

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BRUKER SPATIAL BIOLOGY, INC.,
Petitioner

v.

PRESIDENT AND FELLOWS OF HARVARD COLLEGE and
10X GENOMICS,
Patent Owner

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

**PATENT OWNER RESPONSE
UNDER 37 C.F.R. § 42.120**

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PATENT OWNER'S UPDATED EXHIBIT LIST

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2001	Stahl <i>et al.</i> , “Visualization and analysis of gene expression in tissue selections by spatial transcriptomics,” <i>Science</i> 353(6294):78-82 (July 1, 2016), with attached Supplementary Materials
2002	<i>Withdrawn</i>
2003	International Patent Application Publication No. WO 2012/140224 A1 to Frisen <i>et al.</i> , published October 18, 2012
2004	“No-Heat Flow Cell (FSC2),” Biopetechs Inc., accessible at https://biopetechs.com/product/no-heat-flow-cell-fcs2/ (last accessed May 24, 2024)
2005	“FCS2 Chamber,” Biopetechs Inc., accessible at https://biopetechs.com/product/fcs2-chamber/ (last accessed May 24, 2024)
2006	Prosecution File History of U.S. Patent No. 11,542,554
2007	Fang, <i>et al.</i> , “Three-dimensional single-cell transcriptome imaging of thick tissues,” <i>eLife</i> , August 16, 2023, accessible at https://elifesciences.org/reviewed-preprints/90029 (last accessed May 23, 2024) (“Zhuang 2023”)
2008	Lu, <i>et al.</i> , “Spatially resolved epigenomic profiling of single cells in complex tissues,” <i>Cell</i> 185:4448–4464 (2022)
2009	Su, <i>et al.</i> , “Genome-Scale Imaging of the 3D Organization and Transcriptional Activity of Chromatin,” <i>Cell</i> 182:1641–1659 (2020)
2010	Cox, Jr., <i>et al.</i> , “Organizing your space: The potential for integrating spatial transcriptomics and 3D imaging data in plants,” <i>Plant Physiology</i> 188:703-712 (2022) (“Czymmek 2022”)

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2011	Ghani, <i>et al.</i> , “Optimisation of MERFISH Data Analysis and Visualisation,” May 21, 2019, accessible at https://www.ucl.ac.uk/~ucbpngh/MiniProject3.pdf (last accessed May 28, 2024)
2012	<i>Withdrawn</i>
2013	Docket Report, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), July 8, 2024
2014	Oral Order, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 109, April 25, 2023
2015	Hearing Transcript, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), June 14, 2024
2016	Notice of Service of 10x Genomics, Inc.’s Initial Infringement Contentions, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 68, November 23, 2022
2017	Notice of Service of NanoString Technologies, Inc.’s Initial Invalidity Contentions, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 79, January 3, 2023
2018	Notice of Service of 10x Genomics, Inc.’s Supplemental Infringement Contentions, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 100, March 24, 2023
2019	Notice of Service of NanoString Technologies, Inc.’s Supplemental Invalidity Contentions, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 132, May 17, 2023
2020	Memorandum Opinion and Order, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 269, February 1, 2024

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2021	Notice of Service of NanoString Technologies, Inc.’s Initial Infringement Contentions, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 64, November 23, 2022
2022	Notice of Service of 10x Genomics, Inc.’s Initial Invalidity Contentions, <i>10x Genomics, Inc. v. NanoString Techs., Inc.</i> , Case No. 1:22-cv-00261 (D. Del.), ECF No. 77, December 27, 2022
2023	E-mail Correspondence from Jason J. Rawnsley to Melissa Astell, July 8, 2024
2024	Declaration of Hari Shroff, Ph.D., in Support of Patent Owner’s Response
2025	<i>Curriculum Vitae</i> of Hari Shroff, Ph.D.
2026	Declaration of Julie L. Davis in Support of Patent Owner’s Response
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2028	“Bruker Completes Asset Acquisition of NanoString Business,” Bruker Corporation Press Release, May 6, 2024 (accessible at https://ir.bruker.com/press-releases/press-release-details/2024/Bruker-Completes-Asset-Acquisition-of-NanoString-Business/default.aspx , last accessed December 9, 2024)
2029	“Who We Are,” NanoString Technologies, Inc. (accessible at https://nanosttring.com/about-us/ , last accessed December 9, 2024)
2030	“CosMx™ SMI for Single-Cell Imaging,” NanoString Technologies, Inc. (accessible at https://nanosttring.com/products/cosmx-spatial-molecular-imager/single-cell-imaging-overview/ , last accessed December 9, 2024)

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2031	“NanoString Begins Commercial Shipments of CosMx Spatial Molecular Imager and AtoMx Spatial Informatics Platform,” BusinessWire, December 6, 2022 (accessible at https://www.businesswire.com/news/home/20221206005433/en/NanoString-Begins-Commercial-Shipments-of-CosMx-Spatial-Molecular-Imager-and-AtoMx-Spatial-Informatics-Platform , last accessed December 9, 2024)
2032	NanoString Technologies, Inc. Form 10-K for the fiscal year ended December 31, 2022
2033	“Our mission,” 10x Genomics, Inc. (accessible at https://www.10xgenomics.com/company , last accessed December 9, 2024)
2034	10x Genomics, Inc. Form 10-K for the fiscal year ended December 31, 2023
2035	“10x Genomics Commercially Launches Xenium Platform for In Situ Analysis,” PR Newswire, December 8, 2022 (accessible at https://www.prnewswire.com/news-releases/10x-genomics-commercially-launches-xenium-platform-for-in-situ-analysis-301698748.html , last accessed December 9, 2024)
2036	“Xenium In Situ,” 10x Genomics, Inc. (accessible at https://www.10xgenomics.com/platforms/xenium , last accessed December 9, 2024)
2037	“10x Genomics Begins Commercial Shipments of Xenium Prime 5K Pan-Tissue and Pathways Panel,” PR Newswire, June 20, 2024 (accessible at https://www.prnewswire.com/news-releases/10x-genomics-begins-commercial-shipments-of-xenium-prime-5k-pan-tissue-and-pathways-panel-302177116.html , last accessed December 9, 2024)

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2038	“The First Fully Integrated Single-Cell Spatial Solution,” NanoString Technologies, Inc. (accessible at https://nanosttring.com/products/atomx-spatial-informatics-platform/cosmx-and-atomx-the-first-fully-integrated-single-cell-spatial-solution/ , last accessed December 9, 2024)
2039	“CosMx™ SMI Technology Access Program,” NanoString Technologies, Inc. (accessible at https://nanosttring.com/products/cosmx-spatial-molecular-imager/technology-access-program/ , last accessed December 9, 2024)
2040	“Accelerating the Mastery of Biology to Advance Human Health,” 42 nd Annual J.P. Morgan Healthcare Conference, dated 2024 (accessible at https://s28.q4cdn.com/592666581/files/doc_presentations/2024/01/2024-TXG-JPM-Healthcare-Presentation-vF.pdf , last accessed December 9, 2024)
2041	Chen, K. H., “Spatially resolved, highly multiplexed RNA profiling in single cells,” Dissertation, Harvard University, April 2015
2042	Uchida, A., et al., “Live-cell imaging of neurofilament transport in cultured neurons,” <i>Methods Cell Biol.</i> , 131:21-90 (2016)
2043	Transcript of the Deposition of Kirk Czymmek, Ph.D.
2044	Zhang, B., “Gaussian approximations of fluorescence microscope point-spread function models,” <i>Applied Optics</i> , 46(10):1819-1829 (April 1, 2007)
2045	Waters, J., “Accuracy and precision in quantitative fluorescence microscopy,” <i>The Journal of Cell Biology</i> 185(7):1135-1148 (2009)
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2047	Stallinga, S., et al., “Accuracy of the Gaussian Point Spread Function model in 2D localization microscopy,” <i>Optics Express</i> , 18(24):24461-22476 (November 22, 2010)
2048	McCall, B., “FLUORESCENCE MICROSCOPY/QUANTITATIVE IMAGING: A systems-engineering approach to quantitative fluorescence bioimaging,” laserfocusworld.com, January 21, 2015 (accessible at https://www.laserfocusworld.com/biooptics/bioimaging/fluorescence/article/14190740/fluorescence-microscopy-quantitative-imaging-a-systems-engineering-approach-to-quantitative-fluorescence-bioimaging , last accessed December 9, 2024)
2049	Davis, A., et al., “Characterizing and Diminishing Autofluorescence in Formalin-fixed Paraffin-embedded Human Respiratory Tissue,” <i>Journal of Histochemistry & Cytochemistry</i> , 62(6):405-423 (2014)
2050	Lee, J., et al., “Highly Multiplexed Subcellular RNA Sequencing in Situ,” <i>Science Reports</i> , 343:1360-1363 (March 21, 2014), with supplemental materials
2051	Lubeck, E., et al., “Single cell systems biology by super-resolution imaging and combinatorial labeling,” <i>Nat Methods</i> , 9(7):743-748 (June 2012), with supplemental materials
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2054	Response to Office Action, U.S. Patent Application No. 17/374,000, filed January 26, 2024
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2057	U.S. Patent Application Publication No. 2012/0040397 A1 to Luo et al., published February 16, 2012
2058	U.S. Patent Application Publication No. 2014/0220587 A1 to Green, Jr. et al., published August 7, 2014
2059	Chung, K., et al., “CLARITY for mapping the nervous system,” <i>Nature Methods</i> , 10(6):508-513 (2013)
2060	Xenium Analyzer User Guide, CG000584, Rev. G., 10x Genomics, Inc.
2061	CosMx® SMI Manual, MAN-10161-08, Software v1.4.1, Bruker Spatial Biology, Inc.
2062	Manual Slide Preparation, CosMX™ Spatial Molecular Imager, MAN-10159-01, NanoString Technologies, Inc.
2063	<i>Intentionally Left Blank</i>
2064	Cassini HW.V1 Design Review and Transfer to NPI, June 1, 2022, 10XH-00054907-96 (CONFIDENTIAL – PROTECTIVE ORDER MATERIAL)
2065	CosMx™ Spatial Molecular Imager Grant Support Document, NanoString Technologies, Inc.
2066	<i>Intentionally Left Blank</i>
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2068	<i>Intentionally Left Blank</i>
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2070	“New Insights Into Cleft Palate Unlocked With 10x Genomics’ Single Cell and Spatial Technologies,” 10x Genomics, Inc. Press Release, June 27, 2024

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2071	“10x Genomics Surpasses 100 Xenium Analyzer Shipments,” BioSpace, August 29, 2023 (accessible at https://www.biospace.com/10x-genomics-surpasses-100-xenium-analyzer-shipments , last accessed November 24, 2024)
2072	Peroni, E., “Spatial-transcriptomic profiling: a new lens for understanding myelofibrosis pathophysiology,” <i>Cell Communication and Signaling</i> , 22:510 (2024)
2073	Cassini Phase 2 Design Review – Instrument and Software, July 13, 2022, 10X261-00003121-232 (CONFIDENTIAL – PROTECTIVE ORDER MATERIAL)
2074	Small, A., et al., “Fluorophore localization algorithms for super-resolution microscopy,” <i>Nature Methods</i> , 11(3):267-279 (March 2014)
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2078	Diaspro, A., et al., “Optical nanoscopy,” <i>La Rivista del Nuovo Cimento</i> , 43:385-455 (2020)
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<i>In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.</i> , 676 F.3d 1063 (Fed. Cir. 2012)	59
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<i>Lectrosonics, Inc. v. Zaxcom, Inc.</i> , IPR2018-01129, Paper 33 (P.T.A.B. Jan. 24, 2020) (precedential)	60
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<i>Trivascular, Inc. v. Samuels</i> , 812 F.3d 1056 (Fed. Cir. 2016)	52, 57
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Regulations

37 C.F.R. § 42.100(b)	27
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I. INTRODUCTION

Before November 3, 2015, there was a need in the art for a complete, single-unit, volumetric imaging *system* for determining three-dimensional (“3D”) positional information of nucleic acid molecules in a sample. The invention of U.S. Patent No. 11,542,554 (“the 554 patent”) satisfied that need. Before the volumetric imaging systems of the 554 patent, artisans relied upon homebuilt imaging and fluidics arrangements comprising piecemeal configurations of microscopes, tubes, bottles, tubing, cameras, and other components spread out over an entire laboratory benchtop. Such unwieldy setups were laborious to assemble and maintain, and generally unsuitable for mass production or commercialization. The 554 patent provided the field with a single-unit volumetric imaging system comprising a seamless blend of mechanical components, fluidics system, optics, and computing system engineered for determining 3D positional information of nucleic acid molecules.

Petitioner’s portrayal of the issue in this case is an oversimplified question of whether “3D imaging of nucleic acid molecules” would have been obvious. This is incorrect in view of Petitioner's art combination and the 554 patent’s claimed invention. Various laboratory methods of 3D imaging were known in the art. The 554 patent, however, claims an entire *system* for volumetric imaging on a nanometer scale, not merely a method of 3D imaging. The question before the

Board is not whether a method of 3D imaging would have been obvious, but whether Petitioner's art combination and the relied upon disclosures therein would have rendered obvious the 554 patent's volumetric imaging *system* for determining 3D positional information of nucleic acid molecules. As this Response will show, the answer to that question is a resounding "no."

Petitioner's obviousness case rests on the combination of three primary references: Chen (EX1003), Shen (EX1032), and Cai (EX1004). None of these references—alone or in any combination—teaches or suggests the claimed *system* for 3D imaging. Chen disclosed a highly multiplexed RNA imaging method called MERFISH, and a homebuilt, two-dimensional ("2D")-imaging setup to visualize and identify the RNA molecules. Shen merely disclosed an autofocusing apparatus for microscopy applications. And Cai disclosed RNA imaging methods called smFISH and seqFISH, using a completely different imaging arrangement from that of Chen's homebuilt setup. To reach its conclusion of obviousness, Petitioner relies on hindsight reconstruction to map claim elements to disclosures in its art combination, and then argues in conclusory fashion that a person of ordinary skill in the art ("POSA") would have been motivated to convert Chen's homebuilt 2D-MERFISH imaging arrangement into a *3D MERFISH* system to arrive at the claimed 3D system of the 554 patent, based on certain disclosures in Cai. Petitioner's obviousness theory fails for several reasons.

First, Petitioner's art combination fails to teach or suggest the claimed volumetric imaging system comprising a temperature control apparatus as described in the 554 patent. The 554 patent makes clear the "temperature control" means actively heating *and* cooling, while Petitioner's art combination suggests at most an apparatus for heating.

Second, Petitioner's theory requires that a POSA would have been motivated to convert Chen's homebuilt 2D imaging arrangement into a 3D MERFISH system to arrive at the claimed 3D system, based on Cai. Absent in the Petition, however, is any viable rationale as to *why* the POSA would have modified Chen's 2D imaging arrangement in view of Cai. In particular, Petitioner relies on Cai's purported teachings of using "two or more colors of light" and a "Z-axis motion control system," as rationales for combining Cai with Chen, but both of these aspects are already disclosed in Chen. And a single sentence in Cai cited by Petitioner that refers to 3D "spatial information" is no more informative than the general knowledge in the art that methods of 3D imaging existed. There would have been no reason for a POSA reading Chen and interested in *practicing MERFISH* (as Petitioner contends) to consult the disclosures of Cai.

Third, Petitioner never explains *in the Petition* what purported modifications the POSA would have made to Chen's homebuilt contraption, nor how the artisan would have made them. *See, e.g., Cook Group Inc. v. Boston Scientific Scimed,*

Inc., IPR2017-00435, Paper 94, 31 (P.T.A.B. Dec. 28, 2018) (rejecting petitioner’s obviousness argument because, *inter alia*, “Petitioner has not shown how a [POSA] would have integrated such embodiments, or found them compatible.”). Instead, the Petition’s hindsight approach identifies claim elements purportedly in the art and then summarily concludes the claimed system must have been obvious.

Arguing that a combination of art would have been “intuitive,” “common sense,” or even “obvious” (as Petitioner has done here), without some articulated rationale, “is inadequate to support a finding that there would have been a motivation to combine.” *In re Van Os*, 844 F.3d 1359, 1361 (Fed. Cir. 2017). The Petition’s conclusory rationale “tracks the *ex post* reasoning *KSR* warned of and fails to identify any actual reason why a skilled artisan would have combined the elements in the manner claimed.” *Id.*; *KSR Int’l v. Teleflex Inc.*, 550 U.S. 398 (2007). The Board should accordingly reject the entirety of Petitioner’s arguments. *See, e.g., Kingston Tech. Co., Inc. v. North Star Innovations, Inc.*, IPR2018-01784, Paper 32, 55 (P.T.A.B. Feb. 26, 2020) (rejecting petitioner’s obviousness arguments for “rest[ing] on hindsight” and “us[ing] the challenged patent as a roadmap to reconstruct the claimed invention.”); *Intel Corp. v. VLSI Tech. LLC*, IPR2018-01033, Paper 32, 88-89 (P.T.A.B. Feb. 6, 2020) (similar).

Moreover, as Patent Owner's technical expert Dr. Hari Shroff¹ explains, when viewing the art properly through the lens of a POSA, it is clear that a POSA would have had *no reason* to modify Chen's 2D MERFISH imaging setup into 3D MERFISH in view of Cai's teachings, as Petitioner contends. A POSA would have understood fundamental aspects of single-molecule nucleic acid fluorescence microscopy, such as point-spread function, axial resolution, and signal-to-noise ratio, which would have impacted the artisan's rationales and motivations when reading Petitioner's selected art. Petitioner and its expert fail to address these issues entirely. Indeed, these same fundamental aspects would have quashed a POSA's reasonable expectation of successfully modifying Chen's 2D imaging arrangement into a 3D MERFISH imaging system, because attempting to do so would run the risk of losing the high-multiplex capabilities of MERFISH—the stated objective in Chen and the POSA's goal, according to the Petition.

Petitioner's hindsight reconstruction is further evident in the Petition's failure to address objective indicia of nonobviousness. As shown herein and further explained by Patent Owner's experts, commercial embodiments of the 554 patent's

¹ Patent Owner hereby affirmatively withdraws the expert declaration and CV submitted with Patent Owner's preliminary response (EX2002, EX2012). *See* PTAB Consolidated Trial Practice Guide, November 2019, at 51.

claimed imaging system satisfied a long-felt need in the industry, evident by the rapid adoption and sales growth of these systems in the field once they were launched. The innovation of these systems is also evident by the fact that the commercial embodiments have achieved substantial commercial success and garnered widespread industry praise. This overwhelming objective evidence confirms the patentability of the claimed 3D system. The Board should accordingly reject the Petition in its entirety and find the challenged claims not unpatentable.

II. STATE OF THE ART BEFORE NOVEMBER 3, 2015

Petitioner's hindsight-driven obviousness theory glosses over key aspects of single-molecule imaging that were fundamental to a POSA's understanding and motivations in imaging individual nucleic acid molecules. This is evident not only from Petitioner's selected primary art combination, but also in Petitioner's overall theory of the case. Two of Petitioner's three primary references (Chen and Shen) disclosed imaging setups designed for imaging nucleic acid molecules in two dimensions. Petitioner's third reference (Cai) mentioned three-dimensional spatial information, but in the context of an entirely different imaging setup from Chen and Shen. When considering the state of the art from the view of a POSA, the POSA would not have been motivated to modify Chen's 2D imaging setup in view of Cai and/or Shen. Tellingly, the Petition ignores key aspects of single-molecule fluorescence microscopy such as point-spread function, numerical aperture, axial

resolution, signal-to-noise ratio, and refractive indexes, each of which would have been fundamental in guiding a POSA's motivations and rationales.

A. Level of skill in the art

Before November 3, 2015, a POSA would have had a Ph.D. in molecular, cellular, or developmental biology and/or biophysics, bioengineering, biochemistry, or a related discipline, with at least 2 years of experience in the field of fluorescence imaging of biological materials, including cells and tissues.

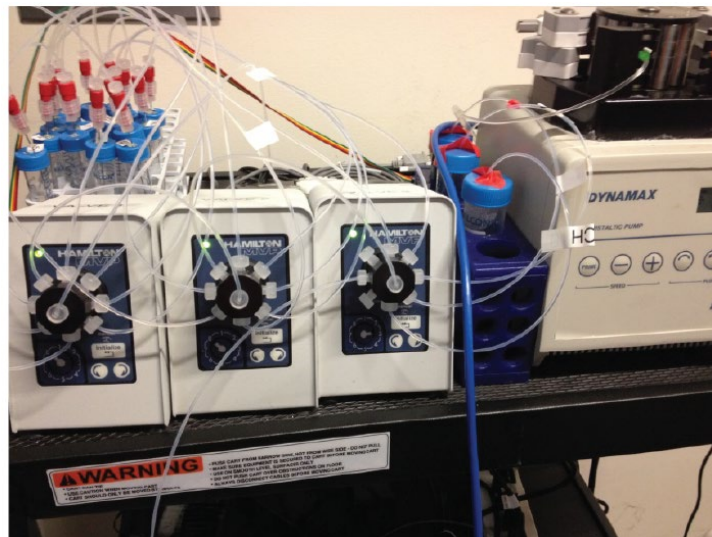
EX2024, ¶11.

A POSA would have understood techniques such as fluorescence imaging technologies and the use of microscopes to collect and record images. EX2024, ¶11. A POSA would also have had some familiarity with gene amplification and sequencing technologies. *Id.*

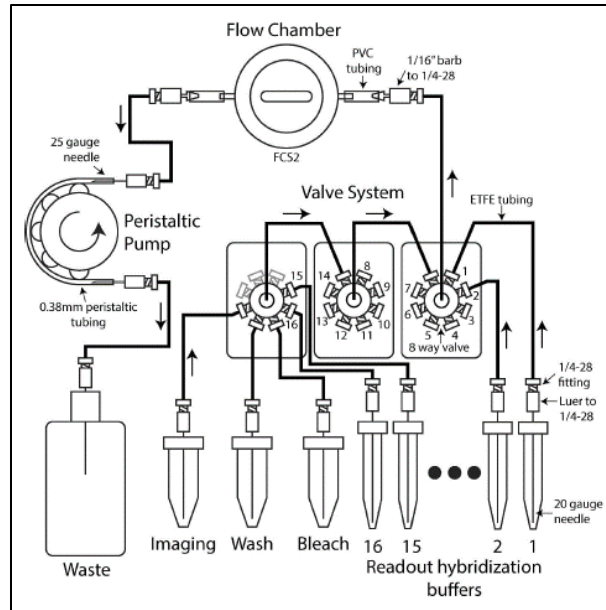
B. Prior art systems for single-molecule imaging were homebuilt arrangements

A POSA would have understood that single-molecule imaging systems of the prior art varied in design, depending on the intended use of the system, but were frequently home-built contraptions that were laborious to assemble and maintain, and generally unsuitable for mass production or commercialization. EX2024, ¶¶20-21, 129. For example, Chen disclosed a homebuilt imaging setup designed for imaging RNA molecules by “multiplexed error robust FISH (MERFISH),” which comprised a “home-built” wide-field microscope (designed

for 2D imaging), a “home-built, autofocusing system,” and a “home-built fluidics system” comprising three valves, a pump, and numerous tubes and bottles for multi-round hybridization buffers and washes. EX1003, 002, 011; *see also* EX1021, FIG. 4. This home-built setup was designed for imaging fixed cells in 2D. *Id.*; EX2024, ¶¶20, 70. The first author of Chen provided a picture of the MERFISH imaging homebuilt fluidics setup in his dissertation, which depicts numerous tubes, tubing, bottles, and pumps:

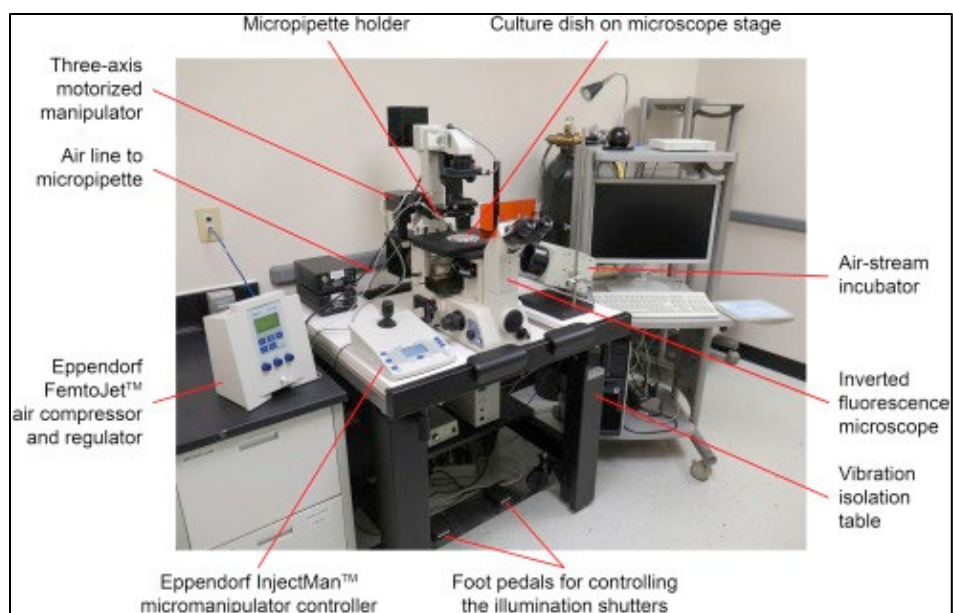


EX2041, FIG. 33. In the image above, only the fluidics portion of the setup is depicted. *Id.* In 2016, Moffitt et al. published a schematic of the same homebuilt setup, depicting 16 different tubes for hybridization buffers, three tubes for imaging, wash, and photobleaching buffers, plus a variety of tubing, valves and a pump:



EX1021, FIG. 4; EX2024, ¶20. Such imaging setups in the prior art were considered unwieldy and unsuitable for mass production or commercial development. EX2024, ¶¶20-21, 129.

Similarly, other prior-art setups designed for 2D imaging were homebuilt. For example, Sivaramakrishnan disclosed a setup for imaging live cells comprising an “FCS2” flowcell that “maintains the cells and media at 37°C.” EX1007, 004, FIG. 8; *see also* EX1027, 098 (describing FCS2 as a “cell growth chamber”). Sivaramakrishnan further disclosed that the FCS2 chamber “was mounted onto the stage of an LSM 510Meta confocal microscope” and further fitted with “an air stream stage incubator.” EX1007, 004. An example of a homebuilt imaging setup comprising an “air stream stage incubator” is depicted below:



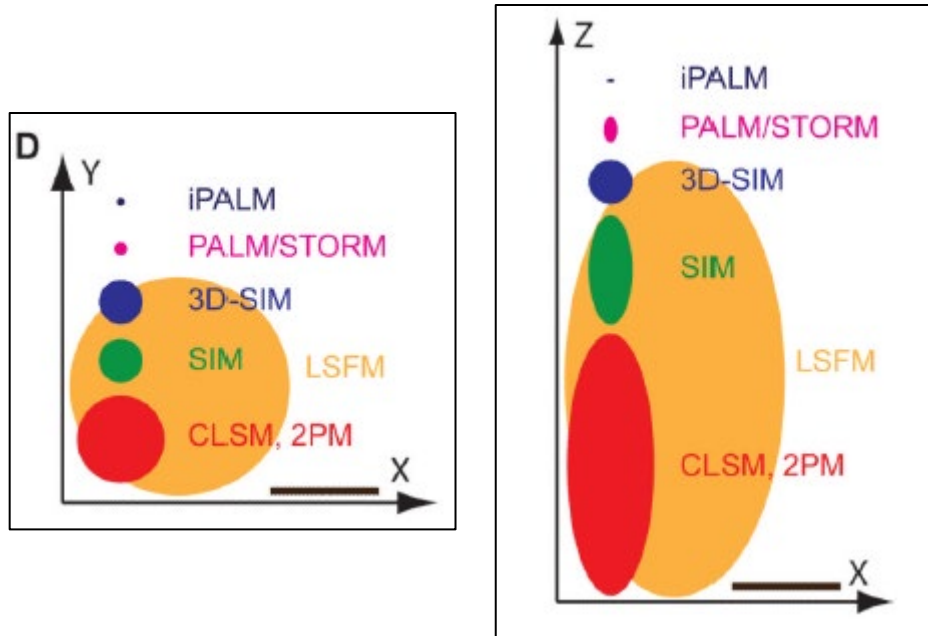
See, e.g., EX2042, FIG. 6; EX2024, ¶21. As Uchida explained, “[b]ecause the fan generates vibration, it must be isolated mechanically from the microscope,” so the authors “place it on an equipment shelf supported above the vibration isolation table top.” EX2042, 32. Thus, these types of homebuilt arrangements were frequently built as ad hoc setups, requiring numerous separate pieces strung together and often spanning an entire benchtop.

C. POSAs understood that axial resolution is more difficult than lateral resolution

Before November 3, 2015, a POSA would have understood that axial resolution (z-axis) in fluorescence microscopy is more difficult than lateral resolution (x-y axis), due in part to the relationship between point-spread function (PSF) and resolution. EX2024, ¶¶22-31. A POSA would have known that when viewing a single fluorescence point (molecule) under a microscope, the diffraction

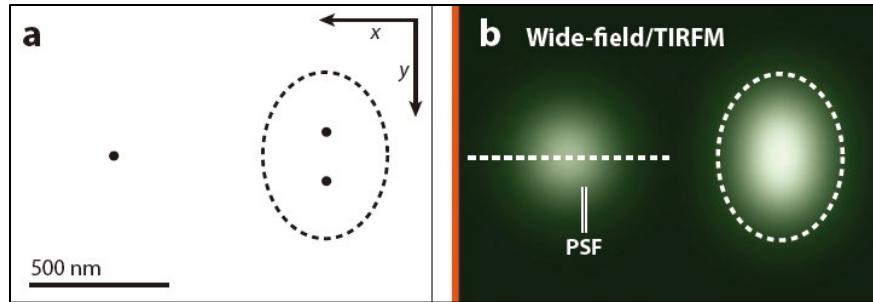
of light causes that point to appear as a bright center with blurry edges, and that the amount of blurring is the PSF. EX2024, ¶22; EX1019, 9; EX1018, 287; EX1027, 22; EX2044, 1819; EX2043, 44:17-18 (“[PSF] will contribute to the blurriness in a signal.”), 48:12-13 (agreeing PSF “play[s] a role” in imaging). The PSF determines the resolution limit of the microscope – i.e., the ability of the microscope to distinguish the smallest distance between two points such that they can still be distinguished. EX2024, ¶¶26-28; EX2043, 43:17-44:14, 45:12-15.

The PSF was also well known to impact axial resolution more substantially than lateral resolution. As Fischer explained, “the resolution along the Z-axis of the microscope is substantially ($\sim 2x$) worse than in the lateral dimensions due to the inherent shape of the point spread function.” EX1019, 001; *see also* EX1018, 004 (“axial resolution ... is typically 2 to 3 times worse than the lateral resolution.”); EX2045, 1141 (“[r]esolution in the z-axis (z) is worse than lateral resolution in the light microscope.”); EX2024, ¶¶24-31, 43, 52, 84, 111. This is depicted in the figure below, showing the “Z-stretch” elongation of the PSF in the z-axis (right) compared to the x-y axes (left):



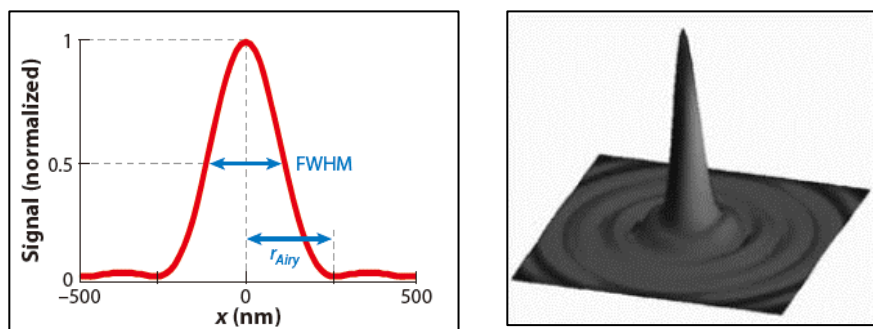
EX1019, FIG. 1; EX2024, ¶24.

POSAs knew that the PSF is influenced by several factors, such as the numerical aperture (NA) of the microscope's objective lens, the wavelength of emission light (e.g., fluorescence), and the refractive index of the sample and imaging medium. EX2024, ¶¶27-28; EX1027, 023; EX1018, 004. For example, in the figure below (left panel), compare the single point on the left with the two points on the right. When imaged under a wide-field microscope (right panel), the two points on the right are unresolvable due to the width of the PSF and the close proximity of the two points:



EX1018, FIG. 1a-b; EX1027, 023; EX2024, ¶¶25-28. This means that the distance between the two points is smaller than the width of the PSF. EX1027, 023; EX2024, ¶¶25-28. The two points on the right cannot be localized to any position because they are unresolvable. *Id.* The single point on the left, however, can be localized by determining the center of the PSF, which represents the localized position of the point. *Id.*

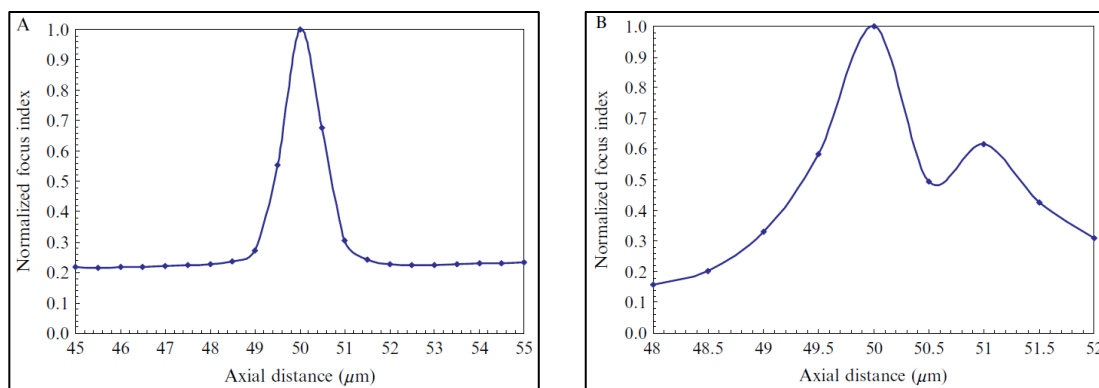
The shape of the PSF is known as an Airy disk, and the resolving power of a microscope's objective lens can be evaluated by measuring the size of the Airy disk. EX1018, 004; EX1027, 023; EX2024, ¶¶26. Two points will be resolvable if the distance between them is at least the radius (r_{Airy} in the figure below, left) of the Airy disk-shaped PSF:



EX1018, FIG. 1c (left); EX1027, FIG. 1.2A (right); EX2024, ¶¶26-28. The center

of the PSF is called the “full-width at half-maximum” (FWHM), and represents the localized position of the fluorescent point. Generally, a higher NA, shorter emission wavelength, and higher refractive index result in a smaller PSF radius and higher resolving power. EX2024, ¶¶26; EX2046, 922. Conversely, a lower NA, longer emission wavelength, and lower refractive index will lower the resolving power. *Id.*

The PSF can be measured by fitting it to a “Gaussian curve” or “Gaussian function”—a mathematical model that describes how a point source of light is spread out in a 2D image plane. EX2024, ¶¶29-31; EX2047, Abstract. A Gaussian curve takes the shape of a bell curve, much like the shape of the Airy disk. *Id.*, FIG. 1. Thus, POSA’s routinely used a Gaussian function as a model “for fitting the position of fluorescent emitters in localization microscopy.” *Id.*, Abstract. POSAs also understood that “[t]hin specimens produce an ideal curve that can be fit readily to a Gaussian [function],” whereas thicker specimens with discernible objects in different planes can produce “multiple peaks ... the curve,” which is “more frequently a problem in images taken using high NA lenses because the DOF [depth of field] is much less than the cell thickness.” EX1032, 007; EX2024, ¶31. For example, in the two curves below, the curve on the left is from a thin specimen and the curve on the right is from a thick specimen in which more than one focal plane provides discernable contrast:



EX1032, FIG. 2; EX2024, ¶31. POSAs also knew that while fitting a PSF to a 2D Gaussian curve was relatively straightforward, fitting a PSF to a 3D function required completely different and more complex computational methods, at least in part due to the “Z-stretch” of the PSF and reduced axial resolution. EX2024, ¶¶24, 31; EX1019, 001; EX1018, 004; EX2045.

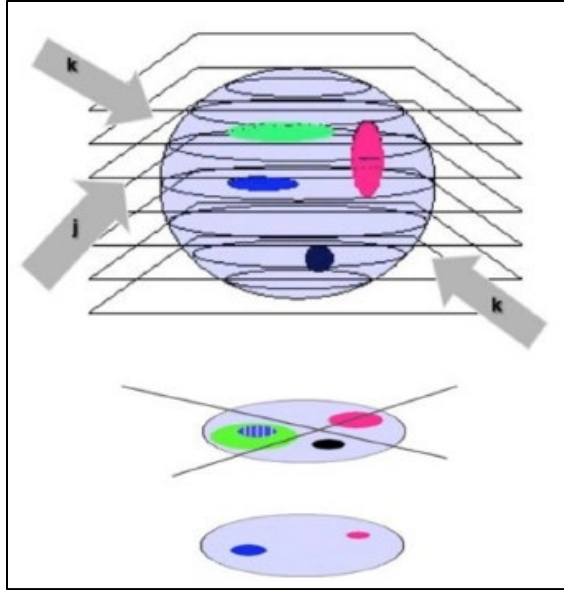
D. POSAs were aware of various additional factors that were important for axial resolution

In addition to the point-spread function and related factors discussed above, various additional factors were critical for axial resolution in single-molecule imaging. EX2024, ¶¶32-38.

Signal-to-Noise Ratio and Signal-to-Background Ratio. In fluorescence imaging, signal-to-noise ratio (SNR) measures how well a signal can be distinguished from noise. EX2024, ¶¶33-36; EX2043, 47:24-25. A high SNR means the signal is easy to see and interpret, while a low SNR means there is more noise and the signal is less clear. EX2045, 1136; EX2024, ¶¶33, 111. Similarly, high amounts of background fluorescence can lower the signal-to-background

(SBR) ratio and likewise affect the ability to resolve individual molecules in close proximity. EX2024, ¶¶33-36; EX2048, 3, 6. Both background and noise often arise from other planes in the sample above and below the focal plane being imaged. As Dr. Shroff explains, the illuminating light is directed to the image focal plane to achieve maximum excitation of fluorophores in the focal plane. EX2024, ¶34. However, fluorophores present in other planes both above and below the focal plane are also excited by the same illuminating light, which appear as out-of-focus background fluorescence in the focal plane. EX2024, ¶¶34-36, 52, 85, 89; EX2045, 1139; EX2046, 920. This background fluorescence from other planes decreases SBR. EX1019, 002; EX2024, ¶105. In some cases, the background from other planes can contribute to over 90% of the fluorescence seen in the focal plane. EX2046, 920.

POSAs knew that SNR and SBR could be improved using optical sectioning. EX2024, ¶36; EX2046, 920; EX1012, 004. In optical sectioning, 2D images of thin slices of the sample are acquired one at a time. In this way, the fluorescence from other planes is removed. *Id.*; EX2024, ¶¶36, 52, 85, 89. This basic process is depicted in the figure below:



EX2024, ¶36; EX2049, FIG. 17. As shown above, while the microscope objective is focused on a single 2D plane (plane j), the light source illuminates the entire specimen, causing background fluorescence from other planes above and below plane j. By optical sectioning, the background fluorescence from other planes is removed, while the focal plane j remains in focus with maximum fluorescence.

EX2024, ¶36; EX2046, 920; EX1012, 004.

Autofluorescence. Another common cause of lowering SNR is sample autofluorescence. EX1010, 022. According to Femino, “[a]utofluorescence is a major source of background inherent in biological samples.....” *Id.*, 024; EX1012, 004; EX2024, ¶37. Autofluorescence often occurs in proteins and lipids present in animal tissues, as well as cultures containing dead or dying cells. EX1012, 004; EX2050, 001; EX2024, ¶37.

Signal density. Another factor known to influence resolution is signal

density. Signal density is a measure of how many individual signals are detected and imaged in a single focal plane. EX2024, ¶38. If there are too many individual signals in close proximity to one another in the plane, the signal density will be high, making it more difficult to resolve individual molecules. *Id.*, ¶¶38, 105. On the other hand, if the individual signals are sparse, the signal density will be lower. EX2024, ¶38. POSAs knew that signal density needs to be “sufficiently low for discerning individual molecules.” EX2050, 1361. POSAs also knew that increasing multiplexing, can increase the signal density and reduce the resolution between individual signals. EX2024, ¶¶38, 105, 111-113. For example, Chen 2015 disclosed that, “[a]s the degree of multiplexing is increased, it is important to consider the potential increase in the density of RNAs that need to be resolved in each round of imaging.” EX1003, 008; *see also* EX2051, 3 (“[w]hen the density of transcripts is high, diffraction-limited fluorescent spots of the same color will overlap and make barcode readout impossible.”). Similarly, Cai recognized that “[b]ecause smFISH [a type of fluorescent imaging] often uses two or more colors for a target, it produces high density of objects in the image, which can increase the complexity of data analysis.” EX1004, 1:67-2:3; EX2024, ¶¶38, 112.

As Dr. Shroff explains, a POSA would have known that these factors become more important for axial resolution than lateral resolution. EX2024, ¶¶38, 105-106, 111-112.

E. MERFISH's touted advance over prior FISH methodologies was its capability for massive multiplexing

Before November 3, 2015, a POSA would have known a variety of fluorescence in-situ hybridization (FISH) techniques for use in fluorescence microscopy. EX2024, ¶¶39-52. The term FISH generally connotes using fluorescent probes to label nucleic acids in a sample, which can be imaged under a fluorescence microscope. *Id.*, ¶39. Relevant to this proceeding are three iterations of FISH methodologies disclosed in Petitioner's references: single-molecule FISH (smFISH), sequential FISH (seqFISH), and multiplexed error robust FISH (MERFISH).

1. smFISH and seqFISH

Lubeck 2012 disclosed methods of using FISH to simultaneously image multiple different mRNA transcripts in yeast cells. EX2051. Lubeck 2012 explained that “the number of molecular species that can be measured simultaneously is limited by the spectral separability of fluorophores.” *Id.*, Abstract. According to Lubeck 2012, their smFISH methods “increase the capacity for multiplex detection of molecules in single cells” by using a combination of spectral barcodes (a combination of fluorophores) and super-resolution microscopy. *Id.*

The authors reported measuring the mRNA levels of 32 genes simultaneously in single *S. cerevisiae* cells. *Id.*, 6-7; EX2024, ¶41. When

performing smFISH, Lubeck 2012 assessed mRNA localization in 2D. EX2051, 3 (“For *two-dimensional imaging* the spatial barcoding scheme necessitates the linearization of mRNA.”)², FIG. 1 (disclosing 2D Gaussian fitting for x-y localization); EX2024, ¶¶40-43. Lubeck 2012’s use of smFISH for 2D imaging is consistent with the knowledge in the art. As Perillo explained, a known “drawback[] of the smFISH technique” is that ““the *data is primarily two dimensional*, due to the lack of axial resolution typical of wide-field imaging.” EX1011, 002; EX2024, ¶¶43, 52, 78, 84.

The same research group subsequently reported a method comprising a “sequential barcoding scheme to multiplex different mRNAs,” named seqFISH. EX2052, 360; EX2024, ¶44. Rather than one round of labeling and imaging (as in Lubeck 2012), in seqFISH, “mRNAs in cells are barcoded by sequential rounds of hybridization, imaging and probe stripping.” *Id.* The sequential rounds of hybridization and imaging generate a series of signals that create barcodes to uniquely identify an mRNA. *Id.*, FIG. 1. The seqFISH methods are also disclosed in Petitioner’s relied-upon Cai reference (EX1004).

Performing seqFISH was known as a long and tedious process, as it requires overnight hybridization of probes, followed by washing and imaging, a four-hour

² Emphasis is added throughout except where otherwise indicated.

stripping procedure to remove the probes, followed by the next round of overnight hybridization. EX2052, 13-14; EX1004, 38:60-39:27. This process is repeated for each round of hybridization, such that an experiment with only six rounds of seqFISH hybridization would take at least 6 days to perform. *Id.* Similar to their 2012 report, Lubeck 2014 disclosed using seqFISH to assess 2D localization of mRNAs yeast and mouse cells. EX2052, FIGs. 1b, S6 (referring to fitting the FISH dots with “*2D Gaussians*.”), S9-S10 (2D images); *see also* EX1004, FIGs. 2B, 8, 10:20-27 (fitting the FISH dots with “*2D Gaussians*”), FIGs. 11-12 (2D images), 10:56-63.

Despite the purported increase in the ability to multiplex, POSAs understood that these prior-art methods “remain[ed] limited by the number of RNA species that can be simultaneously measured in single cells.” EX1003, 002; EX2024, ¶¶46, 102. As Chen explained in 2015, “efforts [] using combinatorial labeling with either color-based barcodes or sequential hybridization have enabled simultaneous measurements of 10 to 30 different RNA species in individual cells.” EX1003, 002 (citing EX2051 and EX2052). Moffitt confirmed seqFISH’s limited multiplexing capability in 2016—two years after Lubeck 2014 published—stating that “even the most advanced multiplexing efforts have only extended this approach to the simultaneous measurement of ~10–30 RNA species.” EX1021, 002 (citing EX2051 and EX2052); EX2024, ¶¶46, 82.

2. Multiplexed Error-Robust FISH

In 2015, Chen et al. disclosed a method of “multiplexed error-robust FISH (MERFISH),” which was a “highly multiplexed smFISH imaging method that substantially increases the number of RNA species that can be simultaneously imaged in single cells.” EX1003, 002; EX2024, ¶¶20, 47-52. As Chen explained, application of smFISH methods were “limited by the number of RNA species that can be simultaneously measured in single cells.” *Id.* Chen expressly mentioned Lubeck 2012 and Lubeck 2014 methods as being limited to “measurements of 10 to 30 different RNA species in individual cells.” *Id.* (citing Lubeck 2012 and Lubeck 2014 as reference no. 17 and 19, respectively). Chen’s MERFISH methods utilized “combinatorial labeling and sequential imaging with error-robust encoding schemes,” which provided the ability to measure over 1,000 RNA species in a single experiment. EX1003, 002, 007-008. According to Chen, performing up to 16 rounds of hybridization, imaging, and photobleaching “was typically completed in ~ 20 hours.” EX1003, 012. This is in marked contrast to the lengthy smFISH and seqFISH methods taught by Lubeck 2012, Lubeck 2014, and Cai. EX2024, ¶¶47, 51, 105.

Chen’s stated goal for its MERFISH methods was to increase *multiplexing*—i.e., the simultaneous detection of multiple different mRNA species. EX1003, 001-002; EX2024, ¶¶48-51, 66, 80-83; *see also* Pet., 30 (referring to “[Chen’s]

objective of ‘rapidly increas[ing] the number of RNA species that can be probed simultaneously.’”). Indeed, Chen discusses approaches to “*further increase* the number of detectable RNA species” as potential next steps for their research. EX1003, 008. The goal of multiplexing was confirmed by the same research group’s publication a year later in 2016, where they reported “an improved MERFISH method that drastically *increases the throughput*.” EX2053, 11047; EX2024, ¶¶49, 82, 104. Additionally, Chen et al. have filed multiple patent applications directed to MERFISH, and repeatedly argued to the USPTO that predecessor Lubeck 2012 and Lubeck 2014/Cai methods are “inadequate” and “not a practical approach to multiplexing.” *See, e.g.*, EX2054, 16; EX2055, 16-17; EX2024, ¶¶49-51, 102.

Chen disclosed constructing a homebuilt MERFISH imaging setup designed to image massively multiplexed mRNA molecules in 2D. EX2024, ¶¶20, 52; EX1003, 011. This is evident from the fact that Chen disclosed examining human fibroblast cells using a wide-field imaging microscope. EX1003, 0011. As Chen explained, “[h]uman primary fibroblasts ... are relatively large and flat, facilitating *wide-field imaging without the need for optical sectioning*.” *Id.*; *see also id.*, 0012; EX2024, ¶¶52, 85-89. In other words, Chen could image the mRNAs in a single 2D focal plane without the need to remove background fluorescence from planes above and below the focal plane. *Id.* POSAs knew that wide-field imaging was

useful for lateral (x-y) localization purposes, but inadequate for providing axial (z-axis) information. *See, e.g.*, EX1012, 004; EX1011, 002; EX2079, 446; EX2024, ¶¶43, 52, 78-79, 84. Thus, before November 3, 2015, a POSA would have known that MERFISH is useful for identifying RNAs in single cells in a highly multiplexed format, and providing 2D (lateral) information of the RNAs. EX2024, ¶¶47-52.

III. THE 554 PATENT

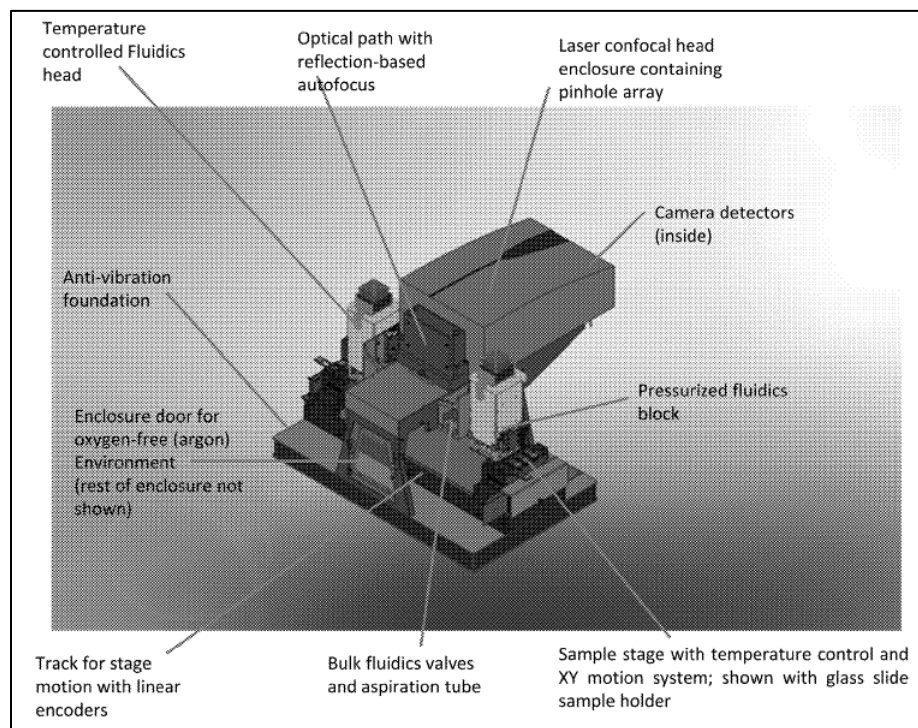
A. The 554 patent's claimed system

The inventors of the 554 patent introduced a novel and complete system for volumetric imaging nucleic acid molecules in a sample having a volume. EX1001, 33:29-34:17; EX2024, ¶¶53-56. As the 554 patent explains, optical detection of single-molecule signals in many prior art systems occurred “in at most two-dimensional planes.” EX1001, 1:48-49. Additionally, as discussed above, prior art imaging “systems” frequently comprised homebuilt piecemeal contraptions that were laborious to construct, challenging to maintain, and unsuitable for mass production or commercialization due to their unwieldy nature and design.

The 554 patent discloses a complete, single-unit, volumetric imaging *system* for determining three-dimensional positional information of nucleic acid molecules in a sample on a nanometer scale – “on the order of 10^{-7} m” (i.e., 100 nanometers). EX1001, 4:43-50, 16:8-15. The automated system comprises an innovative

mechanical design seamlessly paired with a computing system for determining the three-dimensional positional information of a nucleic acid molecule in a sample.

EX1001, 5:29-33 (“an automated sequencing and three-dimensional imaging device is provided which includes a *three-dimensional imaging apparatus*, apparatus for *fluid exchange*, apparatus for *temperature control*, and apparatus for *computational analysis*.”), 16:46-51 (“[t]he device includes *hardware and software functionally assembled*...”). The 554 patent provides a picture of an example system for volumetric imaging in Figure 2:



EX1001, FIG. 2; *see also id.*, FIGs. 3-4; EX2024, ¶¶54-55.

These innovations are reflected in, e.g., independent claim 36 of the 554 patent, which is directed to a *system* comprising specific mechanical components

(e.g., sample holder, temperature control apparatus, XY motion control system, fluidics system, optical system, and Z-axis motion control system) and a computing system component for determining three-dimensional positional information of one or more nucleic acid molecules in the sample. EX1001, 33:29-34:17; *see* Appendix A; EX2024, ¶56.

B. The 554 patent’s prosecution history

During examination, the pending claims were initially rejected as obvious over references Keller (EX1042), Callahan (EX1041), and Frisen. EX2006, 537-549. In response, the Applicant amended the claims to recite, *inter alia*, that the system comprises: a sample holder configured to receive a substrate comprising a “sample *having a volume*”; a *temperature control apparatus operatively coupled* to the sample holder; and a *computing system for determining three-dimensional positional information of one or more nucleic acid molecules*. EX2006, 671-672.

The Examiner allowed the claims, acknowledging that the prior art is “silent with regard to the computing system as currently claimed.” EX2006, 1275; EX1036, 006. The Examiner also acknowledged that “[o]ther references of particular interest”—Luo (EX2057) and Green (EX2058)—do not “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.” EX2006, 1275-

1276.

IV. CLAIM CONSTRUCTION

Claim terms must be given their “ordinary and customary meaning ... as understood by one of ordinary skill in the art” in view of the specification and prosecution history. 37 C.F.R. § 42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc).

Claim 36 recites the phrase “*temperature control* apparatus operatively coupled to said sample holder.” EX1001, 33:33-34. The term “temperature control” is not expressly defined in the 554 patent. However, a POSA reading the plain language of the claims in light of the specification and prosecution history would have understood “temperature control” to mean the ability to actively heat *and* actively cool. EX2024, ¶¶60-64. For example, independent claim 36 recites the term, “*temperature control* apparatus,” and dependent claim 43 further specifies that the temperature control apparatus “comprises a heat sink or a Peltier element” (i.e., components for cooling the sample holder). EX1001, 34:31-32; *see also id.*, 18:6-7 (“thermoelectric cooling using Peltier elements”); Pet., 43 (Petitioner agreeing that Peltier element and heat sink “have *cooling capabilities*.”). And the heat sinks of the 554 patent are heat sinks that actively cool. *See, e.g.*, EX1001, 9:34 (“heat sink in conjunction with a fan”), 18:13-14 (same), 19:61-62 (same), 26:28-29 (“Peltier element and heat sink ... or liquid cooling”), 26:36 (same),

27:22 (“heat sink for Peltier cooler”), FIGs. 3, 5.

This construction is evident throughout the specification. The 554 patent describes using temperature control “such as according to a programmed time and temperature” as in “thermo-cycling for applications such as nucleic acid hybridization, amplification and sequencing”—processes which require both heating and cooling temperatures. EX1001, 18:5-10; EX2024, ¶¶62-63. Figure 5 depicts a “schematic of a stage,” which comprises a “heat sink for peltier for stage temperature control” and a “Thermo-cycling stage.” EX1001, FIG. 5, 27:22-23 (“[a] heat sink for the *Peltier cooler* for stage *temperature control* is provided.”). Figure 3 similarly displays “[a] stage for holding samples, with *temperature control by Peltier element and heat sink* (shown) or *liquid cooling* is provided.” EX1001, 26:35-37, FIG. 3; *see also id.*, 9:35-41 (disclosing a “*radiator and liquid cooling/circulating system* to dissipate heat produced during temperature changes.”); EX2024, ¶63.

Additionally, during prosecution, the Patent Owner amended the claims from reciting “a heating or cooling apparatus” to a “*temperature control* apparatus” as currently claimed, indicating that “temperature control” has a different meaning from “heating or cooling.” EX2006, 671, 677. Indeed, throughout the specification, the 554 patent articulates “temperature control” (i.e., actively heating *and* cooling) separately from a “heating *or* cooling apparatus.” *Compare* EX1001, 18:26-30,

26:11-17, 26:28-39, 27:17-23, *with id.*, 9:38-41, 18:5-21, 19:60-20:2, 30:32-35.

Accordingly, in view of the plain language of the claims, the specification, and the prosecution history, a POSA would construe³ the term “temperature control” to mean the ability to actively heat and cool. EX2024, ¶¶60-64. In the context of claim 36, the “temperature control apparatus” is “operatively coupled to the sample holder” and thus must be capable of actively heating and cooling the temperature of the sample holder. *Id.* This is also the plain and ordinary meaning of the term “temperature control apparatus” – to “control” the temperature of the sample holder, a temperature control apparatus must be able to both heat and cool the sample holder. An apparatus that can only heat the sample holder cannot “control” the temperature of the sample holder because it is not able to cool the sample holder. EX2024, ¶64.

V. PETITIONER FAILS TO DEMONSTRATE THAT ANY OF THE CHALLENGED CLAIMS IS UNPATENTABLE OVER THE CITED ART

The Petition’s obviousness case relies on *ex post* reasoning and hindsight bias, using the 554 patent’s challenged claims as a roadmap to cobble together selected disclosures from the cited art and then asserting conclusory allegations of

³ No claim terms of the 554 patent have been specifically construed in the parallel district court litigation. *See generally*, EX2020.

obviousness. Such an approach to obviousness “is inadequate to support a finding that there would have been a motivation to combine.” *In re Van Os*, 844 F.3d at 1361; *see also KSR Int’l v. Teleflex Inc.*, 550 U.S. 398 (2007) (“[a] factfinder should be aware, of course, of the *distortion caused by hindsight bias* and must be cautious of arguments reliant upon *ex post* reasoning.”). The Board has correctly followed this precedent in other proceedings, and should do the same here. *See, e.g., Kingston Tech. Co., Inc.*, IPR2018-01784, Paper 32, 55 (rejecting petitioner’s obviousness arguments for “rest[ing] on hindsight” and “us[ing] the challenged patent as a roadmap to reconstruct the claimed invention.”); *Intel Corp.*, IPR2018-01033, Paper 32, 88-89 (similar).

Petitioner’s hindsight reconstruction is evident for several reasons: (i) the Petition maps cherry-picked disclosures in the art to the claim elements; (ii) the Petition fails to view the art through the lens of a POSA and instead relies on conclusory allegations of obviousness; (iii) the Petition fails to articulate why or how the POSA would have modified Chen’s imaging setup in view of Cai to arrive at a 3D MERFISH system meeting the elements of claim 36; and (iv) the Petition fails to address objective indicia of nonobviousness. The Board should accordingly reject the Petition and find the challenged claims not unpatentable.

A. Petitioner’s obviousness theory requires a POSA to modify Chen’s 2D MERFISH setup to 3D MERFISH

The foundational premise of Petitioner’s obviousness theory is that a POSA

reading Chen, Cai, and Shen would have been motivated to modify *Chen's 2D MERFISH homebuilt imaging setup* (built for massive multiplexing of RNA molecules and 2D-imaging) in view of Cai and Shen to arrive at a modified version of Chen's imaging setup that now performs *3D MERFISH*, which according to Petitioner meets the limitations of claim 36. For example, Petitioner argues that “[a] POSA would have been *motivated to use [Chen]'s MERFISH method* for 3D investigation.” Pet., 39; *see also id.*, 29 (“it would have been obvious to modify [Chen] in view of Cai to use multiple light sources....”). Dr. Czymmek likewise focuses his analysis on the POSA's alleged modification of Chen's imaging setup to arrive at a 3D MERFISH system allegedly meeting the elements of claim 36, testifying that “a POSA ... would have expected success in *using the system to perform MERFISH as taught in [Chen].*” EX1002, ¶153; *see also id.*, ¶150 (“to perform MERFISH...”), ¶170 (“it would have been obvious to *modify [Chen]* with Cai's teachings using multiple light sources...”), ¶183 (“a POSA *interested in MERFISH ...*”), ¶186 (same), ¶191 (“application of Z-axis scanning and imaging to [Chen]'s MERFISH protocol ...”), ¶207 (“a POSA would have been *motivated to use the MERFISH method* for 3D investigation...”), ¶219 (“it would have been obvious to modify [Chen] to permit a POSA to *use MERFISH to determine three-dimensional positional information...*”), ¶305 (“a POSA *interested in operating [Chen]'s automated system for MERFISH imaging...*”), ¶312 (same).

Thus, Petitioner's obviousness theory indisputably requires modifying Chen's MERFISH 2D imaging setup into a 3D MERFISH system to arrive at the claimed 3D system of the 554 patent. EX2024, ¶¶65-68, 76-77. As shown below and explained in detail by Dr. Shroff, Petitioner's theory falls apart under scrutiny.

B. Petitioner's art combination fails to teach or suggest a system for volumetric imaging comprising a temperature control apparatus, as claimed

As discussed above, the volumetric imaging system of the 554 patent requires a temperature control apparatus with the ability to actively heat *and* cool the temperature of the sample holder. Petitioner alleges that Chen's 2D MERFISH imaging arrangement is "a system that includes temperature control capability" because Chen discloses using "a Biopetechs FCS2 flow chamber." Pet., 24. Petitioner's argument fails for several reasons.

There is no direct evidence that Chen's "FCS2 flow chamber" has any temperature control associated with it at all. EX1003, 011; EX2024, ¶¶69-75. Chen does not disclose using any temperature control associated with the FCS2 flow chamber. EX1003, 011; EX2024, ¶70. This makes sense, because Chen's 2D MERFISH methods were performed on *fixed cells*—there was no need to maintain the cells at 37°C as one would for imaging living cells as disclosed in, e.g., Sivaramakrishnan. EX1003, 011; EX1007, 004; EX2024, ¶70. To satisfy a "suggestion" of this claim element, Petitioner relies on a completely different

reference (Sivaramakrishnan) for its disclosure of an “FCS2 chamber” that “maintains the cells and media at 37°C” for the proposition that Chen’s FCS2 flow cell must also have an apparatus for heating. Pet., 24. But there is no evidence that Chen *necessarily* taught or suggested the same “FCS2 chamber” disclosed in Sivaramakrishnan. *See, e.g.,* EX2004; *Par Pharm., Inc. v. TWI Pharms., Inc.*, 773 F.3d 1186, 1195-96 (Fed. Cir. 2014).

Even if the Board were to conclude that Chen suggested using an FCS2 chamber with heating capability as in Sivaramakrishnan, the alleged temperature control apparatus with the FCS2 chamber is only capable of *heating* (and not cooling). EX2024, ¶71. For example, Petitioner’s “FCS2 instructions” document depicts an FCS2 controller for *heating* and maintaining the chamber at 37°C. EX1006, FIG. 1; EX2024, ¶71; EX1007, 004. The same document instructs the user that “[w]hen the controller is turned on ... it will read the ambient temperature, then *slowly begin to rise* until it *reaches setpoint temperature*.” EX1006, 004; EX2024, ¶71. In other words, the FCS2 temperature regulator maintains the temperature by heating or not heating, but not by heating *and* cooling. *Id.* Dr. Czymmek testified at deposition that with the FCS2, “you have control over the temperature on it, so you can *increase it*.” EX2043, 81:10-11. Petitioner’s alleged temperature control apparatus thus does not meet the claimed “temperature control” limitation. *Id.* Petitioner cites no evidence of a system

comprising a temperature control apparatus capable of heating *and* cooling the sample holder. Pet., 24-25.

Petitioner also relies on Chen’s disclosure of a “Bioptechs objective heater” as an “additional temperature control system that becomes coupled to the stage and sample holder.” Pet., 25. This disclosure fails to meet the claimed temperature control element for the same reason—that is, Chen’s “objective heater” is *only a heater* and is not capable of *cooling* the sample holder, as required by claim 36. EX1003, 011; EX1006, 006; EX2024, ¶72. According to the “FCS2 instructions,” the “Bioptechs Objective Heater” is a “heating band and temperature sensor assembly.” EX1006, 006. Moreover, claim 36 requires that the temperature control apparatus is operatively coupled to the *sample holder*. EX1001, 33:33-34. The Bioptechs objective heater, however, “plugs into a *separate* Temperature Controller,” and thus if anything, is operatively coupled to the *objective* (a claim element recited in claim 36(c)(i)), and *not the sample holder* as required in claim 36(a)(ii). EX1006, 006; EX2024, ¶73.

Petitioner does not rely on any disclosures in Cai or Shen for this claim element. Pet., 24-25. Nor can it. Shen does not disclose temperature control at all, and Cai at most discloses performing enzymatic reactions at either room temperature or 37°C. EX1003, 27:20-3, 55:43-45, 55:64-66; EX1032; EX2024, ¶74. Neither Shen nor Cai taught or suggested temperature control, as claimed. *Id.*

“[O]bviousness requires a suggestion of *all limitations in a claim*.” *CFMT, Inc. v. Yieldup Int’l Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (C.C.P.A. 1974)). And as *KSR* stated, “there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.” *KSR*, 550 U.S. at 418 (citation omitted). Petitioner has failed to provide any rationale for a POSA to have modified Chen’s 2D MERFISH imaging setup to include a temperature control apparatus capable of both heating and cooling the temperature of the sample holder. Pet., 24-25.

The Petition’s discussion of claim 43 cannot cure this failure. When challenging claim 43, Petitioner and its expert merely allege that “it would have been obvious” to add a heat sink or Peltier element, with no underlying support or rationale. Pet., 43; EX1002, ¶¶230-233. “Absent some articulated rationale, a finding that a combination of prior art would have been ‘common sense’ or ‘intuitive’ is no different than merely stating the combination ‘would have been obvious.’ Such a conclusory assertion with no explanation is inadequate to support a finding that there would have been a motivation to combine.” *Van Os*, 844 F.3d at 1361. Such is the case here.

C. Petitioner’s art combination fails to teach or suggest a volumetric imaging system for determining three-dimensional positional information of one or more nucleic acid molecules, as claimed

Petitioner relies exclusively on disclosures in Chen and Cai for this claim

element.⁴ Pet., 42. As discussed below, Chen does not teach or suggest a volumetric imaging system for determining 3D positional information of nucleic acid molecules, and a POSA would not have had any reason to modify Chen's 2D imaging arrangement in view of Cai. Moreover, even if a POSA were so motivated, Petitioner fails to explain with any particularity how a POSA would have modified Chen in view of Cai to arrive at the claimed volumetric imaging system for determining 3D positional information of nucleic acid molecules. EX2024, ¶¶76-114.

1. Chen's stated goal and benefit of MERFISH was increased multiplexing

Chen discloses that predecessor smFISH and seqFISH methods were "limited by the number of RNA species that can be simultaneously measured in single cells." EX1003, 002 (citing EX2051 and EX2052); *see also* EX1003, 001 ("the number of RNA species that can be simultaneously imaged in individual cells [using smFISH] has been limited."); EX2024, ¶80. Chen purported to have solved this problem by developing a "*highly multiplexed* smFISH imaging method" named "*multiplexed* error-robust FISH (MERFISH)." EX1003, 002. Chen reported

⁴ Petitioner does not rely on Shen for any disclosure of determining the 3D positional information of nucleic acid molecules (Pet., 36-39, 42), and for good reason—Shen has no such teaching. EX2024, ¶91.

that their multiplexed methods “*substantially increase[d] the number of RNA species* that can be simultaneously imaged in single cells.” *Id.* Chen also explained that smFISH and seqFISH were impractical for multiplexing due to “the high cost of the massive number of distinct FISH probes needed and the long time required to complete many rounds of hybridization,” which “made it *difficult to probe a large number of RNA species*” using those methods.⁵ EX1003, 002. Chen also disclosed that the investigators “sought to *further increase the throughput* of [their] MERFISH measurement by simultaneously imaging ~ 1000 RNA species.” EX1003, 007. As potential next steps, the authors suggested modifications that “further increase the number of detectable RNA species.” EX1003, 008. Thus, it would have been clear to a POSA that Chen’s objective was 100% focused on 2D *multiplexing*. EX2024, ¶¶80-83. Indeed, Petitioner acknowledges “[Chen]’s objective of ‘rapidly increas[ing] the number of RNA species that can be probed simultaneously.’” Pet., 30 (quoting EX1003, 002).

The same research group confirmed Chen’s goal of multiplexing in subsequent publications. For example, Moffitt reported in 2016 that “MERFISH

⁵ In Chen’s own patent applications directed to MERFISH, the applicant similarly referred to smFISH and seqFISH as “inadequate” and “not a practical approach to multiplexing.” *See, e.g.*, EX2054, 16; EX2055, 16-17.

achieves *large-scale multiplexing*,” and “MERFISH *significantly increases the multiplexing* of smFISH measurements.” EX1021, 002-003; EX2024, ¶82. The same group also later reported “an improved MERFISH method that *drastically increases the throughput* of this technique,” and disclosed “*increas[ing] the throughput* of [MERFISH] by two orders of magnitude.” EX2053, 11047, 11050; EX2024, ¶¶49, 80-83, 104. Thus, a POSA interested in performing MERFISH and reading Chen would have been interested in harnessing MERFISH’s multiplexing capabilities. EX2024, ¶¶80-83.

2. Chen never suggested revamping its 2D MERFISH imaging setup for determining 3D positional information of RNA molecules

Chen disclosed a homebuilt imaging setup designed for imaging RNA molecules *in 2D* with increased multiplexing. EX2024, ¶¶20, 52, 70, 93. This is evident for several reasons. *First*, Chen disclosed imaging the RNA molecules using traditional “wide-field imaging,” which was well known in the art for being *inadequate* to measure z-axis (3D) position coordinates, without the benefit of the 554 patent’s teachings and claimed system. EX2024, ¶¶43, 52, 78-79, 84; EX1012, 004 (“wide-field images can provide clear lateral (x- and y-axis) information but *only limited axial (z-axis) information*.”); EX1011, 002 (disclosing that wide-field imaging data are in 2D due to “the *lack of axial resolution* typical of wide-field imaging.”); EX2079, 446 (characterizing “wide-field epifluorescence” as having

“*poor axial resolution.*”); EX2043, @@.

Second, Chen disclosed a deliberate intention to image the RNA molecules in 2D. EX2024, ¶¶84-90. Chen explained using “[h]uman primary fibroblasts” plated in a layer for MERFISH imaging experiments because these cells are “relatively large and flat, facilitating *wide-field imaging without the need for optical sectioning.*” EX1003, 011-012; EX2024, ¶¶52, 85-89. As Dr. Shroff explains, optical sectioning is a technique to remove out-of-focus light from planes above and below the focal plane, thus providing greater contrast in the image. EX2024, ¶¶36, 89; EX1012, 004 (disclosing that “optical sectioning” “removes out-of-focus blur and permits you to distinguish between the signals in thin ‘optical’ slices”). Thus, a POSA would have understood that given the thin nature of the fibroblast cell layer, Chen imaged the mRNAs in a single focal plane without removing background fluorescence from planes above and below the focal plane (i.e., without optical sectioning) to determine the 2D positional information of the mRNAs in the cells. EX2024, ¶¶36, 52, 85, 89. This is consistent with the understanding in the art that such traditional wide-field imaging has poor axial resolution. EX2024, ¶¶43, 52, 78-79, 84; EX1012, 004; EX1011, 002; EX2079.

Additionally, Chen disclosed that its imaging setup was “configured for oblique incidence excitation.” EX1003, 011. As Dr. Shroff explains, oblique incidence excitation on a thin cell sample such as Chen’s flat cells would simply

reduce potential unwanted fluorescence from planes above and below the focal plane to improve image contrast. EX2024, ¶¶52, 85. Thus, a POSA reading Chen would have known that Chen simply had no interest in imaging other focal planes, which would have been necessary for 3D imaging. Thus, Chen disclosed using a custom-built imaging setup, made of a “home-built microscope,” a “home-built, autofocus system,” and a “home-built fluidics system,” that was designed to support increased multiplexing of RNA molecules in 2D. EX2024, ¶¶20, 52, 80-83.

Third, all of Chen’s reported data and analyses depict multiplexed, single-molecule mRNA imaging in 2D. EX1003, 004-005, 011-012, FIGs. 2, 4, 5; EX2024, ¶¶86-87. While Petitioner argues that Chen’s “goal” was “identify[ing] distinct spatial distribution patterns of RNAs” (Pet., 39), it ignores the fact that the spatial distribution analyzed and reported in Chen is *2D spatial distribution*. For example, when Chen discusses “mapping spatial distributions of RNAs,” it depicts *2D images* of cells (e.g., Figures 4-5) and *2D spatial location* of the RNA molecules. EX1003, 005 (“RNA transcripts enriched *in the perinuclear region*, some enriched *in the cell periphery*, and some scattered *throughout the cell*.”); EX2024, ¶86. Chen further discloses measuring the RNA molecules in a “*40 by 40 μm imaging area*” (i.e., x-y dimensions). EX1003, 012, 011. And Chen discloses identifying and localizing the fluorescent spots in each image “using a multi-

Gaussian-fitting algorithm assuming a Gaussian with a uniform *width of 167 nm*” (i.e., an x-y localization). EX1003, 012; EX2024, ¶87.

Petitioner alleges that Chen suggests using MERFISH to determine 3D positional information of the RNA molecules because Chen discloses “optical sectioning” by “collecting stacks of images at different focal depths through the entire depth of the cells.” Pet., 38; EX1003, 012. This is simply not correct. A POSA would not have understood this disclosure in Chen to suggest going from 2D MERFISH to 3D MERFISH. EX2024, ¶88. As Dr. Shroff explains, this disclosure in Chen relates to a quality-control experiment that was designed to apply “conventional smFISH” to “*quantify* the fraction of individual RNA species that was outside the axial range of [Chen’s] imaging geometry”—*not* to determine the 3D positional information of those RNA molecules. EX1003, 012; EX2024, ¶88. In other words, in this smFISH experiment, Chen disclosed imaging RNA molecules in other planes above and below the focal plane *and counting those RNA molecules* to determine what fraction of the total RNAs might be missed *in the imaged- and analyzed-2D focal plane*. *Id.* Chen concluded from this smFISH experiment that “only a small fraction” of the RNA molecules “were outside [the] imaging range of a fixed focal plane without z-scanning.” EX1003, 012; EX2024, ¶88. Even Dr. Czymmek admits that this statement in Chen is “in connection with its smFISH validation experiments.” EX1002, ¶¶128, 183. Thus,

Chen confirmed that its homebuilt imaging arrangement was capturing the relevant RNAs in the cell, validating Chen's 2D MERFISH approach. EX2024, ¶88.

Chen stated that, “[f]rom an imaging perspective, any optical sectioning technique could be used in MERFISH to allow the imaging of RNAs in thicker cells or tissues.” EX1003, 012. Here, Chen merely suggested optical sectioning to remove the background fluorescence arising from other planes above and below the 2D focal plane when imaging thicker cells or tissues compared to the flat fibroblast cell layer used in Chen's MERFISH method. EX2024, ¶89. A POSA would have known that wide-field imaging, even with oblique incidence excitation, illuminates planes above and below the focal planes. EX2024, ¶89; EX2046, 920; EX1012, 004. In fact, Chen itself disclosed that its “excitation geometry illuminated the full depth of [the] cells.” EX1003, 012; EX2024, ¶89. Because thicker cells or tissues than the flat fibroblast cell layer of Chen would encounter illumination of several additional planes, the fluorescent signals generated when imaging thicker cells or tissues would have higher out-of-focus fluorescent signals in the 2D focal plane, increasing the background and noise, and thus decreasing the SNR and SBR. EX2024, ¶89; EX2046, 920; EX1012, 004. To address this, Chen recommended adding optical sectioning to *remove* fluorescence from the planes above and below the 2D focal plane when imaging thicker cells or tissues. EX2024, ¶89; EX1003, 012. Accordingly, Chen never suggested

modifying its 2D MERFISH methods to somehow arrive at 3D MERFISH. *See supra*, Section V.A.

3. A POSA would not have had any reason to look to Cai

No doubt recognizing that neither Chen nor Shen taught or suggested a volumetric imaging system comprising a computing system for determining 3D positional information of nucleic acid molecules, Petitioner's presents a fallback position that, "to the extent that [Chen] is deemed to not disclose the claimed 3D feature, it would have been obvious to modify [Chen] in view of Cai to include this feature." Pet., 39. And yet, as discussed below, a POSA reading Chen in view of the state of the art would not have had any reason to look to Cai in the first place. EX2024, ¶¶92-107. Petitioner's basis for bringing Cai into its obviousness theory is rooted in hindsight bias. *Kingston Tech.*, IPR2018-01784, Paper 32, 55; *Intel Corp.*, IPR2018-01033, Paper 32, 88-89.

Moreover, even if a POSA reading Chen were to consult the relied-upon disclosures in Cai, nowhere does the Petition explain with any particularity what modifications the POSA would have allegedly made to convert Chen's 2D MERFISH imaging setup into 3D MERFISH imaging. EX2024, ¶¶108-109. And as Dr. Shroff explains, even if a POSA were motivated to modify Chen's 2D MERFISH imaging setup (and they would not be, as discussed below in Section V.C.3.c)), the artisan would not have had a reasonable expectation of successfully

performing MERFISH imaging in 3D (according to Petitioner's obviousness theory) while maintaining Chen's stated goal of *multiplexing*. . EX2024, ¶¶92, 110-114.

a. Cai's imaging system has a different design and function from Chen's homebuilt setup

Petitioner relies on a disclosure from Cai related to a purported Z-axis motion control system in a light sheet microscope depicted in Figure 17. Pet., 32-33 (citing EX1004, FIG. 17, 47:43-45). Cai's disclosures of a light sheet microscope are no different than a POSA's general knowledge in the art that light sheet microscopes existed. EX2024, ¶¶93-98.

Petitioner's reliance on Cai's light sheet microscope system with Z-axis motion control fails to consider the viewpoint of a POSA. A POSA would have understood that Cai's light sheet microscope is completely different from Chen's 2D MERFISH imaging setup and was not designed for MERFISH imaging. For example, a POSA would have known that the "perpendicular excitation/detection geometry required by LSFM [light sheet fluorescence microscopy] *forces the use of long working distance*, somewhat *low NA* (typically 1.0 or less) objective lenses." EX1019, 007; EX2024, ¶94. Cai confirmed this geometry, disclosing that its light sheet microscope system provided "*large FOV* [field of view] imaging ... with 20× *low NA objectives*." EX1004, 44:34-35; *see also id.*, 47:52-53 ("20× water immersion objective"), 42:17-19 ("20× air objective"), 43:29-35 ("large

FOV imaging with 20× air objectives”), FIG. 17 (“objective 20×”); EX2024, ¶94.

To implement this imaging setup with such a large working distance and low NA objective in a FISH method, Cai further disclosed using a “hybridization chain reaction” (HCR) “to *amplify* [the] FISH signal.” EX1004, 43:29-35; EX2024, ¶95.

A POSA would have immediately recognized that Cai’s imaging setup is in stark contrast with Chen’s 2D MERFISH imaging setup designed for imaging thousands of single-molecule RNAs in a single cell in 2D. EX2024, ¶96. Indeed, Chen disclosed that its imaging system utilized a “*1.45 NA, 100× oil immersion objective*”—i.e., imaging a *small FOV* with a *high NA*, the opposite of Cai’s system. EX1003, 011; EX2024, ¶96; *see also* EX1021, 25 (“[f]or published MERFISH measurements, we used a *1.45 NA, 100× oil immersion* Olympus objective.”); *see also* EX1012 (a “high NA also comes at the expense of reduced working distance”); EX1019, 002 (“longer working distances for objective lenses ... comes at the cost of lowering the numerical aperture”). The two imaging setups are of a completely different design and function. EX2024, ¶¶96, 98. Chen’s imaging arrangement was designed for massive multiplexing over 1000 RNA species simultaneously. EX1003, 001-002; EX2024, ¶¶48, 80. Cai’s arrangement was designed for imaging single molecules (β -actin mRNA) after amplifying the RNA molecules. EX1004, 47:30-56; EX2024, ¶96.

In addition to incompatible FOV and NA, the POSA would also have

recognized that Cai’s light sheet microscope was designed to match the refractive index of the CLARITY cleared brain tissue samples. EX2024, ¶¶97. A POSA would have known that it is important to match the refractive index of the objective lens with the refractive index of the sample and sample medium to avoid aberration and maintain high resolution. *See, e.g.,* EX2059, 510 (discussing the importance of developing “*objectives ... that are matched to the refractive index of clarified tissue* to minimize aberration and maintain both high resolution and long working distance.”); EX1019, 002 (“*Refractive index mismatches ... further add to spherical aberration, inducing errors in Z localization.*”); EX2024, ¶¶27-28, 97; EX2043, 53:16-54:13 (agreeing matching the refractive indices is important “[f]or the best performance” and mismatching causes “spherical aberration”). Thus, the POSA would have viewed Cai’s light sheet imaging setup (comprising an objective lens with a refractive index matched to that of CLARITY cleared brain tissue) as unsuitable for practicing Chen’s MERFISH methods of simultaneously imaging thousands of single-molecule RNAs in a single cell, using a different refractive index. EX2024, ¶¶97-98. Petitioner and its expert fail to address this issue entirely.

b. Petitioner fails to articulate any credible reasons to modify Chen’s setup in view of Cai

Petitioner’s obviousness theory relies on hindsight and three purported “teachings” in Cai that the POSA would have allegedly combined with Chen’s homebuilt MERFISH setup: (i) Cai’s disclosure of Z-axis motion control system;

(ii) Cai’s disclosure of using two or more fluorescent colors; and (iii) Cai’s single sentence referring to “three-dimensional spatial information.” Pet., 29-30, 32-34, 39. For obviousness, Petitioner must “explain *why* a person of ordinary skill in the art would have combined elements from specific references *in the way the claimed invention does*.” *ActiveVideo Networks, Inc. v. Verizon Communications, Inc.*, 694 F.3d 1312, 1328 (Fed. Cir. 2012) (emphasis in original). None of the aforementioned disclosures in Cai would have motivated a POSA to modify Chen’s imaging setup, and Petitioner fails to articulate any credible basis showing otherwise. EX2024, ¶¶99-107.

First, Chen’s system already purportedly had a “home-built autofocusing system,” and Petitioner provides no explanation why the POSA would have allegedly modified it with a *different* (and redundant) autofocus system. EX1003, 011; Pet., 31; EX2024, ¶100. The POSA reading Chen and interested in practicing MERFISH with Chen’s imaging setup had no reason to look to Cai (or Shen) for a Z-axis motion control system. EX2024, ¶100.

Nor would Cai’s purported disclosure of using “two or more light sources configured to emit two or more colors of light” have provided any motivation to a POSA reading Chen. Pet., 29. Petitioner first argues that Chen itself discloses the “two or more light sources” element of claim 36(c)(ii), relying solely on Chen’s disclosure of solid state lasers for excitation of the “Cy5-labeled readout probes,

the fiducial beads, and nuclear counterstains.” Pet., 29; EX1003, 011. This is not a disclosure of “two or more light sources,” as claimed, because claim 36(c) requires that the two or more light sources are configured to emit two or more colors of light, and the two or more colors (detected as light signals in claim 36(e)) are “associated with one or more nucleic acid molecules.” EX1001, 33:45-67; EX2024, ¶101. Only *one* of Chen’s “colors of light” (Cy5) is associated with the imaged RNA molecules. EX2024, ¶101; EX1003, 011. The fluorescence emitted from the fiduciary bead and nuclear counterstain do not meet the element of claim 36.

Petitioner’s fallback position for this claim element relies on Cai. According to Petitioner, “[a] POSA would have been motivated to use two or more colors of light as taught by Cai with the method of [Chen]” to increase MERFISH’s multiplexing capabilities. Pet., 30. But Chen already suggested using “two-color imaging in each round” to “increase the throughput of [their] MERFISH measurement.” EX1003, 007; EX2024, ¶102. In fact, that is precisely what Chen’s authors did one year later when they reported improvements in their system to increase the throughput of 2D MERFISH. EX2053, 11048 (“to reduce measurement time, we used multicolor imaging.”); EX2024, ¶¶102. Thus, the POSA reading Chen and allegedly *interested in using two or more colors in MERFISH* would not have had any reason to consult Cai’s purported disclosures of

two or more colors of light. After all, Chen disparages the smFISH and seqFISH methods taught in Cai for their “limited” multiplexing capabilities. EX1003, 001-002; EX2024, ¶102; *see also* EX2054, 16 (; EX2055, 16-17. A POSA reading Chen and interested in MERFISH simply had no reason to consult Cai.

Finally, Petitioner also relies on a bare, single statement in Cai that, “in some embodiments, an imaging step provides three-dimensional spatial information of a target.” Pet., 39 (citing EX1004, 21:50-51). Cai provides no further elaboration on this statement, as its next sentence refers to a different embodiment that “quantifies a target.” EX1004, 21:51-52; EX2024, ¶103. Nor does Petitioner cite any additional disclosure from Cai in this regard. Pet., 32-34, 39. Thus, Cai’s bare statement is no different than a POSA’s general knowledge in the art that various methods of 3D imaging existed. EX2024, ¶103. The same can be said for Cai’s generic disclosure of “super-resolution microscopy” techniques such as “PALM/STORM, SSIM/GSD/STED.” EX1004, 21:16-22; DOI, 43-44; EX2024, ¶103. The question before the Board is not whether 3D imaging of nucleic acid molecules would have been obvious, but whether the 554 patent’s *complete system* for volumetric imaging comprising a computing system for determining the 3D positional information of nucleic acid molecules was obvious in view of Chen's MERFISH method, Shen, and Cai.

c. A POSA would have had no reason to revamp Chen's 2D MERFISH imaging setup to 3D MERFISH

Even if putting aside Petitioner's failure to demonstrate any credible reasons to modify Chen's imaging arrangement, the evidence shows that a POSA would *not* have had any reason to revamp Chen's massively multiplexed 2D imaging setup into a volumetric 3D imaging system while still satisfying MERFISH's goal of *multiplexing*. EX2024, ¶¶104-107. Chen is clear not only that its goal was multiplexing, but that its homebuilt MERFISH imaging arrangement was designed for multiplexing in 2D. EX2024, ¶¶20, 52, 70.

Chen warns that, “[a]s the degree of multiplexing is increased, it is important to consider the potential *increase in the density of RNAs that need to be resolved* in each round of imaging.” EX1003, 008; EX2024, ¶¶38, 105, 111. A POSA would have known that as signal density increases in wide-field microscopy as taught in Chen, the resolution, SBR, and SNR will decrease, while the complexity of data analysis increases. EX2024, ¶¶38, 105; EX2050, 1361 (disclosing that signal density needs to be “sufficiently low for discerning individual molecules.”); EX1004, 2:1-3 (referring to an “*increase [in] the complexity of data analysis*” when there is a “*high density of objects in the image*.”); EX2051, 3 (“[w]hen the density of transcripts is high, diffraction-limited fluorescent spots of the same color will overlap and make barcode readout impossible.”); EX2043, 51:19-52:4 (“resolving individual entities, if they’re closely spaced, needs to be considered”),

55:10-24. The POSA would have further understood that this complexity becomes even worse for axial (z-axis) resolution if attempting to image multiple planes through the sample for 3D MERFISH imaging, because the thousands of RNA molecules imaged in one 2D plane would now be multiplied to numerous planes through the depth of the sample. This is complicated even further by poor axial resolution due to the “Z-stretch” elongation of the PSF in the axial dimension. EX2024, ¶¶24, 31, 111; EX1019, FIG. 1; EX2043, 44:8-14 (“[PSF] looks like an hourglass or football shape in the z-axis”). This would not only make imaging and data analysis extremely computationally complex and burdensome, it would defeat the purpose of Chen’s MERFISH imaging approach—multiplexing—because of the loss in resolution between single RNA molecules. Additionally, as Dr. Shroff explains, a POSA would have known that Chen’s use of wide-field imaging would lower SNR and SBR by extinguishing fluorescent signals (photobleaching) outside of the focal plane in thick samples, causing a decrease in signal over time for attempted 3D MERFISH. EX2024, ¶105. Adding the purported “more colors of light” and a “Z-axis motion control system” from Cai simply would *not* have motivated a POSA to modify Chen’s 2D MERFISH imaging arrangement because doing so would have “destroy[ed] the basic objective” of Chen’s ability to massively multiplex and analyze the data. *Trivascular, Inc. v. Samuels*, 812 F.3d 1056, 1068 (Fed. Cir. 2016).

Thus, Cai’s bare statement—in combination with Chen and Shen—would not have suggested to a POSA the 554 patent’s *claimed system*. EX2024, ¶103. Nor would Cai’s statement have provided a POSA with the motivation or rationale to modify *Chen’s 2D MERFISH setup* to *3D MERFISH* to arrive at the *claimed 3D system* of the 554 patent. EX2024, ¶103; *see infra*, Section V.C.4.

Even if this generic statement in Cai were interpreted as a suggestion to perform 3D imaging, the POSA would have recognized it at most as a suggestion to use Cai’s imaging setup for practicing Cai’s smFISH HCR amplification methods. EX2024, ¶103; EX1004, 43:29-35. Petitioner’s obviousness theory, however, requires a POSA to modify *Chen’s homebuilt 2D MERFISH setup* to *3D MERFISH*. *See supra*, Section V.A. And as discussed below, Petitioner does not explain with any particularity what those technical modifications would have been or how they would have been made. Pet., 32-34, 39, 42. *Cook Group*, IPR2017-00435, Paper 94, 31 (“Petitioner has not shown how a [POSA] would have integrated such embodiments, or found them compatible.”).

To the extent Petitioner attempts to rely on other so-called “3D FISH” references (not listed in Ground 1) as providing a POSA’s motivation to modify Chen’s 2D MERFISH, those references fail for similar reasons in that they either do not suggest 3D imaging at all, are not prior art, or do not account for the impact of PSF, SNR, SBR, and signal density on axial resolution one would encounter if

attempting to determine 3D positional information of *highly multiplexed* nucleic acid molecules (i.e., MERFISH). EX2024, ¶¶106, 114. For example, Larsson disclosed collecting images as z-stacks specifically to create “a maximum intensity projection” (i.e., 2D images). EX1008, 005; EX2024, ¶¶106, 113. Femino disclosed basic 3D FISH but only in the context of a single RNA species (not MERFISH). EX1010. Perillo likewise only disclosed imaging a single RNA species (“RunX2”) in FISH images (not MERFISH). EX1011, FIG. 4; EX2024, ¶¶106, 113. Perillo mentioned “multiplexing” in a different context, in relation to multiple colors, and only one of those colors is associated with the FISH signal. EX1011, 004 (“DAPI for nucleus, *Cy3 for the FISH signal*, and Cy5 for a membrane stain.”); EX2024, ¶¶106, 113. Brileya disclosed performing 3D FISH on *whole biofilms*, not individual nucleic acid molecules and not MERFISH. EX1039, 003, FIG. 5; EX2024, ¶¶106, 113. Tirichine performed 3D FISH on *whole plant nuclei*—not individual nucleic acid molecules and not MERFISH. EX1049, FIG. 1-2; EX2024, ¶¶106, 113. Zessin disclosed 3D super resolution microscopy of histone H2B and *chromosomal DNA* – neither FISH nor MERFISH. EX1052, 002, FIG. 4; EX2024, ¶¶106, 113. And Shah is a 2018 publication and is not prior art to the 554 patent. EX1018, 001.

Thus, a POSA would not have had a reason to modify Chen’s imaging setup for 2D MERFISH to arrive at 3D MERFISH in view of Cai and the general

knowledge in the art.

d. Petitioner fails to explain with any particularity the modifications a POSA would have made to Chen’s system to allegedly arrive at the claimed system

Even if a POSA were motivated to modify Chen’s MERFISH imaging setup in view of Cai and Shen, the Petition fails to articulate what those modifications would have been or how a POSA would have made them. EX2024, ¶¶108-109. In its Decision on Institution, the Board cited *MCM Portfolio LLC v. Hewlett Packard Co.*, 812 F.3d 1284 (Fed. Cir. 2015), for the notion that “obviousness does not require features of a secondary reference to be bodily incorporated into the structure of the primary reference.” DOI, 44. While “bodily incorporation” may not be required for obviousness, the test still requires “some *articulated reasoning* with some *rational underpinning* to support the legal conclusion of obviousness.” *KSR*, 550 U.S. at 418. Indeed, the Petition must show “*how* a [POSA] would have integrated such embodiments, or found them compatible.” *Cook Group*, IPR2017-00435, Paper 94, 31.

Petitioner has not met its burden. Rather, all Petitioner has done here is cherry-pick disclosures from the art and then, in conclusory fashion, argue that the claimed system would have been obvious. But that is not obviousness; that is hindsight reconstruction. *See, e.g., TQ Delta, LLC v. CISCO Systems, Inc.*, 942 F.3d 1352, 1361 (Fed. Cir. 2019) (expressing concerns of “allowing the challenger

to use the challenged patent as a roadmap to reconstruct the claimed invention using disparate elements from the prior art—i.e., the impermissible *ex post* reasoning and hindsight bias that *KSR* warned against.” (citations omitted)); *InTouch Technologies, Inc. v. VGO Communications, Inc.*, 751 F.3d 1327, 1351-1352 (Fed. Cir. 2014) (rejecting expert testimony that relied on the challenged patent “as [a] roadmap for putting ... pieces of a jigsaw puzzle together.” (internal quotations omitted). *Kingston Tech.*, IPR2018-01784, Paper 32, 55 (finding that petitioner’s obviousness arguments “rest on hindsight” and “used the challenged patent as a roadmap to reconstruct the claimed invention.”).

Petitioner never explains *in the Petition* what modifications the POSA would have made to Chen’s 2D MERFISH arrangement to convert it to a volumetric imaging system for 3D MERFISH beyond (i) adding more fluorescence colors for *increased multiplexing* and (ii) adding a Z-axis motion control system. Pet., 32-34, 39, 42. As discussed above, these proposed modifications are already disclosed in Chen, and thus the POSA would not have had any reason to combine Chen with Cai (or Shen, for that matter). Even if, *arguendo*, a POSA were to make those modifications to Chen’s system, the POSA would have still performed MERFISH imaging in 2D because that is what Chen taught. EX2024, ¶¶108-109. Adding more colors and a Z-axis motion control system to Chen’s system would not have changed Chen’s stated objective of multiplexing, nor would it have changed

Chen's method of massive multiplex imaging of single-molecule RNAs in 2D.

Accordingly, Petitioner's grounds do not make a case for obviousness, they make a case for hindsight. "A factfinder should be aware, of course, of the *distortion caused by hindsight bias* and must be cautious of *arguments reliant upon ex post reasoning*." *KSR*, 550 U.S. at 421; *TQ Delta*, 942 F.3d at 1361; *InTouch*, 751 F.3d at 1351-1352.

Moreover, as discussed above, a POSA would *not* have sought to refit Chen's homebuilt 2D MERFISH imaging setup into a volumetric imaging system for 3D MERFISH. Doing so would "destroy the basic objective" of Chen's MERFISH system because it would *reduce the ability to multiplex* due to decreases in resolution, SBR, and SNR and increases in data complexity and requisite computing power. EX2024, ¶¶105, 109. *See Trivascular*, 812 F.3d at 1068 (rejecting Trivascular's argument that the prior art references need not be "bodily incorporated" because "TriVascular's proposed substitution would destroy the basic objective of the barbs, which is to penetrate surrounding tissue.").

D. A POSA would have had no reasonable expectation of successfully modifying Chen's setup to arrive at the claimed system

Petitioner argues that a POSA would have had a reasonable expectation of successfully "using MERFISH in 3D" because "(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." Pet., 39.

As discussed above, Petitioner's arguments do not account for factors such as PSF, SBR, SNR, signal density, and their impact on axial resolution, or the exacerbated difficulties of axial resolution if attempting to image massively multiplexed nucleic acid molecules. EX2024, ¶¶105-106. Petitioner's argument for reasonable expectation of success is no different. EX2024, ¶¶110-114.

None of Petitioner's so-called "3D FISH" references would have provided a POSA with a reasonable expectation of successfully modifying Chen's imaging setup for determining 3D positional information of nucleic acid molecules using *MERFISH* because Petitioner's references either do not suggest 3D imaging at all (EX1008), are not prior art (EX1020), or fail to account for the impact of PSF, SNR, SBR, and signal density on axial resolution one would encounter if attempting to determine 3D positional information of *highly multiplexed* nucleic acid molecules (EX1010, EX1011, EX1039, EX1049, EX1052). EX2024, ¶¶106, 113.

Petitioner's attempt to equate MERFISH with "conventional FISH" (Pet., 39) grossly oversimplifies the two methods and ignores the massive multiplexing aspect of MERFISH. As Chen explained, MERFISH "*substantially increases* the number of RNA species that can be simultaneously imaged in single cells," and reported simultaneously imaging over 1,000 RNA species. EX1003, 002, 007; EX2024, ¶¶47-48, 80. Petitioner's obviousness theory is founded on a POSA

interested in performing MERFISH using Chen’s setup, and thus the artisan would have sought to achieve Chen’s same objective: massive multiplexing. EX2024, ¶¶80-83. The POSA therefore would not have had a reasonable expectation of successfully modifying Chen’s imaging setup to attempt MERFISH in 3D for the same reasons the artisan would not have been motivated to modify it in the first place: doing so would destroy the basic objective of Chen’s ability to massively multiplex RNAs. EX2024, ¶¶111-112.

E. Petitioner failed to meet its burden on dependent claims 37-70.

This defect in the Petition applies not only to Petitioner’s failure to meet its burden on claim 36 but also to dependent claims 37-70. EX2024, ¶115.

F. Objective indicia further confirm the nonobviousness of the claimed system

An obviousness analysis requires assessing all four *Graham* factors, including objective indicia. *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1075 (Fed. Cir. 2012). Petitioner ignored objective indicia in its obviousness analysis—further exposing Petitioner’s hindsight approach to obviousness. *Id.* at 1079 (“[t]he objective considerations, when considered with the balance of the obviousness evidence in the record, guard as a check against hindsight bias.”); *Graham v. John Deere*, 383 U.S. 1, 17-18 (1966).

As discussed below, the innovation of the 554 patent’s claimed system is

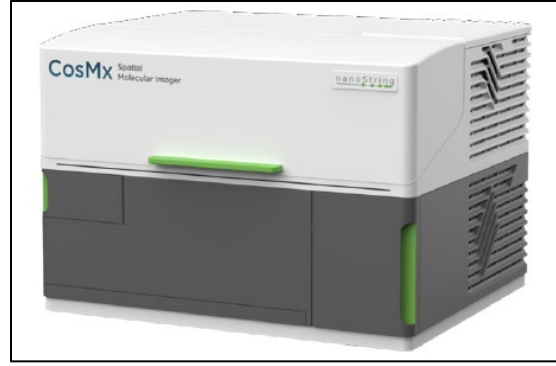
further evident from extensive objective indicia associated with 10x's Xenium and Bruker's CosMx imaging systems, both of which are embodiments of the 554 patent's claimed system.

1. Nexus is presumed because Xenium and CosMx are commercial embodiments of the 554 patent's claimed imaging system

“[T]here is a presumption of nexus for objective considerations when the patentee shows that the asserted objective evidence is tied to a specific product and that product ‘is the invention disclosed and claimed in the patent.’” *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1329-1330 (Fed. Cir. 2016); *Quanergy Systems, Inc. v. Velodyne Lidar USA, Inc.*, 24 F.4th 1406, 1418 (Fed. Cir. 2022). Here, a nexus is presumed because both Xenium and CosMx are commercial embodiments of the 554 patent's claimed system. EX2024, ¶¶53-56, 116-128. Both commercial products are complete, single-unit volumetric imaging systems for determining three-dimensional positional information of nucleic acid molecules, and are thus “coextensive (or nearly coextensive)” with the claimed system of the 554 patent. *Lectrosonics, Inc. v. Zaxcom, Inc.*, IPR2018-01129, Paper 33 at 33 (P.T.A.B. Jan. 24, 2020) (precedential).



10x's Xenium

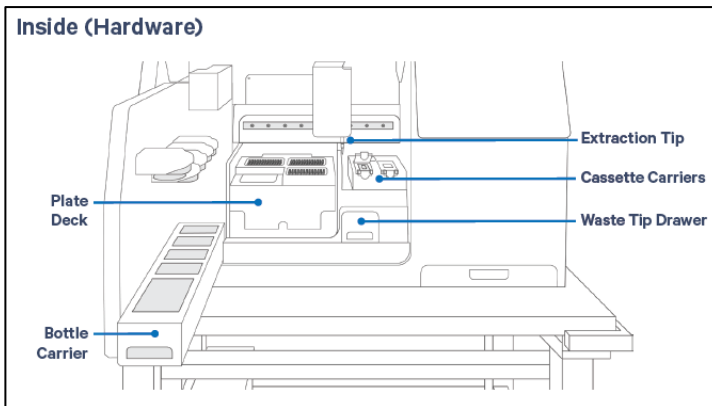


Bruker's CosMx

EX2060, 1; EX2061, 9; EX2024, ¶¶56, 119.

For example, the system of claim 36 comprises a “sample holder configured to receive a substrate comprising a sample having a volume.” EX1001, 33:31-32.

10x's Xenium utilizes a cassette carrier, which is configured to receive a substrate (the cassette) comprising a sample having a volume (tissue sample). This is demonstrated in the figures below from the Xenium User Guide:



EX2060, 17, 29, 70; EX2024, ¶120. Likewise, CosMx utilizes slides, which are configured to receive a substrate comprising a sample having a volume (tissue sample). EX2061, 10; EX2062, 132; EX2024, ¶120.

The system of claim 36 comprises a temperature control apparatus

operatively coupled to the sample holder. EX1001, 33:33-34. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

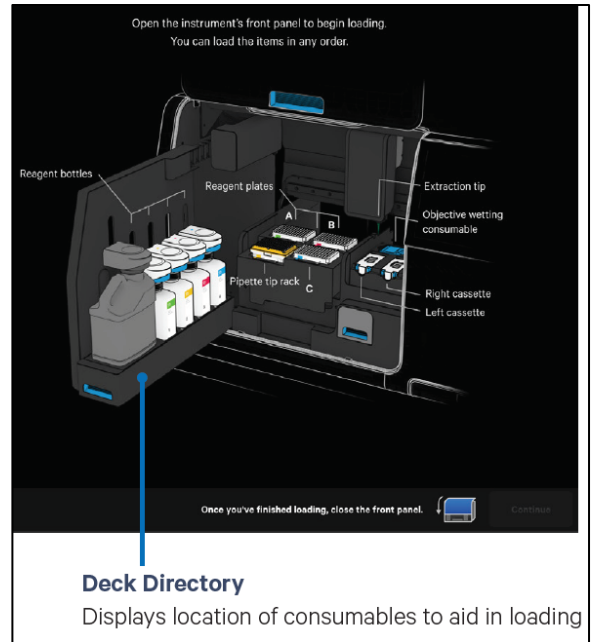
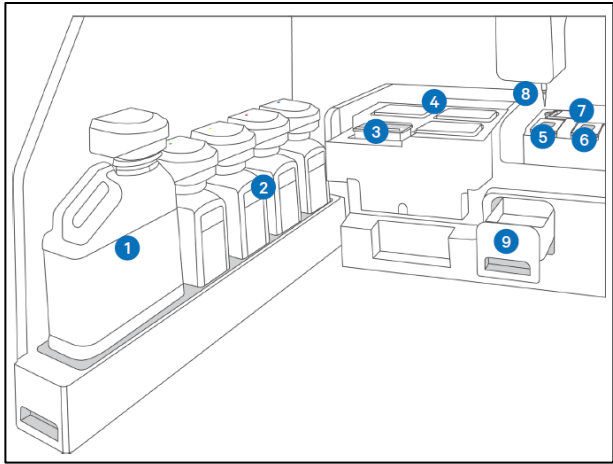
[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

The system of claim 36 also comprises a “fluidics system,” comprising “one or more reservoirs” and a “fluid dispenser.” EX1001, 33:35-42. 10x’s Xenium comprises a fluidics system comprising one or more reservoirs and a fluid dispenser. This is shown below:

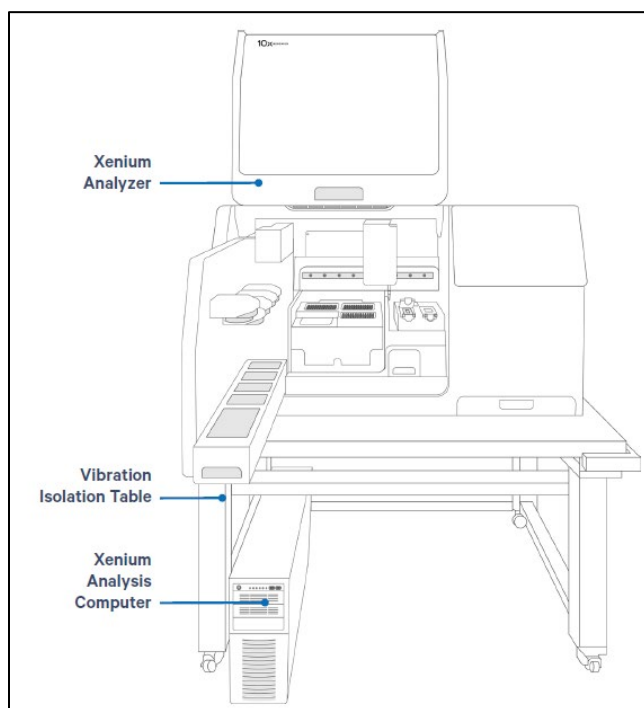


EX2060, 18, 66-67; *see also id.*, 77, 85 (discussing cleaning the instrument's "fluidic system."); EX2024, ¶123. Similarly, CosMx also comprises a fluidics system comprising "bulk reagent bottles" that must be "loaded before starting a run," and are placed in color-coded reservoirs in the instrument. EX2061, 12, 51-54; EX2024, ¶123.

The system of claim 36 also comprises an "optical system," comprising "two or more light sources," "one or more detectors," and a "Z-axis motion control system." EX1001, 33:43-54. 10x's Xenium User Guide discloses that Xenium's optical system "captures vertical stacks of images at every cycle (of fluorescent probe hybridization, imaging, and probe removal) and in every channel for multiple fields of view," and that the process begins with a "sample scan." EX2060, 26, 72; EX2024, ¶¶124-125. Likewise, CosMx includes a range of

excitation and emission spectra that are used during “scanning” of the flowcells for image acquisition. EX2061, 14, 48-49, 55-57; EX2024, ¶124.

The system of claim 36 also comprises a “computing system” for “determining three-dimensional positional information of [] one or more nucleic acid molecules.” EX1001, 33:55-34:17. Xenium comprises a “Xenium Analysis Computer,” depicted in the User Guide figure below:



EX2060, 16; EX2024, ¶126. The User Guide discloses that the software of the Xenium Analysis Computer analyzes multiple fields of view and then “processe[s], correct[s] and stitche[s] to build a single seamless image representing the tissue section.” EX2060, 26; EX2024, ¶127. According to the Guide, “[t]he pipeline detects puncta in every cycle and every image in order to observe all potential

mRNA. These puncta are decoded into gene IDs, and each decoded transcript is assigned a quality score.” *Id.*; EX2024, ¶127. CosMx also comprises a computing system for determining three-dimensional positional information of nucleic acid molecules. *See, e.g.*, EX2061, 56 (“FOVs are selected and saved, the CosMx SMI instrument begins Reporter Cycling and Data Acquisition”).

Accordingly, Xenium and CosMx embody the claims of the 554 patent, and nexus is presumed. *WBIP*, 829 F.3d at 1329-1330; *Quanergy Sys.*, 24 F.4th at 1418; EX2024, ¶¶119-128. To the extent Petitioner argues against a presumption of nexus, finding a nexus here is still appropriate because, as shown below, “the evidence of secondary considerations is the ‘direct result of the unique characteristics of the claimed invention.’” *Fox Factory, Inc. v. SRAM, LLC*, 944 F.3d 1366, 1374 (Fed. Cir. 2019).

2. Xenium and CosMx have achieved substantial commercial success

The commercial success of Xenium and CosMx, including gross revenue, consumer demand, and the rapidity of their adoption in the field, demonstrate that the claimed imaging system is non-obvious. EX2026, ¶¶29-39. For example, as Ms. Julie Davis explains in her expert declaration, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

As Ms. Davis explains, the rapid sales growth of these products further evinces their commercial success. EX2026, ¶36-37; EX2040, 5-8. For example a JP Morgan Healthcare Presentation discloses that more than 250 Xenium instruments were sold in the first year since launching. *Id.* Without the 554 patent's technology, Xenium would not have experienced the same level of success. EX2026, ¶37; EX2024, ¶129.

The fact that the Xenium and CosMx products also practice other patented inventions does not detract from this commercial success and its nexus to the 554 patent. “[M]ultiple patents do not necessarily detract from evidence of commercial success of a product or process, which speaks to the merits of the invention, not to how many patents are owned by a patentee.” *Merck Sharp & Dohme Corp. v. Hospira, Inc.*, 874 F.3d 724, 731 (Fed. Cir. 2017); *Fox Factory*, 944 F.3d at 1377 (“[w]here a product embodies claims from two patents, a presumption of nexus can be appropriate only if the claims of both patents generally cover the same invention.”).

3. The system of the 554 patent satisfied a long-felt, but unmet need

The rapid adoption of the Xenium and CosMx systems once they became

commercially available demonstrates a long-felt need in the art for a system for volumetric imaging. EX2026, ¶¶@@; EX2040, 5, 8. As Dr. Shroff explains, prior to the commercial availability of these products, few, if any labs had a complete system for volumetric imaging. EX2024, ¶129. At best, artisans had to rely on homebuilt arrangements made from piecemeal compilations of components, which were laborious to construct and not achievable outside of specialized labs. EX2024, ¶¶20-21, 129. That changed rapidly once CosMx and Xenium became commercially available in late 2022. EX2024, ¶129. Researchers around the world rapidly purchased these volumetric imaging systems, demonstrating that the field had longed for such a system until the 554 patent's system became commercially available. EX2024, ¶129.

4. Xenium has garnered widespread industry praise

Xenium has also garnered widespread industry praise. EX2024, ¶¶130-131. For example, Dr. Jeremie Oliver Piña of the NIH recently lauded Xenium's ability to provide "greater depth and breadth than prior studies could..." EX2070, 2. Dr. Piña further extolled the system's innovative blend of hardware and software, stating that "[t]he intuitive design of [Xenium's] *bench workflow and post-run analysis software* may be the most valuable aspects of this high-throughput *in situ* technology to *accelerate science*." *Id.* Similarly, Dr. Luciano Martelotto of University of Adelaide exclaimed, Xenium is "a cut above the rest," because it

“streamlines the process from tissue handling to data interpretation, making it faster and easier to get insights into biology.” EX2071, 2. Peroni et al. praised Xenium for its capabilities in accurately determining three-dimensional positional information of nucleic acid molecules, noting that “[Xenium’s] ability to provide *high-resolution spatial data* makes Xenium *an invaluable tool* for advancing precision medicine.” EX2072, 13. Dr. Simon Gregory of Duke University similarly praised Xenium for “providing [his team] with unprecedented insight into the molecular pathology of disease at an *incredible resolution*.” EX2071, 1.

These objective indicia weigh heavily in favor of non-obviousness, and further underscore the fact that Petitioner’s obviousness theory is founded on hindsight bias.

VI. CONCLUSION

Petitioner failed to meet its burden of persuasion in this proceeding. The Board should reject the Petition and find all of the challenged claims not unpatentable.

Respectfully submitted,

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VII. APPENDIX A: LISTING OF CHALLENGED CLAIMS

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.

CERTIFICATE OF WORD COUNT (37 C.F.R. § 42.24(d))

1. This Patent Owner Response complies with the type-volume limitation of 14,000 words, comprising 13,712 words, excluding the parts exempted by 37 C.F.R. § 42.24(a)(1).
2. This Patent Owner Response complies with the general format requirements of 37 C.F.R. § 42.6(a) and has been prepared using Microsoft® Word 2016 in 14-point Times New Roman font.

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CERTIFICATE OF SERVICE (37 C.F.R. § 42.6(e))

I certify that the above-captioned **PATENT OWNER RESPONSE**
UNDER 37 C.F.R. § 42.120 and Exhibits 2024-2055, 2057-2062, 2064-2065, and
2070-2081 were served in their entireties on December 10, 2024, upon the
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