

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'S SUR-REPLY

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PATENT OWNER'S UPDATED EXHIBIT LIST

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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Withdrawn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)
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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
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2024	Declaration of Hari Shroff, Ph.D., in Support of Patent Owner’s Response
2025	<i>Curriculum Vitae</i> of Hari Shroff, Ph.D.
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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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2032	NanoString Technologies, Inc. Form 10-K for the fiscal year ended December 31, 2022
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2036	“Xenium In Situ,” 10x Genomics, Inc. (accessible at https://www.10xgenomics.com/platforms/xenium , last accessed December 9, 2024)
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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
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2053	Moffitt, J., et al., “High-throughput single-cell gene-expression profiling with multiplexed error-robust fluorescence in situ hybridization,” <i>PNAS</i> , 113(39):11046-11051 (September 27, 2016)
2054	Response to Office Action, U.S. Patent Application No. 17/374,000, filed January 26, 2024
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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.
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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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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)
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2082	Transcript of the Second Deposition of Kirk Czymmek, Ph.D
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<i>In re Van Os</i> , 844 F.3d 1359 (Fed. Cir. 2017)	8
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I. INTRODUCTION

The POR demonstrated that Petitioner cannot meet its burden of persuasion because Petitioner’s art combination not only fails to teach or suggest all of the elements of the challenged claims, but Petitioner’s entire obviousness theory is rooted in hindsight. POR, 29-66. There is no dispute that Petitioner’s obviousness theory requires a POSA to modify Chen’s 2D MERFISH imaging setup for use in 3D MERFISH, which Petitioner alleges meets all the elements of claim 36. *See e.g.*, POR, 30-32; EX2024, ¶¶67-68. Petitioner’s Reply confirms this theory. *See e.g.*, Reply, 10 (“as detailed in the Petition, a POSA would have wanted to *adapt Chen’s MERFISH method for 3D...*”¹), 19 (referring to “issues [with] *adapting MERFISH to 3D...*”); EX1086, ¶¶10 (discussing obviousness of “*adapt[ing] MERFISH to 3D volumetric imaging...*”), 13 (discussing a POSA “seeking to *adapt MERFISH to 3D...*”), 14 (similar).

“[T]he expedited nature of IPRs bring with it an obligation for petitioners to *make their case in their petition* to institute.” *Intelligent Bio-Sys. v. Illumina Cambridge*, 821 F.3d 1359, 1369 (Fed. Cir. 2016); *see also, Axonics, Inc. v. Medtronic, Inc.*, 75 F.4th 1374, 1380 (Fed. Cir. 2023). The Supreme Court explained in *SAS Institute, Inc. v. Iancu*, “the *petitioner’s petition* ... is supposed to

¹ Emphasis has been added throughout, unless otherwise indicated.

guide the life of the litigation.” 584 U.S. 357, 366 (2018); *see also, id.*, 367.

Petitioner is therefore bound by the Petition’s 3D MERFISH theory involving *highly* multiplexed imaging. Any argument that a POSA would be motivated to perform *less* multiplexing than 3D MERFISH or to use a more advanced and complex imaging setup compared to the Petition’s proposed modified Chen setup, is new argument that should be rejected. Petitioner is foreclosed from “rel[ying] on an entirely new rationale to explain why one of skill in the art would have been motivated to combine [the art]...” *Intelligent Bio-Sys.*, 821 F.3d at 1370.

In its Reply, Petitioner improperly attempts to portray *10x* as somehow unable to meet a burden of *proving nonobviousness*, e.g., alleging that *10x*’s expert Dr. Shroff did not “*persuasively state* what is inventive about the claims.” Reply, 1. Petitioner ignores the long-held Federal Circuit precedent that patent owners in IPRs have no burden to prove nonobviousness: “In an *inter partes* review, *the burden of persuasion is on the petitioner* to prove ‘unpatentability by a preponderance of the evidence,’ 35 U.S.C. § 316(e), and *that burden never shifts to the patentee.*” *Dynamic Drinkware, LLC v. National Graphics, Inc.*, 800 F.3d 1375, 1378-1379 (Fed. Cir. 2015); *see also, In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1377 (Fed. Cir. 2016) (finding “that the Board *improperly shifted* the burden to ... the patentee, *to prove nonobviousness.*”).

It is Petitioner who failed to meet its burden here, not 10x. As discussed below, Petitioner's Reply attempts to backfill the Petition's defective arguments, belatedly raises new arguments, and mischaracterizes 10x's arguments in an attempt to recast the Petition's obviousness rationales. The Board should reject Petitioner's arguments and find the challenged claims not unpatentable.

II. PETITIONER CANNOT SALVAGE THE DEFICIENCIES IN ITS ART COMBINATION

Petitioner's art combination fails to teach or suggest: 1) a system for volumetric imaging comprising a temperature control apparatus as defined and claimed in the 554 patent, and 2) a volumetric imaging system for determining three-dimensional positional information of one or more nucleic acid molecules, as claimed. POR, 32-46. The Petition relies on Chen's 2D MERFISH imaging setup as its primary reference, but Chen at most teaches using an apparatus that *only actively heats* and provides no suggestion for determining 3D positional information of the RNA molecules. *Id.*

A. Petitioner's construction of "temperature control" is incorrect

The POR explained that based on the claim language, specification, and file history, a POSA would have understood the term "temperature control" in claim 36 to mean "the ability to actively heat and actively cool." POR, 27-29; EX2024, ¶¶60-64. In its Reply, Petitioner and its expert, Dr. Czymmek, argue that temperature control "means an ability to adjust or *control* temperature." EX1086,

¶18 (emphasis added); Reply, 2. Petitioner’s circular construction is unhelpful to determine the meaning of claim terms, as it uses the construed term (control) to define the term. *See e.g., Rhode & Schwarz GmbH & Co., KG v. Tektronix, Inc.*, IPR2018-00643, Paper 41, 14 (P.T.A.B. Sept. 10, 2019) (declining to adopt a construction that “is circular” and “relies on the [claim term] itself.”); *Comcast Cable Comm’s., LLC v. Rovi Guides, Inc.*, IPR2017-01048, Paper 36, 17 (P.T.A.B. Oct. 16, 2018) (“we view each of Rovi’s proposed constructions as circular and unhelpful.”).

Dr. Czymmek further argues that “temperature control” encompasses “using *only* active heating.” EX1086, ¶18 (emphasis in original). This construction ignores the 554 patent’s specification, which makes clear that a temperature control apparatus possesses the ability to actively heat *and actively cool* the sample holder. A POSA reads claim terms “in the context of the entire patent, *including the specification.*” *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc). Dr. Shroff confirmed he applied this legal standard in his analysis, testifying that “a POSA would have understood the meaning of temperature control *in light of the language of the patent specification and the claims.* And I give -- I give examples and I’m happy to read in paragraph 62.” EX1082, 79:14-18; EX2024, ¶60. Petitioner’s accusation that Dr. Shroff was provided incorrect legal standards is meritless and inflammatory. Reply, 2.

Further, Petitioner distorts 10x's construction by omitting the word "ability," implying that 10x's construction requires an apparatus that in fact generates constant active heating and active cooling, rather than an apparatus with *the ability* to actively heat and actively cool. For example, Petitioner argues that "[n]othing in the claim language requires 'temperature control' to *include* both active heating and active cooling," and discusses whether temperature control in claim 36 "*always requires* active cooling." Reply, 2-3. This is incorrect.

A POSA reading the claim language and the specification would understand that temperature control means the *ability* to actively heat and actively cool. POR, 27-29; EX2024, ¶¶60-64. This is clearly why the specification describes the apparatus as heating *or* cooling—the apparatus possesses *the ability* to actively heat *and* to actively cool. Likewise, the specification's description that the stage "may" be heated or cooled describes a temperature control apparatus that possesses the *ability* to actively heat and actively cool—it need not do both simultaneously. EX1001, 18:5-7, Reply, 3-4.

Finally, Petitioner's argument that 10x imports "mere examples" from the specification into the claims is incorrect. Reply, 3. The temperature control apparatuses described in the specification repeatedly and consistently have the ability to actively heat and actively cool. POR, 27-28. These are *not* merely "preferred embodiments or specific examples in the specification"—rather, these

are *the only* temperature control apparatuses described. *Littelfuse v. Mersen USA EP Corp*, 29 F.4th 1376, 1381 (Fed. Cir. 2022). And even if they were considered “preferred embodiments,” “the patentee’s choice of preferred embodiments can shed light on the intended scope of the claims.” *Astrazeneca AB v. Mut. Pharm. Co.*, 384 F.3d 1333, 1340 (Fed. Cir. 2004). The 554 patent inventors’ deliberate choice to repeatedly and consistently describe temperature control apparatuses the same way provides a clear indication of the inventors’ intended claim scope: a temperature control apparatus must have the ability to actively heat and actively cool.

B. It is undisputed that Chen’s FCS2 flowcell and objective heater lack the capability to actively cool

Petitioner relies on two disclosures in Chen to meet the temperature control apparatus element of claim 36: Chen’s FCS2 flowcell and objective heater.

Petition, 24-25; Reply, 4-6. First, Petitioner’s reliance on Su’s FCS2 flow cell serial number fails to show that Chen discloses FCS2 with “temperature control,” even under Petitioner’s construction. Reply, 4-5. Su cites Chen for “custom-built fluidics system,” *not* for “FCS2 flow chamber.” EX2009, e7. Chen’s “FCS2 flow chamber” is a separate and distinct feature from “home-built fluidics system composed of three computer-controlled eight-way valves . . . and a computer-controlled peristaltic pump.” EX1003, 011; POR, 32-33.

Moreover, neither device in Chen has the ability to actively heat *and actively*

cool, as required by the claims under the proper construction. Indeed, it is undisputed that neither of Chen's devices possesses the ability to actively cool. Reply, 5; EX1086, ¶¶29-30. Dr. Czymmek further admitted at deposition that "[t]he FCS2 system is understood in the art by a POSA to be *used for heating*." EX2082, 24:8-14; *see also, id.*, 32:1-7 (testifying that the FCS2 system "would *turn off or reduce the current*" (i.e., passively cool) to lower the temperature), 33:9-34:1 ("turning it off means *it's not actively cooling it*, correct"), 35:8-20 ("[i]f it's *not pulling away the heat in an active way* using an electronic mechanism, *it would be passive*."). Thus, a POSA would have understood that Chen's FCS2 flowcell and/or objective heater at most provides a capability to actively heat and *not* actively cool, and therefore Chen does not teach or suggest the temperature control apparatus of claim 36.

Petitioner's argument that it would have been obvious to add a Peltier element to Chen's system is conclusory and unsupported. Pet., 43; Reply, 6. The Petition offers no explanation why a POSA would have been motivated to modify Chen's 2D MERFISH imaging setup to include an apparatus, such as a Peltier element, with the ability to actively heat *and actively cool*. POR, 35; Pet., 43. Petitioner's Reply offers nothing more than the same conclusory allegations of obviousness, without explanation of why a POSA would have been motivated to construct a system capable of actively cooling fixed cell samples for MERFISH

imaging (as in Chen). Reply, 6. “Such a conclusory assertion with no explanation 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).

C. Chen only discloses 2D MERFISH

There is also no dispute that Chen’s MERFISH data are all 2D data. *See e.g.*, Reply, 6 (“[t]he authors of Chen acknowledged that *their initial experiments involved only 2D data...*”), 19 (“the Chen paper *described results with 2D MERFISH...*”); EX1086, ¶¶32 (“the authors of Chen are stating that *the experiments they performed generated 2D data...*”), 40 (“[t]he Chen publication reports *2D data with MERFISH...*”). Thus, the issue before the Board is whether a POSA would have been motivated to modify Chen’s 2D MERFISH setup into a system for 3D MERFISH with a reasonable expectation of success. And as the POR explained, neither Chen nor any of Petitioner’s other cited art would have so motivated a POSA. POR, 38-54.

Petitioner continues to erroneously rely on Chen’s disclosure of z-scanning or “tiling” as a suggestion of determining the 3D positional information of the RNA molecules. Reply, 7-9. First, Petitioner’s allegation that 10x ignored Chen’s tiling disclosure is baseless. As 10x previously explained, z-scanning (which is same as tiling adjacent focal planes or z-stacking) in Chen is used as a test to confirm counting all of the RNA molecules in the 2D focal plane. POR, 16-17, 23,

29; EX1003, 012; EX2082, 54:10-56:22. Chen expressly discloses that “[t]iling ... could be used to improve the *counting accuracy*.” EX1003, 010, 012; EX2082, 56:25-58:6. Dr. Czymmek testified at deposition that Chen’s intent was merely RNA counting:

Q. So counting the spots is, the spots are RNA species. Is that what you said?

A. Yes.

Q. Okay.

A. That’s one intent of spatial elements is to know *how many messages per cell*, yeah.

EX2082, 57:12-18.

Moreover, as the POR explained, Chen’s disclosure of merely z-scanning/tiling adjacent focal planes/z-stacking) is not a suggestion to *determine the 3D positional information* of the nucleic acid molecules imaged in the planes.

POR, 41-43; EX2024, ¶¶88-89. Similarly, Dr. Shroff testified that z-stacking is not the same as determining 3D positional information:

Q. Now, in November 2015, were people of ordinary skill in the art aware of the concept of stacking focal [planes] to do 3D microscopy?

A. What I would say is that, in general, the idea of taking a stack to acquire a volume was known. But of course in particular, and

with reference to our discussion here, *what was not known is the know-how to any multiplexed RNA environment obtained axial coordinates*, as I discussed at length in my declaration.

You know, the part that is specific to this patent, namely, *element E of the '554 patent, determining the three-dimensional positional information* of said one or more nucleic acid molecules *in the context of the entire claim 36*.

EX1082, 121:8-22. Petitioner conveniently cherry-picked the first sentence from Dr. Shroff's answer above. Reply, 8 (citing only EX1082, 121:8-13). A POSA simply would not have considered Chen's disclosure of z-scanning/z-stacking/tiling adjacent focal planes for *counting* RNA molecules to suggest *determining 3D positional information* of the RNA molecules. POR, 41-43; EX2024, ¶¶88-89.

Similar to Chen's tiling or z-scanning disclosures, its optical sectioning disclosure also does not teach or suggest determining 3D positional information of nucleic acid molecules. EX1003, 010, 012; POR, 41-43. As Dr. Shroff explained, optical sectioning was known in the art as a method for removing out-of-focus light from other planes, and is distinct from determining 3D positional information of nucleic acid molecules. EX2024, ¶¶36, 88-89. Dr. Shroff confirmed this at deposition, explaining that "optical sectioning, as I detail in my declaration, is distinct from three-dimensional imaging. They're two separate concepts." EX1082,

246:17-248:3. Consistent with this, Chen expressly discloses “optical sectioning methods to *reduce the fluorescence background*.” EX1003, 010, 012; POR, 41-43; EX2024, ¶¶88-89.²

Petitioner’s reliance on the 237 patent (EX1073) does not help its case. Reply, 9. 10x cited prosecution statements from Chen’s own patent applications as confirming what was *taught in Chen EX1003*: predecessor smFISH and seqFISH methods were considered “‘inadequate’ and ‘not a practical approach to multiplexing.’” POR, 37, n.5 (quoting EX2054, 16; EX2055, 16-17). Petitioner’s reliance on the 237 patent specification as disclosing “how MERFISH can be used” is misplaced because the 237 patent does not suggest performing 3D MERFISH using *Chen’s modified system*, as required by Petitioner’s obviousness theory (nor does Petitioner contend that it does). Additionally, on cross-examination, Dr. Czymmek could not deny that the 237 patent discloses only 2D MERFISH data, just like Chen. EX2082, 180:19-186:12. Finally, even if the 237

² To the extent the Board accepts Bruker’s purported position that optical sectioning and tiling in Chen are synonymous, any optical sectioning disclosure in Chen still fails to teach or suggest determining 3D positional information of nucleic acid molecules for the same reasons that Chen’s tiling or z-scanning disclosures fail. Reply, 8; EX2024, ¶¶88-89; EX2082, 54:18-56:21.

patent were considered as somehow suggesting 3D MERFISH imaging with Chen's modified system (it does not), the 237 patent is not part of the Petition's unpatentability grounds, nor has Petitioner established that it is prior art to the 554 patent.

III. A POSA WOULD HAVE HAD NO REASON TO MODIFY CHEN IN VIEW OF CAI AND SHEN

As the POR explained, the Petition's grounds are rooted in hindsight, evidenced by the Petition's cherry-picked disclosures from the art, conclusory allegations of obviousness, failure to articulate why or how a POSA would have modified Chen, and failure to address objective indicia of nonobviousness. *See e.g.*, POR, 30. Unable to substantively rebut this, Petitioner's Reply offers a single conclusory sentence that 10x's arguments pertaining to hindsight bias "should be rejected" (Reply, 10) and introduces several new theories for the first time in reply.

A. Petitioner's obviousness theory regarding what could be done is legally erroneous

Petitioner's Reply continues to advance improper hindsight rationales, repeatedly arguing that a POSA *could have* modified Chen's setup for use in 3D MERFISH, rather than demonstrating that a POSA *would have* been so motivated based on the state of the art at the time. *See e.g.*, Reply, 12 ("it *could be done* depending on the sample type, correct?"), 13 ("it was known that widefield *could be used* for 3D microscopy"), 14 (discussing "a minimum number of targets that

could be analyzed with MERFISH”), 18 (arguing that a POSA “*could have* simply selected a microscopy modality with a sufficiently low PSF.”).

But the test for obviousness asks not what *could* be done, rather what a POSA *would be motivated* to do. In *Personal Web Technologies, LLC v. Apple, Inc.*, 848 F.3d 987, 993-994 (Fed. Cir. 2017), the Federal Circuit rejected the Board’s motivation to combine analysis because the “reasoning seems to say no more than that a skilled artisan, once presented with the two references, would have understood that they *could be* combined. *And that is not enough*: it does not imply a motivation to pick out those two references and combine them to arrive at the claimed invention.” *Id.*; *see also, Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015). Such is the case here.

Petitioner at best argues that a POSA *could have* combined Chen, Cai, and Shen, failing to show that the artisan *would have* combined them to arrive at the claimed volumetric imaging system. Dr. Shroff testified consistently on this topic at his deposition—testimony that Petitioner conveniently omitted from its quoted text. For example, when asked if widefield imaging is “*ever* used with this concept of making Z stacks,” Dr. Shroff answered that “[w]idefield imaging *can be* used in the context of taking a Z stack,” but “[o]f course *that’s not what was done in Chen*, in the context of trying to get 3D information.” EX1082, 212:17-213:2; Reply, 12 (quoting only EX1082, 212:17-20).

Petitioner's rationale of what *could be* or *might be* done "does not meet the requirements for a sustainable obviousness determination," and should be rejected. *Personal Web*, 848 F.3d at 994.

B. Chen's stated goal and benefit of MERFISH was increased multiplexing, which weighs against modifying Chen to perform 3D MERFISH

There is no dispute that Chen's goal of MERFISH was increased multiplexed imaging. EX1003, 001-002; POR, 36-38; Reply, 14. As Dr. Shroff explained, Chen's multiplexing goal was consistent with Chen's disclosure of only 2D MERFISH imaging. EX2024, ¶¶84-90. Dr. Shroff also explained that, given Chen's goal of high multiplexing with MERFISH, a POSA would have had *no* reason to modify Chen's 2D imaging setup into a 3D system due to increased imaging challenges associated with determining axial resolution in highly multiplexed imaging, including resolution, signal density, SNR, SBR, and PSF. *Id.*, ¶¶99-107.

Unable to dispute the facts, Petitioner responds by *changing its argument* to now say that a POSA would have been motivated to image only a small number of targets using Chen's MERFISH imaging setup. *See e.g.*, Reply, 14 ("there is nothing about MERFISH that would preclude analyzing a small number of targets, nor would a POSA deem this unnecessary or uninteresting"); EX1086, ¶50 ("to the extent a POSA believed that 3D imaging may have presented challenges for large

numbers of targets with MERFISH, he or she simply would have analyzed fewer targets”), ¶15 (similar).

Petitioner’s new single-plex or low-plex theory is contrary to the fundamental multiplexing goal and benefit of MERFISH—a highly multiplexed methodology that allows “thousands of RNA species to be imaged,” and according to the Petition’s original theory, the POSA is interested in *practicing MERFISH*. EX1003, 001; POR, 36-38. Chen discloses that predecessor FISH methods, smFISH and seqFISH, were “limited by the number of RNA species that can be simultaneously measured in single cells” and that Chen solved this problem by developing a “*highly multiplexed smFISH imaging method*” (MERFISH). EX1003, 002. Chen’s MERFISH methods “*substantially increase[d] the number of RNA species that can be simultaneously imaged in single cells.*” *Id.*

As Dr. Czymmek testified, Chen’s 140-RNA MERFISH experiment (performed in 2D) imaged up to 35 different RNA species *per round* of readout probe hybridization (with 16 total rounds) as estimated by counting the number of “1s” in each column (i.e., each hybridization round) in Chen’s codebook.³ *See*

³ Compare EX1003, 003 and FIGs. 2-6 with EX1073, 48:43-48 (Example 5) and FIGs. 6-10; *see also*, EX2082, 170:2-176:19. The 237 patent’s Example 5 and Figure 20 provide the codebooks used in the 140-RNA experiment. EX1073,

EX2082, 177:11-179:12; EX1073, FIGs. 20A-D (Codebook 1), E-H (Codebook 2).

This is not a “small number of targets” (Reply, 14), and is consistent with Chen’s goal of high multiplexing in 2D—a goal the POSA would not simply abandon if truly interested in performing MERFISH. EX2024, ¶¶80-83, 104-107. Thus, a POSA interested in *performing MERFISH* would *not* be interested in analyzing “a small number of targets” (as in predecessor FISH methods) because that contradicts the purpose of MERFISH.

Moreover, Petitioner cannot change its obviousness theory in its Reply to now argue that a POSA would have sought to practice only *FISH* (and not MERFISH) in 3D for imaging a single nucleic acid species. *Henny Penny Corp. v. Frymaster LLC*, 938 F.3d 1324, 1329–31 (Fed. Cir. 2019) (rejecting a new theory of unpatentability raised in the petitioner’s reply). As in *Henny Penny*, the Board should “hold[] [Petitioner] to its word and disregard[] its new theory first raised in reply.” *Id.* at 1331; *see also Wasica Fin. GmbH v. Cont'l Auto. Sys., Inc.*, 853 F.3d 1272, 1286 (Fed. Cir. 2017); *Intelligent Bio-Sys.*, 821 F.3d at 1369-1370.

Petitioner accordingly fails to show that a POSA *would have* been motivated to modify Chen’s 2D imaging setup to arrive at the claimed volumetric imaging system, as required under the proper obviousness standard. POR, 46-54; EX2024,

48:43-48, FIG. 20.

¶¶99-107; *see also* Section IV below.

C. Petitioner fails to explain *why* a POSA would have looked to Cai and/or Shen

The Petition never explained how or why a POSA would have modified Chen’s 2D imaging setup in view of Cai or Shen. POR, 46-49; *ActiveVideo Networks, Inc. v. Verizon Communications, Inc.*, 694 F.3d 1312, 1328 (Fed. Cir. 2012). In its Reply, Petitioner attempts to belatedly explain its purported art combination, stating that “the theory of the Petition is ... that *Cai’s z-axis motion control should be applied to Chen* to enable stacking of focal planes and hence 3D imaging.” Reply, 12. Dr. Czymmek likewise testified that “the *specific feature* I am proposing to use from Shen and/or Cai *is a z-axis motion control system.*” EX1086, ¶42. But even accepting this backfilled explanation, Petitioner still provides no *reason why* a POSA would have combined these references. “[T]here *must be a motivation to make the combination* and a reasonable expectation that such a combination would be successful, otherwise a skilled artisan would not arrive at the claimed combination.” *In re Stepan Co.*, 868 F.3d 1342, 1346 n.1 (Fed. Cir. 2017); *Virtek Vision Int’l ULC v. Assembly Guidance Sys. Inc.*, 97 F.4th 882, 887 (Fed. Cir. 2024) (discussing “the *requirement that there must exist a motivation to combine*” the art).

As 10x previously explained, Chen’s 2D MERFISH setup was already equipped with “a home-built autofocus system,” which both Petitioner and its

expert previously asserted is a Z-axis motion control system. Petition, 31; EX1002, ¶¶179-184; POR, 47; EX1003, 011. Dr. Czymmek confirmed that, in his opinion, *Chen alone* teaches the Z-axis motion control system of claim 36. EX2082, 61:13-21, 64:17-19. Neither Petitioner nor its expert provides any explanation *why* the POSA would have allegedly modified Chen with a different (and redundant) Z-axis motion control system from Cai or Shen, when Chen's setup admittedly has a Z-axis motion control system. EX1003, 011. 10x raised this point in its POR, and Petitioner failed to rebut it. POR, 47; Reply, 11-12.

Petitioner also relies on new embodiments disclosed in Cai, now referring to Cai's Figures 16 and 18 as "extensive actual 3D imaging data." Reply, 10. Neither the Petition nor the POR cite or discuss Cai Figure 16 or 18. Pet., 29-30, 32-34; POR, 44-46. Petitioner's belated shift to Cai Figures 16 and 18 is yet another new and improper argument that is "foreclosed by statute, [the Board's] precedent, and Board guidelines." *Wasica*, 853 F.3d at 1286 (citing 35 U.S.C. § 312(a)(3)); *Henny Penny*, 938 F.3d at 1331; *Intelligent Bio-Sys.*, 821 F.3d at 1369-1370.

Moreover, even if considering Cai's data in Figures 16 and 18, Cai still provides no motivation to modify Chen's setup to arrive at a 3D MERFISH imaging system meeting the elements of claim 36. Cai's Figure 16 does not involve any nucleic acid probes for imaging, and Figure 18 involves imaging a single target (β -actin) with a single fluorescence color. EX1004, 11:17-24, 11:27-

32; EX2082, 82:22-83:7, 84:12-85:2. And both images were generated with Cai's light sheet microscope – a completely different imaging setup than Chen. EX2082, 81:25-82:21; POR, 44-46. While Petitioner argues that “bodily incorporat[ion]” is not required for obviousness (Reply, 11), the test still requires “*some articulated reasoning with some rational underpinning* to support the legal conclusion of obviousness.” *KSR Int’l Co. Teleflex, Inc.*, 550 U.S. 398, 418 (2007). The Petition is devoid of any such reasoning or rational underpinning. POR, 54-56.

IV. A POSA WOULD HAVE HAD NO REASONABLE EXPECTATION OF SUCCESS

The Petition also failed to establish that a POSA would have had a reasonable expectation of success. POR, 56-58. In particular, the Petition failed to account for the numerous issues a POSA would have encountered, whether imaging in 2D or attempting to image in 3D. As Dr. Shroff previously testified, “[a] POSA would have understood that the greater multiplexity of Chen’s 2D MERFISH arrangement would lead to increased imaging challenges, especially for any attempts at 3D imaging.” EX2024, ¶105. These challenges include issues with signal density (expressly acknowledged by Chen – *see e.g.*, EX1003, 008), resolution, SNR, SBR, and PSF. EX2024, ¶104. A POSA would have expected to encounter these issues whether imaging in 2D or 3D, but the issues become “even worse when attempting to image multiple planes in a sample for 3D imaging, as overlapping PSFs from large populations of fluorescently-labelled nucleic acids are

more problematic in 3D than 2D (due to the elongation of the PSF in the axial dimension) and a reduction in SNR and SBR from increasing numbers of out-of-focus molecules (whose background signal would make it harder to precisely localize molecules in each in-focus plane).” EX2024, ¶105.

In Reply, Petitioner alleges that these issues are only meaningfully relevant when imaging thicker samples, and that the POSA could simply image “thin samples, including even a single cell,” and fewer RNA molecules (less multiplexing), completely ignoring the purpose of Chen’s MERFISH. Reply, 14-17; EX1086, ¶¶14-15. Petitioner further argues that the POSA could select different microscopy modalities, such as iPALM, PALM/STORM, SIM, or 3D-SIM to overcome any issues encountered. Reply, 17-19; EX1086, ¶13. As discussed below, Petitioner’s arguments are new theories raised for the first time in its Reply and should be rejected by the Board. Moreover, Petitioner’s new theories are technically incorrect.

A. Petitioner’s argument regarding sample thickness ignores issues with PSF, SBR, and SNR when imaging thin or small samples

Dr. Czymmek alleges that the foregoing imaging issues are not relevant for thin or small samples, testifying that “[w]hatever difficulties may have arisen for axial resolution when performing 3D imaging of very thick samples, *these deep tissue issues are not relevant for thin or small samples* which still have 3D volumes.” EX1086, ¶14; Reply, 16 (discussing “thin samples or single cells”). On

cross-examination, however, Dr. Czymmek could not defend his position and admitted that issues of PSF, SBR, and SNR are indeed relevant *even when imaging thin or small samples*. EX2082, 88:12-92:21 (discussing issues of coverslip distance, numerical aperture, refractive index of mounting, refractive index of immersion media, scattering properties of the sample, light wavelength, deconvolution methods, and aperture size that can influence PSF when imaging thin or small samples), 94:12-100:2 (discussing issues of aperture size, signal amplification, probe brightness, exposure time, light intensity, and photobleaching that can influence SNR when imaging thin or small samples), 100:3-104:16 (discussing issues of autofluorescence and non-specific probe binding that can influence SBR when imaging thin or small samples). Thus, Petitioner's argument that "the claims are not limited to any particular sample thickness" (Reply, 16) is irrelevant, because the POSA would have expected imaging difficulties when attempting to modify Chen's 2D MERFISH setup into a 3D MERFISH system, regardless of sample thickness or size. EX2024, ¶¶104-105.

B. Petitioner's argument regarding single-plex or low-plex imaging ignores its original theory for obviousness and is an improper new argument

As discussed above, there is no dispute that the goal and advantage of MERFISH was increased multiplexed imaging. EX1003, 001-002; POR, 36-38; Reply, 14. The POR explained that a POSA would not have had a reasonable

expectation of successfully modifying Chen's 2D imaging setup to attempt MERFISH in 3D while retaining MERFISH's massively multiplexed RNA imaging. POR, 56-58; EX2024, ¶¶110-114. Petitioner argues that multiplexing is irrelevant because "the claims require detecting only 'one' nucleic acid molecule." Reply, 14; EX1086, ¶15. As discussed above in Section III.B, Petitioner's new low-plex or single-plex argument not only belies the Petition's original obviousness theory requiring a POSA to practice the highly multiplexed MERFISH methods in 3D, but is impermissible new argument. *Wasica*, 853 F.3d at 1286; *Henny Penny*, 938 F.3d at 1331; *Intelligent Bio-Sys.*, 821 F.3d at 1369-1370.

C. Petitioner's argument regarding selecting a different "microscopy modality" is an improper new argument

Petitioner argues that, "[i]f a POSA were concerned about the PSF, he or she could have simply selected a microscopy modality with a sufficiently low PSF," referring to "SIM, IPALM, PALM, STORM, and 3D SIM." Reply, 18; EX1086, ¶13. Petitioner's suggestion of implementing these microscopy modalities in its modification of Chen is yet another new theory that should be rejected. *Wasica*, 853 F.3d at 1286; *Henny Penny*, 938 F.3d at 1331; *Intelligent Bio-Sys.*, 821 F.3d at 1369-1370.

The Petition never argued that a POSA would implement other microscopy modalities beyond Chen's widefield microscope setup. To wit, the Petition

unequivocally relied on *Chen*'s widefield imaging setup for its obviousness theory (“[Chen]’s system is built around an Olympus IX-71 optical microscope ... As such, [Chen] discloses an optical system”), with the purported modification of adding Cai’s (or Shen’s) Z-axis motion control (“the theory of the Petition is ... that *Cai’s z-axis motion control should be applied to Chen.*”). Pet., 28; Reply, 12. The only mention of other microscopy modalities in the Petition or first Czymmek declaration is a passing reference in the Background section, not as part of the arguments and certainly not in the discussion of modifying Chen. Pet., 5; EX1002, ¶45.

Moreover, even if considering these new modalities raised for the first time in the Reply, Petitioner never explains how a POSA would have implemented them with Chen’s imaging setup. Reply, 18. As Dr. Czymmek admitted at deposition, iPALM is “a more exotic technique that not many folks are using,” and it has a “specific configuration” with “some very limited geometry.” EX2082, 115:22-116:8, 119:18-25. He also admitted that both iPALM and 3D-SIM and require complex, specific optical configurations. EX2082, 119:18-121:15, 153:24-154:17, 156:20-158:5. Dr. Czymmek further testified that each of iPALM, PALM, and STORM requires unique subsets of dyes or fluorophores, special buffers, and completely different probes from those taught in Chen. EX2082, 116:18-24, 127:7-9, 132:9-133:9, 133:10-21, 136:4-14, 137:8-10, 139:1-12. Additionally, PALM

methods image “genetically encoded florescent [*sic*] proteins,” and do not even image nucleic acid molecules. *Id.*, 129:25-130:5. These modalities were also known for being tedious and time-consuming, and several run the risk of unintentionally photobleaching the samples, eliminating detectable fluorescence. *Id.*, 141:14-25, 146:20-147:8, 154:18-156:6, 162:7-163:17; EX1019, 020 (Table 1). None of these limitations is discussed in the Petition or Reply, nor how the POSA would have implemented any of these complicated, non-routine microscopy modalities in Chen’s 2D MERFISH setup to modify it into a 3D MERFISH system. Even Petitioner’s expert was uncomfortable testifying about iPALM and STORM methods because he “didn’t study” them. EX2082, 116:19-117:20, 137:12-139:1. The Board should accordingly reject Petitioner’s new arguments.

V. OBJECTIVE INDICIA OF NONOBVIOUSNESS CONFIRM PATENTABILITY OF THE CHALLENGED CLAIMS

As 10x’s POR showed, nonobviousness of the 554 patent’s volumetric imaging system is further evident from the objective indicia of commercial success, long-felt need, and industry praise. POR, 58-67. In Reply, Petitioner fails to provide its own economics expert to rebut Ms. Davis’s testimony and criticizes Dr. Shroff’s nexus analysis. Reply, 21-25.

Nexus is presumed “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). Here, Petitioner's Reply fails to credibly rebut the presumption of nexus. Petitioner's only criticism of Dr. Shroff's nexus analysis on Xenium relates to whether Xenium utilizes two or more light sources, as recited in claim 36.

Reply, 21-22. Petitioner argues that Xenium may not use two or more light sources because "[a] single light source *can be* used to generate multiple optical channels."

Reply, 22. The fact that a single light source "can be" used does not mean that is the case in Xenium. Indeed, as Dr. Shroff testified, "[t]he multiple channels of the Xenium system indicate that multiple sources of light are used in the system."

EX2024, ¶124; EX2060, 73. A clear nexus exists between the 554 patent claims and Xenium, and Petitioner's Reply fails to show otherwise.

Petitioner also fails to substantively rebut 10x's economics expert Ms. Julie Davis's extensive testimony demonstrating that Xenium is a commercial success. Dr. Czymmek admitted he is not an expert in economics, finance, or accounting. EX2082, 196:3-16. Petitioner provided no economics expert to rebut the merits of Ms. Davis's opinions.

VI. CONCLUSION

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

Respectfully submitted,

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

1. This Patent Owner Sur-Reply complies with the type-volume limitation of 5,600 words, comprising 5,586 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'S SUR-REPLY** and Exhibit 2082 were served in their entireties on April 25, 2025, upon the following parties via electronic mail:

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