should not be covered during tissue placement • If tissue must cover fiducials, avoid top and bottom '+' of frame 10x GENOMICS © 2021 10X GENOMICS, INC. 0001 FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. 11

U.S. Patent 11,098,303	10x Accused Products
	Xenium Sample Preparation Cassette Xenium Cassette: • The Xenium Cassette encases the Xenium Slide and creates a leakproof well for adding reagents ◦ Cassette includes an attached Xenium Gasket to create a seal • Cassette top is off-white in color • Cassette bottom is black in color and has notches for proper alignment on Xenium thermocycler adaptors as well as the on the Xenium analyzer <i>See also</i> 10X595-000000162 at 10X595-00000211-222; 10X595-00000223-266; 10X595-00000267-356; 10X595-00000610 at 10X595-00000621 (depicting sample preparation); 10X595-00000669 at 10X595-00000677 (disclosing the use of a tissue preparation guide to Section FFPE tissues onto Xenium slides); 10X595-00000690 at 10X595-00000709 (depicting sample preparation of tissues onto Xenium slides); 10X595-00000690 at 10X595-00000727-734 (depicting the sample preparation workflow); <i>see also</i> 10X595-00000784 at Slides 16-45 (describing the sample preparation workflow).
Claim 5: The method of claim 1,	<i>See</i> Claim 1, which is incorporated herein by reference in its entirety.
comprising determining the fluorescent	In addition, the use of 10x's Accused Products includes a method comprising determining the fluorescent signal entity by imaging at least a portion of the sample using a light microscope, or an equivalent thereof. <i>See, e.g.,</i> Claim 1(pre), which is incorporated herein by reference in its entirety. <i>See also, e.g.:</i>

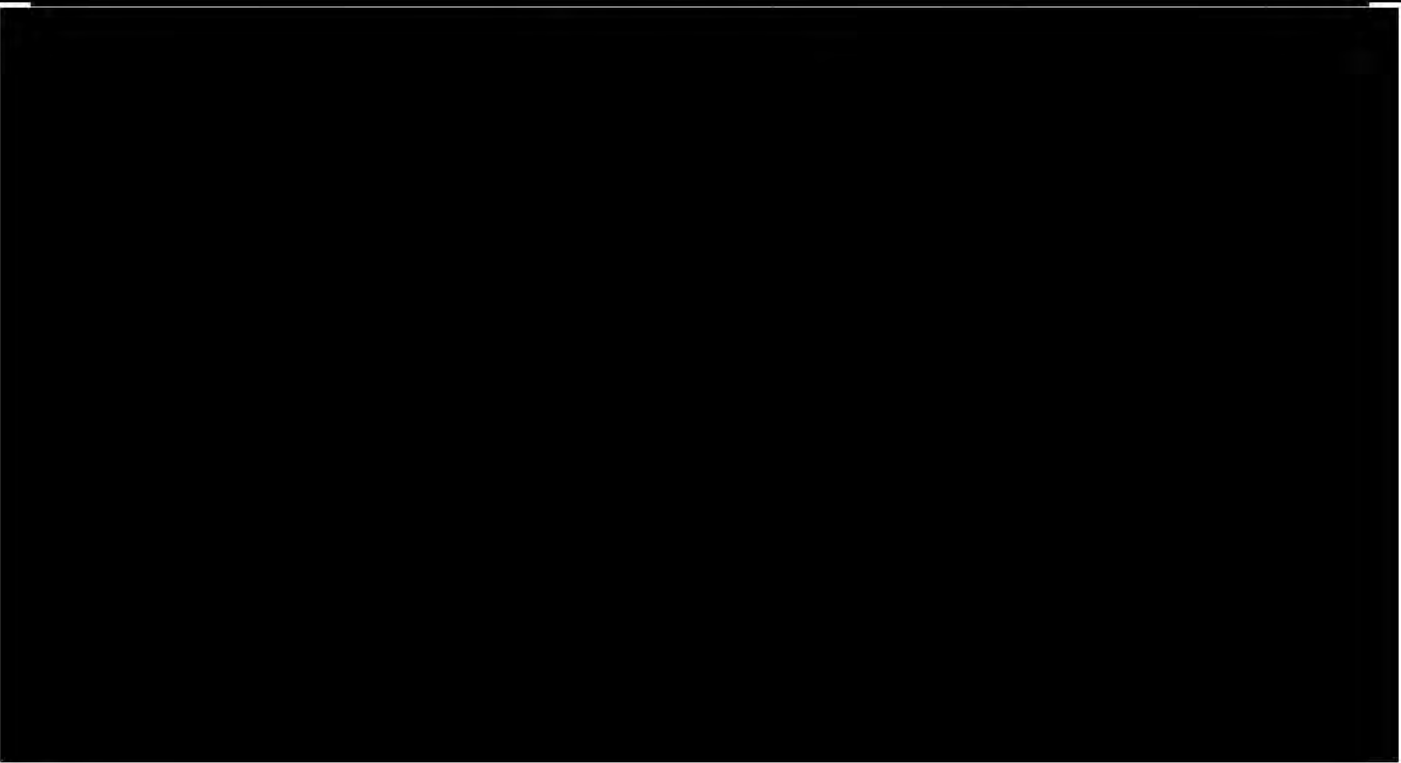
U.S. Patent 11,098,303	10x Accused Products
signal entity by imaging at least a portion of the sample using a light microscope.	In situ analysis of cells in their true context (10X595-00000052) at 10X595-00000054 (“On the Xenium Analyzer, microscope images of the tissue detect the location of each fluorescent probe . . .”); <i>see also</i> (10X595-00001004) at 10X595-00001006 (same); Cassini – Imaging System (10X595-00000076-96) at 10X595-00000080-81 (depicting the imaging system of 10x’s Accused Products, including the use of microscopy); Xenium Analyzer Overview (10X595-00000785) at Slides 7-8, 15 (depicting the imaging system of 10x’s Accused Products, including the use of microscopy). Janesick (2022) at 10X595-000000001 (states that authors used “automated microscopy-based in situ technology using a 313-plex gene panel (Xenium In Situ)”; <i>id.</i> at 10X595-000000004 (“The novel Xenium In Situ platform used a microscopy based read-out.”).
Claim 6: The method of claim 1,	<i>See</i> Claim 1, which is incorporated herein by reference in its entirety.
further comprising determining an abundance and/or spatial distribution of the different nucleic acid targets at locations within the sample.	In addition, the use of 10x’s Accused Products includes a method further comprising determining an abundance and/or spatial distribution of the different nucleic acid targets at locations within the sample, or an equivalent thereof. <i>See, e.g.,</i> Claim 1(pre), which is incorporated herein by reference in its entirety. <i>See also, e.g.,:</i>

U.S. Patent 11,098,303	10x Accused Products
Claim 7: The method of claim 1,	See Claim 1, which is incorporated herein by reference in its entirety.
further comprising removing the readout nucleic acid probes unhybridized to each of the	In addition, the use of 10x's Accused Products includes a method further comprising removing the readout nucleic acid probes unhybridized to each of the plurality of first hybridized complex prior to step (c), or an equivalent thereof. <i>See, e.g.</i>, Claims 1(b) and 1(c), which are incorporated herein by reference in their entirety. <i>See also, e.g.</i>: Xenium Analyzer (10X595-00000042), at 10X595-00000044 ("In the event that one part of the probe experiences an off-target binding event, ligation will not occur, suppressing off-target signals and ensuring high specificity.");

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products
plurality of first hybridized complex prior to step (c).	Xenium Protocol Walkthrough (10X595-00000784) at Slide 47 (“Excess probes are washed away”); Slide 51 (“Post-hybridization wash: Unbound probes are washed by incubating with Xenium post-hyb wash buffer 30 min at 37°C.”)
Claim 8: The method of claim 1,	<i>See</i> Claim 1, which is incorporated herein by reference in its entirety.
wherein the valid codewords form an error-checking or an error-correcting code space.	In addition, the use of 10x’s Accused Products includes a method wherein the valid codewords form an error-checking or an error-correcting code space, or an equivalent thereof. <i>See, e.g.</i> , Claims 1(i) and 1(j), which are incorporated herein by reference in their entirety. <i>See also, e.g.,</i> : 

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products
	

U.S. Patent 11,098,303	10x Accused Products
Claim 9: The method of claim 1,	
wherein in said comparing the codewords to the valid codewords in	

U.S. Patent 11,098,303	10x Accused Products
the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one or more errors.	

U.S. Patent 11,098,303	10x Accused Products
Claim 10: The method of claim 1,	
wherein in said comparing the codewords to the valid codewords in	

U.S. Patent 11,098,303	10x Accused Products
the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one error.	

U.S. Patent 11,098,303	10x Accused Products
Claim 11: The method of claim 1,	
wherein in said comparing the codewords to the valid codewords in	

U.S. Patent 11,098,303	10x Accused Products
the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has two errors.	

U.S. Patent 11,098,303	10x Accused Products
Claim 12: The method of claim 1,	
wherein said comparing the codewords to the valid codewords in the codebook	

U.S. Patent 11,098,303	10x Accused Products
in step (j) comprises comparing the values at each of the positions of the codewords.	

OUTSIDE COUNSEL EYES' ONLY

U.S. Patent 11,098,303	10x Accused Products

CERTIFICATE OF SERVICE

The undersigned hereby certifies that on November 23, 2022, a copy of the foregoing document was served on the persons listed below in the manner indicated:

BY EMAIL

Kenneth L. Dorsney
Cortlan S. Hitch
Morris James LLP
500 Delaware Avenue, Suite 1500
Wilmington, DE 19801
kdorsney@morrisjames.com
chitch@morrisjames.com

Geoffrey M. Raux
Ruben J. Rodrigues
Michael J. Tuteur
Foley & Lardner LLP
111 Huntington Avenue
Suite 2500
Boston, MA 02199

BOST-F-10Xv.Vizgen@foley.com

*Attorneys for President and Fellows of
Harvard College*

Karen Jacobs
Cameron P. Clark
Morris, Nichols, Arsht & Tunnell LLP
1201 North Market Street
P.O. Box 1347
Wilmington, DE 19899
kjacobs@morrisnichols.com
cclark@morrisnichols.com

Samantha Jameson
Kiley White
Ron Pabis
Tensegrity Law Group LLP
8260 Greensboro Dr., Suite 260
McLean, VA 22102

Matthew Powers
Paul Ehrlich
Stefani Smith
Robert Gerrity
Li Shen
Tensegrity Law Group LLP
555 Twin Dolphin Drive, Suite 650
Redwood Shores, CA 94065

10x_Vizgen_Service@tensegritylawgroup.com

Attorneys for 10X Genomics, Inc.

YOUNG CONAWAY STARGATT & TAYLOR, LLP

/s/ *Pilar G. Kraman*

James L. Higgins (No. 5021)

Pilar G. Kraman (No. 5199)

Rodney Square

1000 North King Street

Wilmington, DE 19801

(302) 571-6600

jhiggins@ycst.com

pkraman@ycst.com

Attorneys for Vizgen, Inc.