

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

PETITION FOR *INTER PARTES* REVIEW
OF U.S. PATENT NO. 11,542,554

TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
II. TECHNOLOGY BACKGROUND.....	4
A. Cellular Imaging.....	4
B. Microscopy Techniques for Imaging Nucleic Acids in Cells	5
C. Automation of Imaging Systems.....	7
III. OVERVIEW OF THE '554 PATENT	8
A. Challenged Claims	9
B. Prosecution History	12
IV. LEVEL OF ORDINARY SKILL IN THE ART OF THE '554 PATENT	13
V. THE CITED PRIOR ART	14
A. Primary Combination Relied Upon Herein.....	14
1. Zhuang	14
2. Cai	15
3. Shen.....	17
B. Secondary References Relied Upon Herein.....	18
VI. MANDATORY NOTICES	20
A. Real Party-in-Interest	20
B. Related Matters.....	20
C. Lead and Back-Up Counsel and Service Information	21
VII. GROUNDS FOR STANDING.....	21

VIII. STATUTORY GROUNDS FOR THE CHALLENGES	21
IX. CLAIM CONSTRUCTION	22
X. GROUND 1: OBVIOUSNESS IN VIEW OF ZHUANG, CAI, AND SHEN	22
A. Claim 36	22
1. A system comprising:	22
a. (a) a stage comprising:.....	23
i. (a)(i) a sample holder configured to receive a substrate comprising a sample having a volume;	23
ii. (a)(ii) a temperature control apparatus operatively coupled to said sample holder; and	24
iii. (a)(iii) An XY motion control system;.....	25
b. (b) a fluidics system comprising:	26
i. (b)(i) one or more reservoirs configured to contain one or more fluid reagents; and	26
ii. (b)(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	27
c. (c) an optical system comprising:.....	28
i. (c)(i) an objective;	28
ii. (c)(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.....	29
iii.	(c)(iii) one or more detectors configured to receive light signals from said volume;	30
d.	(d) a z-axis motion control system configured for z-motion of an objective focal plane through said volume; and	31
e.	(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:	35
i.	(e)(i) 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;	36
ii.	(e)(ii) 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;.....	40
iii.	(e)(iii) identifying said one or more nucleic acid molecules based on said plurality of optically-encoded signals; and	41
iv.	(e)(iv) determining three-dimensional positional information of said one or more nucleic acid molecules.	42
B.	Claim 37: The system of claim 36, wherein said substrate comprises a flow cell.....	42
C.	Claim 38: The system of claim 37, wherein said flow cell is a closed flow cell.....	42
D.	Claim 39: The system of claim 38, wherein said flow cell is a closed flow cell comprises a fluid inlet and a fluid outlet, and wherein said fluid inlet is fluidically coupled to said fluid dispenser.	42
E.	Claim 43: The system of claim 36, wherein said temperature control apparatus comprises a heat sink or a Peltier element.	43
F.	Claim 44: The system of claim 36, further comprising a thermal control system.	43
G.	Claim 46: The system of claim 36, wherein said fluidics system further comprises one or more valves downstream of said one or more reservoirs and upstream of said fluid dispenser, wherein, upon actuation, said one or more valves facilitate dispensation of fluid to said sample holder.....	44
H.	Claim 47: The system of claim 46, wherein said one or more valves comprise a rotary valve	45
I.	Claim 54: The system of claim 36, further comprising an objective wetting apparatus.....	45

J.	Claim 56: The system of claim 36, wherein said two or more light sources comprise one or more lasers.	45
K.	Claim 59: The system of claim 36, wherein said optical system further comprises one or more emission filters configured to selectively filter one or more wavelengths of light emitted from said volume to said one or more detectors.	46
L.	Claim 60: The system of claim 36, wherein said optical system is mechanically, electrically, or electromechanically coupled to a controller system configured to synchronize one or more imaging events within said system.....	46
M.	Claim 63: The system of claim 44, wherein said temperature control apparatus further comprises a temperature sensor configured to provide a feedback signal to said thermal control system.	47
N.	Claim 64: The system of claim 59, wherein at least one of said one or more emission filters is configured to selectively filter light having a wavelength of about 510 nm, about 570 nm, about 620 nm, or about 680 nm to said one or more detectors from said volume to said one or more detectors.	49
O.	Claim 65: The system of claim 36, wherein said two or more light sources excite fluorescence in unique color channels such that each light source of said two or more light sources is configured to excite fluorescence in a unique color channel of said unique color channels.	50
P.	Claim 66: The system of claim 65, wherein each optically-encoded signal of said plurality of optically-encoded signals comprises at least one of said unique color channels.....	51
Q.	Claim 67. The system of claim 36, wherein at least one of the plurality of optically-encoded signals comprises two or more colors detected in series.....	51
R.	Claim 68: The system of claim 36, wherein said two or more light sources comprise four light sources.....	52

S.	Claim 69: The system of claim 36, wherein said optical system is configured for wide-field microscopy.	52
XI.	GROUND 2: OBVIOUSNESS OF CLAIM 40 IN VIEW OF ZHUANG, CAI AND SHEN, IN FURTHER VIEW OF XIAO AND EHRLICHER.....	52
XII.	GROUND 3: OBVIOUSNESS OF CLAIMS 41 AND 70 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF TIRICHINE	53
XIII.	GROUND 4: OBVIOUSNESS OF CLAIMS 42 AND 70 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF BRILEYA	54
XIV.	GROUND 5: OBVIOUSNESS OF CLAIM 45 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF FANG-YEN	55
XV.	GROUND 6: OBVIOUSNESS OF CLAIM 48 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF RICHARD	56
XVI.	GROUND 7: OBVIOUSNESS OF CLAIM 49 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF NIKON	57
XVII.	GROUND 8: OBVIOUSNESS OF CLAIMS 50 AND 51 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF NIKON AND SKAFTE-PEDERSEN	58
XVIII.	GROUND 9: OBVIOUSNESS OF CLAIM 52 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF SANDS	59
XIX.	GROUND 10: OBVIOUSNESS OF CLAIM 53 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF SANDS AND BOOKER	60
XX.	GROUND 11: OBVIOUSNESS OF CLAIMS 55, 57, AND 58 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF WESSELS.....	62

XXI. GROUND 12: OBVIOUSNESS OF CLAIM 61 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF THE SKILL AND KNOWLEDGE OF A POSA	64
XXII. GROUND 13: OBVIOUSNESS OF CLAIM 62 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF NIMAN AND/OR WINER.....	65
XXIII. THE BOARD SHOULD REACH THE MERITS OF THIS PETITION	66
A. The Facts and Law Weigh Against Discretionary Denial under § 325(d)	66
B. <i>Fintiv</i> Does Not Support Discretionary Denial.....	68
1. Factor 1: Likelihood of a Stay	69
2. Factor 2: Proximity of Trial Date to Final Written Decision	69
3. Factor 3: Investment in Parallel Proceeding	71
4. Factor 4: Overlap in Issues With Parallel Proceeding	71
5. Factor 5: Identity of the Parties.....	71
6. Factor 6: Other Factors	72
XI. CONCLUSION.....	73
CERTIFICATE OF SERVICE	81

TABLE OF AUTHORITIES

	Page(s)
 Cases	
<i>10x Genomics, Inc. et al. v. NanoString Techs., Inc.</i> , No. 21-cv-653 (D. Del. Filed May 6, 2021).....	70
<i>10X Genomics, Inc. v. NanoString Technologies, Inc.</i> , 1-22-cv-00261 (D. Del. Filed Feb. 28, 2022).....	20
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<i>OpenSky Indus., LLC v. VLSI Tech. LLC</i> , IPR2021-01064, Paper 102 (P.T.A.B. Oct. 4, 2022).....	68, 72
<i>Samsung Elecs. Co., LTD v. Mojo Mobility, Inc.</i> , IPR2023-01089, Paper 11 (PTAB, Jan. 11, 2024)	70
 Statutes	
35 U.S.C. Section.....	21
35 U.S.C. § 102(a)(1).....	<i>passim</i>
35 U.S.C. § 102(a)(2).....	15
35 U.S.C. § 311(c)(1).....	69

PETITIONER'S EXHIBIT LIST

Exhibit #	Description
Ex. 1001	U.S. Patent No. 11,542,554 (“the ’554 Patent”)
Ex. 1002	Declaration of Dr. Kirk Czymmek, Ph.D. under 37 C.F.R. § 1.68
Ex. 1003	Kok Hao Chen et al., <i>Spatially resolved, highly multiplexed RNA profiling in single cells</i> , 348(6233) Science 412 (Research Article Summary) – aaa6090-1 - aaa6090-14 (2015) (“Zhuang”)
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Ex. 1005	Olympus Lifescience, <i>Instructions IX71/IX51 Inverted Research Microscope/Inverted Basic Microscope</i> , (2005) at https://www.ucc.ie/en/media/academic/anatomy/imagingcentre/icdocuments/OLYMPUSIX71_manual.pdf (“Olympus Manual”)
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Ex. 1009	Emmanuelle Querido and Pascal Chartrand, “Chapter 12 Using Fluorescent Proteins to Study mRNA Trafficking in Living Cells,” 85 <i>Methods In Cell Biology</i> , Elsevier Inc. 273-292 (2008) (“Querido”)
Ex. 1010	Andrea M. Femino et al., “[13] Visualization of Single Molecules of mRNA in Situ,” 361 <i>Methods in Enzymology</i> , Elsevier Science (USA) 245-304 (2003) (“Femino”)

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Ex. 1012	Alison J. North, <i>Seeing is believing? A beginners’ guide to practical pitfalls in image acquisition</i> , 172 The Journal of Cell Biology 9-18 (2006) (“North”)
Ex. 1013	US Patent Application Publication No. US 2015/0267251 A1 to Long Cai et al. (“Cai reference”)
Ex. 1014	Paul James, <i>Water Objectives A personal exploration.....all is not what it seems</i> , Microscopy UK 1-8 (2004) at www.microscopy-uk.org.uk/mag/indexmag.html ? http://www.microscopy-uk.org.uk/mag/artoct04/pjwater.html (“James”)
Ex. 1015	David L. Spector et al., “[75] Observation of Live Cells in the Light Microscope,” <i>Cells A Laboratory Manual Volume 2: Light Microscopy and Cell Structure</i> , Cold Spring Harbor Laboratory Press, 75.1-75.13 (1998) (“Spector”)
Ex. 1016	Hamilton, <i>HVXM 8-5 Valve Laboratory Products</i> , (November 7, 2023) at https://www.hamiltoncompany.com/laboratory-products/valves/36766 (“Hamilton website”)
Ex. 1017	Clare O’Connor, <i>Fluorescence In Situ Hybridization (FISH)</i> , 1(1):171 Nature Education (2008) at https://www.nature.com/scitable/topicpage/fluorescence-in-situ-hybridization-fish-327/ (“O’Connor”)
Ex. 1018	Derek Toomre and Joerg Bewersdorf, <i>A New Wave of Cellular Imaging</i> , 26 Annu. Rev. Cell Dev. Biol. 285-287 (2010) (“Toomre”)
Ex. 1019	Robert S. Fischer et al., <i>Microscopy in 3D: a biologist’s toolbox</i> , 21(12) Trends Cell Biol. 682-691 (2011) at https://pubmed.ncbi.nlm.nih.gov/22047760/ (“Fischer”)

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Ex. 1029	Christopher Fang-Yen et al., <i>Video-rate tomographic phase microscopy</i> , 16(1) Journal of Biomedical Optics 011005-1 – 011005-5 (2011) (“Fang-Yen”)
Ex. 1030	Marc J.P. Richard et al., <i>Cellular mechanisms by which lipoic acid confers protection during the early stages of cerebral ischemia: A possible role for calcium</i> , 69 Neuroscience Research 299-307 (2011) (“Richard”)

Exhibit #	Description
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Ex. 1033	Olympus Lifescience, <i>Inverted Research System Microscopes IX71/IX81 IX2 Series Manual Olympus IX-71</i> , (2009) at https://afns-labs.ualberta.ca/wp-content/uploads/sites/58/2018/05/Olympus-IX81-brochure.pdf.pdf
Ex. 1034	Information Disclosure Statement (IDS) dated August 6, 2021, submitted by Applicant during prosecution of U.S. Patent Application No. 17/395,534 before the United States Patent and Trademark Office.
Ex. 1035	Non-Final Rejection issued by the United States Patent and Trademark Office dated March 4, 2022, during prosecution of U.S. Patent Application No. 17/395,534
Ex. 1036	Notice of Allowance issued by the United States Patent and Trademark Office dated June 21, 2022, during prosecution of U.S. Patent Application No. 17/395,534
Ex. 1037	Pending and Amended claims filed by the Applicant on February 14, 2022, during prosecution of U.S. Patent Application No. 17/395,534 before the United States Patent and Trademark Office
Ex. 1038	Applicant's Arguments and Remarks made in an Amendment, Amendment/Request for ReConsideration-After Non-Final Rejection and Claims filed by the Applicant on June 3, 2022, during prosecution of U.S. Patent Application No. 17/395,534 before the United States Patent and Trademark Office
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Ex. 1042	U.S. Patent Application Publication No. US 2015/0098126 A1 to Phillip Johannes Keller and Raghav Kumar Chhetri (“Keller”)
Ex. 1043	Nikon MicroscopyU, <i>Culture Chambers for Live-Cell Imaging</i> (August 2015) at https://web.archive.org/web/20150810165805/http://www.microscopyu.com:80/articles/livecellimaging/culturechambers.html . (“Nikon”)
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Ex. 1049	Leïla Tirichine et al., <i>3D fluorescent in situ hybridization using Arabidopsis leaf cryosections and isolated nuclei</i> , 5:11 Plant Methods 1-7 (2009) (“Tirichine”)
Ex. 1050	Johannes T. Wessels et al., <i>Light-Emitting Diodes in Modern Microscopy—From David to Goliath?</i> , 81A Cytometry Part A 188-197 (2012) downloaded at https://onlinelibrary.wiley.com/doi/10.1002/cyto.a.22023 (“Wessels”)
Ex. 1051	Michael H. Winer et al., <i>Application of a three-dimensional (3D) particle tracking method to microfluidic particle focusing</i> , 14 Lab Chip 1443-1451 (2014) (“Winer”)

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Ex. 1052	Patrick JM Zessin et al., <i>A hydrophilic gel matrix for single-molecule super-resolution microscopy</i> , 2:4 Optical Nanoscopy 1-8 (2013) (“Zessin”)
Ex.1053	Olympus Lifescience, <i>Research Inverted System Microscope IX71/IX81 IX2 Series</i> , (2005) at https://www.olympus-lifescience.com/data/olympusmicro/brochures/pdfs/ix71.pdf?rev=EAB E
Ex. 1054	Ilja Gerhardt et al., <i>Detection of Single Molecules Illuminated by a Light-Emitting Diode</i> , 11 Sensors 905-916 (2011) (“Gerhardt”)
Ex. 1055	Akifumi Hattori et al., <i>Single-molecule Imaging with an Inexpensive UV-LED Light Source</i> , 38 Chemistry Letters 234-235 (2009) (“Hattori”)
Ex. 1056	Jason S. Kuo et al., <i>High-power blue/UV light-emitting diodes as excitation sources for sensitive detection</i> , 25 Electrophoresis 3796-3804 (2004) (“Kuo”)
Ex. 1057	Thermo Fischer Scientific, <i>FluoSpheres™ Carboxylate-Modified Microspheres</i> , (January 9, 2024) at https://www.thermofisher.com/order/catalog/product/F8809
Ex. 1058	Metadata for Ex.1005
Ex.1059	Metadata for Ex.1033
Ex. 1060	Metadata for Ex.1053
Ex. 1061	Stipulation and Order Amending Scheduling Order, <i>10X Genomics, Inc. and President And Fellows of Harvard College v. Nanostring Technologies Inc.</i> , C.A. No. 22-261-MFK (December 20, 2023 D. Del.) (No. 231)
Ex. 1062	Plaintiffs, 10x Genomics, Inc. and President and Fellows of Harvard College’s Complaint, <i>10X Genomics, Inc. and President And Fellows of Harvard College v. Nanostring Technologies Inc.</i> , C.A. No. 22-261-MFK (February 28, 2022 D. Del.) (No. 1)

Exhibit #	Description
Ex. 1063	Plaintiffs, 10x Genomics, Inc. and President and Fellows of Harvard College's Second Amended Complaint, <i>10X Genomics, Inc. and President And Fellows of Harvard College v. Nanostring Technologies Inc.</i> , C.A. No. 22-261-MFK (March 1, 2023 D. Del.) (No. 92)
Ex. 1064	Defendant, NanoString Technologies, Inc.'s First Amended Answer, Affirmative Defenses, and Counterclaims to 10x and Harvard's Second Amended Complaint, <i>10X Genomics, Inc. and President And Fellows of Harvard College v. Nanostring Technologies Inc.</i> , C.A. No. 22-261-MFK (July 18, 2023 D. Del.) (No. 157)
Ex. 1065	Curriculum Vitae of Kirk J. Czymmek

I. INTRODUCTION

Nanostring Technologies, Inc. (“Petitioner”) requests review of claims 36–70 of United States Patent No. 11,542,554 B2 (“the ’554 patent”) (EX1001), which issued on January 3, 2023, and is assigned to President and Fellows of Harvard College (“Patent Owner”). The ’554 patent is exclusively licensed to 10x Genomics, Inc.

The ’554 patent is entitled “Method and Apparatus for Volumetric Imaging,” and pertains to methods for imaging nucleic acids, such as DNA and RNA, in biological samples. Claim 36, the sole challenged independent claim, is lengthy and detailed. But length and detail should not be mistaken for inventiveness, particularly in this case.

Claim 36 of the ’554 patent can be viewed as having two basic parts, wherein the first part consists of recited elements (a)-(e) and the second part consists of software instructions associated with element (e), which refers to a “computing system.” These two parts—whether considered individually or together—are not meaningfully distinct from the prior art.

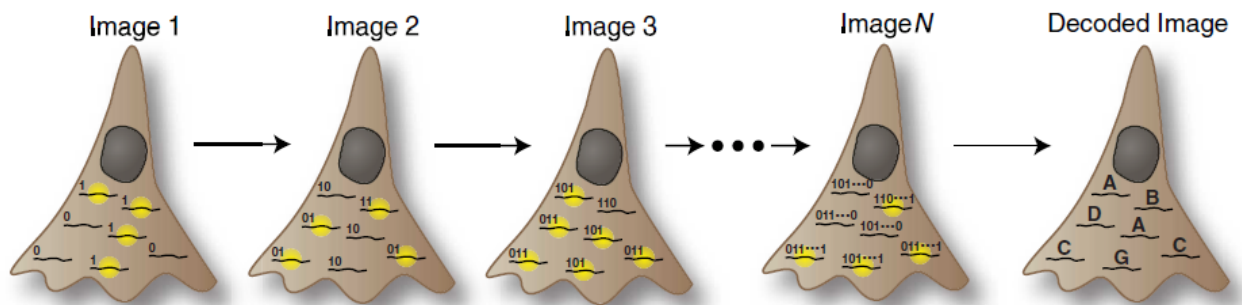
Elements (a)-(e) recite nothing more than standard, well-known components for optical imaging of biological samples, such as a microscope stage, sample holder, temperature control system, light source, objective lens, and computing system. Nothing about any of these components is inventive or new, nor is there anything

novel in how the recited components are arranged in the claims. The '554 patent acknowledges this, stating the “practice of the methods disclosed herein may employ conventional biology methods, software, computers and computer systems.” EX1001 at 29:36-38. Likewise, during prosecution, Patent Owner did not dispute that the prior art disclosed an imaging system with the components set forth in elements (a)-(e).

To the extent any point of novelty was identified during prosecution, it had nothing to do with the conventional imaging components recited in elements (a)-(e), but rather the software instructions supplied to the “computing system” in element (e). These software steps, however, involve little more than imaging the sample multiple times to generate a collection of signals from the analytes in the sample, and then decoding the images to determine the identity and position of the analytes. This exact process, however, had previously been achieved through use of the very imaging components set forth in the claims of the '554 patent.

Specifically, well before the alleged invention of the '554 patent, the Zhuang research group had published an approach known as “Multiplexed Error-Robust Fluorescence in Situ Hybridization,” or MERFISH for short. As set forth in the Zhuang reference relied upon in this Petition, MERFISH is a “single-molecule imaging approach that allows the copy numbers and spatial localizations of thousands of RNA species to be determined in single cells.” EX1003.002.

To this end, Zhuang teaches a system based on the use of an Olympus IX-71 microscope. Just like the alleged invention set forth and claimed in the '554 patent, Zhuang's MERFISH system involved repeatedly introducing to a sample fluorescently labeled probes that can be attached to target analytes in the sample. Targets "can be simultaneously imaged in single cells" during multiple "rounds of hybridization and imaging." EX1003.002-.003. This is depicted in Figure 5A of Zhuang, which shows the multiple targets within a cell being imaged in multiple rounds:



EX1003 at Fig. 1A. In each cycle, an image of the sample is received, and code "1" represents detection of a signal and "0" represents no signal. The sequence of codes is then used to determine which targets are present at which position.

As proven herein and in the accompanying declaration of Dr. Kirk Czymmek, Zhuang in combination with other references renders claims 36-70 obvious. Unfortunately, during prosecution, the Examiner gave Zhuang no consideration and Cai no meaningful consideration, and the '554 patent erroneously issued. The

combinations of asserted references set forth renders claims 36-70 obvious. The Board should now take this opportunity to correct this error and cancel claims 36-70 of the '554 patent.

II. TECHNOLOGY BACKGROUND

As detailed by Dr. Kirk Czymmek, the technology underlying the alleged invention of the '554 patent was not new, but rather constituted well-known conventional approaches to biological imaging. EX1002 ¶¶ 44-67.

A. Cellular Imaging

Microscopes have long been sophisticated instruments for scientific research, including for three-dimensional imaging of fixed and live cells. EX1002 ¶¶ 44-55. The Olympus IX-71 microscope from early 2000s is exemplary, comprising various standard components—a movable stage for holding the sample, a light source for illumination, and optical equipment for visualization. *Id.* ¶¶ 46-49. By 2015, microscope systems had evolved to include computing for automated imaging and data analysis, and to include various other modifications, such as temperature control and fluidics systems. *Id.* ¶¶ 51-52. Zhuang, as detailed below, used a modified version of the Olympus IX-71 microscope for MERFISH imaging.

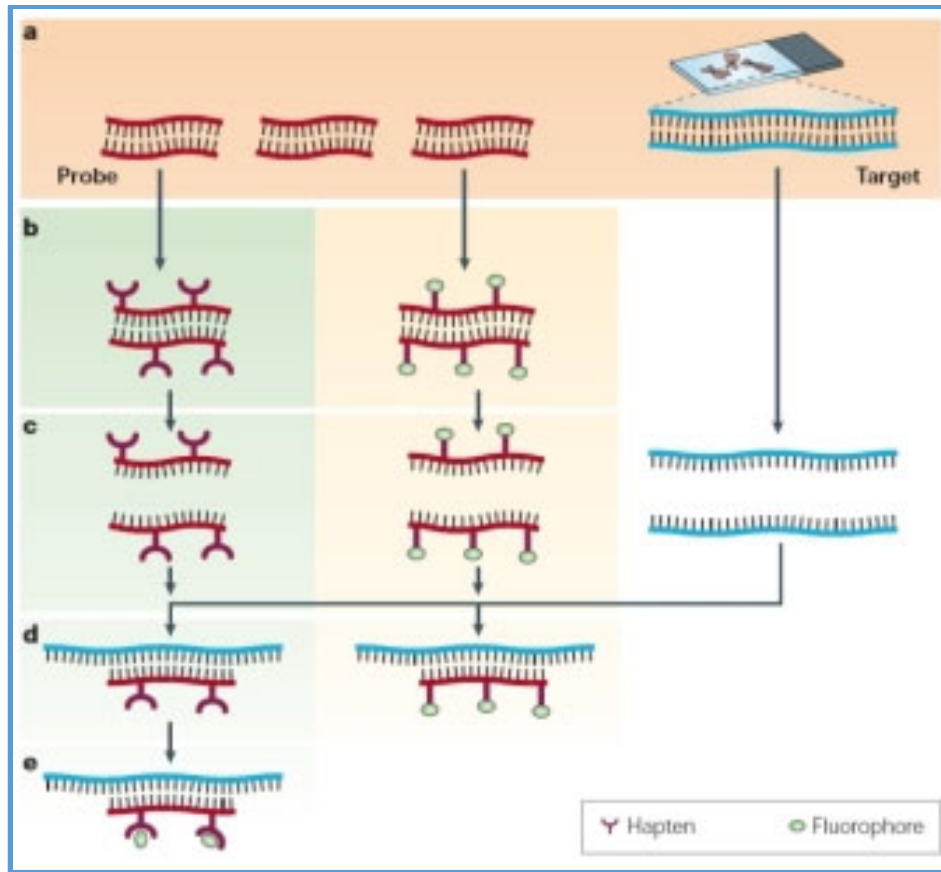
Before 2015, live-cell imaging utilized numerous microscopy techniques, including confocal and super-resolution approaches, with some relying on imaging using fluorescent probes, thus also allowing detection and localization of target

molecules or structures. EX1002 ¶ 44; EX1018.002. These well-established techniques included *three-dimensional* cellular imaging, providing a picture in three dimensions of the distribution of analytes within samples, such as structured illumination microscopy, STORM, PALM, spinning disk confocal microscopy, and light sheet fluorescence, which rely on illuminating labels and capturing cross-sectional images at multiple focal planes within samples. EX1018.008; EX1019.003-.007. Using a “stack”, these cross-sectional images provide a three-dimensional image of the biological sample. EX1002 ¶ 45.

B. Microscopy Techniques for Imaging Nucleic Acids in Cells

Before 2015, numerous fluorescent probe-based microscopy techniques had been developed for nucleic acid (DNA and RNA) imaging. EX1002 ¶¶ 56-60. Such techniques were of importance to researchers because locations of nucleic acids encoding proteins in cells and tissues provide useful information for biological research and medical diagnostics. Fluorescence in situ hybridization (“FISH”), sequential fluorescence in situ hybridization (“seqFISH”), and multiplexed error robust fluorescence in situ hybridization (“MERFISH”) are three such related techniques, each discussed briefly below.

The following image illustrates the basic concept underlying FISH:



EX1017 at Fig. 1; EX1002 ¶ 58.

FISH uses labeled nucleic acid probes complementary to target nucleic acids in the cell(s) or tissue that hybridizes, either directly to the target nucleic acid (as shown above), or indirectly (by hybridizing to a nucleic acid that is hybridized to the target nucleic acid), thus revealing the target's presence and location in cell(s)/tissue upon detection of the label. EX1017.001; EX1004 at 14:27-31. FISH is “widely known and practiced by persons having ordinary skill in the art.” *Id.* at 14:27-29.

seqFISH, a variant of FISH, employs fluorescently-labeled nucleic acid probes like FISH, but uses sequential rounds of probe hybridization and fluorescence

imaging to capture three-dimensional images of cells or tissues. EX1020.002-.003; EX1002 ¶ 59. Sequential hybridization and imaging, with detection of fluorescence signal at different locations, provides a series of signals for each location where hybridization occurs, providing a series of signals (“barcodes”) that are then used to distinguish among different target nucleic acid molecules in the sample, providing three-dimensional spatial information about the target mRNA or DNA in the cell or tissue. EX1020.003-.004.

MERFISH follows similar principles to seqFISH but is multiplexed, *i.e.*, it uses *simultaneous* sequential hybridization with numerous labeled probes, enabling imaging and identification of thousands of RNA species simultaneously, while similarly providing spatial context. EX1003.001; EX1021 at Abstract, .003-.005, .018; EX1002 ¶ 60.

C. Automation of Imaging Systems

Automated microscopic imaging systems were common and commercially available before 2015, and included automation of the system for capturing images and for image analysis, increasing efficiency by minimizing labor and enhancing experimentation. EX1002 ¶¶ 61, 64-66; EX1022.001-.002; EX1018.006. This automation encompassed image capture, microscope apparatus, and image analysis. EX1002 ¶ 61. Automation for capturing images includes maintaining focus, *i.e.*, autofocus, during imaging. *Id.* ¶¶ 62-64; EX1032.001-.003. Automation also

includes automated image processing using software to process image data for analysis and visualization of data, including data as to the identity and 3D location of nucleic acids of interest, allowing analysis to construct three-dimensional representations, with such software including commercial and open-source programs prior to 2015. EX1002 ¶¶ 65-67; EX1022.005.

III. OVERVIEW OF THE '554 PATENT

The '554 patent is directed to an apparatus for imaging nucleic acids within a cell. EX1001 at Abstract. The general approach set forth and claimed in the '554 patent is no different from the prior art relying on sequential hybridization and imaging as in MERFISH and seqFISH.

The '554 patent describes the use of fluorescently labeled oligonucleotides that are introduced to cells and sequentially hybridized to target nucleic acids in the cell, allowing imaging of the target nucleic acids. *Id.* at 12:23-30 (“[T]he nucleic acids, which may represent bits of information, are readily accessed by oligonucleotides, such as labeled oligonucleotide probes” and the matrix used in sample preparation “has optical properties suitable for...deep three dimensional imaging”), 15:64-67 (“[N]ucleic acids within the matrix can be interrogated using methods known to those of skill in the art including fluorescently labeled oligonucleotide/DNA/RNA hybridization.”), 16:25-27 (“The automated...three-

dimensional imaging device may be used for detecting optical signals by fluorescence microscopy.”).

The fluorophores on the oligonucleotides may have one of a number of different colors, and each target is labeled with one or more oligonucleotides. The colors are detected in serial or in parallel, with serial detection including rounds of detection once new probes are hybridized. *Id.* at 9:42-45 (“[T]he automated...three-dimensional imaging device described herein comprises a color multiplexer for detection of one or more distinct colors of light.”), 9:61-63 (“[T]he automated...three-dimensional imaging device...is used to detect two or more colors of light in serial or parallel.”).

The ’554 Patent further describes automation of imaging systems. *Id.* at 8:19-20, 12:41-44, 21:22-31, 23:60-63. This can include automation of reagent delivery and imaging devices, such as computerized systems for “controlling and coordinating the timing of fluidic, optical, and motion-related events.” *Id.* at 12:41-44, 23:60-63. Automated imaging systems in the ’554 patent may include use of computational algorithmic methods of processing digital image data and use of image-based software programs. *Id.* at 8:19-20, 21:22-31.

A. Challenged Claims

Claims 36-70 of the ’554 patent are challenged in this Petition. Briefly, the challenged claims are directed to standard systems for determining the three-

dimensional positional information for nucleic acid molecules within a sample volume that include an optical detection system and a computing system. Independent claim 36 is reproduced below, and a full listing of claims 36-70 is submitted herewith as an Appendix.

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.

The system set forth above in elements (a)-(e) is wholly conventional. EX1002 ¶ 25. The stage, fluidics system, optical system, and Z-axis motion control system are wholly conventional. *Id.* ¶¶ 25-26. The computing system is also wholly conventional and is simply configured to perform a method of detecting, identifying, and determining the location of nucleic acids using the optical detection system that was also known. *Id.* ¶¶ 26-28.

Dependent claims 37-70 add limitations to particular types of apparatus that are likewise wholly conventional and were well-known in the prior art. *See infra* Appendix A.

B. Prosecution History

The '554 patent issued from U.S. Application No. 17/395,534 filed August 6, 2021. It was subject to a single Non-Final Rejection where then-pending claims 9-38 were rejected on the grounds of nonstatutory obviousness-type double patenting and for obviousness in view of Keller (US 2015/0098126) (EX1042) and Callahan (US 2011/0216953) (EX1041). EX1035.009-.021; EX1002 ¶ 30.

The Applicant amended the rejected pending claims, and added new claims 39-45, where claim 9 (issued claim 1) and claim 45 (issued claim 36) each recite a computing system and program instructions. EX1038; EX1001 at claims 1, 36. The amended claims were allowed over Keller because the Examiner found that Keller “is silent with regards to a computing system” configured as it was in Claim 36 (in effect as the rejection was not applied to new claim 45), and other references that allegedly failed to “teach[] the systems as currently claimed, including the computing system configured for determining three-dimensional positional information of the one or more nucleic acid molecules using a plurality of optically-encoded signals.” EX1002 ¶¶ 33-39; EX1001 at claims 1, 36; EX1035.009-.011, .014-.015; EX1036.006-.007; EX1038.002, .005-.013. The components of the microscope system were found as all taught by the cited prior art. EX1002 ¶¶ 37-39; EX1036.006-.007; EX1035.009-.011, .014-.015.

Neither Zhuang nor Shen were before the Examiner. EX1002 ¶¶ 40-41. Cai was submitted in an IDS, but the Examiner gave Cai no substantive consideration. *Id.* ¶ 40.

IV. LEVEL OF ORDINARY SKILL IN THE ART OF THE ’554 PATENT

A person having ordinary skill in the art (“POSA”) relevant to the ’554 patent as of November 3, 2015, would have had a Ph.D. in Molecular, Cellular, or Developmental Biology and/or Biophysics, Bioengineering, Biochemistry, or a

related discipline with 2 years of experience in the field of fluorescence imaging of biological materials, including cells and tissues. EX1002 ¶¶ 68-70.

A POSA would have understood the following technologies and techniques: fluorescence imaging technologies; the use of microscopes to collect and record images; and the processing of images and data to obtain 2D or 3D spatial information relating to cells or tissues. *Id.* ¶ 69. A POSA would also have had some familiarity with gene amplification and sequencing technologies. *Id.* ¶ 70.

V. THE CITED PRIOR ART

A. Primary Combination Relied Upon Herein

This Petition relies on the combination of Zhuang, Cai, and Shen in Ground 1 challenging independent claim 36 and dependent claims 37-39, 43, 44, 46, 47, 54, 56, 59, 60, and 63-69.

1. Zhuang

Zhuang was published in April 2015. EX1003; EX1002 ¶ 72. Zhuang is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).

Zhuang teaches MERFISH, a multiplexed method of spatial determination of nucleic acids, utilizing fluorescent probes. EX1003.002 (Abstract), .003; EX1002 ¶¶ 73-74. Zhuang teaches an automated system based on a modified version of the Olympus IX-71 microscope that includes fluidics and optical systems to carry out the MERFISH imaging method. EX1003.003, .011; EX1002 ¶¶ 75-76. Zhuang

discloses using fluid reagents comprising fluorescently labeled probes, and successive imaging rounds that capture the light signals emitted by the probes in each round. EX1003 at Fig. 1, .011; EX1002 ¶ 76. Zhuang further discloses using a computing system to control the imaging and to analyze the average number of light signals so as to reveal information on the identity and location of the nucleic acid species in the sample. EX1003.011; EX1002 ¶¶ 76-77. As Zhuang explains, the “entire system was fully automated, so that imaging and fluid handling were performed for the entire experiment without user intervention.” EX1003.011; EX1002 ¶ 78.

2. Cai

Cai issued as U.S. Patent No. 10,457,980, from Application No. 14/435,735, the national phase of PCT/US2014/036258, that was filed on April 30, 2014. EX1004; EX1002 ¶ 79. Cai is therefore prior art under 35 U.S.C. § 102(a)(2).

Cai teaches seqFISH, described above. *See supra* Section II.B.; EX1002 ¶¶ 80-85. Cai discloses the use of oligonucleotide probes with a detectable label that are able to diffuse into a sample and hybridize with nucleic acid molecules of interest, allowing imaging. EX1004 at 47:57-61; EX1002 ¶¶ 81-84. Detectable labels can be a fluorophore or fluorescent dye, such that the “detectable signal is a certain color.” EX1004 at 4:40-45, 15:33-45; 16:26-37; EX1002 ¶ 84. Signals generated by sequential rounds of hybridization and imaging can be used to identify

targets, where a sequence of color signals obtained represents a “barcode” unique to each target. EX1002 ¶¶ 85, 171; EX1004 at 9:28-48, 15:23-32; EX1002 ¶ 85.

Cai describes various exemplary systems for seqFISH, including automated systems. EX1002 ¶¶ 86-87; EX1004 at 45:32-62, 42:20-21, 44:28-33, 45:57-62, 46:34-48. These exemplary systems provide sequential 2D (X-Y axis) scans along the Z-axis, i.e., scanning the sample by moving objective in Z-axis, as depicted in Figure 17, providing three-dimensional imaging.

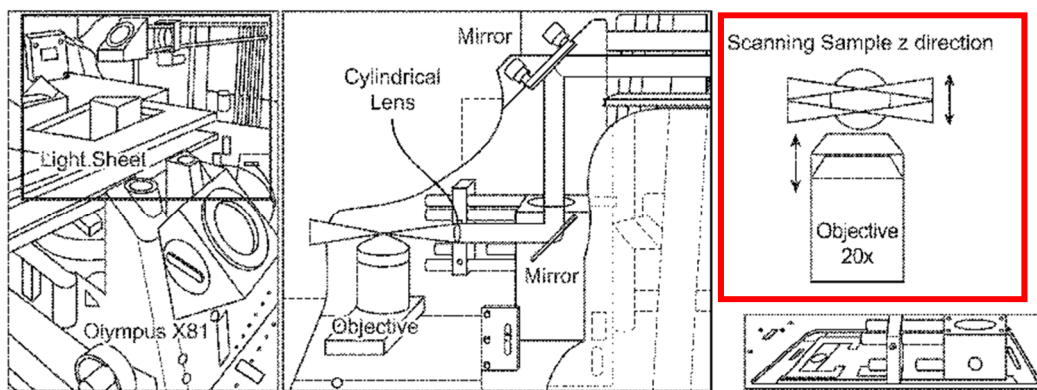


FIG. 17

Cai (EX1004) at Fig. 17 (annotated), 47:30-47, 47:43-45 (“the objective is moving synchronically with the illumination sheet, allowing scanning the sample along z-axis”); EX1002 ¶¶ 87-88. These exemplify three-dimensional scanning and imaging of three-dimensional samples in the provided hybridization chamber. EX1004 at 47:43-47; EX1002 ¶¶ 87-88.

Cai discloses a computing system with software for automating imaging and for data processing. EX1002 ¶¶ 89-90. EX1004 at 46:4-33 (“[S]oftware packages for data analysis in Python and Matlab” provide automated software capable of image analysis for three-dimensional cellular imaging), 45:22-31 (teaching, in “Automation,” that software automation tools “can be applied to efficiently scale up, for example, to map 100 genes and/or reduce human labor and/or errors,” and that “integrated analysis can be independently and optionally combined with fast hybridization cycle time and imaging time”), 45:63-46:3 (teaching use of software to automate “fluidics, image acquisition, and/or integrated analysis [that] can be independently and optionally combined with fast hybridization cycle time and imaging time,” including control of “valves, stages, light sources, camera, and/or microscopes”); EX1002 ¶¶ 75-76.

3. Shen

Shen published in 2006. EX1002 ¶ 91; EX1032.001. Shen is therefore prior art under 35 U.S.C. § 102(a)(1).

Shen teaches an automated autofocus mechanism that functions by providing automated z-axis motion for use in microscope imaging systems. EX1002 ¶¶ 92-95; EX1032.001-.002. Shen discloses use of its system with an Olympus IX71 microscope, a motorized stage, and the necessary software to control stage movement and control. EX1002 ¶¶ 92-93, 95; EX1032.002-.003 Fig. 1. Shen

also discloses that linear encoders and stepper motors can be used in such apparatuses to provide z-axis motion in implementing microscope autofocus. EX1002 ¶¶ 92-93; EX1032.004.

B. Secondary References Relied Upon Herein

While this Petition relies principally on the combination of Zhuang, Cai, and Shen, the following references are utilized herein in challenging the remaining claims—claims 40-42, 45, 48-53, 55, 57, 58, 61, 62, and 70—not subject to Ground 1:

- **Xiao:** published in 2009. EX1027; EX1002 ¶ 96. Xiao is therefore prior art to the '554 patent under post-AIA ¶ 102(a)(1).
- **Ehrlicher:** published in 2007. EX1028; EX1002 ¶ 98. Ehrlicher is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Tirichine:** published in 2009. EX1049; EX1002 ¶ 100. Tirichine is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Brileya:** published in 2014. EX1039; EX1002 ¶ 102. Brileya is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).

- **Fang-Yen:** published in 2011. EX1029; EX1002 ¶ 104. Fang-Yen is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Richard:** published 2010. EX1030; EX1002 ¶ 106. Richard is thus prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Nikon:** published no later than August 10, 2015, as it was a publicly-accessible webpage as of that date. EX1043; EX1002 ¶ 108. Nikon is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Skaft-Pedersen:** published in 2011. EX1048; EX1002 ¶ 110. Skaft-Pedersen is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Sands:** published in 2005. EX1046; EX1002 ¶ 112. Sands is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Brooker:** published 2011. EX1040; EX1002 ¶ 114. Brooker is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).

- **Niman:** published 2013. EX1044; EX1002 ¶ 116. Niman is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Wessels:** published in 2012. EX1050; EX1002 ¶ 118. Wessels is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).
- **Winer:** published in 2014. EX1051; EX1002 ¶ 120. Winer is therefore prior art to the '554 patent under post-AIA 35 U.S.C. § 102(a)(1).

VI. MANDATORY NOTICES

A. Real Party-in-Interest

The Real Party-in-Interest is NanoString Technologies, Inc.

B. Related Matters

As of the filing date of this Petition, the 11,542,554 Patent is involved in litigation in *10X Genomics, Inc. v. NanoString Technologies, Inc.*, 1-22-cv-00261 (D. Del. Filed Feb. 28, 2022) (“Related Matter”).

C. Lead and Back-Up Counsel and Service Information

Petitioner provides the following designation of Lead and Back-Up Counsel.

Lead Counsel	Back-Up Counsel
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VII. GROUNDS FOR STANDING

Petitioner certifies that the '554 patent is available for *inter partes* review and that Petitioner is not barred or estopped from requesting *inter partes* review challenging the patent claims on the grounds identified in this Petition.

VIII. STATUTORY GROUNDS FOR THE CHALLENGES

Ground	35 U.S.C. Section (post-AIA)	Claims	References
1	§ 103	36-39, 43, 44, 46, 47, 54, 56, 59, 60, 63-69	Zhuang, Cai, Shen
2	§ 103	40	Zhuang, Cai, Shen, Xiao, Ehrlicher
3	§ 103	41, 70	Zhuang, Cai, Shen, Tirichine
4	§ 103	42, 70	Zhuang, Cai, Shen, Brileya

5	§ 103	45	Zhuang, Cai, Shen, Fang-Yen
6	§ 103	48	Zhuang, Cai, Shen, Richard
7	§ 103	49	Zhuang, Cai, Shen, Nikon
8	§ 103	50, 51	Zhuang, Cai, Shen, Nikon, Skafte-Petersen
9	§ 103	52	Zhuang, Cai, Shen, Sands
10	§ 103	53	Zhuang, Cai, Shen, Sands, Brooker
11	§ 103	55, 57, 58	Zhuang, Cai, Shen, Wessels
12	§ 103	61	Zhuang, Cai, Shen, knowledge of POSA
13	§ 103	62	Zhuang, Cai, Shen, Niman, and/or Winer

IX. CLAIM CONSTRUCTION

For purposes of this Petition, Petitioner does not believe that any claims require construction in view of the prior art being presented. To the extent Patent Owner identifies terms for construction, Petitioner reserves the right to respond.

X. GROUND 1: OBVIOUSNESS IN VIEW OF ZHUANG, CAI, AND SHEN

A. Claim 36

1. A system comprising:

To the extent the preamble is limiting, Zhuang discloses a complete system for MERFISH imaging that includes optics, fluid handling, and data analysis. EX1003; EX1002 ¶¶ 124-131. As Zhuang explains, the “entire system was fully

automated, so that imaging and fluid handling were performed for the entire experiment without user intervention.” EX1003.011; EX1002 ¶¶ 124-131.

a. (a) a stage comprising:

Zhuang explains that the “sample was imaged on a home-built microscope constructed around an Olympus IX-71 body....” EX1003.011. This microscope system, like all microscope systems, includes a stage. EX1002 ¶¶ 132-135. Indeed, such a stage is necessary to act as a platform for the sample so that it can be imaged using the optics system. *Id.* ¶ 135. As documented immediately below, Zhuang further discloses or renders obvious in combination with Cai the specific components that are recited in the claims as being part of the “stage.”

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

Zhuang discloses a “Biotech’s [sic] FCS2 flow chamber” designed to hold a sample having a volume. EX1003.011. This Biotech’s flow cell included within it a substrate, a sample coverslip with deposited sample cells. *Id.* (“Cells were plated on...coverslips (Biotech’s, 0420-0323-2)...”); *id.* (“The sample coverslip was assembled into a Biotech’s [sic] FCS2 flow chamber....”).

Zhuang further discloses the sample having a volume. Specifically, Zhuang discloses cellular samples. Such samples consisting of cells have a “volume.” EX1003.012 (explaining that “cells [were optically sectioned] by collecting stacks

of images at different focal depths through the entire depth of the cells”); EX1002 ¶ 139. Indeed, the ’554 patent describes individual “cells” as a sample type that can be interrogated in “3D.” EX1001 at 4:44-50, Fig. 1. Moreover, Zhuang explains that using optical sectioning “allow[s] the imaging of RNAs in *thicker* cells or tissues.” EX1003.012 (emphasis added). Referring to imaging in “thicker” samples, Zhuang contemplates samples that have a “volume.” EX1002 ¶ 140.

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

As noted above, Zhuang discloses that its stage includes a Biopetechs FCS2 flow chamber, which is a system that includes temperature control capability. EX1002 ¶¶ 142-143; EX1003.011. This is taught, for instance, in Sivaramakrishnan, published in 2009 and addressing similar types of experiments with the Biopetechs technology. EX1002 ¶ 142; EX1007.004 (“The Biopetechs FCS2 chamber maintains the cells and media at 37°C.”).

Zhuang further discloses that its optical system makes use of an oil immersion objective “heated to 37°C with a Biopetechs objective heater.” EX1003.011; EX1002 ¶ 143. A skilled artisan would understand that an oil immersion objective is used in a system wherein oil is applied to the specimen on the microscope stage, and the stage is raised, immersing the objective in oil. EX1002 ¶ 144. As such, the objective is in thermal contact with the stage holding the sample, such that the

objective heater is an additional temperature control system that becomes coupled to the stage and sample holder. *Id.*

As Dr. Czymmek explains, a skilled artisan would understand from Zhuang's use of a Biopetechs FCS2 flow cell and objective heater that it is providing comprehensive temperature control to the sample holder on the stage so that the experiments can be carried out at 37°C. *Id.*

iii. (a)(iii) An XY motion control system;

Zhuang describes a system that is “fully automated so that imaging and fluid handling were performed for the entire experiment without user intervention” and that was built upon on “Olympus IX-71” microscope “configured for oblique incidence excitation.” EX1003.011. As Dr. Czymmek explains, the Olympus IX-71 is an inverted microscope comprising a stage that is configured for X-Y positioning of the sample. EX1002 ¶¶ 145-146.

To the extent the Board finds, or Patent Owner argues, that Zhuang does not disclose an XY motion control system, it would have been obvious to a POSA to include such a system. EX1002 ¶ 147. Systems to automate X-Y movement of the stage of the Olympus IX-71 microscope were well-known as early as 2006, long before the priority date of the '554 Patent. EX1002 ¶ 148. Shen, for example, teaches an automated microscope system using the Olympus IX-71 with a “motorized x-y stage with linear encoding feedback,” which a POSA would have

understood is an X-Y motion control system. EX1032.002; EX1002 ¶¶ 148-150. Given Zhuang's teaching of a "fully automated" system, a POSA would have been motivated to look to Shen to find ways to automate the Olympus IX-71 Zhuang uses for MERFISH. EX1002 ¶¶ 151-152. A POSA would have had a reasonable expectation of success in applying Shen's teachings to Zhuang's system, given that Shen automates the type of microscope that Zhuang uses for the same goal, e.g., fluorescent imaging. *Id.* ¶ 153.

b. (b) a fluidics system comprising:

Zhuang expressly discloses that its automated platform comprises a "fluidics system." EX1003.011. This fluidics system includes not just the Biopetechs flow chamber, but also "three computer controlled eight-way valves...and a computer-controlled peristaltic pump...." *Id.* Zhuang teaches its fluidics system is fully automated and does not require any human intervention. *Id.*; EX1002 ¶ 155. As documented below, Zhuang teaches specific components set forth in the claims are part of the "fluidics system." *See also* EX1002 ¶¶ 154-156.

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

Zhuang discloses fluid reagents for use with its system, including solutions of labeled readout probe, readout wash buffer, imaging buffer, and photobleaching buffer:

One milliliter of...fluorescently labeled *readout probe in readout hybridization buffer*...was flown across the sample, flow was stopped, and the sample was incubated....Then...*readout wash buffer*...was flown across the sample, flow was stopped, and the sample was incubated....Two milliliters of *imaging buffer*...was flown across the sample (50)....

After imaging, the fluorescence of the readout probes was extinguished via photobleaching. The sample was washed with...*photobleaching buffer*...and each imaged region of the sample was exposed to 200 mW of 641-nm light for 3 s.

EX1003.011 (emphasis added). Zhuang explains these reagents are applied to the sample in “multiple successive rounds” of “[s]equential hybridization, imaging, and bleaching.” *Id.* Given that fluid reagents are repeatedly introduced to the sample in the flow chamber, Zhuang teaches the fluidic system includes reservoirs for holding these fluids. EX1002 ¶¶ 158-159.

- ii. **(b)(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**

Zhuang discloses a fluid dispenser in its fluidics system that repeatedly introduces fluid reagents to the flow chamber. EX1003.011; EX1002 ¶ 160. Zhuang discloses a peristaltic pump as part of its fluidics system, explaining that this system includes “a computer-controlled *peristaltic pump*...and three computer-

controlled 8-way valves....” EX1003.011 (emphasis added); EX1002 ¶ 161. Zhuang further states that fluidics system that includes this pump is for the purpose of controlling “flow through” the chamber of fluid reagents. *Id.*; EX1002 ¶¶ 160-162.

c. (c) an optical system comprising:

As documented above, Zhuang’s system is built around an Olympus IX-71 optical microscope. For example, Zhuang discloses that its sample is “imaged on a home-built microscope constructed around an Olympus IX-71 body and a 1.45 NA, 100× oil immersion objective and configured for oblique incidence excitation.” EX1003.011. As such, Zhuang discloses an optical system. EX1002 ¶¶ 163-164. As set forth further below, Zhuang either discloses or renders obvious in combination with Cai use of the additional features that are recited in the claims as being part of this “optical system.” *Id.*

i. (c)(i) an objective;

As noted above, Zhuang’s system is built around an Olympus IX-71 microscope, which included an objective. As Zhuang, explains, the “sample was imaged on a home-built microscope constructed around an Olympus IX-71 body and a 1.45 NA, 100× oil immersion objective and configured for oblique incidence excitation.” EX1003.011; EX1002 ¶¶ 165-166.

- ii. **(c)(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**

Zhuang teaches two or more light sources, configured to deliver light of different colors to the sample volume. EX1002 ¶¶ 167-169. Specifically, Zhuang teaches the use of three different lasers at three different wavelengths of 641 nm, 561, and 405 nm, which corresponds to red, green, and violet light, respectively. EX1003.011. As Zhuang states, “[i]llumination was provided at 641, 561, and 405 nm by using [three different] solid-state lasers...for excitation of our Cy5-labeled readout probes, the fiducial beads, and nuclear counterstains, respectively.” *Id.*; EX1002 ¶ 168.

To the extent that Zhuang is deemed to not disclose the claimed two or more light sources configured to emit two or more colors of light, it would have been obvious to modify Zhuang in view of Cai to use multiple light sources. EX1002 ¶ 170.

Cai teaches profiling nucleic acids by sequential barcoding, wherein probes labeled with different detectable moieties (e.g., fluorophores or colors) are used in different cycles. EX1004 at 13:4-14:15. For example, using “four-color FISH,” “[t]welve genes were encoded by 2 rounds of hybridization” in an embodiment of

Cai. *Id.* at 9:34-38; 9:45-48 (“Exemplary barcodes. mRNA 1: Yellow-Blue-Yellow; mRNA 2: Green-Purple-Green; mRNA 3: Purple-Blue-Purple; and mRNA 4: Blue-Purple-Blue.”). As Dr. Czymmek explains, Cai’s disclosure of different color fluorophores teaches using different colors of excitation light. EX1002 ¶ 172. Thus, Cai discloses using multiple colors of light configured to excite multiple fluorophores and emit multiple colors of light. Indeed, such multicolor detection was standard in the art at the relevant time frame. *Id.* ¶¶ 171-172.

A POSA would have been motivated to use two or more colors of light as taught by Cai with the method of Zhuang because it would have further increased the multiplexity of barcoding, which would have furthered Zhuang’s objective of “rapidly increas[ing] the number of RNA species that can be probed simultaneously.” EX1003.002; EX1002 ¶¶ 173-174. A skilled artisan would have expected success in employing two or more colors of light because, as Dr. Czymmek explains, such an approach was standard at the time and had been implemented routinely, including as set forth in Cai. EX1002 ¶ 175.

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

Zhuang teaches the use of an EMCCD camera to detect light signals from the sample volume:

Fluorescence was separated with a QuadView (Photometrics) by using the dichroics...and the emission filters...and imaged with an EMCCD camera....The camera was configured so that a pixel corresponds to 167 nm in the sample plane.

EX1003.011; EX1002 ¶¶ 176-178.

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

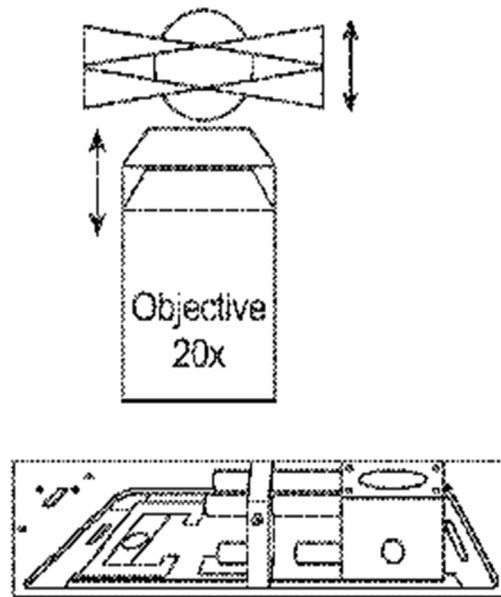
Zhuang includes a Z-axis motion control system for moving a focal plane through the volume of the cell. As discussed above, Zhuang's system is built upon an Olympus IX-71 inverted microscope. EX1003.011. As Dr. Czymmek explains, this system include an up-down control system so that the objective could be moved up and down, which would move the focal plane through the sample volume. EX1002 ¶¶ 180-181. Likewise, the system included an "up/down" fine adjustment knob to control the stage, which would have the same effect. *Id.*

Moreover, one of skill in the art would understand that Zhuang utilized yet an additional Z-axis motion control system. As Zhuang states, "[c]onstant focus was maintained throughout the imaging process with a home-built, autofocusing system." EX1003.011. In order to maintain a constant focus throughout an imaging process, a person of skill would understand that a Z-axis motion control system for adjusting the focal plane would be necessary. EX1002 ¶¶ 182-183.

Shen provides an example of such automation. As early as 2006, Shen acknowledges, “automatic focusing of microscope images [was] an essential part of modern high-throughput microscopy.” EX1032.001. Shen used a piezo-electric transducer to permit autofocus, but taught many different systems for automating Z-axis movement. *Id.* As Dr. Czymmek explains, these technologies were common long before the alleged invention date of the ’554 Patent. EX1002 ¶ 185. A POSA would have been motivated to combine the relevant teachings in Shen with Zhuang because Zhuang explicitly requires an autofocus system for the MERFISH method. EX1003.011; EX1002 ¶ 186. A POSA would have had a reasonable expectation of success, because Shen teaches modifying the Olympus IX-71 microscope, the same one used in Zhuang, in the same way to accomplish the same goal. EX1002 ¶ 187.

To the extent Zhuang is deemed to not disclose the claimed Z-axis motion control system, it would have been obvious to modify Zhuang in view of Cai to include such a feature. Cai’s Figure 17, reproduced below, demonstrates the use of such a system:

Scanning Sample z direction



EX1004 at Fig. 17 (right-hand portion). Cai describes that in this configuration, “the objective is moving synchronically with the illumination sheet, *allowing scanning the sample along z-axis.*” EX1004 at 47:43-45 (emphasis added). As Dr. Czymmek confirms, such Z-axis motion control systems were standard in the art at the relevant time frame. EX1002 ¶ 188.

A POSA would have been motivated to include the Z-axis motion control system of Cai with the system of Zhuang because it would have enabled one to more effectively scan the sample in three dimensions at different focal plans, furthering Zhuang’s objective of determining “[k]nowledge of the expression profile and

spatial landscape of the transcriptome in individual cells....” EX1003:002 (Abstract), .005 (“As an imaging-based approach, MERFISH also allowed us to investigate the spatial distributions of many RNA species simultaneously.”). Indeed, Zhuang teaches that “[t]iling images of adjacent areas *and adjacent focal planes* could be used to improve the counting accuracy.” EX1003.012 (emphasis added). To collect these images across the different focal depths, a POSA would have understood a system for z-axis motion control would be needed so that the focal plane could be moved through the volume of the sample. EX1002 ¶¶ 189-190.

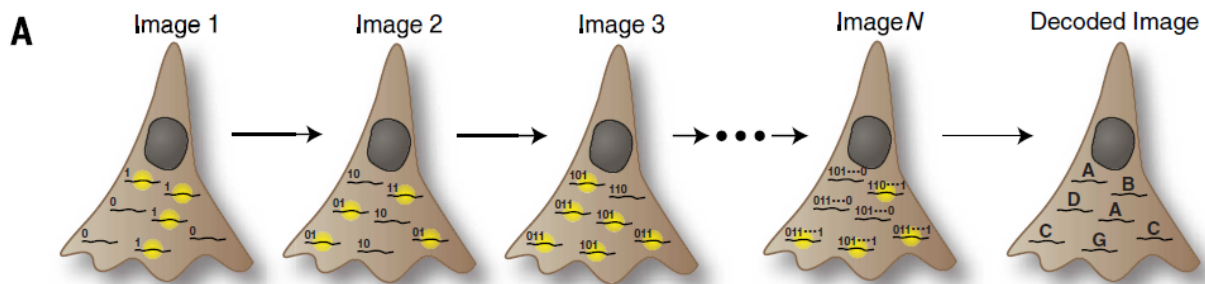
A skilled artisan would have expected success in including a Z-axis motion control system in Zhuang for this purpose because such systems were standard at the time and had been implemented routinely, including as set forth in Cai. EX1002 ¶¶ 188-192. Cai for instance, expressly contemplates that “an imaging step provides three-dimensional spatial information of a target.” EX1004 at 21:50-51. As another example, in 2010, Larsson and co-workers reported on the use of this approach in an *in situ* hybridization method that utilized fluorescent detection. EX1008.005 (“For SKBR3 and SKOV3 cells, images were collected as z-stacks to ensure that all RCPs were imaged.”); EX1002 ¶¶ 191-192.

- e. **(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:**

Zhuang teaches a computing system associated with its MERFISH system. Specifically, Zhuang teaches that the “entire system was fully automated, so that imaging and fluid handling were performed for the entire experiment without user intervention.” EX1003.011. Likewise, with respect to the application of reagents to the flow chamber, Zhuang teaches that “flow through this chamber was controlled via a homebuilt fluidics system composed of three computer controlled eight-way valves...a computer-controlled peristaltic pump...” *Id.*; EX1002 ¶ 194. A POSA would understand that this disclosure of computer automation would have been achieved via a computing system with program instructions. EX1002 ¶¶ 193-195. As documented below, this computing system includes instructions to carry out the various steps recited in connection with step (e).

- i. **(e)(i) receiving a plurality of images of at least a portion of said volume from a plurality of readout 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;**

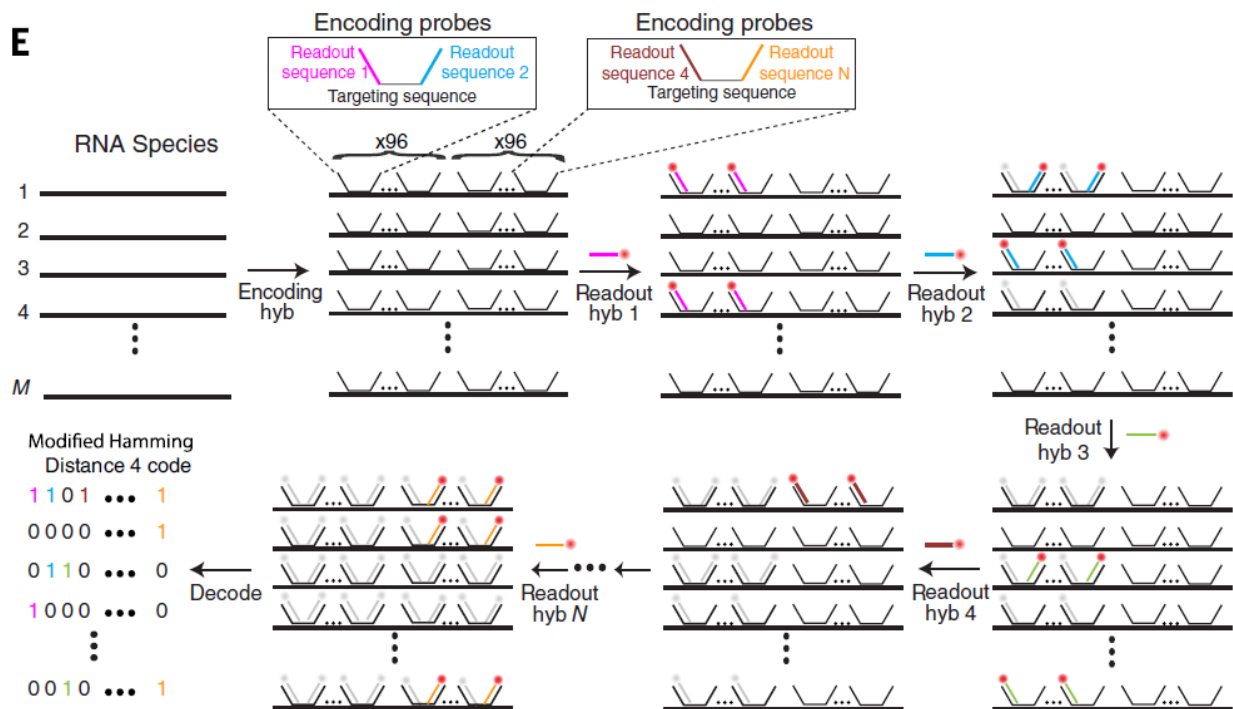
Zhuang teaches receiving a plurality of images of the sample volume from a plurality of readout cycles, wherein said plurality of images comprises a plurality of light signals received from said volume during said plurality of readout cycles. Zhuang discloses embodiments of the MERFISH method wherein the sample “can be simultaneously imaged” during multiple “rounds of hybridization and imaging.” EX1003.002, .003; EX1002 ¶ 197. This is depicted in Figure 1A of Zhuang:



EX1003.003 (Fig. 1A). Here, in each readout cycle, an image of the sample is received, wherein code “1” represents detection of a light signal and “0” represents no light signal. *Id.*; EX1002 ¶ 198. After N readout cycles, N images are received from the sample, which N images provide a string of light signals associated with

each nucleic acid molecule. EX1002 ¶¶ 199-200. Also as shown by the “Decoded Image,” the string of light signals is used to decode the identity of the RNA species. *Id.*; EX1003.012, Fig. 1A.

Zhuang discloses that the light signals are associated with the nucleic acid molecules within the sample volume. As shown in Figure 1A, each detected light signal is associated with a nucleic acid molecule (yellow spots) within the sample volume. EX1003.003-.004, .012; EX1002 ¶¶ 199-200. This process is depicted by Figure 1E of Zhuang:



EX1003.003 (Fig. 1E); EX1002 ¶¶ 201-202. Specifically, each RNA species is hybridized with sequence-specific encoding probes that contain “a specific

combination of readout sequences.” EX1003.003 (Fig. 1, legend) (“Encoding hyb”); EX1002 ¶ 201. Successive hybridization and imaging of fluorescently-labeled readout probes provide specific combinations of readout sequences that form barcodes that identify the RNA species. EX1002 ¶¶ 201-202. In each readout cycle, a unique readout probe is introduced to the sample volume and associated with the RNA species via the encoding probes when its complementary readout sequence is present, which leads to a detection of a light signal during imaging. *Id.*; EX1003.003 (Fig. 1, legend) (“N subsequent rounds of hybridization with the fluorescent readout probes are used to probe the readout sequences (hyb 1, hyb 2, ..., hyb N).”).

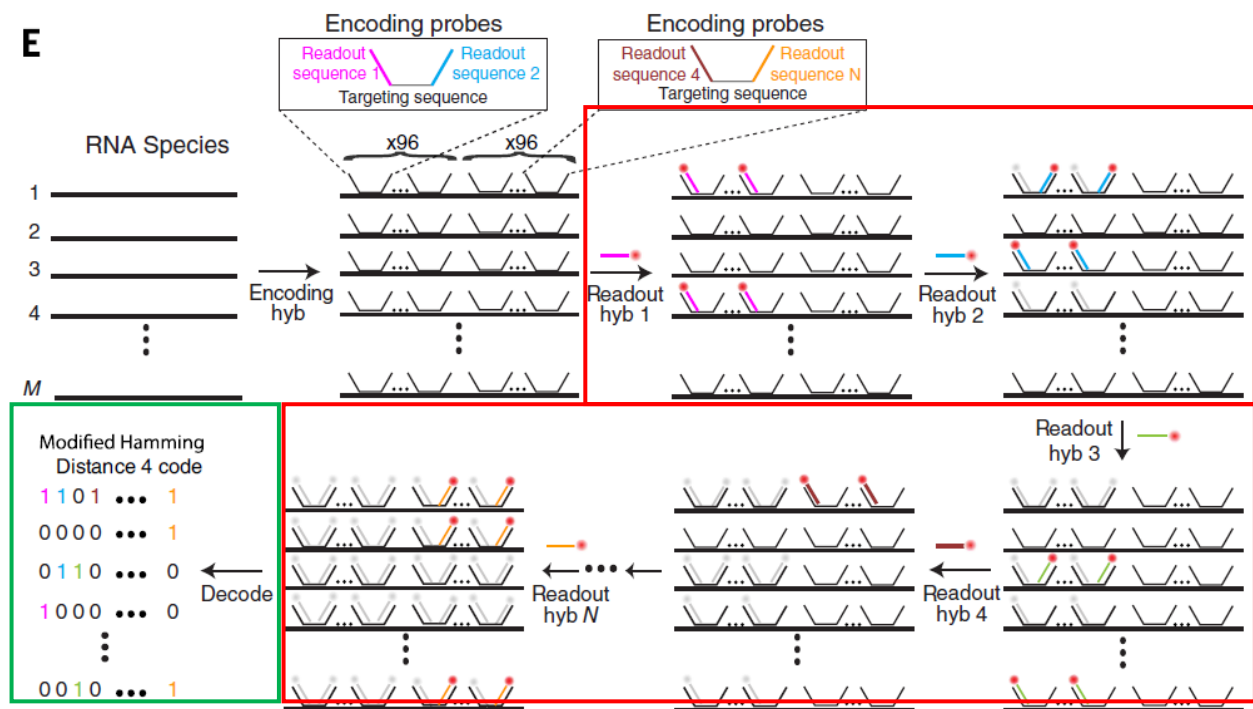
Zhuang discloses that the light signals have a three-dimensional (3D) spatial position within the sample volume. As discussed above, the light signal is detected “*in situ*” only when a readout probe hybridizes to the nucleic acid molecule. EX1003.002. Hence, the position of the light signals also reflects the spatial position of the nucleic acid molecules. EX1002 ¶¶ 203-204. This spatial position can be a 3D position within the sample volume. For example, Zhuang describes “optically section[ing] [] cells by collecting stacks of images at different focal depths through the entire depth of the cells.” EX1003.012. Thus, Zhuang teaches using the MERFISH method to obtain the 3D spatial position of nucleic acid species through the entire depth of the sample volume. EX1002 ¶¶ 203-204.

To the extent that Zhuang is deemed to not disclose the claimed 3D feature, it would have been obvious to modify Zhuang in view of Cai to include this feature. EX1002 ¶ 205. Cai teaches profiling nucleic acids by SeqFISH to obtain spatial information, including “three-dimensional spatial information.” EX1004 at 21:50-51; EX1002 ¶ 206. Specifically, as documented above, Cai teaches the claimed Z-axis motion control system, which enables 3D interrogation. *See supra* Section X.A.1.d.

A POSA would have been motivated to use Zhuang’s MERFISH method for 3D investigation in order to obtain meaningful spatial information in the z-dimension, furthering Zhuang’s goal of “identify[ing] distinct spatial distribution patterns of RNAs.” EX1003.002; EX1002 ¶ 207. A skilled artisan would have reasonably expected success in using MERFISH in 3D because, as Dr. Czymmek explains, (1) 3D FISH was standard at the time and had been implemented routinely, including as set forth in Cai, and (2) MERFISH is based on the same imaging method as conventional FISH—to the extent it uses sequential hybridization to increase the multiplexity, this feature does not limit 3D detection. EX1002 ¶ 208.

- ii. (e)(ii) 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;

Zhuang in Figure 1E illustrates the determination of optically encoded signals (boxed in green) from the light signals obtained from the multiple read-out cycles (boxed in red):



EX1003.003 (Fig. 1E) (annotated). After N readout cycles, a string of light signals (e.g., 1100...0, 0000...1, etc.) are generated for each RNA species, as shown in the green box. EX1002 ¶¶ 210-211. These strings of light signals are optically-

encoded signals that interpret the codeword/barcode generated by the readout sequence. Thus, they are associated with the nucleic acid molecules and can be used to identify the RNA species. *Id.*

Further, Zhuang teaches these optically encoded signals comprise two or more colors of light detected by said one or more detectors in series. EX1002 ¶ 212. As explained above in Section X.A.c.ii, Zhuang discloses two or more light sources that emit two or more colors that can be used to activate the fluorophore-labeled readout probes. *Id.* A POSA would have understood that these fluorophores upon activation would further emit light of two or more colors which would be detected by one or more detectors in series and further reflected in the optically encoded signals. *Id.* To the extent that Zhuang is deemed to not disclose the claimed method using two or more colors of light, it would have been obvious to modify Zhuang in view of Cai to include such a feature. *See supra* Section X.1.c.ii; EX1002 ¶ 213.

iii. (e)(iii) identifying said one or more nucleic acid molecules based on said plurality of optically-encoded signals; and

Zhuang decodes the identity of the RNA species based on the optically-encoded signals. *See supra* X.A.1.e.ii; EX1002 ¶¶ 214-215.

- iv. **(e)(iv) determining three-dimensional positional information of said one or more nucleic acid molecules.**

Zhuang, by itself or in view of Cai, determines the 3D positional information of the RNA species. *See supra* X.A.1.e.i; EX1002 ¶¶ 216-219.

B. Claim 37: The system of claim 36, wherein said substrate comprises a flow cell.

Zhuang discloses this limitation. Zhuang discloses a “Biotech’s [sic] FCS2 flow chamber.” EX1003.011. According to Zhuang, “the flow through this chamber [was] controlled via a home-built fluidics system.” *Id.* Thus, a POSA would have understood this flow chamber comprises a flow cell. EX1002 ¶¶ 220-221.

C. Claim 38: The system of claim 37, wherein said flow cell is a closed flow cell.

Zhuang discloses this limitation. A POSA would have known that the Bioptechs’s FCS2 flow chamber comprises a closed flow cell. EX1002 ¶¶ 222-224.

D. Claim 39: The system of claim 38, wherein said flow cell is a closed flow cell comprises a fluid inlet and a fluid outlet, and wherein said fluid inlet is fluidically coupled to said fluid dispenser.

Zhuang discloses this limitation. A POSA would have understood that the Bioptechs’s FCS2 flow chamber is a closed flow cell comprising a fluid inlet and a fluid outlet. EX1002 ¶¶ 222-224, 226. For instance, Zhuang discloses its home-

built fluidics system controls the flow “*through*” the chamber, which indicates the presence of an inlet and an outlet. EX1003.011 (emphasis added); EX1002 ¶ 227. Further, as discussed above, Zhuang teaches its fluid dispenser is capable to transfer fluid to its flow cell. Sections X.A.1.b and X.A.1.b.ii *supra*; EX1002 ¶ 228. A POSA would have understood that the fluid dispenser is fluidically coupled to the inlet. EX1002 ¶ 229.

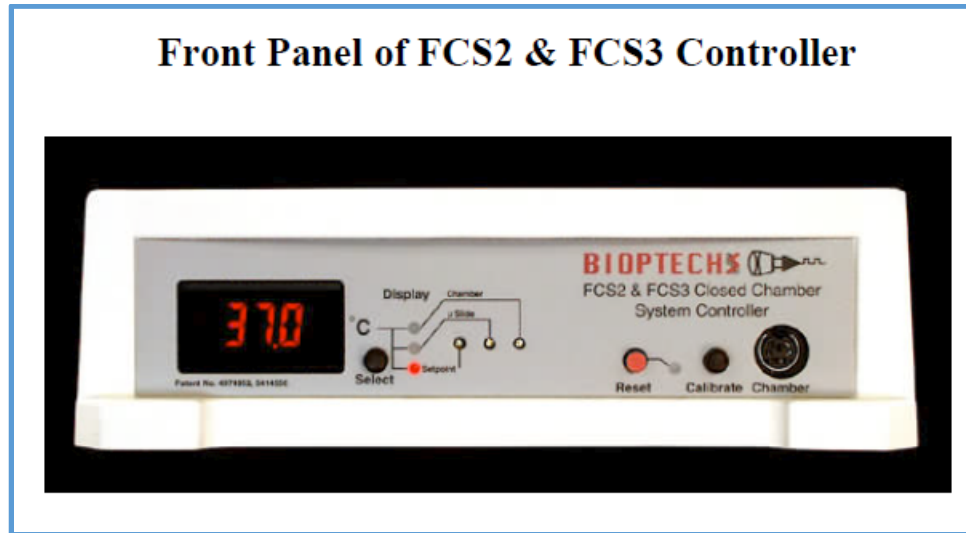
E. Claim 43: The system of claim 36, wherein said temperature control apparatus comprises a heat sink or a Peltier element.

Zhuang renders this limitation obvious in view of the knowledge of a POSA. EX1002 ¶¶ 230-233. A heat sink and a Peltier element were well known temperature control devices in the art and were readily accessible at the relevant time. EX1002 ¶ 233. It would have been obvious for a POSA to choose a heat sink or a Peltier element for temperature control because, for example, these components have cooling capabilities. *Id.*

F. Claim 44: The system of claim 36, further comprising a thermal control system.

As documented above, the Zhuang reference discloses the use of Bioprotech's FCS2 flow chamber. Zhuang discloses using thermal control, thus disclosing to a POSA that the system included a thermal control system. EX1003.011; EX1002 ¶¶ 234-236 (discussing evidence that the FCS2 flow chamber was well known to include thermal control system). This evidence of what would have been well-

known to a POSA includes instructions for the FCS2 system, depicting a setpoint temperature:



EX1006 (Fig. 1); EX1002 ¶ 236.

- G. Claim 46: The system of claim 36, wherein said fluidics system further comprises one or more valves downstream of said one or more reservoirs and upstream of said fluid dispenser, wherein, upon actuation, said one or more valves facilitate dispensation of fluid to said sample holder**

Zhuang teaches this limitation. EX1002 ¶¶ 237-239. Zhuang discloses that its fluidics system comprises, in part, “three computer controlled eight-way valves (Hamilton, MVP and HVXM 8-5).” EX1003.011. Further, Zhuang discusses an automated fluidics system, which Dr. Czymmek describes as requiring the use of valves that would necessarily be downstream of the reservoir and upstream of the dispenser. EX1002 ¶ 239.

H. Claim 47: The system of claim 46, wherein said one or more valves comprise a rotary valve

Zhuang teaches this limitation. EX1002 ¶¶ 240-242. According to Dr. Czymmek, a POSA would have known the Hamilton MVP and HVX 8-5 valves are rotary valves, further evidenced by Hamilton’s own description on its website. EX1002 ¶ 241.

I. Claim 54: The system of claim 36, further comprising an objective wetting apparatus.

Zhuang teaches this limitation. EX1002 ¶¶ 243-247. Zhuang teaches both the use of an oil immersion objective and a fully automated system. EX1003.011; EX1002 ¶¶ 244-245. A POSA would have thus understood Zhuang to disclose an objective wetting apparatus in disclosing a fully automated system using an oil immersion objective, i.e., an objective that is wetted in operation. EX1002 ¶ 246. A POSA would have further understood this to be wholly consistent with the meaning of “objective wetting apparatus” as set forth in the ’554 patent. *Id.*; EX1001 at 22:19-36.

J. Claim 56: The system of claim 36, wherein said two or more light sources comprise one or more lasers.

Zhuang discloses this limitation. *See supra* Section X.A.1.c.ii; EX1002 ¶¶ 248-250.

- K. Claim 59: The system of claim 36, wherein said optical system further comprises one or more emission filters configured to selectively filter one or more wavelengths of light emitted from said volume to said one or more detectors.**

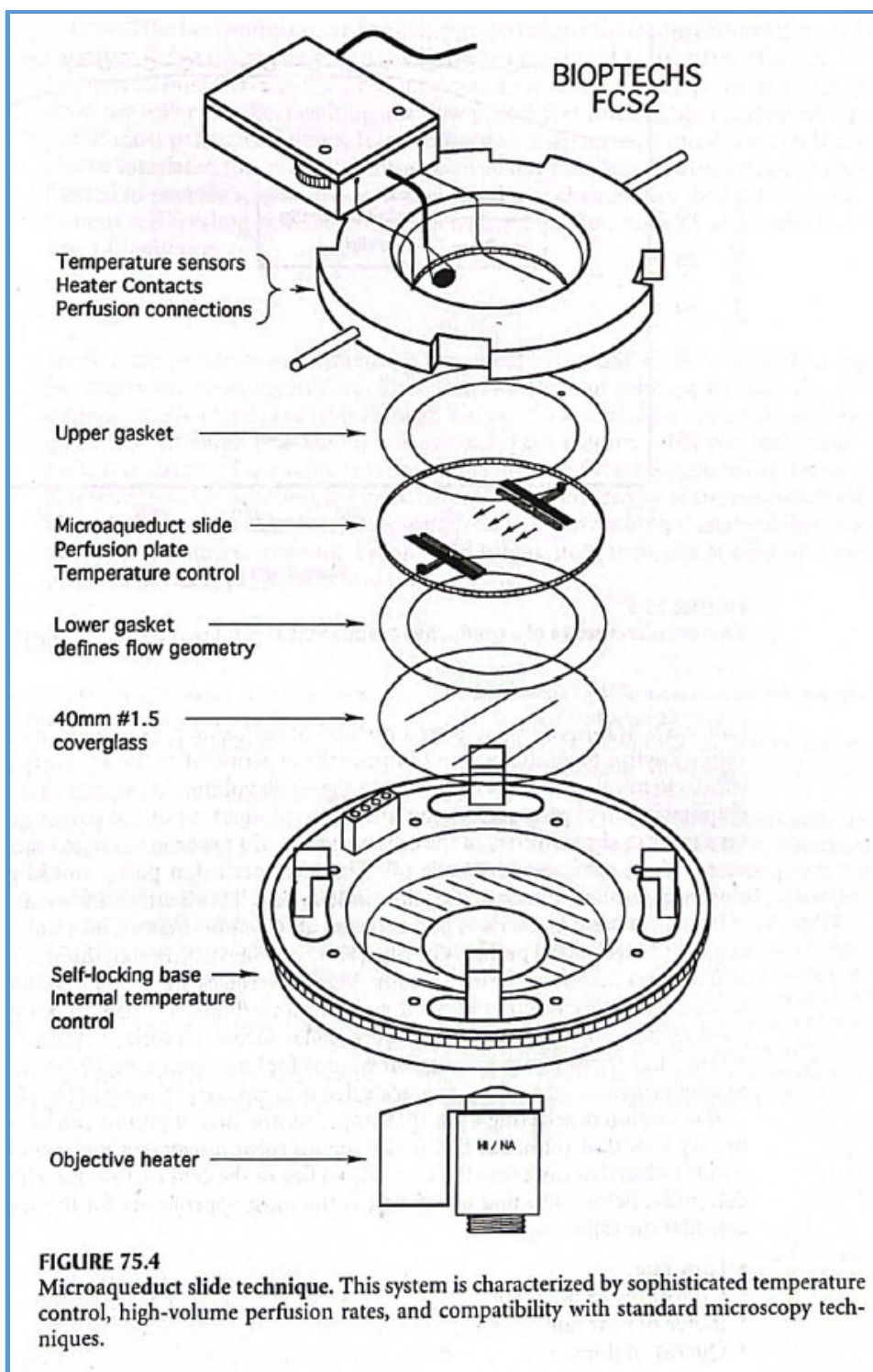
Zhuang discloses this limitation. Zhuang discloses the use of “emission filters ET525/50m, WT5955m-2f, ET700/75m, HQ7701p (Chroma)” in advance of the EMCCD camera. EX1003.011; EX1002 ¶¶ 251-253.

- L. Claim 60: The system of claim 36, wherein said optical system is mechanically, electrically, or electromechanically coupled to a controller system configured to synchronize one or more imaging events within said system.**

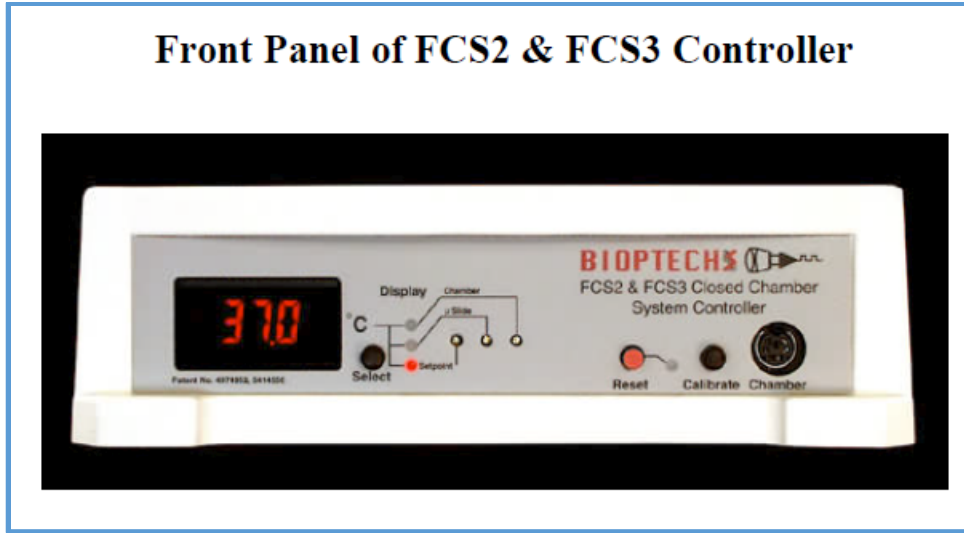
Zhuang discloses this limitation. As documented above, Zhuang teaches a computing system associated with its MERFISH system. *See supra* Section X.A.1.e. Specifically, Zhuang teaches that the “entire system was fully automated, so that imaging and fluid handling were performed for the entire experiment without user intervention.” EX1003.011. A POSA would understand that this disclosure of computer automation would have been achieved via the optical system mechanically, electrically, or electromechanically coupled to a controller system configured to synchronize one or more imaging events within the system. EX1002 ¶¶ 254-256.

- M. Claim 63: The system of claim 44, wherein said temperature control apparatus further comprises a temperature sensor configured to provide a feedback signal to said thermal control system.**

As documented above, the Zhuang reference discloses the use of Biopetechs FCS2 flow chamber, including a temperature control apparatus. *See supra* X.F. An expanded view of the FCS2 system with the conductive temperature sensors shown at the top is depicted in the figure below:



EX1015.017. at 8. The instructions for the FCS2 system further evidence that it includes a thermal control system:



EX1006 (Fig. 1). A POSA would have understood that Zhuang’s system including a temperature control apparatus and thermal control system would have necessarily included a temperature sensor in the temperature control apparatus. This is further evidenced by the instructions explaining that there is a feedback loop to maintain the temperature; “[i]t is important that the flat side of the surface probe is making uniform contact with the microaqueduct slide because the *feedback loop that controls the microaqueduct temperature* is reliant on this thermal contact point.” *Id.*, .005 (emphasis added); EX1002 ¶¶ 257-261.

- N. Claim 64: The system of claim 59, wherein at least one of said one or more emission filters is configured to selectively filter light having a wavelength of about 510 nm, about 570 nm, about 620 nm, or about 680 nm to said one or more detectors from said volume to said one or more detectors.**

Zhuang discloses the use of “emission filters ET525/50m, WT59550m-2f, ET700/75m, HQ7701p (Chroma)” in advance of the EMCCD camera.

EX1003.011. A POSA would know these filters to be configured to selectively filter light having a wavelength of about 510 nm, about 570 nm, about 620 nm, or about 680 nm to detectors from the sample volume of the detectors. EX1002 ¶¶ 262-264.

- O. Claim 65: The system of claim 36, wherein said two or more light sources excite fluorescence in unique color channels such that each light source of said two or more light sources is configured to excite fluorescence in a unique color channel of said unique color channels.**

Zhuang explains that “Illumination was provided at 641, 561, and 405 nm by using solid-state lasers (MPB communications, VFL-P500-642; Coherent, 561-200CWCDRH; and Coherent, 1069413/AT) for excitation of our Cy5-labeled readout probes, the fiducial beads, and nuclear counterstains, respectively.”

EX1003.011. Thus, there are unique color channels. EX1002 ¶ 267. For instance, 641 nm is a red color channel whereas 561 nm is an orange/yellow color channel. *Id.* As Zhuang explains, the 641 nm color channel excites fluorescence in the Cy5-labeled readout probes, and the 561 nm color channel excites fiducial beads, which Zhuang explains are “carboxylate-modified orange fluorescent beads.” EX1003.011.

Further, as documented above, to the extent Zhuang is deemed not to disclose the use of two or more light sources, it would have been obvious to do so in view of Cai, which discloses using multiple colors of light configured to excite multiple

fluorophores and emit multiple colors of light in unique color channels. *See supra* Section X.1.c.ii.; EX1002 ¶ 269. For example, Cai discloses four-color FISH that comprises yellow, green, blue, and purple probes. *See supra* Section X.1.c.ii.; EX1004 at 9:45-48. A POSA would have understood that each of these colors corresponds to a unique color channel. EX1002 ¶¶ 268-269.

P. Claim 66: The system of claim 65, wherein each optically-encoded signal of said plurality of optically-encoded signals comprises at least one of said unique color channels.

As documented above, the optically-encoded signal comprises fluorescent signal detected when the readout probe is hybridized to the RNA species. *See supra* Sections X.A.1.e.i-ii. In Zhuang, the readout probes are labeled with Cy5, which is excited by the 641 nm color channel. EX1003.011; *supra* Section X.O. Thus, a POSA would have understood that the optically-encoded signal comprises at least one of the unique color channels. EX1002 ¶¶ 270-271. In Cai, the barcodes likewise comprise light signals detected with different colors (*i.e.*, in different unique color channels. *See supra* Section X.O.; EX1002 ¶¶ 272.

Q. Claim 67. The system of claim 36, wherein at least one of the plurality of optically-encoded signals comprises two or more colors detected in series.

Disclosed. *See supra* Sections X.1.c.ii., X.1.e.ii.; EX1002 ¶¶ 273-274.

R. Claim 68: The system of claim 36, wherein said two or more light sources comprise four light sources.

Disclosed. *See supra* X.A.1.c.ii., X.A.1.e.ii. Specifically, Cai teaches four-color SeqFISH, which a POSA would have understood to require four light sources. EX1004 at 9:28-48; EX1002 ¶¶ 275-279.

S. Claim 69: The system of claim 36, wherein said optical system is configured for wide-field microscopy.

Zhuang explains that they “imaged cells using *wide-field imaging* with oblique-incidence illumination....” EX1003.012 (emphasis added); EX1002 ¶¶ 280-281.

XI. GROUND 2: OBVIOUSNESS OF CLAIM 40 IN VIEW OF ZHUANG, CAI AND SHEN, IN FURTHER VIEW OF XIAO AND EHRLICHER

Zhuang, Cai and Shen in view of Xiao and Ehrlicher render obvious the system of claim 37 where the flow cell is an open flow cell, as recited in claim 40. EX1002 ¶ 282.

As documented above, Zhuang expressly identifies using the “Biotech FCS2 flow chamber” in its system. *See supra* Section X.1.C.; EX1002 ¶ 283.

Xiao discloses the Biotech FCS2 flow chamber can be used as an open flow cell where the chamber is not sealed. EX1027.099; EX1002 ¶ 284. Ehrlicher similarly teaches the use of the Biotech FCS2 flow chamber as an open flow cell

in disclosing either an open or closed mode can be used as required. EX1028.002, .009 (Fig. 2), .011-12; EX1002 ¶ 284.

A POSA would have been motivated to combine the teachings of Zhuang, Cai, and Shen with the teachings of Xiao and Ehrlicher on the function and utilization of the Biotech FCS2 flow chamber in an open flow cell mode as these references comprehensively explain the operation and application of Zhuang's flow chamber, particularly in an open-dish format for monitoring live cells to a POSA's interest. EX1027.098-99; EX1028.011-12; EX1002 ¶ 285. A POSA would further have had a reasonable expectation of success in combining these references because the related prior art, including Xiao and Ehrlicher, was well-known in the art. EX1002 ¶ 285.

XII. GROUND 3: OBVIOUSNESS OF CLAIMS 41 AND 70 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF TIRICHINE

Claim 41 depends from claim 36 and recites the use of a water immersion objective. Claim 70 depends from Claim 36 and further recites that the numerical aperture of the objective is about 1.0.

While Zhuang teaches the use of an oil immersion objective, as Dr. Czymmek explains, this is only one type of objective and others were known in the art. EX1002 ¶ 287. A water immersion objective was not only known, but was known for use specifically for FISH experiments. EX1002 ¶ 288. As Trichine teaches,

a water immersion objective was used for FISH on 3D isolated nuclei on the model organism *Arabidopsis thaliana*, a type of plant. *Id.*; EX1049.001, .004. This water immersion objective has an NA of 1.2. EX1049.004. Further, a POSA would have known that water immersion objectives would have had lower numerical apertures than oil immersion objectives as taught in Zhuang, such as Trichine's use of an objective with a 1.2 NA, which is about 1.0. EX1002 ¶ 288.

As Dr. Czymmek explains, a POSA would have been motivated to use a water immersion objective as taught in Trichine as a mere design choice. That is, a POSA would have been aware of different types of objectives and different reasons to use those objectives. EX1002 ¶ 289. A water immersion objective, for example, could be useful for a thick, largely aqueous, live specimen. *Id.* A POSA would have also had a reasonable expectation of success in using a water immersion objective in Zhuang, as FISH experiments could be performed with a water immersion objective and a skilled artisan would have expected using them in a MERFISH experiment to be successful. *Id.* ¶ 290.

XIII. GROUND 4: OBVIOUSNESS OF CLAIMS 42 AND 70 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF BRILEYA

Claim 42 depends from claim 36, and further recites that the objective is a water dipping objective. Claim 70 depends from Claim 36 and further recites that the numerical aperture of the objective is about 1.0.

As Dr. Czymmek describes, the use of different objectives were well-known to a POSA. EX1002 ¶¶ 293-294. Brileya, for instance, used a water dipping objective in confocal laser scanning microscopy in 3D-FISH. EX1039.003. Brileya also uses an objective with a numerical aperture of 0.9, which is roughly 1.0. *Id.*, .003; EX1002 ¶ 294. This is consistent with what would have been the typical value of the numerical aperture of a water dipping objective. EX1002 ¶ 294.

As Dr. Czymmek explains, a POSA would have been motivated to use a water dipping objective as a design choice in the experiment. EX1002 ¶¶ 295-296. If, for example, a scientist wished to use an open flow cell to allow a sample access to oxygen and micromanipulation at extremely high magnification, they would have sought to use a water dipping objective. *Id.* ¶ 296. Further, a POSA would have had a reasonable expectation of success in combining the use of a water dipping objective with MERFISH, as Brileya shows that such a system can be used in FISH experiments to study mixed groups of cells with different biomarkers. *Id.* ¶¶ 297-298.

XIV. GROUND 5: OBVIOUSNESS OF CLAIM 45 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF FANG-YEN

Claim 45 depends from claim 36, and further recites that the pump is a syringe pump. To the extent Zhuang does not disclose or render obvious the use of a syringe pump, this would have been obvious in further view of Fang-Yen.

As Dr. Czymmek describes, the use of pumps for transfer of fluid reagents to and from a sample holder were well-known to a POSA. EX1002 ¶¶ 300-301. Fang-Yen, for instance, used a syringe pump to inject culture medium into the sample holder. EX1029.002. A POSA would have viewed the substitution of Fang-Yen's syringe pump into Zhuang's MERFISH system as simply substituting one known element for another to obtain predictable results. EX1002 ¶ 303. Further, a POSA would have had a reasonable expectation of success given that both references teach using pumps to deliver fluid reagents to the same kind of chamber, and Fang-Yen's syringe pump allows for accurate observations of dynamic cellular processes. EX1002 ¶ 304.

XV. GROUND 6: OBVIOUSNESS OF CLAIM 48 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF RICHARD

Claim 48 depends from claim 46, and further recites that the "said one or more valves comprise a solenoid or a microfluidic valve." To the extent Zhuang does not disclose or render obvious the use of a solenoid or microfluidic valve, this would have been obvious in further view of Richard.

As Dr. Czymmek describes, the use of solenoid and microfluidic valves for transfer of fluid reagents to and from a sample holder were well-known to a POSA. EX1002 ¶¶ 307-308. Richard, for instance, uses a solenoid valve to transfer perfusion solutions to and from the sample holder. EX1030.003. A POSA would

have been motivated to use a solenoid valve because it would have been the simple substitution of one known element for another to allow for the predictable result of accurate and rapid control over the flow of fluid reagents into a sample. EX1002 ¶¶ 309-310. A POSA would have had a reasonable expectation of success in using a solenoid valve for delivery of fluid for cell imaging. EX1002 ¶¶ 311-312.

XVI. GROUND 7: OBVIOUSNESS OF CLAIM 49 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF NIKON

Claim 49 depends from claim 40 and recites that “said fluidics system further comprises an aspiration system comprising: [] an aspiration tube configured to remove fluid from said open flow cell and a pressure source operatively coupled to said aspiration tube.” Zhuang in view of Nikon renders this claim obvious. EX1002 ¶ 313.

For instance, in 2015, a webpage published by Nikon describes the use of an aspirator to remove fluid from an open flow cell in connection with live-cell imaging. EX1043.001, .007; EX1002 ¶¶ 313-314. As Dr. Czymmek explains, a POSA would have understood that the aspirator comprises an aspiration tube configured to remove fluid from the flow cell and a pressure source (particularly, a vacuum source) operatively coupled to the aspiration tube. EX1002 ¶ 314. Indeed, it was well-known to use an aspiration system configured to remove fluid from an open flow cell for reagent exchange before 2015. *Id.* ¶¶ 314-315.

A POSA would have been motivated to use an aspiration system and have had a reasonable expectation of successfully using such system in 2015 because the system was known to work with open flow cells to provide rapid fluid removal without disturbing the biological sample as shown in Nikon. *Id.* ¶ 315.

XVII.GROUND 8: OBVIOUSNESS OF CLAIMS 50 AND 51 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF NIKON AND SKAFTE-PEDERSEN

Claim 50 depends from claim 49 and recites that “said aspiration system further comprises a motor operatively coupled to said aspiration tube, wherein said motor is configured to adjust a motion axis of said aspiration tube to control an interface between said aspiration tube and said open flow cell.” Claim 51 depends from claim 50 and recites the use of a stepper motor. Zhuang in view of Nikon and Skaft-Pedersen renders these claims obvious. EX1002 ¶¶ 316-319.

As Dr. Czymmek explains, it would have been obvious to a POSA to adjust the motion axis of the aspiration tube to control an interface between the aspiration tube and the open flow cell in order to control the amount of fluid to be aspirated. EX1002 ¶ 317. Specifically, the use of a motor would have allowed this adjustment to be automated. *Id.* Further, it would have been obvious to a POSA to use a stepper motor for this purpose. *Id.* ¶¶ 318-319.

For instance, a 2012 article describes the use of a stepper motor in connection with a “programmable microfluidic cell culture system with real-time microscopy

access” and explains that the stepper motor helps achieve “a smooth motion” “with a minimum footprint and weight for microscopy compatibility.” EX1048.004; EX1002 ¶ 318. As Dr. Czymmek explains, a POSA would have understood that the stepper motor was suitable for controlling the motion of an aspiration tube. *Id.*

A POSA would have been motivated to use a motor to control the motion axis of the aspiration tube in 2015 as it allows a POSA to control the amount of fluid to be removed from the open flow cell in an automated system. *Id.* ¶ 319. A POSA would also have had a reasonable expectation of success as using a motor to adjust the position of fluidic system components was common. *Id.* Further, a POSA would have been motivated to use a stepper motor and reasonably expected it to work for this purpose in 2015 because a stepper motor was known to provide smooth positioning and be compatible with microscopy. *Id.*

XVIII. GROUND 9: OBVIOUSNESS OF CLAIM 52 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF SANDS

Claim 52 depends from claim 36 and recites the use of a “Z-axis linear motor configured for Z-motion of said objective focal plane through said volume.” To the extent Zhuang does not disclose the use of a linear motor for the z-axis control, this would have been obvious to a POSA. EX1002 ¶¶ 320-324.

As Dr. Czymmek explains, for instance, in 2009, the brochure for Olympus microscopes described the use of microscopes that were “fully motorized” and that

included a “high-precision z-motor.” *Id.* ¶¶ 321-322. As of 2015, it was extremely common to use precision z-motors in connection with microscopy. *Id.* Further, Sands described the use of such a motor in a system for automated imaging of tissues using confocal microscopy to generate three dimensional images. EX1046.001-.002; Fig. 3; EX1002 ¶ 323.

As Dr. Czymmek explains, a POSA would have been motivated to use a linear motor in 2015, as in Sands, and would have reasonably expected success, because such motors provide precise positioning enabling three dimensional-imaging of biological samples. EX1002 ¶324. Shen specifically teaches the use of a linear motor configured for control of the stage, using an “electric stepper motor attachment *with linear-encoded feedback.*” *Id.* ¶ 322 (emphasis added); EX1032.004 (emphasis added). Thus, use of a Z-axis linear motor would have been well within the ambit of a POSA, and even required for Zhuang’s MERFISH method. EX1002 ¶ 324.

XIX. GROUND 10: OBVIOUSNESS OF CLAIM 53 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF SANDS AND BOOKER

Claim 53 depends from claim 52 and further recites the use of a “linear encoder configured to provide Z-motion feedback to said Z-axis linear motor.” Briefly, a linear encoder encodes position, and the encoded position can be provided to the Z-motor of claim 52 so as to adjust the position. EX1002 ¶ 325.

To the extent Zhuang, Cai, and Shen in view of Sands does not include a linear encoder with Z-motion feedback, this would have been obvious in further view of Brooker. *Id.* ¶ 326. Zhuang teaches autofocusing, explaining that “[c]onstant focus was maintained throughout the imaging process with a home-built, autofocusing system.” *Id.*; EX1003.011. As Dr. Czymmek explains, to maintain focus with a microscope, a person of skill in the art would have been motivated to utilize a linear encoder position and provide it to the motor in order to adjust position. EX1002 ¶ 326. This was a standard manner of autofocusing, as reflected, for instance, in Brooker, which teaches an autofocusing apparatus for a microscope. EX1040; EX1002 ¶ 326. To implement the autofocusing mechanism, Brooker explains that “position feedback is facilitated using...linear encoder scales embedded within the stage construction.” EX1040 ¶ 23.

Another example of an autofocusing system is taught in Shen, which uses an “electric stepper motor attachment *with linear-encoded feedback.*” EX1032.004 (emphasis added); EX1002 ¶ 327. As Dr. Czymmek explains, a POSA would have found it obvious to use a linear encoder as taught in Shen to precisely control the distance between objective and the sample, permitting autofocus. *Id.*

A POSA seeking to implement an autofocusing system of the type set forth in Zhuang in 2015 would have been motivated to use a linear encoder to provide feedback to a Z-motor because such systems were standard components that were

known to be capable of effectively providing feedback in the context of microscope autofocusing, as reflected in Brooker. *Id.* ¶ 328. A POSA would have expected success in using such an approach for precisely the same reason. *Id.*

XX. GROUND 11: OBVIOUSNESS OF CLAIMS 55, 57, AND 58 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF WESSELS

Claim 55 depends from claim 36 and further recites “wherein said two or more light sources comprise one or more light emitting diodes (LEDs).” Claim 57 depends from claim 36 and further recites that the optical system comprises “one or more excitation filters configured to selectively filter one or more wavelengths of light from said one or more light sources to said volume.” Claim 58 depends from claim 57 and further recites that filter(s) is configured to filter light having a wavelength of about 480 nm, 530 nm, 590 nm, or 650 nm.

Zhuang, as described above, teaches an optical system. *See supra* Section X.A.1.c. This optical system is designed to provide monochromatic light to the light source, such as at “641, 561, and 405 nm by using solid-state lasers” to excite fluorescent probes. EX1003.011; EX1002 ¶ 330.

While Zhuang teaches the use of lasers, analogous prior art, such as Wessels, teaches the use of LEDs. EX1002 ¶ 331. By 2015, LEDs were already considered “ideal light sources for microscopy.” *Id.*; EX1050.001. For example, Wessels describes a system where an “LED replaces the arc lamp at the same

position” with “a conventional filter block containing a dichroic mirror (excitation filter/dichroic mirror/emission filter).” EX1050.005-.006 (Fig. 1). A POSA would have understood this to teach both the use of LEDs as a light source in fluorescence microscopy and the use of an excitation filter in fluorescence microscopy using LEDs. EX1002 ¶¶ 332-333; 341-342. Wessels also teaches specifically using light sources providing light at 470 nm, 525 nm 530 nm, 590 nm, and 650 nm. EX1050.009 (Table 3); EX1002 ¶ 346. A POSA would have recognized these wavelengths are exactly or “about” the wavelengths recited in Claim 58. EX1002 ¶¶ 345-346.

A POSA would have been motivated to use LEDs and the filter system as taught by Wessels in the optical system as taught Zhuang, Cai, and Shen to accomplish the delivery of monochromatic light to the sample, as in claim 55 requiring an LED(s) and claim 57 requiring an emission filter(s). EX1002 ¶¶ 335-336; 341-342. Further, a POSA would have been motivated to use filters to specifically deliver light of a wavelength of about 480 nm, 530 nm, 590 nm, or 650 nm, given these wavelengths are expressly taught in Wessels. EX1002 ¶¶ 346-347. Given the use of LEDs and excitation filters was well-known and conventional, with numerous references in the art, a POSA would have had a reasonable expectation of success. EX1002 ¶¶ 335-36; 343; 347

XXI. GROUND 12: OBVIOUSNESS OF CLAIM 61 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF THE SKILL AND KNOWLEDGE OF A POSA

Claim 61 depends from Claim 36 and further recites that the system comprises a vibration control rigid foundation. To the extent Zhuang, Cai, and Shen do not disclose the use of such vibration control, it would have been obvious to a POSA. EX1002 ¶¶ 349-353.

As described above, Zhuang teaches the use of an automated system for imaging or determination of nucleic acids, built around the Olympus IX-71 microscope. As Dr. Czymmek explains, a POSA would have known before 2015 that the Olympus IX-71 included a vibration control rigid foundation. EX1002 ¶¶ 350-351. A 2009 brochure for the microscope contains numerous references to maintaining stability and rigidity regardless of vibrations. EX1033.009, .010, .018; EX1002 ¶ 351. Thus, a POSA would have understood that Zhuang's microscope would have included a vibration control rigid foundation, and such would have been within the knowledge of a POSA. *Id.* ¶ 352. The inclusion of a vibration control rigid foundation would have further been obvious to improve image quality during automated imaging. *Id.* ¶ 353.

XXII. GROUND 13: OBVIOUSNESS OF CLAIM 62 IN VIEW OF ZHUANG, CAI, AND SHEN, IN FURTHER VIEW OF NIMAN AND/OR WINER

Claim 62 depends from Claim 36 and further recites “a fluidics controller operatively coupled to the fluid dispenser and configured to automate or control dispensation of said one or more fluid reagents to said sample holder.” To the extent Zhuang and Cai do not disclose use of a fluidics controller system, it would have been obvious to use one as taught by references such as Niman and Winer. EX1002 ¶¶ 355-358.

As described by Dr. Czymmek, fluidics controller systems were commercially available and saw widespread use long before 2015. *Id.* ¶¶ 356-357. For example, Niman teaches use of the Fluigent fluidics system, which allowed for “pressure-driven flow and control of differential pressures to inlets and waste outlets.” EX1044.003. Yet another example was Winer, who used the same Fluigent system for flow pressure control. EX1051.003.

Not only were these systems available, but they were used in conjunction with fluorescence microscopy. EX1002 ¶ 357. Niman used the system to study “time-dependent change in concentration during switching of solutions” in the context of single-molecule and single-cell studies. *Id.*; EX1044.001. Winer used the system to perform “three-dimensional...particle tracking” and determining precisely the 3D locations of “cell-sized particles in microscale flows.” EX1051.001.

A POSA would have been motivated to use such commercially available systems because of the need for fluidics control in automating imaging systems, as taught by Zhuang and Cai. EX1002 ¶ 358. Further, a POSA would have had a reasonable expectation of success in combining the references, given the state of the art and known successes with such systems. *Id.*

XXIII. THE BOARD SHOULD REACH THE MERITS OF THIS PETITION

A. The Facts and Law Weigh Against Discretionary Denial under § 325(d)

The *Advanced Bionics* test does not support discretionary denial of institution under § 325(d). *See Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH*, IPR2019-01469, Paper 6 (P.T.A.B. Feb. 13, 2020) (precedential).

Neither Zhuang nor Shen were cited in the file history or before the Examiner. EX1001 at code (56); EX1002 ¶ 40. Cai was merely nominally of record during examination, based on inclusion in an Information Disclosure Statement (IDS) submitted by the Applicant to the Office in an August 6, 2021, IDS (EX1034 at 14, 17); EX1002 ¶ 40. The Examiner gave Cai no substantive consideration during examination. EX1002 ¶ 40. Further references were neither cited in the file history nor before the Examiner. *Id.* ¶ 43.

First and foremost, the Board should not exercise its discretion under § 325(d) to deny institution because neither Zhuang nor Shen was before the Office. As set

forth above, Zhuang teaches virtually every element of independent claim 36 of the '554 patent, along with the features of several dependent claims. To the extent there is anything inventive in the '544 patent, it is the software instructions associated with element (e) of the claim. Zhuang teaches precisely these software instructions in connection with a system for imaging nucleic acids in tissue that is insubstantially different from what is described and claimed in the '554 patent. As to Cai, while nominally before the Examiner being listed in an IDS submission, that fact only evidences material error by the Examiner in failing to rely on Cai and reject the then-pending claims. Indeed, the Examiner gave Cai no substantive consideration and certainly did not consider the specific combination of Zhuang, Cai, and Shen that is detailed herein.

To the extent Patent Owner contends Zhuang and/or Shen is cumulative to prior art that was before the Office, this would only confirm that the Examiner made a material error during Examination, misapprehending or overlooking the equivalent of the references' teachings. Specifically, as set forth in the Reasons for Allowance for the '554 Patent, the Examiner determined that ***none*** of the prior art taught "the systems as currently claimed, *including the computing system configured for determining three-dimensional positional information of the one or more nucleic acid molecules using a plurality of optically-encoded signals.*" EX1036.006-.007 (emphasis added). If prior art of record teaches these elements, the Examiner's

determination that there was *none* shows material error, and institution should not be denied under § 325(d).

B. *Fintiv* Does Not Support Discretionary Denial

The parallel district-court proceedings do not justify discretionary denial under the factors identified in *Apple Inc. v. Fintiv, Inc.*, IPR2020-00019, Paper 11 (PTAB Mar. 20, 2020) (precedential) (“*Fintiv*”), as applied in *CommScope Tech. LLC v. Dali Wireless, Inc.*, IPR2022-01242, Paper 23 (P.T.A.B. Feb. 27, 2023) and *OpenSky Indus., LLC v. VLSI Tech. LLC*, IPR2021-01064, Paper 102 at 49 (P.T.A.B. Oct. 4, 2022) (precedential).¹

Fintiv, and cases and guidance applying *Fintiv*, weigh additional considerations other than whether the statutory requirements for institution are met in determining whether the Board should exercise its discretion to not institute review. The Board rightfully can account for unique circumstances, including those impacting the timing of proceedings that would make discretionary denial

¹ See also the USPTO Memorandum, Interim Procedure for Discretionary Denials in AIA Post-Grant Proceedings with Parallel District Court Litigation (“Memorandum”) (June 21, 2022), available at https://www.uspto.gov/sites/default/files/documents/interim_proc_discretionary_denials_aia_parallel_district_court_litigation_memo_20220621_.pdf.

unwarranted and unjust, and those relating to the complexity of the subject matter, for which the benefit of an expert tribunal is particularly warranted.

1. Factor 1: Likelihood of a Stay

Even to the extent a stay is unlikely here, this should not weigh against institution in the specific circumstances at issue here. As documented below, most notably in connection with the *Fintiv* Factor 2, the circumstances here are unique and *inter partes* review is especially warranted in the interest of fairness and justice given the strong merits of the Petition.

2. Factor 2: Proximity of Trial Date to Final Written Decision

While a final written decision is unlikely to issue prior to trial in the parallel district court proceedings, currently scheduled for September 16, 2024 (EX1061 at 2), this consideration does not support denying institution under the facts here.

Petitioner was prohibited by statute from filing this IPR until October 2023, nine months after the '554 patent issued in January 2023. *See* 35 U.S.C. § 311(c)(1). As of October 2023, the parallel litigation, filed in February 2022, had already been pending for roughly nineteen months based on several unrelated

patents.² EX1062. When the '554 patent was eventually asserted against Petitioner in March 2023 (EX1063), the relevant parallel litigation had already been pending for roughly a year.

While the timeline is such that trial will likely occur before a final written decision, this would have been the case even if Petitioner had filed on the earliest day allowed. Given the statutory constraints prohibiting Petitioner from filing until nearly 19 months after being sued in district court, it would be unjust to conclude that the proximity of trial to final written decision weighs in favor of denying institution pursuant to § 325(d).

Further, the Board has declined to consider trials in parallel proceedings coming before deadlines for final written decisions to bar institution. *See, e.g., Samsung Elecs. Co., LTD v. Mojo Mobility, Inc.*, IPR2023-01089, Paper 11 at 17-18, 25 (PTAB, Jan. 11, 2024); -01089, Paper 11 at 15-17, 24 (P.T.A.B. Jan. 11, 2024).

² Petitioner and Patent Owner's exclusive licensee (10x Genomics) were also involved in another proceeding that did not involve the '554 patent, that went to trial in November 2023. *10x Genomics, Inc. et al. v. NanoString Techs., Inc.*, No. 21-cv-653 (D. Del. Filed May 6, 2021).

3. Factor 3: Investment in Parallel Proceeding

This consideration should not weigh against institution. While the parallel district court litigation was first filed in February 2022, this patent was only asserted against Petitioner in March 2023 and could not have been challenged in an IPR until October 2023. Furthermore, the amount of investment at this time is minimal, and that by the time of an institution decision remains speculative. EX1061.

4. Factor 4: Overlap in Issues With Parallel Proceeding

This factor weighs against discretionary denial. Only one challenged claim (independent claim 36) is at issue in the District Court. The limitations recited in the other thirty-four non-overlapping claims (dependent claims 37-70) add significant, albeit well-known, features. *Supra* Sections X-XXII (including grounds relying on additional references for features only recited in dependent claims). Petitioner would be prejudiced if institution of the non-overlapping claims was denied because Patent Owner could assert these claims against Petitioner's future products and/or customers in the future whereas the § 315(b) time bar would apply to Petitioner. *See Fintiv* at 12-13.

5. Factor 5: Identity of the Parties

This factor is neutral because it is often the case for a patent asserted in district court and challenged at the Board.

6. Factor 6: Other Factors

Other circumstances impacting the Board’s exercise of discretion—including the merits—strongly favors institution. As set forth above, the Petition sets forth “[c]ompelling, meritorious challenges...in which the evidence, if unrebutted at trial, would plainly lead to a conclusion that one or more claims are unpatentable by a preponderance of the evidence.” *OpenSky* at 49 (citing Memorandum). For the reasons set forth herein, grounded on the evidence of record, it is highly likely that the Petitioner will prevail with respect to at least one challenged claim and Petitioner sets forth a compelling, meritorious challenge. *Id.* Thus, the Board should institute *inter partes* review because “compelling, meritorious challenges will be allowed to proceed at the PTAB even where district court litigation is proceeding in parallel.” *Id.* (quoting Memorandum at 4).

Further, this case is unique, especially as to timing where the ’554 patent did not issue, and was not asserted, until after the district court proceeding was well underway. Petitioner reasonably could not have filed this petition early enough to fulfill factors 1-3 as applied in the quite-different typical case in which parallel proceedings are not already well underway before patent issuance. These factors, thus, in the interests of fairness and justice, should not weigh against institution of review.

Moreover, review is warranted because the challenged claims are lengthy and involve complex technology particularly suited for comprehensive focused review by an expert tribunal in their entirety rather than limited review by a lay jury of the single asserted claim of the '554 patent among twenty asserted claims from ***six total*** patents where other weighty issues must also be addressed. EX1063.001, .011-.031; EX1064.013-48.

XI. CONCLUSION

For the reasons above, Petitioner respectfully requests *inter partes* review of the '554 patent's claims 36-70.

Dated: January 30, 2024

Respectfully submitted,

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APPENDIX A: LISTING OF CHALLENGED CLAIMS

No.	Limitation
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.
37	The system of claim 36, wherein said substrate comprises a flow cell.
38	The system of claim 37, wherein said flow cell is a closed flow cell.
39	The system of claim 38, wherein said closed flow cell comprises a fluid inlet and a fluid outlet, and wherein said fluid inlet is fluidically coupled to said fluid dispenser
40	The system of claim 37, wherein said flow cell is an open flow cell.
41	The system of claim 36, wherein said objective is a water immersion objective.
42	The system of claim 36, wherein said objective is a water dipping objective.
43	The system of claim 36, wherein said temperature control apparatus comprises a heat sink or a Peltier element.
44	The system of claim 36, further comprising a thermal control system.
45	The system of claim 36, wherein said pump is a syringe pump.
46	The system of claim 36, wherein said fluidics system further comprises one or more valves downstream of said one or more

	reservoirs and upstream of said fluid dispenser, wherein, upon actuation, said one or more valves facilitate dispensation of fluid to said sample holder.
47	The system of claim 46, wherein said one or more valves comprise a rotary valve.
48	The system of claim 46, wherein said one or more valves comprise a solenoid valve or a microfluidic valve.
49	The system of claim 40, wherein said fluidics system further comprises an aspiration system comprising: (1) an aspiration tube configured to remove fluid from said open flow cell and a pressure source operatively coupled to said aspiration tube.
50	The system of claim 49, wherein said aspiration system further comprises a motor operatively coupled to said aspiration tube, wherein said motor is configured to adjust a motion axis of said aspiration tube to control an interface between said aspiration tube and said open flow cell.
51	The system of claim 50, wherein said motor is a stepper motor.
52	The system of claim 36, wherein said Z-axis motion control system comprises a Z-axis linear motor configured for Z-motion of said objective focal plane through said volume.
53	The system of claim 52, further comprising a linear encoder configured to provide Z-motion feedback to said Z-axis linear motor.
54	The system of claim 36, further comprising an objective wetting apparatus.
55	The system of claim 36, wherein said two or more light sources comprise one or more light emitting diodes (LEDs).
56	The system of claim 36, wherein said two or more light sources comprise one or more lasers.
57	The system of claim 36, wherein said optical system further comprises one or more excitation filters configured to selectively filter one or more wavelengths of light from said one or more light sources to said volume.
58	The system of claim 57, wherein at least one excitation filter of said one or more excitation filters is configured to selectively filter light having a wavelength of about 480 nm, about 530 nm, about 590 nm, or about 640 nm from said one or more light sources to said volume.

59	The system of claim 36, wherein said optical system further comprises one or more emission filters configured to selectively filter one or more wavelengths of light emitted from said volume to said one or more detectors.
60	The system of claim 36, wherein said optical system is mechanically, electrically, or electromechanically coupled to a controller system configured to synchronize one or more imaging events within said system.
61	The system of claim 36, further comprising a vibration control rigid foundation.
62	The system of claim 36, further comprising a fluidics controller operatively coupled to said fluid dispenser and configured to automate or control dispensation of said one or more fluid reagents to said sample holder.
63	The system of claim 44, wherein said temperature control apparatus further comprises a temperature sensor configured to provide a feedback signal to said thermal control system.
64	The system of claim 59, wherein at least one of said one or more emission filters is configured to selectively filter light having a wavelength of about 510 nm, about 570 nm, about 620 nm, or about 680 nm to said one or more detectors from said volume to said one or more detectors.
65	The system of claim 36, wherein said two or more light sources excite fluorescence in unique color channels such that each light source of said two or more light sources is configured to excite fluorescence in a unique color channel of said unique color channels.
66	The system of claim 65, wherein each optically-encoded signal of said plurality of optically-encoded signals comprises at least one of said unique color channels.
67	The system of claim 36, wherein at least one of the plurality of optically-encoded signals comprises two or more colors detected in series.
68	The system of claim 36, wherein said two or more light sources comprise four light sources.
69	The system of claim 36, wherein said optical system is configured for wide-field microscopy.

70	The system of claim 36, wherein a numerical aperture of said objective is about 1.0.
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CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24 et seq., the undersigned certifies that this document complies with the type-volume limitations. This document contains 13,939 words as calculated by the “Word Count” feature of Microsoft Word 2016, the word processing program used to create it.

Respectfully submitted,

WEIL, GOTSHAL AND MANGES LLP

Dated: January 30, 2024

/Derek C. Walter/
Derek C. Walter
Lead Counsel for Petitioner
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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.105 and § 42.6(e), the undersigned certifies that on January 30, 2024, a complete and entire copy of this **PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 11,542,554 WITH PETITIONER'S EXHIBIT LIST, POWER OF ATTORNEY, AND EXHIBITS 1001-1065** will be sent via FedEx on January 31, 2024, for next business day delivery to the following:

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