

EXHIBIT 9

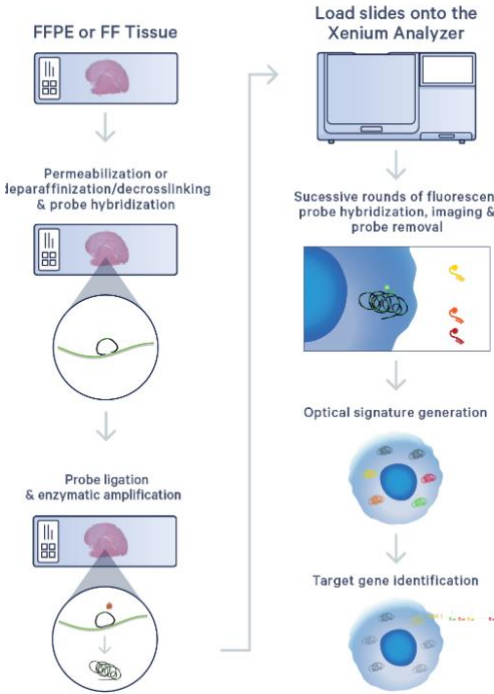
**Exemplary Infringement of the Zhuang '303 Patent by the Xenium In Situ Analysis Technology,
Either Literally or Under the Doctrine of Equivalents**

U.S. Patent 11,098,303	Exemplary Xenium In Situ Analysis Technology
Claim 1: A method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization, comprising:	To the extent the preamble of the Zhuang '303 Patent is limiting, 10x's Xenium In Situ Analysis Technology is used to perform a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. The Xenium In Situ platform can be used to identify multiple nucleic acid targets (<i>e.g.</i>, expressed genes) in a tissue sample using <i>in situ</i> hybridization of probes that recognize targets. The Xenium Website¹ provides, <i>e.g.</i>: 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, or regional gene expression. Researchers can then use

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.

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	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

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

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	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) 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

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

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	Xenium In Situ Analysis Technology. 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.” 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, further demonstrates the use of the Xenium In Situ Analysis Technology 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) et al. 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 Xenium In Situ Analysis Technology 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 Xenium In Situ Analysis Technology employs 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 In Situ Analysis Technology is further evidenced by patent applications assigned to 10x as, on information and belief, 10x seeks to obtain patent protection on this technology.

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	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. On information and belief, 10x’s Xenium In Situ Analysis Technology utilizes the method for detecting 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) (all highlighting in Shah added for emphasis): 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 On information and belief, 10x’s Xenium In Situ Analysis Technology utilizes, <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 10x’s Xenium In Situ Analysis Technology. (See Counterclaims at ¶¶ 32-51). On information

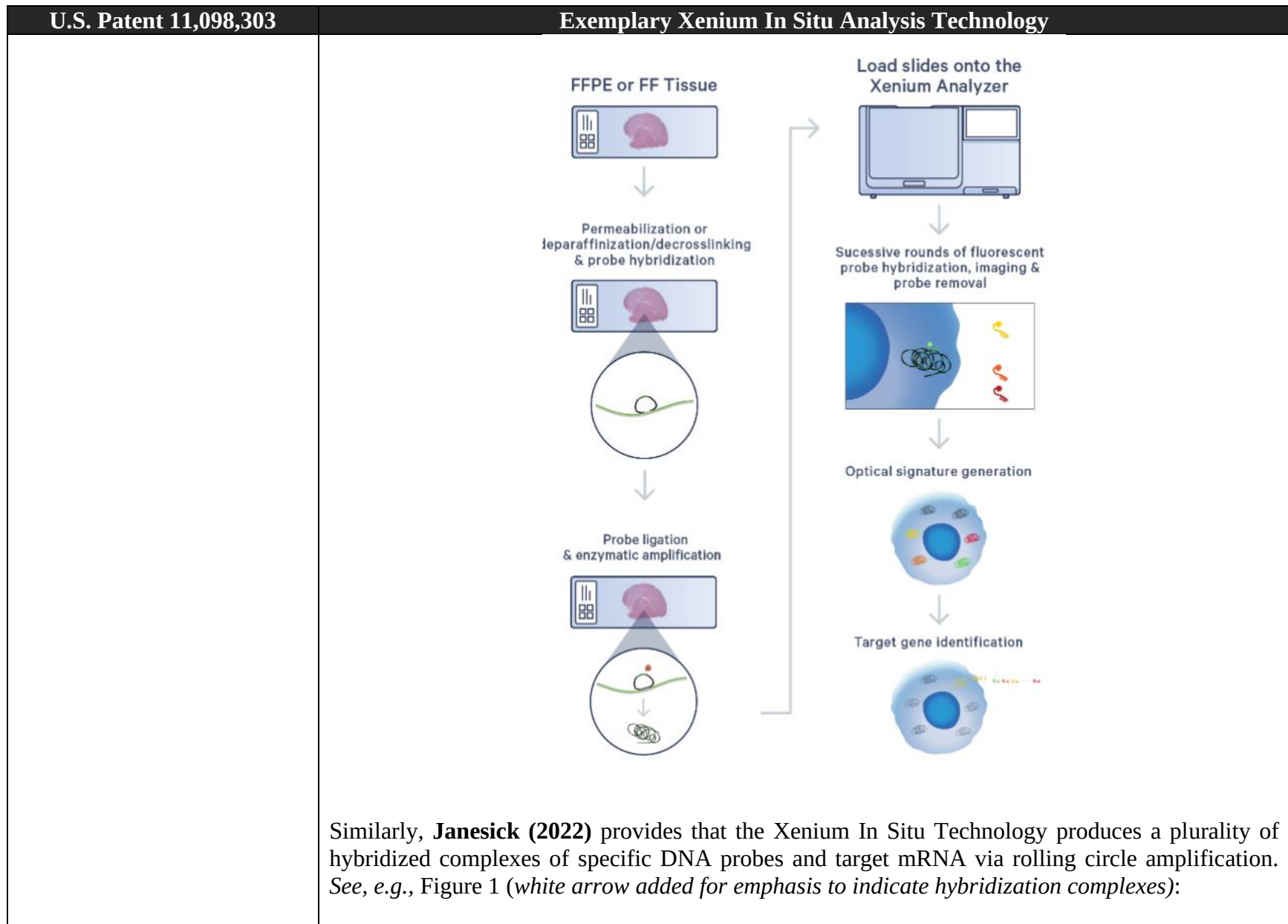
⁴ Preyas Shah is the inventor listed on U.S. Patent Pub. No. 2022/0084628. In Janesick (2022), Shah is listed as a team member who contributed to the development of 10x’s technologies. On information and belief, Preyas Shah contributed to the development of 10x’s Xenium In Situ Analysis Technology.

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	and belief, publications by CartaNA or its employees, including Mats Nilsson, disclose aspects of the Xenium In Situ Analysis Technology. 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.
(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 Xenium Website, Xenium Video, and Xenium Website (Nov. 2022) describe the production of 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:

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.

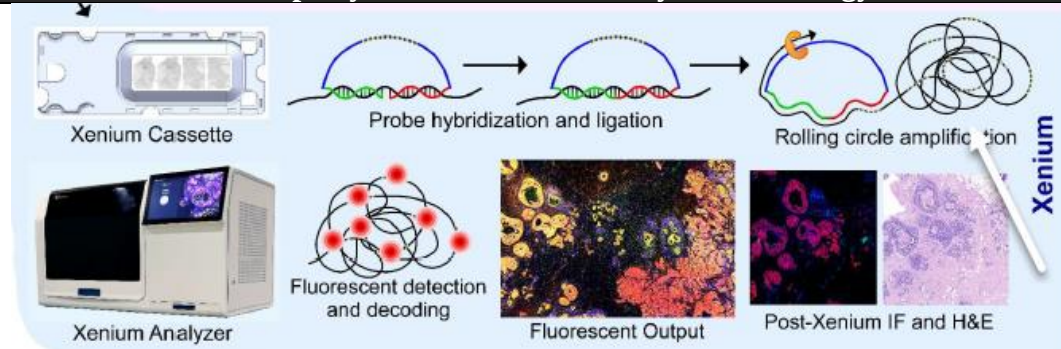
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	“Next, the RNA is labeled with circularizable DNA probes. These probes contain two regions that hybridize to the target RNA, as well as a gene-specific sequence that we refer to as the barcode.”  Barcode The Xenium Video (at 0:32, excerpt below) shows the formation of a hybridized complex between portions of the encoding probe and the nucleic acid target.  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>).

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	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>



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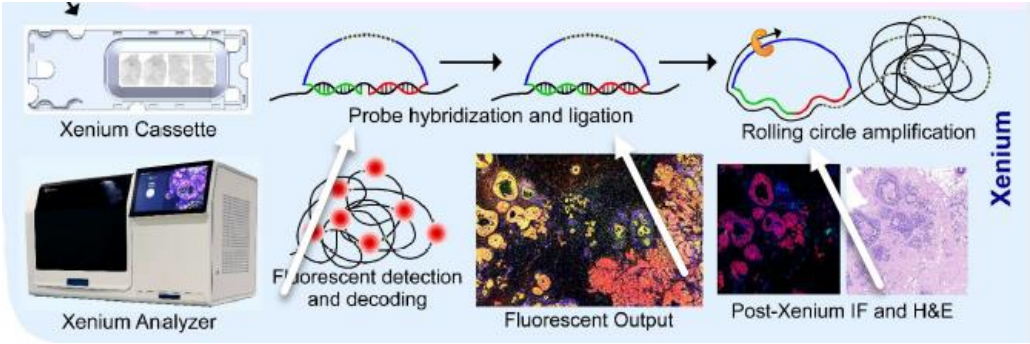
Janesick (2022) states in the text of Figure 1 (*italics added for emphasis*): “A 5 μm tissue section was sectioned onto a Xenium slide, followed by **hybridization and ligation of specific DNA probes to target mRNA, followed by rolling circle amplification**. The slide was placed in the Xenium Analyzer instrument for multiple cycles of fluorescent probe hybridization and imaging.”

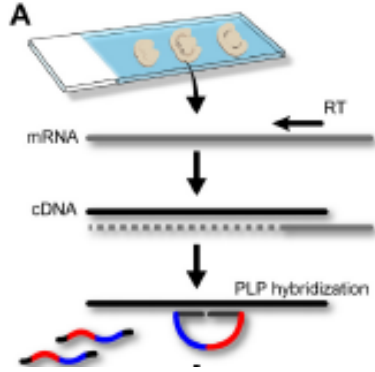
Similarly, **Shah [0277]** provides, *e.g.*, that “[p]robes (such as padlock probes) ... are utilized to detect target gene transcripts.”

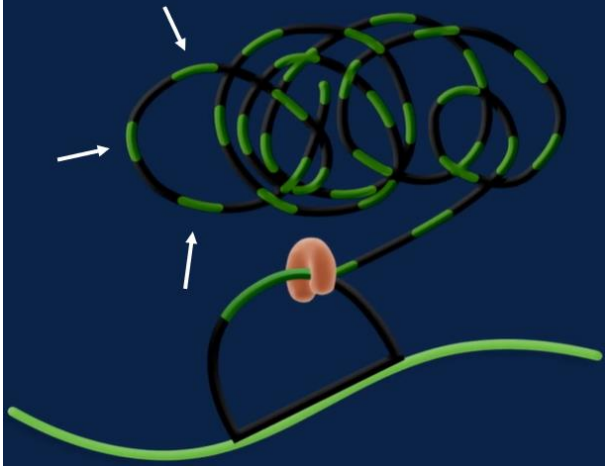
Example 1: In Situ Detection of Target Gene Transcripts

[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

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wherein each of the plurality of encoding nucleic acid probes comprises (i) a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid target of the plurality of different nucleic acid targets and	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. 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:

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	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>  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 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.” Janesick (2022) also states that the probes used for the Xenium In Situ Analysis Technology “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.” 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., Gyllborg (2020)</i> at 4 and Figure 1(A) (excerpted below) provides (<i>emphasis added</i>): Similar to previous established ISS methods, PLPs [padlock probes] are designed to target specific cDNA sequences 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

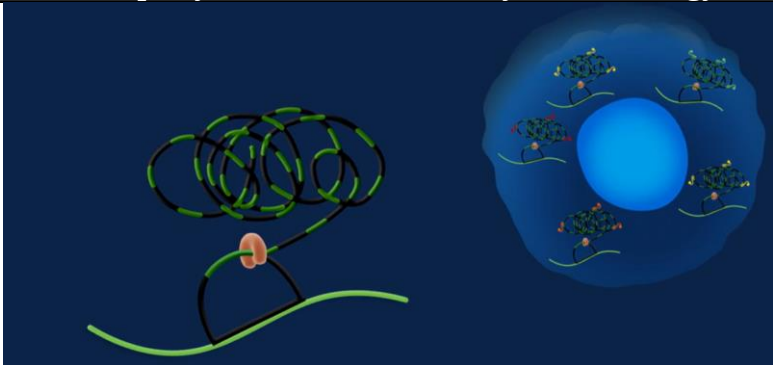
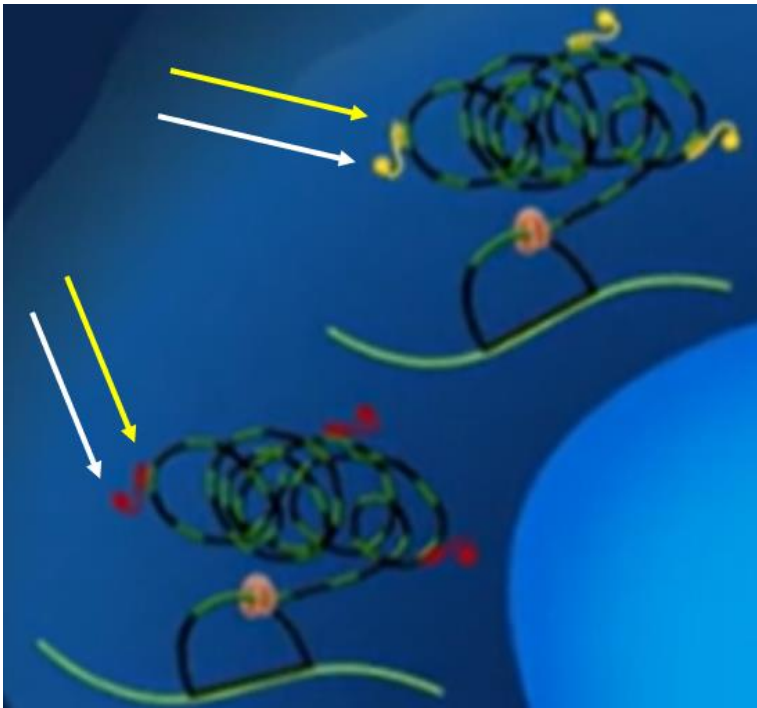
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	to target a 30 nt gene unique cDNA sequence split into two 15 nt arms, and a backbone with a 20 nt ID sequence and a 20 nt anchor sequence, resulting in a final 70 nt long PLP ...  Gyllborg (2020) at 4 further describes hybridization of encoding nucleic 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. five PLPs targeting five regions of one gene transcript 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 probes of the different encoding nucleic acid probes located in its different locations;	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, added for emphasis (excerpt at 0:58).

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	 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. five PLPs targeting five regions of one gene transcript 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. 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. on

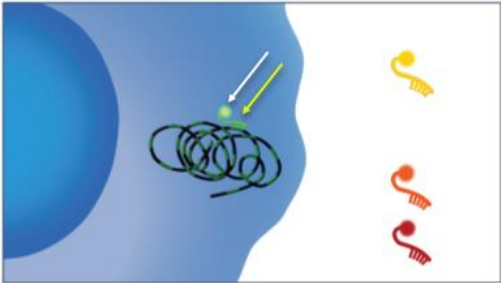
⁶ U.S. Patent Publication No. 2021/0198723 to Kühnemund & Verheyen (“Methods of Detecting an Analyte”). The applicant of the ’723 publication’s application is CartaNA AB. As mentioned above, 10x’s Xenium In Situ Analysis Technology utilizes, *e.g.*, technology developed at CartaNA and/or by CartaNA employees such as Mats Nilsson. On information and belief, 10x’s Xenium In Situ Analysis Technology utilizes the methods disclosed in the ’723 Publication.

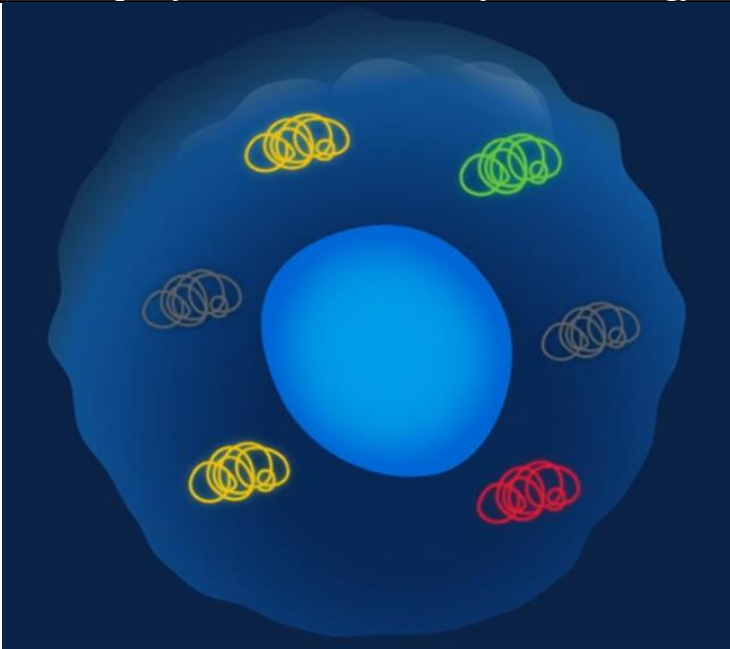
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	information and belief, similarly discloses that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes located in its different locations, <i>e.g.</i>, at [0147]: [0147] Firstly, multiple target nucleic 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, <i>e.g.</i> 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 Xenium In Situ Analysis Technology “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 state that: <i>The mRNAs were targeted by the 313 probes described above</i> 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.
(b) producing a plurality of readout probe-hybridized complexes by exposing each of the plurality of first	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:

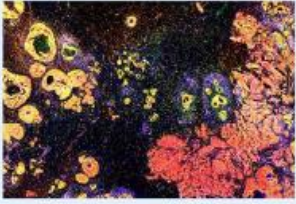
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hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal;	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.”

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	 Exemplary portion enlarged (yellow and white arrows added for emphasis): 

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	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, <i>e.g.</i>, at 1:40 (<i>arrows added for emphasis</i>): 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

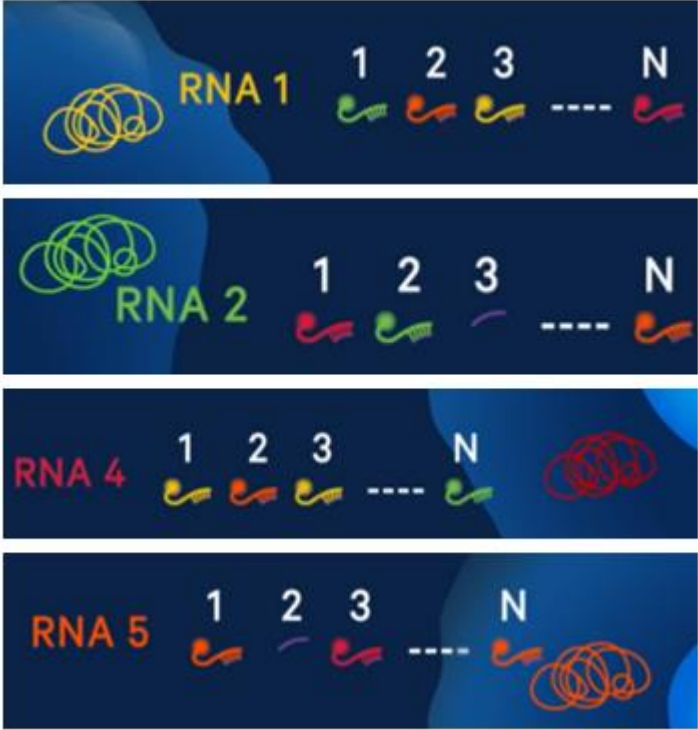
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	readout sequence (<i>indicated by added yellow arrow</i>) and a portion that comprises a fluorescent signal (<i>indicated by white arrow</i>). These two portions of the readout probe design can be seen below: Sucessive rounds of fluorescent probe hybridization, imaging & probe removal 
(c) detecting a fluorescent 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 fluorescent probe hybridization, imaging, and removal.” 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):

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	 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.” It further shows that a fluorescent signal can be detected from multiple target complexes (excerpted below):

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	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.” 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.”
(d) removing the fluorescent signal from each of the plurality of readout probe-hybridized complexes by	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.

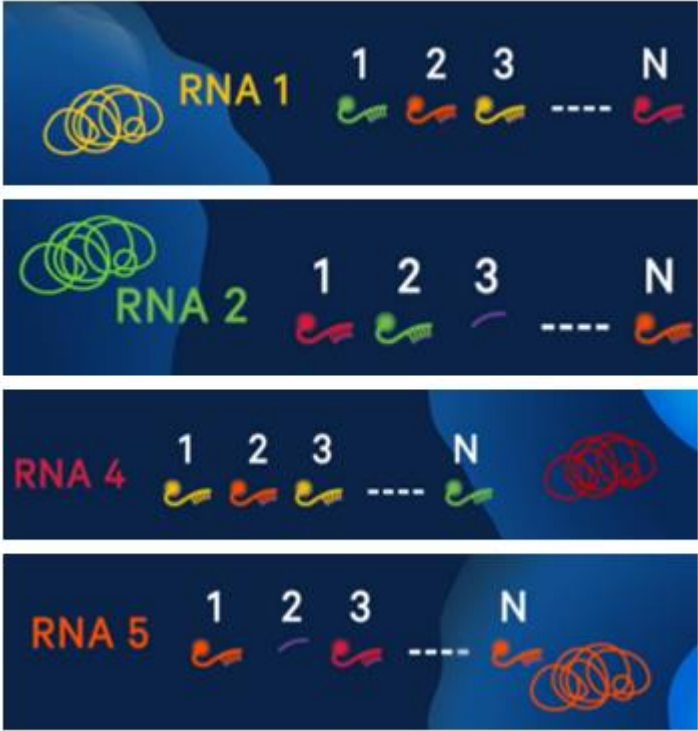
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inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes;	The Xenium Website provides, <i>e.g. (emphasis added)</i>: “[t]he slide is then placed in the 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.”
(e) after step (d), producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read	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.</i>, the Xenium Website provides that (<i>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.” The Xenium Website (Nov. 2022) further provides that: “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.”

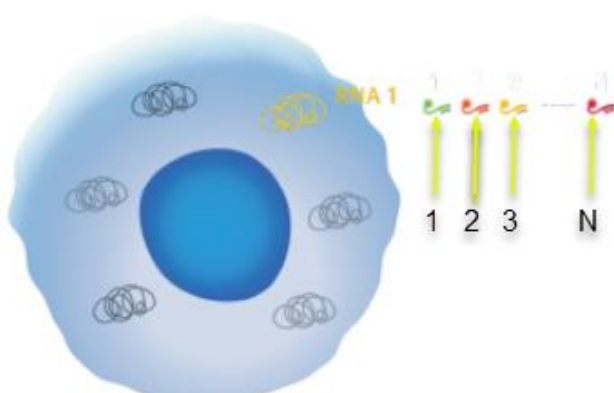
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sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal;	Janesick (2022) also discloses e.g. (emphasis added): “On the Xenium Analyzer, image acquisition was performed in cycles. The reagents, <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).

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	 The diagram illustrates the sequential hybridization process for Xenium In Situ Analysis Technology. It shows four horizontal panels, each representing a different RNA probe (RNA 1, RNA 2, RNA 4, and RNA 5) being hybridized to a target RNA molecule (represented by a blue squiggly line). Each panel displays a sequence of readout probes labeled 1, 2, 3, ..., N, which are associated with different color fluors. The probes are shown as small, colored, Y-shaped structures. The sequence of probes is: 1 (green), 2 (orange), 3 (yellow), ..., N (red) for RNA 1; 1 (red), 2 (green), 3 (purple), ..., N (orange) for RNA 2; 1 (yellow), 2 (orange), 3 (yellow), ..., N (green) for RNA 4; and 1 (orange), 2 (purple), 3 (red), ..., N (orange) for RNA 5. The probes are shown hybridizing to the target RNA molecule, with the color of the probe indicating the specific RNA being analyzed. 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).

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	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:

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	C Barcode Bridge-Probe Library ID Sequence 1 (ID1) 43221 ID Sequence 2 (ID2) 12434 ID Sequence 3 (ID3) 12224 ... Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5
(f) detecting a fluorescent signal from each of the different plurality of readout probe hybridized complexes;	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.</i>, the Xenium Website provides that “the sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and removal.” (emphasis added); the Xenium Website (Nov. 2022) provides “samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal” (emphasis added).
(g) removing the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes;	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 successive rounds of fluorescent probe hybridization imaging, and removal.” (emphasis added); the Xenium Website (Nov. 2022) provides “samples undergo successive rounds of fluorescent probe hybridization, imaging, and removal.” (Emphasis added).

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

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	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>). Target gene identification  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).

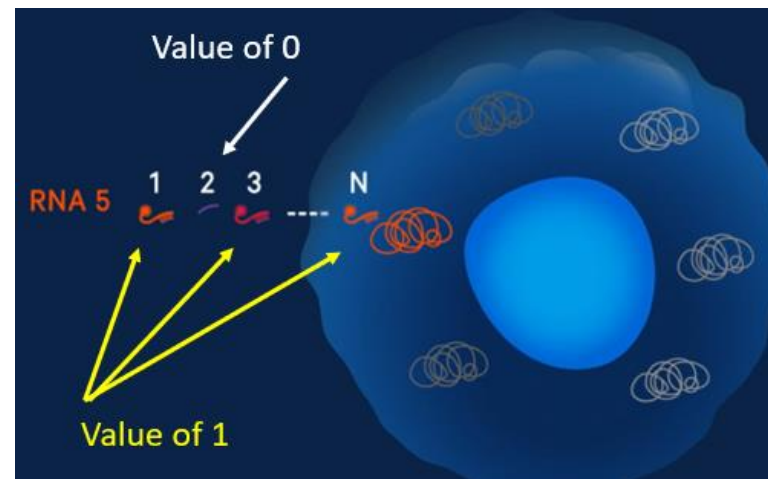
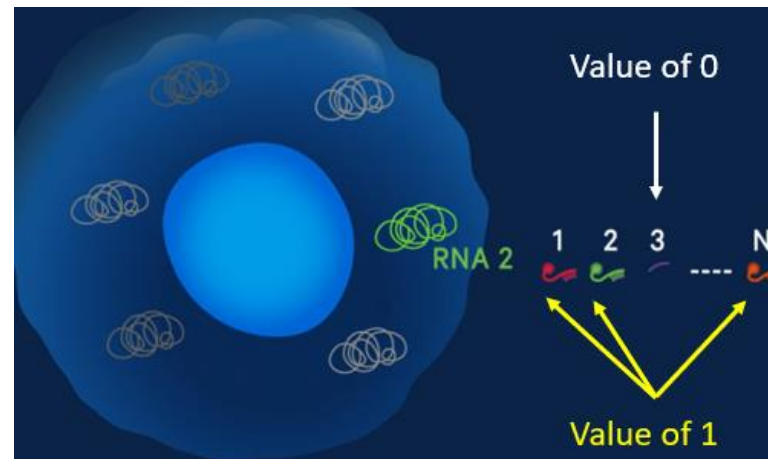
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	probes to bind with the target gene transcripts. Any number of optional processing steps can be performed either pre- or post-probe hybridization (e.g., fixation, permeabilization, washes, hydrogel embedding, probe ligation, amplification, such as 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 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	On information and belief, either literally or under the doctrine of equivalents, the Xenium In Situ Analysis Technology involves producing codewords (e.g., an “optical signature”) 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. For example, 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.

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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 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).

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The **Xenium Video** states (at 1:43-1:51) (emphasis added): “Each barcode, and therefore each RNA label, is turned **on or off** in a specific color at each cycle of this process.” As indicated by the yellow and white arrows and text (added for emphasis), these sequences are comprised of either a fluorescent signal (“1”) or the failure to detect a fluorescent signal (“0”).



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	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,</i> spot localization accuracy, and signal purity. 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”).

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	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 ***** [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, <i>e.g.</i>, the fluorescent signal may not be detected from one of the plurality of readout probe-hybridized complexes during at least one decoding cycle (<i>e.g.</i>, a value of “0”).

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	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 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 (emphasis added): 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.
(j) matching the codewords with valid codewords in a codebook by comparing the codewords to the valid codewords in the codebook, and if one of the codewords is not matched with one of the valid codewords in the	On information and belief, either literally or under the doctrine of equivalents, the Xenium In Situ Analysis Technology involves an error detection or correction system, as, e.g., 10x “expects the Xenium Analyzer will deliver targeted gene expression information at subcellular resolution at high sensitivity . . .”⁷ The Xenium Website (Nov. 2022) also states that “[t]he 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.”

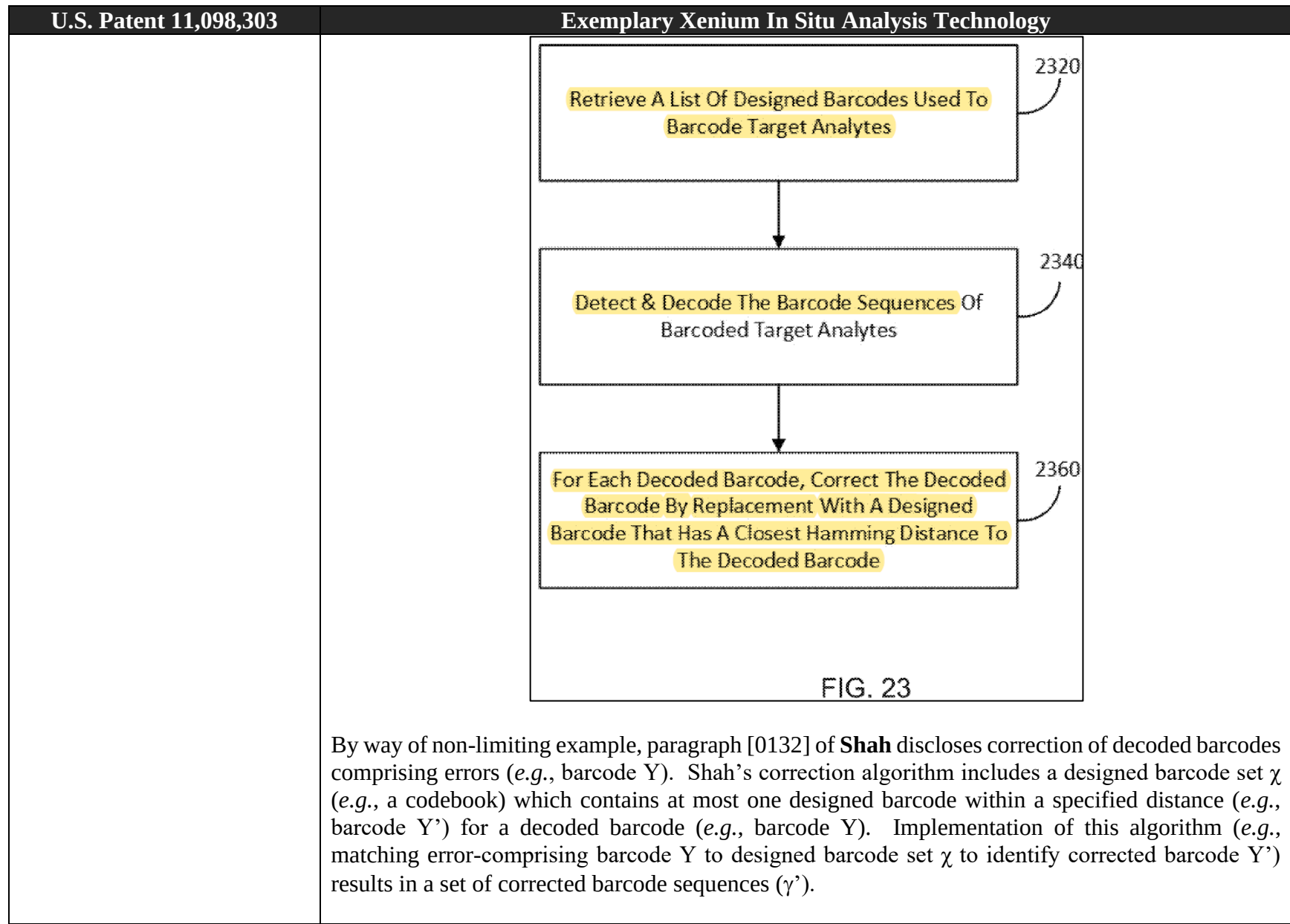
⁷ 10x Genomics Showcases Robust Innovation Pipeline at Xperience 2022 Event (Feb. 22, 2022), <https://www.prnewswire.com/news-releases/10x-genomics-showcases-robust-innovation-pipeline-at-xperience-2022-event-301487919.html> (emphasis added).

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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; and	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. On information and belief, 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.</i> <i>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 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. 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

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	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” (e.g., 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 (e.g., a designed barcode described herein) in a sparse decoding process. In some instances, target gene transcripts ***** 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

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	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 $\omega_v = \{\omega, \eta\}$. 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. For example, Shah [0252] and Figure 23 disclose that, e.g., if one of the codewords is not matched with one of the valid codewords in the codebook, applying an error detection or correction system,

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	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 1616, 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. *****



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	[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 data-bbox="1058 849 1545 1187"> 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 $Y' =$ neighbor of Y within distance k in χ; if neighbor found then Insert Y' into γ' else Insert Y into γ'; 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).

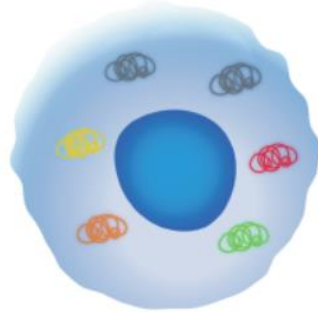
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	1. A computer-implemented method for error correction of decoded target barcode sequences comprising: obtaining an image for each decoding cycle of a plurality of decoding cycles to obtain a series of images; detecting, in each image of the series 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. 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). *****

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	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. As described in, <i>e.g.</i>, Qian (2020),⁸ CartaNA technology involves error correction using a “codebook” defined by probe barcodes. Qian (2020) provides (at 102, Methods Section 107-108) (emphasis added): <i>In total 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

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, 10x’s Xenium In Situ Analysis Technology utilizes, *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 10x’s Xenium In Situ Analysis Technology.

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 .”

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	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.
(k) determining the plurality of nucleic acid targets in the sample based on results from the matching step.	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: The figure is a spatial map of transcripts across a tissue section. It features a central blue circle. Surrounding this central circle are six smaller, colored circles, each labeled with a specific RNA target. The labels and their corresponding colors are: RNA 1 (yellow), RNA 2 (green), RNA 3 (purple), RNA 4 (red), RNA 5 (orange), and RNA 6 (cyan). The circles are arranged in a circular pattern around the central blue circle. 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:

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	Optical signature generation  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. 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. 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. Shah [0277] similarly discloses using, e.g., decoded and/or corrected barcodes to identify target gene transcripts in the biological sample.

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	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.