

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NANOSTRING TECHNOLOGIES, INC.,
Petitioner

v.

PRESIDENT AND FELLOWS OF HARVARD COLLEGE,
Patent Owner

Case IPR2024-00526
U.S. Patent No. 11,542,554

DECLARATION OF LAURA WALLER, PH.D.

Mail Stop "PATENT BOARD"
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

10x Genomics EX2002
NanoString v. Harvard
IPR2024-00526

TABLE OF CONTENTS

I.	INTRODUCTION	1
II.	SUMMARY OF MY OPINIONS	2
III.	MY BACKGROUND AND QUALIFICATIONS	4
IV.	MY UNDERSTANDING OF THE RELEVANT LEGAL PRINCIPLES	9
	A. Person of Ordinary Skill in the Art (“POSA”).....	9
	B. Obviousness.....	10
V.	THE 554 PATENT	11
VI.	CLAIM CONSTRUCTION	14
VII.	OVERVIEW OF ZHUANG AND CAI	15
	A. Zhuang’s system used MERFISH to determine two-dimensional positional information of RNA molecules in individual cells.....	15
	B. Dr. Czymmek’s relied-upon teachings in Cai did not disclose a system that determined three-dimensional positional information of nucleic acid molecules in a sample	21
VIII.	THE PRIOR ART RELIED UPON BY DR. CZYMMEK DOES NOT TEACH OR SUGGEST A SYSTEM FOR DETERMINING THREE-DIMENSIONAL POSITIONAL INFORMATION OF ONE OR MORE NUCLEIC ACID MOLECULES IN A SAMPLE.....	23
	A. Zhuang does not teach or suggest a system for determining three-dimensional positional information of one or more nucleic acid molecules	24
	B. The relied-upon disclosures in Cai do not teach or suggest a system for determining three-dimensional positional information of nucleic acid molecules.....	35
	C. Dr. Czymmek does not account for significant differences between Zhuang’s and Cai’s systems when discussing a POSA’s motivation to combine the references with a reasonable expectation of success.....	38

I, Laura Waller, hereby declare as follows:

I. INTRODUCTION

1. I am over the age of 18 and competent to make this Declaration.

2. I have been retained as an expert witness on behalf of 10x Genomics, Inc. (“10x”) for the above-captioned *inter partes* review (IPR) of U.S. Patent No. 11,542,554 (“the 554 patent”; EX1001). I understand that 10x is the exclusive licensee of the 554 patent. I understand that this Declaration accompanies 10x’s Patent Owner Preliminary Response (“POPR”). I am being compensated for my time in connection with this IPR.

3. I understand that the 554 patent resulted from U.S. Patent Application No. 17/395,534 (“the 534 application”) filed on August 6, 2021. I understand that the 534 application is a continuation of U.S. Patent Application No. 16/386,337 filed on April 17, 2019 (issued as U.S. Patent No. 11,118,220), which is a continuation of U.S. Patent Application No. 15/969,118 filed on May 2, 2018 (issued as U.S. Patent No. 10,266,888), which is a continuation of PCT Application No. PCT/US2016/060279 (“the 279 application”) filed on November 3, 2016. I understand that the 279 application claims benefit to U.S. Provisional Application No. 62/250,182 filed on November 3, 2015.

4. I understand November 3, 2015, is the 554 patent’s earliest possible priority date. I refer to this date throughout this Declaration.

5. I understand that NanoString Technologies, Inc. (“NanoString”) has challenged claims 36-70 of the 554 patent in this IPR. I understand that NanoString has submitted a declaration by Dr. Kirk Czymmek (EX1002) in support of its challenge.

6. My Declaration addresses certain aspects of opinions provided by Dr. Czymmek. In preparing for my Declaration, I have reviewed the 554 patent (EX1001), its prosecution history (EX2006), and each of the documents cited herein, in light of the general knowledge in the art before November 3, 2015.

7. In formulating my opinions, I have relied upon my experience, education, and knowledge in the relevant art. I have also considered the viewpoint of a person of ordinary skill in the art (“POSA”), as defined below in Section IV.A before November 3, 2015.

II. SUMMARY OF MY OPINIONS

8. I understand that Dr. Czymmek has opined that combinations of certain references would have rendered obvious claims 36-70 of the 554 patent. EX1002. I understand that, as part of his declaration, Dr. Czymmek has opined that Zhuang (EX1003) alone or with Cai (EX1004) discloses a system meeting the following element of claim 36: “determining three-dimensional positional information of said one or more nucleic acid molecules.” EX1002, ¶216-219.

9. I disagree with Dr. Czymmek's opinion. As discussed herein, and contrary to Dr. Czymmek's opinion, Zhuang and Cai, alone or in combination, do not teach or suggest a system for determining three-dimensional positional information of one or more nucleic acid molecules. Dr. Czymmek's opinion in his declaration is based on a misapplication of the disclosures in at least Zhuang and Cai. As I explain below, a POSA would have understood that Zhuang's disclosed methods are limited to two-dimensional image analysis and the relied-upon disclosures in Cai relate to a low-resolution image analysis using a specific light sheet microscope system. Contrary to Dr. Czymmek's opinion, these disclosures would not have taught or suggested to a POSA a system for determining three-dimensional positional information of nucleic acids, as claimed in the 554 patent.

10. I have also been asked to consider Dr. Czymmek's opinion on whether "it would have been obvious to modify Zhuang [with Cai's disclosure] to permit a POSA to use MERFISH to determine three-dimensional positional information about one or more nucleic acid molecules." EX1002, ¶219. As discussed herein, I disagree with Dr. Czymmek's opinions regarding the combination of Zhuang and Cai for several reasons. For example, Dr. Czymmek's declaration does not account for significant differences between the imaging systems of Zhuang and Cai when discussing reasons a POSA would have allegedly combined these references with a reasonable expectation of success. A POSA would have recognized that the

differences between these imaging systems, including different resolutions and working distances, are material differences. These differences, which went completely unaddressed by Dr. Czymmek, would have impacted a POSA's motivations and expectations.

III. MY BACKGROUND AND QUALIFICATIONS

11. I am the Charles A. Desoer Endowed Professor of Electrical Engineering and Computer Sciences at the University of California, Berkeley since July 2021.

12. I have over 14 years of experience in the field of computational imaging, bioimaging, phase microscopy, super-resolution microscopy, light field cameras, metrology and inspection, inverse problems in imaging, optical coherence theory, and 3D imaging and display. Within these areas of research, I have specific experience imaging biological samples and developing new 3D microscopy technology for both label-free (phase) and fluorescence microscopy. I have also worked on single-shot 3D coded aperture microscopy and intensity diffraction tomography, as well as optical coherence tomography, multi-photon computer generated holography, structured illumination microscopy and other 3D imaging methods for microscopes.

13. My current research focuses on optics, signal processing, computer algorithms, bioimaging, and system design. My lab develops methods for designing imaging systems and algorithms jointly in terms of hardware and

software to produce improved bioimaging systems. We focus in particular on microscopy, bioimaging, multi-dimensional imaging and inverse problems that incorporate wave-optical effects (*e.g.*, phase, diffraction, coherence) with optical, X-ray or electron microscopes. We have developed 3D refractive index (phase) measurement models and inverse problems, and focused on two-photon microscopy for fast 3D neural activity tracking using calcium fluorescence methods. My research group has also demonstrated time-resolved structured illumination microscopy for super-resolution video imaging.

14. I have provided a copy of my *curriculum vitae* as EX2012.

15. I graduated with a Bachelor's degree in Electrical Engineering from the Massachusetts Institute of Technology ("MIT") in 2004. I also received by Master of Engineering in Electrical Engineering and Computer Science from MIT in 2005. From August 2007 to November 2007, I was a Researcher in the Advanced Nanostructures Lab at the Walter Schottky Institute at the Technical University of Munich. I then received my Ph.D. in Electrical Engineering and Computer Science from MIT in 2010.

16. From 2004-2010, I was a Research Assistant in the 3D Optical Systems Group at the Department of Mechanical Engineering at MIT and a Singapore-MIT Alliance for Research and Technology student, where I worked on several different

imaging techniques, including imaging biological samples and 3D imaging techniques including digital holography and phase tomography.

17. From 2010-2012, I was a Postdoctoral Research Associate and a part-time lecturer in Physics in the Department of Electrical Engineering at Princeton University. In July 2012, I joined the Department of Electrical Engineering and Computer Sciences at the University of California, Berkeley, where I served as an Assistant Professor from 2012 to 2016, and then the Ted Van Duzer Endowed Associate Professor from 2016 to 2021 until I was promoted to my current position in July 2021.

18. I have authored more than 90 peer-reviewed publications that discuss optics and methods of computational imaging. Among these papers are my work on developing techniques of 3D imaging and fluorescence microscopy (such as, Xue, Ren, and Waller, “Three-dimensional bi-functional refractive index and fluorescence microscopy (BRIEF),” *Biomedical Optics Express* 13(11), 5900-5909 (2022); Yanny *et al.*, “Miniscope3D: optimized single-shot miniature 3D fluorescence microscopy,” *Light: Science and Applications* 9:171 (2020); Yeh, Chowdhury, and Waller, “Speckle-structured illumination for 3D phase and fluorescence computational microscopy,” *Biomedical Optics Express* 10(7), 3635-3653 (2019), which was the top download in July 2019; and Liu, Zhong, and

Waller, “Multiplexed phase-space imaging for 3D fluorescence microscopy,” *Optics Express* 25(13), 14986-14995 (2017)).

19. I have also published numerous articles pertaining to 3D imaging techniques, such as Xue *et al.*, “Three-dimensional Multi-site Random Access Photostimulation (3D-MAP),” *elife* 11:e73266 (2022); Tanksalvala *et al.*, “Non-Destructive, High-Resolution, Chemically Specific, Three-Dimensional Nanostructure Characterization using EUV Complex-Imaging Reflectometry,” *Science Advances* 7(5), eabd9667 (2021); Javidi *et al.*, “Roadmap on 3D Integral Imaging: Sensing, Processing and Displays,” *Optics Express* 28(22), 32266-32293 (2020), which was the top download in November 2020; Chen *et al.*, “Multi-Layer Born multiple-scattering model for 3D phase microscopy,” *Optica* 7(5), 394-403 (2020); Ren *et al.*, “A multiple scattering algorithm for three dimensional phase contrast atomic electron tomography,” *Ultramicroscopy* 208, 112860 (2020); Chowdhury *et al.*, “High-resolution 3D refractive index microscopy of multiple-scattering samples from intensity images,” *Optica* 6, 1211-1219 (2019), which was selected as the journal cover image; Liu *et al.*, “3D imaging in volumetric scattering media using phase-space measurements,” *Optics Express* 23(11), 14461-14471 (2015), which was also selected for *Virtual Journal of Biomedical Optics*.

20. I have also published my research in the field of fluorescent microscopy, such as Wang *et al.*, “Image denoising for fluorescence microscopy by self-

supervised transfer learning,” *Optics Express* 29(25), 41303-41313 (2021); Phillips *et al.*, “High-throughput fluorescence microscopy using multi-frame motion deblurring,” *Biomedical Optics Express* 11(1), 281-300 (2020); Kuo *et al.*, “On-chip fluorescence microscopy with a random microlens diffuser,” *Optics Express* 28(6), 8384-8399 (2020); and Yeh, Chowdhury, and Waller, “Computational structured illumination for high-content fluorescent and phase microscopy,” *Biomedical Optics Express* 10(4), 1978-1998 (2019).

21. I also routinely attend and present at technical conferences around the world. I have presented my research at over 360 conferences, and have been the keynote speaker at several of these conferences. I have also presented my research at over 200 invited seminars at institutions around the world.

22. Throughout my career, I have also mentored and supervised over 30 graduate students and over 20 postdoctoral researchers and visiting scholars.

23. I have also received several awards and commendations listed on my CV. For example, in 2022, I was named the Charles A. Desoer Endowed Chair in Engineering and received the Chan-Zuckerberg Initiative Biohub Investigator Award. In 2021, I received the Optical Society of America Adolph Lomb Medal. In 2020, I was named an American Institute for Medical and Biological Engineering fellow. In 2019, I was an Optical Society of America Fellow. In 2018, I received the International Society for Optics and Photonics Early Career

Achievement Award. In 2017, I received the Chan-Zuckerberg Initiative Biohub Investigator Award. In 2016, I was a National Academy of Engineering Frontiers of Engineering participant, I was an Agilent Early Career Professor Award Finalist for Big Data Technologies and Imaging, I received the Carol D. Soc Distinguished Graduate Student Mentorship Award for Junior Faculty, I was awarded the Qualcomm Innovation Fellowship, and I was named the Ted Van Duzer Endowed Professor (from 2016-2020). In 2014, I was named a Packard Fellow of the David and Lucile Packard Foundation, I received the Moore Foundation Data-driven Discovery Investigator Award, I received the NSF Career award, and I was named a Bakar Fellow. In 2012, I received the Optical Society of America Ivan P. Kaminow Outstanding Early Career Professional Prize. I have also received more than 30 conference presentation awards.

IV. MY UNDERSTANDING OF THE RELEVANT LEGAL PRINCIPLES

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

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

25. I understand that patent law analyses are performed from the viewpoint of a POSA. I understand that a POSA is a hypothetical person who is presumed to be aware of all pertinent art, thinks along conventional wisdom in the art, and is a

person of ordinary creativity. Solely for the purposes of the opinions in this

Declaration, I apply Dr. Czymmek's definition of a POSA. EX1002, ¶¶68-70.

B. Obviousness

26. I understand that obviousness of claims 36-70 of the 554 patent is at issue in this IPR. I understand that an obviousness analysis involves comparing a claim to the prior art to determine whether the claimed subject matter would have been obvious to a POSA in view of the prior art and general knowledge of the prior art.

27. I understand that obviousness must be assessed from the viewpoint of a POSA at the time of the invention. I further understand that to show obviousness a party must perform the following factual inquiries: (a) determining the scope and content of the prior art; (b) ascertaining the differences between the claimed invention and the prior art; and (c) resolving the level of skill in the art. I understand that, for a finding of obviousness, a POSA needs to have (i) a reason or motivation to modify or combine the prior art to arrive at the claimed subject matter and (ii) a reasonable expectation of doing so successfully.

28. I understand that combining or modifying the teachings of the prior art to achieve the claimed subject matter is a way to establish obviousness. I understand that the reason to combine prior-art references can come from a variety of sources, not just the prior art itself or the specific problem the patentee was trying to solve. And I understand that the references themselves need not provide a specific hint or

suggestion of the alteration needed to arrive at the claimed invention; the analysis may include recourse to logic, judgment, and common sense available to a person of ordinary skill that does not need to be explicit in any reference.

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

30. I also understand that when considering the obviousness of the claimed subject matter, any objective evidence that supports non-obviousness of the claimed subject matter should also be considered. The objective evidence of non-obviousness can include evidence of, for example, failure of others, unexpectedly superior results, skepticism followed by praise in the industry, long-felt but unmet need, copying by others, and commercial success.

V. THE 554 PATENT

31. The 554 patent relates to methods and devices for volumetric imaging (*i.e.*, three-dimensional imaging) of molecules, such as nucleic acids, within a fixed biological specimen. EX1001, 1:53-59, 2:27-36. For example, in one instance, the patent provides a “three-dimensional imaging device (*i.e.*, volumetric imaging device) ... that can measure, resolve and optionally localiz[e] light signals in three-dimensional space to measure fluorescently- or optically-encoded nucleic acid sequencing” EX1001, 5:19-25.

32. I understand that the 554 patent issued with 70 claims, however, only claims 36-70 are at issue in this IPR. I understand that claim 36 is the sole independent claim in this IPR. I understand that claims 37-70 are dependent claims, which depend directly or indirectly from claim 36. I understand that a dependent claim contains all the elements of the claim from which it depends. Thus, I understand that claims 37-70 contain all the elements of claim 36.

33. Claims 36-70 are directed to a system for determining three-dimensional positional information of one or more nucleic acid molecules in a sample, wherein the system comprises a stage, a fluidics system, an optical system, a z-axis motion control system, and a computing system. Independent claim 36 recites:

36. A system comprising:

(a) a stage comprising:

(i) a sample holder configured to receive a substrate comprising a sample having a volume;

(ii) a temperature control apparatus operatively coupled to said sample holder; and

(iii) an XY motion control system;

(b) a fluidics system comprising:

(i) one or more reservoirs configured to contain one or more fluid reagents; and

(ii) a fluid dispenser operationally coupled to a pump, which pump is configured to provide said one or

more fluid reagents from said one or more reservoirs to said sample holder; and

(c) an optical system comprising:

(i) an objective;

(ii) two or more light sources, wherein each light source of said two or more light sources is configured to deliver light to said volume, wherein said two or more light sources are configured to emit two or more colors of light; and

(iii) one or more detectors configured to receive light signals from said volume;

(d) a Z-axis motion control system configured for Z-motion of an objective focal plane through said volume; and

(e) a computing system comprising at least one computer readable storage medium having program instructions stored thereon, which program instructions are executable by at least one processor of said computing system to cause said at least one processor to perform a method comprising:

receiving a plurality of images of at least a portion of said volume from a plurality of read-out cycles, wherein said plurality of images comprises a plurality of light signals received from said volume during said plurality of readout cycles, wherein said plurality of light signals is associated with one or more nucleic acid molecules within said volume, wherein each light signal

of said plurality of light signals has a three-dimensional spatial position within said volume;

determining a plurality of optically-encoded signals from said plurality of light signals obtained from said plurality of read-out cycles, wherein each optically-encoded signal is associated with a nucleic acid molecule of said one or more nucleic acid molecules, wherein said plurality of optically-encoded signals comprises said two or more colors of light detected by said one or more detectors in series;

identifying said one or more nucleic acid molecules based on said plurality of optically-encoded signals; and

determining three-dimensional positional information of said one or more nucleic acid molecules.

EX1001, 33:29-34:16.

34. Thus, claim 36 and its dependent claims 37-70 require a “system” that performs a method comprising “determining three-dimensional positional information of said one or more nucleic acid molecules” in a sample. EX1001, 33:29-34:16.

VI. CLAIM CONSTRUCTION

35. Dr. Czymmek states that no express claim construction is required. EX1002, ¶¶122-123. Solely for the purposes of the opinions in this Declaration, I likewise do not apply any specific claim construction.

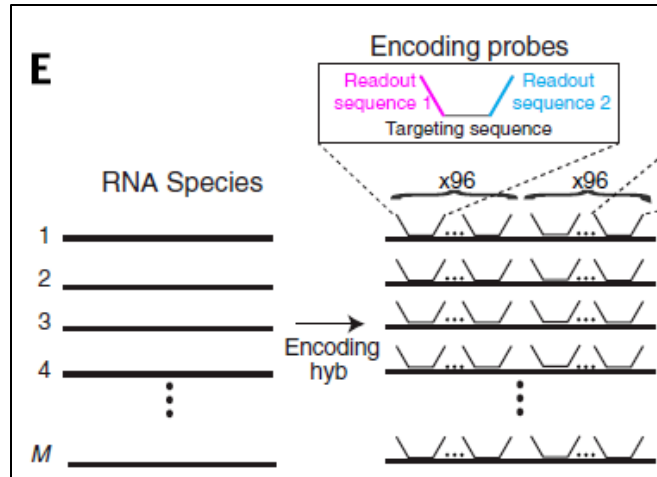
VII. OVERVIEW OF ZHUANG AND CAI

36. Dr. Czymmek considers Zhuang (EX1003) and Cai (EX004) as prior-art references. I summarize teachings of these references below.

A. **Zhuang's system used MERFISH to determine two-dimensional positional information of RNA molecules in individual cells**

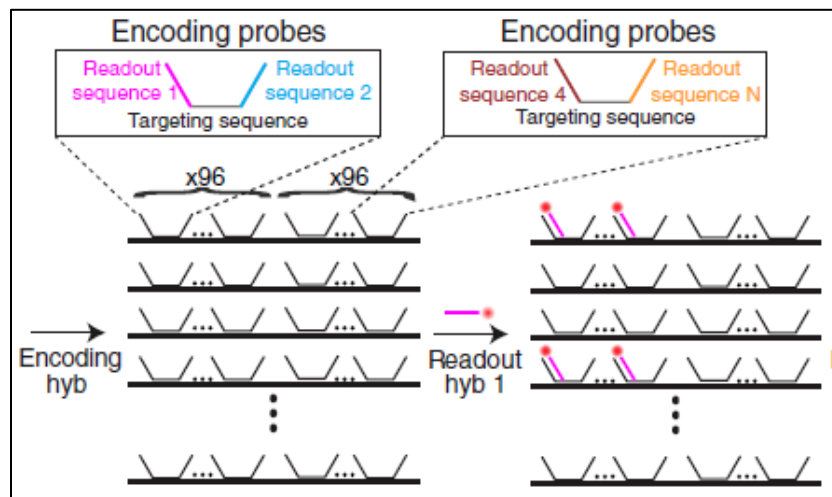
37. Zhuang disclosed a system for imaging RNA molecules and determining their two-dimensional positional information in individual cells using “multiplexed error-robust fluorescence in situ hybridization” (“MERFISH”). EX1003, Abstract, 001, 011, 012. Zhuang described MERFISH as “a single-molecule imaging approach that allows the copy numbers and spatial localizations of thousands of RNA species to be determined in single cells.” EX1003, Abstract, 001.

38. MERFISH used fluorescently labeled nucleic acid probes to detect the target RNA molecules in the cells. EX1003, 001, 003, FIG. 1. Specifically, in MERFISH, the target RNA molecules in cells were tagged with “encoding probes” containing (1) a “central RNA-targeting region” with complementary sequences that bind to the target RNAs; and (2) two readout sequences that bind to a second set of fluorescently-labeled probes (“readout probes”), which flank the RNA-targeting region. EX1003, 003, FIG. 1. Zhuang showed this first labeling step with encoding probes in Figure 1E, referred to as “Encoding hyb”:



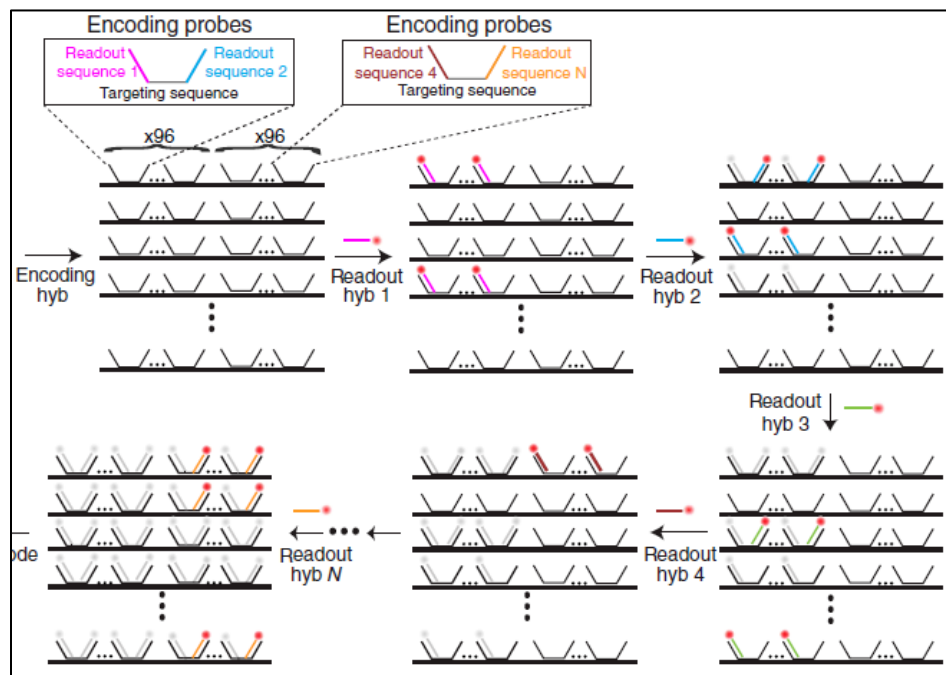
EX1003, FIG. 1. Zhuang explained that this encoding probe labeling step “convert[ed] the RNA into a specific combination of readout sequences (Encoding hyb).” EX1003, 003, FIG. 1.

39. Next, Zhuang disclosed that fluorescently labeled “readout probes” were hybridized to the readout sequences of the encoding probes, which were already hybridized to the target RNA. EX1003, 003, FIG. 1. This hybridization of readout probes to the encoding probes fluorescently labeled the target RNAs. Zhuang depicted this step in Figure 1E, referred to as “Readout hyb 1”:



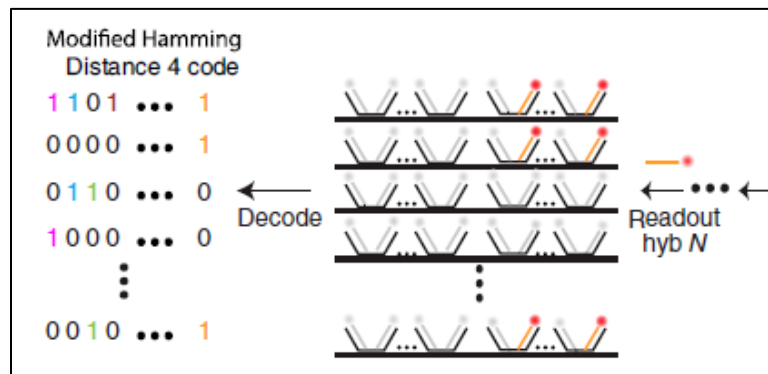
EX1003, FIG. 1.

40. Zhuang disclosed that “a]fter each round of hybridization with the fluorescent readout probes, cells were imaged by means of conventional wide-field imaging with an oblique-incidence illumination geometry.” EX1003, 003, FIG. 1. After imaging, the first round of fluorescently labeled readout probes were “inactivated by photobleaching.” EX1003, 003, FIG. 1. Zhuang disclosed that the readout probe hybridization process was repeated for additional “successive rounds of hybridization and imaging, each with a different readout probe,” to “identify the readout sequences bound to each RNA and to decode the RNA.” EX1003, 001, 003, FIG. 1. Zhuang’s Figure 1E showed these additional rounds (“N rounds”) of sequential hybridization:



EX1003, FIG. 1.

41. Zhuang disclosed that the fluorescent signals obtained from imaging the sample after each round of successive hybridization were used to “construct[] binary words from the observed fluorescent spots based on their on-off patterns.” EX1003, 003. These binary words (*i.e.*, codewords made of up “0” and “1”) were matched to a specific RNA sequence, allowing “the copy numbers and spatial localizations of thousands of RNA species to be determined in single cells.” EX1003, 003, Abstract, FIG. 1. Zhuang disclosed exemplary binary codewords in Figure 1E:



EX1003, FIG. 1.

42. Zhuang expressly disclosed imaging in two dimensions. EX1003, 011, 012. Zhuang disclosed that its MERFISH-based system used “relatively large and flat” human primary fibroblast cells plated on a coverslip as a sample. EX1003, 011. Because the fibroblast cells were large and flat and were plated on the coverslip in a layer, the majority of RNAs and fluorescent signals from the RNAs were in a single focal (z-axis) plane. Accordingly, Zhuang explained that the cells

were imaged “*without* the need for optical sectioning” and “*without* ... z-scanning.” EX1003, 011, 012 (emphasis added). A POSA would have understood this to mean that Zhuang’s MERFISH-based system only used a single focal plane for determining the RNA spatial position (two dimensions), and did not determine the spatial position in three dimensions.

43. In addition, Zhuang explained that the imaging data was analyzed using a two-dimensional computational algorithm. EX1003, 012. Specifically, Zhuang’s system in each image “identified and localized” fluorescent spots corresponding to individual RNA molecules by inputting a “width of 167 nm” as a parameter in a computational fitting algorithm. EX1003, 012. The use of the parameter “width” during analysis indicated that Zhuang’s system determined x- and y-axis (lateral) positional coordinates, *i.e.*, two-dimensional positional information, of the target cellular RNA molecules in the sample. EX1003, 012.

44. Thus, Zhuang’s system identified the “spatial landscape” or “spatial distribution” of RNA molecules in the cells in two dimensions, and not three dimensions. Indeed, the art commonly used the term “spatial” to indicate two-dimensional analysis. EX2001, Abstract; EX2003, 11:31-33. For example, Stahl *et al.* disclosed a “*spatial* transcriptomics” strategy for “visualization and quantitative analysis of the transcriptome with *spatial* resolution in individual tissue sections” that provides “RNA-sequencing data with maintained *two-dimensional* positional

information” from the tissue. EX2001, Abstract (emphasis added). Similarly, Frisen *et al.* disclosed a method for “partial or global transcriptome analysis of a tissue sample with two-dimensional *spatial* resolution.” EX2003, 11:31-33 (emphasis added).

45. Zhuang further explained that because its MERFISH-based system imaged the cells “without optical sectioning and z-scanning,” a quality-control experiment was performed to determine the fraction (quantity) of labeled RNAs that might be “outside the axial range of [] imaging geometry,” *i.e.*, outside of the basal focal plane targeted by Zhuang’s MERFISH-based system. EX1003, 012. The quality-control experiment showed that “only a small fraction” of RNA molecules resided “outside the imaging range of a fixed focal plane” of the MERFISH-based method. EX1003, 012. This confirmed that optical sectioning and z-scanning *were not needed* for the disclosed MERFISH-based system. Additionally, Zhuang’s quality control experiment did not involve determining three-dimensional positional information of the RNAs in the cells, as Zhuang only disclosed assessing the fraction of RNAs that might be present in different focal planes. EX1003, 012.

46. A POSA would not have understood that Zhuang’s quality control experiment teaches or suggests determining three-dimensional positional information of the RNAs at least because (i) the experiment only assessed the

fraction (quantity) of RNAs in other focal planes; and (ii) the experiment did not determine any *positional information* of those RNAs.

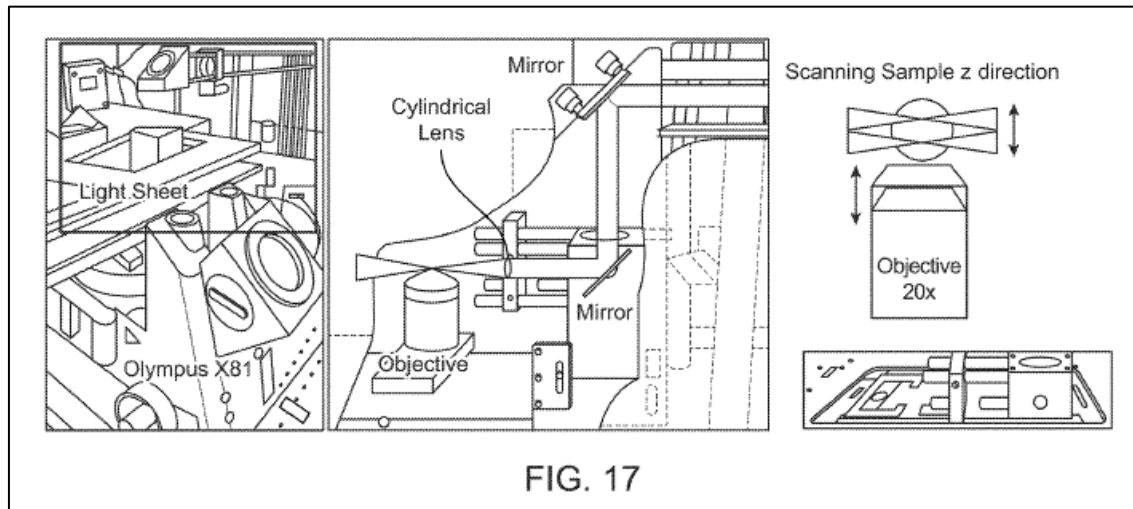
47. Thus, Zhuang disclosed a MERFISH-based system to determine *two-dimensional*—not *three-dimensional*—positional information of RNA molecules in individual cells.

B. Dr. Czymmek’s relied-upon teachings in Cai did not disclose a system that determined three-dimensional positional information of nucleic acid molecules in a sample

48. Cai (EX1004) described methods for “detecting and/or quantifying nucleic acids in cells, tissues, organs or organisms.” EX1004, Abstract. When discussing Cai’s teachings and combining them with the disclosures in Zhuang, Dr. Czymmek relies on Cai’s disclosure of a light sheet microscope for imaging thick 100 μ M brain slices by “scanning the [brain] sample along [the] z-axis.” EX1002, ¶188; EX1004, FIG. 17, 47:42-45.

49. Before November 3, 2015, a POSA would have known that light sheet microscopy uses a thin sheet of light to illuminate a sample from the side, perpendicular with the objective lens. For example, Cai disclosed that “the present invention provides a light sheet microscope, wherein the sample is illuminated from the side.” EX1004, 47:31-33. Thus, a light sheet microscope illuminates only one select plane of the volumetric sample. Dr. Czymmek relies upon Cai’s “exemplary setup of [a] light sheet microscope [] illustrated in FIG. 17.” EX1004,

47:36-37; EX1002, ¶188. Cai's Figure 17 depicting a light sheet microscope is reproduced below:



EX1004, FIG. 17.

50. Cai disclosed that Figure 17's light sheet microscope used two mirrors and a "cylindrical lens" to generate a "plane of light sheet," which "illuminates the sample from the side, and is perpendicular to the detection objective." EX1004, 47:31-40. Cai explained that the configuration of the "plane of light sheet" allowed the objective to move "synchronously with the illumination sheet," which in turn allowed for "scanning the sample along the z-axis." EX1004, 47:40-47. This allowed a POSA to identify and select the optimal focal plane with the lowest out-of-focus background in the image. Accordingly, the art commonly referred to light sheet microscopy as "*selective plane* illumination microscopy," or "SPIM." EX1004, 46:49-53 (emphasis added). Cai explained that its disclosure utilized "imaging technologies that *minimize or prevent out-of-focus background*," and that

“SPIM is used for *thicker slices* that have *higher out-of-focused background*.”

EX1004, 47:15-18 (emphasis added).

51. Cai disclosed using the light sheet microscope system of Figure 17 with a 20x water immersion objective to image β -actin mRNA in thick brain slices tagged with “hybridization chain reaction” (HCR) probes. EX1004, 47:48-56, FIG. 18.

Cai described imaging 200 optical sections (*i.e.*, focal planes) to image all of the β -actin mRNA in the thick brain slices. EX1004, 47:48-56, FIG. 18.

52. Cai did not teach or suggest determining the three-dimensional positional information of the β -actin mRNA molecules in the thick brain slices. Instead, Cai only disclosed that “[t]he β -actin mRNA is *highly expressed*, accounting for the *large number* of dots in the cell bodies.” EX1004, 47:53-55 (emphasis added).

53. Thus, the disclosures of Cai upon which Dr. Czymmek relies did not disclose a system for determining three-dimensional positional information of nucleic acid molecules in a sample.

**VIII. THE PRIOR ART RELIED UPON BY DR. CZYMMEK DOES NOT
TEACH OR SUGGEST A SYSTEM FOR DETERMINING THREE-
DIMENSIONAL POSITIONAL INFORMATION OF ONE OR MORE
NUCLEIC ACID MOLECULES IN A SAMPLE**

54. Claim element 36(e) requires “determining three-dimensional positional information of said one or more nucleic acid molecules.” EX1001, 34:15-16. Dr. Czymmek argues that Zhuang (EX1003) discloses this claim element and, to the

extent Zhuang is found or argued not to disclose determining three-dimensional positional information, Cai (EX1004) discloses it. EX1002, ¶¶216-18, 219 (citing EX1002, Section XI.A.1.e.(1)). Dr. Czymmek also argues that “it would have been obvious to modify Zhuang [with Cai’s disclosure] to permit a POSA to use MERFISH to determine three-dimensional positional information about one or more nucleic acid molecules.” EX1002, ¶219 (citing EX1002, Section XI.A.1.e.(1)).

55. I disagree with Dr. Czymmek’s conclusions for the reason I explain below. Briefly, neither Zhuang nor Cai, alone or in combination, teaches or suggests a system for determining three-dimensional positional information of nucleic acid molecules, as required by claim 36(e). Additionally, Dr. Czymmek does not address the significant differences between Zhuang’s and Cai’s systems or explain why or how a POSA would have modified Zhuang’s MERFISH-based system with Cai’s system to determine three-dimensional positional information of nucleic acid molecules as required by claim 36(e) with a reasonable expectation of success.

A. Zhuang does not teach or suggest a system for determining three-dimensional positional information of one or more nucleic acid molecules

56. A POSA would have understood that Zhuang discloses a MERFISH-based system for imaging different RNA species to determine their two-

dimensional positional information in cells. EX1003, 001, 002, 011, 012. As I explain in Section VII.A above, Zhuang's MERFISH-based system used fluorescently labeled nucleic acid probes to detect the target RNA molecules in the cells. EX1003, 001, 003, FIG. 1. Zhuang discloses that its system images a layer of "relatively large and flat" human primary fibroblast cells plated on a coverslip. EX1003, 011. Because Zhuang's system images a layer of flat fibroblast cells, a POSA would have understood that the majority of target cellular RNAs and fluorescent signals from the RNAs are in a single focal plane (which Zhuang refers to as the "basal focal plane"). EX1003, 012. Zhuang confirms this when explaining that the layer of relatively large and flat fibroblast cells "facilitat[ed]" imaging "*without* the need for optical sectioning" and "*without* ... z-scanning" in its system. EX1003, 011, 012.

57. Moreover, the imaging data analysis described in Zhuang would have confirmed to a POSA that the system does not determine three-dimensional positional information of RNA molecules. Zhuang discloses that the fluorescent spots (corresponding to individual RNA molecules tagged with a MERFISH fluorescent probe) are "identified and localized in each image by using a multi-Gaussian-fitting algorithm [] assuming a Gaussian with a uniform width of 167 nm." EX1003, 012. A POSA would have understood from this disclosure that Zhuang's system uses an algorithm that considers only "width" as a parameter to

identify and localize RNA molecules in each image because Zhuang does not mention using a height parameter for imaging analysis. EX1003, 012.

58. Before November 3, 2015, it was well known in the bioimaging field that the “width” parameter indicates lateral (x- and y- axis) distances, while the “height” parameter indicates axial (z-axis) distance. Because Zhuang teaches to only use “width” as a parameter in the algorithm for image analysis (there is no “height” parameter in Zhuang), a POSA would have understood that Zhuang’s disclosed methods only considered lateral (x- and y- axis) resolution, without axial (z-axis) resolution. Consequently, a POSA would have understood that Zhuang teaches a system for determining *two-dimensional* positional information for RNA molecules during image analysis. To determine three-dimensional positional information, Zhuang would have had to fit to both the lateral distance and axial distance, which it does not.

59. Thus, while Zhuang discloses using its system to assess “spatial distribution” of RNA molecules, a POSA would have understood that Zhuang is referring to two-dimensional spatial resolution. Zhuang’s use of the term “spatial” in the context of two-dimensional spatial resolution is consistent with the use of the term in the art. EX2001, Abstract; EX2003, 11:31-33. For example, Frisen *et al.* discloses a method for “partial or global transcriptome analysis of a tissue sample with two-dimensional *spatial* resolution.” EX2003, 11:31-33 (emphasis added).

60. In fact, Zhuang’s explicit teaching that its two-dimensional MERFISH imaging system performs “*without the need* for optical sectioning” and “*without* optical sectioning and z-scanning” would have dissuaded a POSA from seeking to apply Zhuang’s MERFISH-based system for determining three-dimensional positional information. EX1003, 011, 012 (emphasis added). Zhuang expressly teaches that there is no need to take steps towards optical sectioning and z-scanning using the MERFISH-based system, and thus no reason to determine three-dimensional positional information using the MERFISH-based system. EX1003, 011, 012.

61. Dr. Czymmek does not address these explicit disclosures in Zhuang describing a system for determining *two-dimensional* positional information of RNA molecules. Instead, Dr. Czymmek argues that Zhuang discloses determining three-dimensional positional information because Zhuang describes “optically sectioning cells ‘by collecting stacks of images at different focal depths through the entire depth of the cells.’” EX1002, ¶¶204, 216-218; EX1003, 012. I disagree with Dr. Czymmek’s opinion because Dr. Czymmek mischaracterizes this disclosure in Zhuang.

62. This statement in Zhuang relates to a conventional smFISH-based (*not* MERFISH-based) quality-control experiment for confirming that there was *no need* to image the cell sample in focal planes other than the single focal plane in

which Zhuang's MERFISH-based system images the cells. EX1003, 011, 012.

Nowhere does Zhuang state or suggest that the quality-control experiment determines three-dimensional positional information RNA molecules or should be used to determine three-dimensional positional information. I note that even Dr. Czymmek admits that this statement in Zhuang is made "in connection with its smFISH *validation experiments*," not as a suggestion of determining three-dimensional positional information. EX1002, ¶¶128, 183 (emphasis added).

63. I have included below an excerpt from Zhuang containing the single statement (highlighted in italics) on which Dr. Czymmek relies:

Because we imaged cells using wide-field imaging with oblique-incidence illumination, without optical sectioning and z-scanning, we quantified the fraction of individual RNA species that was outside the axial range of our imaging geometry for six different RNA species using conventional smFISH. *For this purpose, we optically sectioned these cells by collecting stacks of images at different focal depths through the entire depth of the cells.* We aligned the images in consecutive focal planes and then computed for each cell the fraction of RNAs that were detected in the three-dimensional stack but not in the basal focal plane. We found that only a small fraction ... of RNA molecules were outside the imaging range of a fixed focal plane without z-scanning.

EX1003, 012 (emphasis added).

64. Zhuang explains in the above excerpt that because its MERFISH-based system images the fibroblast cells “without optical sectioning and z-scanning,” its authors performed a quality-control experiment to “*quantify[] the fraction*” of individual RNA species that was “outside the axial range of [the system’s] imaging geometry.” EX1003, 012 (emphasis added). Thus, a POSA would have understood that Zhuang discloses quantifying the fraction of RNA molecules that was outside of the single basal focal plane targeted by Zhuang’s system to confirm that the system captured the majority of the relevant fluorescent signals from the RNAs. EX1003, 012.

65. To perform this quality-control experiment, Zhuang discloses that the authors “optically sectioned the[] cells by collecting stacks of images at different focal depths through the entire depth of the cells.” EX1003, 012. Zhuang then discloses “align[ing]” the stacks of images collected from “consecutive focal planes” along the z-axis to “compute[] for each cell the *fraction* of RNAs” in the collected image stacks, but *not* for determining the three-dimensional positional information of nucleic acids. EX1003, 012 (emphasis added).

66. Zhuang expressly discloses that “only a small fraction” of the RNA molecules were “outside the imaging range of a fixed focal plane” targeted by Zhuang’s system, confirming that optical sectioning and z-scanning *were not needed* in Zhuang’s MERFISH-based system. EX1003, 011, 012. Thus, a POSA

would *not* have considered Zhuang's quality control experiment to teach or suggest determining three-dimensional positional information of the RNAs at least because (i) the experiment only assesses the fraction (quantity) of RNAs in other focal planes; and (ii) the experiment does not determine or suggest determining any positional information of those RNAs. EX1003, 011, 012.

67. Moreover, Zhuang's mere disclosure of optical sectioning and/or z-scanning would not have taught or suggested to a POSA to determine three-dimensional positional information of nucleic acid molecules, as claimed. A POSA would have known that optical sectioning is a technique of acquiring images of thin slices (planes) from a thick specimen by removing the contribution of out-of-focus light in each image plane. *See, e.g.*, EX1012, 004 (stating that "optical sectioning" "removes out-of-focus blur and permits you to distinguish between the signals in thin 'optical' slices"). This removal of unwanted light enhances the contrast and improves image quality.

68. Similarly, a POSA would have understood that "z-scanning" simply allows for imaging a sample in different focal planes along the z-axis. For determining three-dimensional positional information, the data obtained from optical sectioning and z-scanning must be further analyzed, and even then it may not determine three-dimensional positional information. For example, Cai expressly discloses using the data obtained after z-scanning to create two-

dimensional images of cells. *See* EX1004, FIG. 12, 10:56-63 (“Images were *2D maximum projections created from z stacks* of 11 images taken every 1.5 μM .” (emphasis added)).

69. Zhuang further discloses that “any optical sectioning technique *could be* used in MERFISH to allow the imaging of RNAs in thicker cells or tissues.” EX1003, 012 (emphasis added). When discussing this sentence in Zhuang, Dr. Czymmek states only that a “POSA would have understood that ‘thicker’ tissue samples imaged using an optical sectioning technique would refer to a sample having a 3D volume.” EX1002, ¶140. Dr. Czymmek does not argue that this sentence in Zhuang teaches or suggests determining three-dimensional positional information of nucleic acids. EX1002, ¶¶216-219. Regardless of whether a tissue sample has a 3D volume, this statement in Zhuang would not have suggested to a POSA to determine three-dimensional positional information of nucleic acid molecules. Instead, a POSA would have understood this statement to refer to imaging cells or tissues that are thicker than Zhuang’s relatively flat, cellular layer sample. In such “thicker cells or tissues,” the labeled target RNAs could reside in multiple focal planes and an imaging system can encounter significant unwanted signals from other focal planes contaminating signals from the target focal plane. Therefore, the statement notes that when imaging thicker cells or tissues in the

future with Zhuang’s MERFISH-based system, optical sectioning could potentially be used to remove the contaminating signals from other focal planes. EX1003, 012.

70. Several publications after Zhuang EX1003’s 2015 publication (and after November 3, 2015) confirm that Zhuang teaches a system for determining two-dimensional—and not three-dimensional—positional information of RNA molecules in cells. For example, in 2023, Zhuang EX1003’s research group published an article (“Zhuang 2023”) disclosing a method for “three-dimensional (3D) single-cell transcriptome imaging of thick tissue specimens” by integrating MERFISH with confocal microscopy for optical sectioning. EX2007, Abstract. Zhuang 2023 explicitly states that MERFISH disclosed in Zhuang EX1003 (referred to as “Chen et al., 2015b” in Zhuang 2023) was “extended to allow spatially resolved 3D-genome imaging and epigenomic profiling of individual cells” in articles published *several years after* Zhuang EX1003. EX2007, 1 (citing a 2022 publication by Lu *et al.* (EX2008) and a 2020 publication by Su *et al.* (EX2009)). By explaining that MERFISH was “extended” years after Zhuang EX1003 to spatially resolved 3D-genome imaging, Zhuang 2023 confirms that the MERFISH-based system disclosed in Zhuang EX1003 did not determine three-dimensional positional information of RNA molecules in the sample.

71. Moreover, the reviewers of Zhuang 2023 characterized the eventual use of MERFISH to determine three-dimensional position information of nucleic acids

as a “technical milestone.” For example, during peer-review of Zhuang 2023, one of the reviewers (Reviewer #2) stated that Zhuang 2023’s new MERFISH three-dimensional methods “present[] a *technical milestone*.” EX2007, 27-28 (emphasis added). Similarly, Reviewer #1 stated that “expanding the capacity of MERFISH to thick tissues [in Zhuang 2023] represents a major technical advance to enable 3D spatial transcriptomics.” EX2007, 27.

72. Similarly, Dr. Czymmek’s own publication from 2022 (“Czymmek 2022”) confirms that the MERFISH-based system disclosed in Zhuang EX1003 in 2015 does not determine three-dimensional positional information. EX2010. Czymmek 2022 is a review article, entitled “Organizing your space: The potential for integrating spatial transcriptomics and 3D imaging in plants.” EX2010. In this article, Dr. Czymmek and co-authors “review advances in [spatial transcriptomics] and [three-dimensional] microscopy approaches and describe how these technologies could be combined to provide high resolution, spatially organized plant tissue transcript mapping.” EX2010, Abstract.

73. When discussing Zhuang’s system (referred to as “Chen et al., 2015b” in Czymmek 2022) and other spatial transcriptomics approaches, Czymmek 2022 first notes MERFISH’s limitations on the number of genes that can be analyzed, and then discusses the potential to *now* generate the data in 3D in 2022:

Targeted methods such as single molecule, fluorescent in situ hybridization (smFISH), yield precise and spatial gene expression data at a subcellular resolution (*Chen et al., 2015b, 2018; Merritt et al., 2020*). However, these methods are limited in the number of genes that can be analyzed per experiment ... Excitingly, there is also *potential to generate these data in 3D*, which requires reassembling the tissue from a serial array of 2D optical or physical tissue sections.

EX2010, 704 (emphasis added).

74. Czymmek 2022's explanation that there was a "*potential*" to generate data from MERFISH in 3D *seven years after* Zhuang EX1003's 2015 publication confirms that Zhuang EX1003 did not teach or suggest a system for determining three-dimensional positional information.

75. A 2019 report by Ghani and Isogai ("Ghani"), entitled, "Optimisation of MERFISH Data Analysis and Visualisation," provides additional confirmation that Zhuang's MERFISH-based system was only in 2D. EX2011. Ghani reports using MERFISH to study "the cellular diversity of hypothalamic tissue." EX2011, Abstract. It describes Zhuang EX1003's MERFISH-based system as a method of "sequential labelling and stripping of fluorophores to a fixed sample" that "exploits principles of digital communications to do robust quantification of gene expression." EX2011, 1. Ghani states that at the time of its publication in 2019,

about four years after Zhuang EX1003’s publication, MERFISH was “currently done in 2D” and Ghani in 2019 developed methods to “accelerate” using 3D MERFISH:

MERFISH analysis is currently done in 2D, where the *z-data is discarded* after generating maximum intensity projections to decrease computation time at the cost of sub-optimal single molecule localisation. We hypothesized that using machine learning techniques and GPU-computing would minimise this trade-off and accelerate 3D MERFISH analysis.

EX2011, 2 (emphasis added).

76. Accordingly, a POSA would *not* have understood Zhuang to teach or suggest a system for determining three-dimensional positional information of one or more nucleic acid molecules, as required by claim 36(e). Later publications, such as Zhuang 2023, Czymmek 2022, and Ghani 2019, confirm this understanding in the art.

B. The relied-upon disclosures in Cai do not teach or suggest a system for determining three-dimensional positional information of nucleic acid molecules

77. Cai’s disclosures that Dr. Czymmek relies on do not teach or suggest a system for determining three-dimensional positional information of nucleic acid molecules, as claimed.

78. Dr. Czymmek argues that if Zhuang “is found or argued to not disclose determining 3D positional information,” it is disclosed in Cai. EX1002, ¶¶219 (citing EX1002, Section XI.A.1.e.(1)), 205. Dr. Czymmek relies on two disclosures in Cai: (1) Cai’s purported disclosure of a “Z-axis motion control system” in Figure 17 and at 47:42-45 that according to Dr. Czymmek, “permit[s] three-dimensional interrogation”; and (2) Cai’s single-sentence at 21:50-51 to obtain “three-dimensional spatial information.” EX1002, ¶¶205-206 (citing EX1002, Section XI.A.1.d), 188; EX1004, 21:50-51, 47:42-45, FIG. 17. As I discuss below, Dr. Czymmek mischaracterizes these disclosures in Cai.

79. Cai’s Figure 17 and disclosure at 47:42-45 relate to using a light sheet microscope to image thick 100 μ M brain slices by “scanning the [brain] sample along z-axis.” EX1004, 11:25-32, 47:42-45, 47:48-50, FIGs. 17, 18. Indeed, Cai expressly describes light sheet microscopy as “*selective plane* illumination microscopy (SPIM).” EX1004, 46:49-53 (emphasis added). The light sheet microscope’s purported z-axis motion control system would simply move the objective (and the sheet of light) along the z-axis to adjust the focal plane for imaging. As I explain above in Section VIII.A, mere scanning of focal planes along the z-axis (*i.e.*, z-scanning) does not determine three-dimensional positional information. Thus, even if Cai’s light sheet microscope system had a z-axis motion control apparatus, that apparatus would not have determined three-dimensional

positional information of nucleic acid molecules in the sample. Dr. Czymmek does not provide any explanation how a POSA would have used a z-axis motion control system to determine three-dimensional positional information. EX1002, ¶¶206, 219.

80. The second disclosure in Cai that Dr. Czymmek relies on also does not disclose a system for determining three-dimensional position as required in claim 36(e). EX1002, ¶206. This limited disclosure in Cai only states that “[i]n some embodiments, an imaging step provides three-dimensional spatial information of a target.” EX1004, 21:49-51. Cai does not provide any further disclosure of a system for determining three-dimensional positional information of nucleic acid molecules, nor does Dr. Czymmek cite any such disclosure in Cai or explain how this single sentence in Cai would suggest specific modifications to Zhuang’s or Cai’s systems to determine three-dimensional positional information of nucleic acids. EX1002, ¶¶205-206, 216-219.

81. Accordingly, a POSA would *not* have understood Cai to teach or suggest a system for determining three-dimensional positional information of one or more nucleic acids, as claimed.

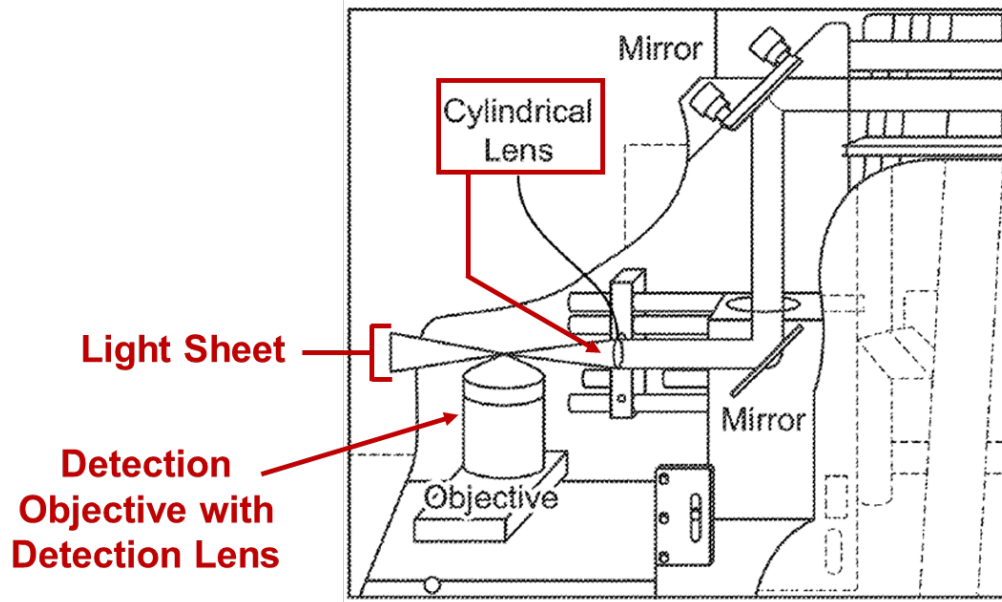
C. Dr. Czymmek does not account for significant differences between Zhuang's and Cai's systems when discussing a POSA's motivation to combine the references with a reasonable expectation of success

82. Dr. Czymmek argues that “it would have been obvious to modify Zhuang [with Cai's disclosure] to permit a POSA to use MERFISH to determine three-dimensional positional information about one or more nucleic acid molecules.” EX1002, ¶219 (citing EX1004, 21:50-51; EX1002, Section XI.A.1.e.(1)); *see also* EX1002, ¶205. However, Dr. Czymmek fails to address significant differences between Zhuang's and Cai's systems and explain why and how a POSA would have modified Zhuang's system with Cai's light sheet microscope for determining three-dimensional positional information of nucleic acid molecules, with a reasonable expectation of success.

83. For example, Zhuang's system images individual RNA molecules in cells and therefore requires (and uses) a high-resolution “1.45 NA, 100x oil immersion” objective. EX1003, 011. The NA (Numerical Aperture) of a lens in a microscope objective was known to impact the working distance (*i.e.*, the space between the sample and lens) of the optical system. EX1012, 10. For example, a higher NA (higher resolution) lens was known to result in an optical system having a smaller working distance. EX1012, 10 (stating that “high NA also comes at the expense of reduced working distance (how far the objective lens can focus into your sample)”); EX1019, 002 (stating that “longer working distances for objective

lenses ... comes at the cost of lowering the numerical aperture”). Thus, a POSA would have known that Zhuang’s disclosure of a 1.45 NA (high NA) objective lens taught an optical system with a small working distance. EX1003, 011-012.

84. In contrast, Cai discloses that its system images brain sections using a light sheet microscope with a low-resolution “20x water immersion” detection objective. EX1004, FIGs. 17, 18, 47:30-56. Cai further discloses that its light sheet microscope “illuminates the sample from the side” using a plane of light (a “light sheet”) that is “perpendicular to the detection objective.” EX1004, 47:36-40, FIG. 17. Thus, Cai discloses a system that includes two optical setups. EX1004, FIG. 17. The *first* optical setup is *perpendicular* to the sample and comprises a low-resolution “20x water immersion” *detection objective* (containing a *detection lens*) for imaging the sample. EX1004, FIG. 17; 47:30-56. The *second* optical setup is *parallel* to the sample and the detection objective, and it comprises a “cylindrical lens” (also known as an “illumination lens” in the art) for focusing a light sheet to illuminate the sample. EX1003, FIG. 17. This is depicted in the annotated excerpt of Cai’s Figure 17 below.



EX1003, FIG. 17 (annotated).

85. A POSA would have generally known that light sheet microscopes like the exemplary microscope in Cai's Figure 17 utilize larger working distances and lower NA to account for the optical machinery and physical geometry needed to create the plane of light as well as the typically larger specimens being imaged. For example, as the authors in one of Dr. Czymmek's exhibits stated, light-sheet microscopy was known to "force[] the use of *long working distance* [and] somewhat *low NA* (typically 1.0 or less) objective lenses." EX1019, 007 (emphasis added). Indeed, Cai describes using "*20x low NA objectives*." EX1004, 44:34-35 (emphasis added). Further, Cai expressly discloses using a light sheet microscope, such as Figure 17's exemplary microscope, with a 20x water immersion objective to image "100 μ m brain slices." EX1003, 47:30-56, FIGs. 17, 18. A POSA would

have known that Cai's disclosure to use a light sheet microscope to image the brain slices taught that the microscope uses a low NA cylindrical lens. Thus, a POSA reading Cai would have known that Cai's light sheet microscope system uses a low NA detection lens and a low NA cylindrical lens, both of which use a large working distance.

86. Dr. Czymmek fails to address these material differences between the systems of Zhuang and Cai, and explain how these differences would have impacted a POSA's supposed motivation to combine these systems with a reasonable expectation of success. Nowhere does Dr. Czymmek explain which parts of Cai's low NA/large working distance light sheet microscope a POSA would have incorporated into Zhuang's high NA/small working distance MERFISH-based system to modify Zhuang's system to arrive at the claimed system for determining three-dimensional positional information of nucleic acids.

87. Nor does Dr. Czymmek explain how a POSA would have modified Zhuang's system with a light sheet microscope to arrive at a system that has both a high NA detection lens (like Zhuang's system) and high-resolution illumination lens (for the light sheet system), all with the corresponding very small working distance, and yet maintain sufficient physical space to fit all of the necessary optical parts (such as, the detection objective, condenser, cylindrical lens) and other parts (such as, the stage with the sample holder) in the small space between

the sample and the two lenses. A POSA would have known that significant physical modifications to a microscope's geometry can undermine its performance. For example, as one of Dr. Czymmek's own exhibits states, "[t]he *working distance can be increased* by removing the condenser CV," but in making such a modification, the "illumination becomes *inappropriate*." EX1005, 028.

88. Thus, Dr. Czymmek fails to explain why and how a POSA would have modified Zhuang's system with Cai's system to arrive at a system for determining three-dimensional positional information of nucleic acid molecules with a reasonable expectation of success, as required in claim 36(e).

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

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

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Laura Waller', with a stylized flourish at the end.

Laura Waller, Ph.D.

Date: 5/28/24

22327646