Bruker Spatial Biology, Inc.,
Petitioner
v.
President and Fellows of Harvard College and
10x Genomics, Inc.,
Patent Owner

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

Patent Owner's Demonstratives for Oral Hearing

May 16, 2025

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

10x EX2086
Bruker v. 10x
IPR2024-00526

Overview

- The 554 patent (slides 3-5)
- Technical background (slides 6-15)
- Petitioner's obviousness theory requires 3D MERFISH (slides 16-21)
- The asserted art fails to teach or suggest the claimed volumetric imaging system (slides 22-31)
- A POSA would not have been motivated to modify Chen in view of Cai and Shen (slides 32-49)
- A POSA would not have had a reasonable expectation of success (slides 50-59)
- Petitioner's art combination fails to teach or suggest a system comprising a temperature control apparatus as claimed (slides 60-71)
- Objective indicia further confirms non-obviousness of the claimed system (slides 72-75)

The 554 Patent

Volumetric imaging system

(12) **United States Patent**
Daugharthy et al.

(10) **Patent No.: US 11,542,554 B2**
(45) **Date of Patent: *Jan. 3, 2023**

(54) **METHOD AND APPARATUS FOR VOLUMETRIC IMAGING**

(71) Applicant: **President and Fellows of Harvard College**, Cambridge, MA (US)

(72) Inventors: **Evan R. Daugharthy**, Cambridge, MA (US); **Richard C. Terry**, Carlisle, MA (US); **Je-Hyuk Lee**, Allston, MA (US); **George M. Church**, Brookline, MA (US); **Benjamin W. Pruitt**, Cambridge, MA (US)

(73) Assignee: **President and Fellows of Harvard College**, Cambridge, MA (US)

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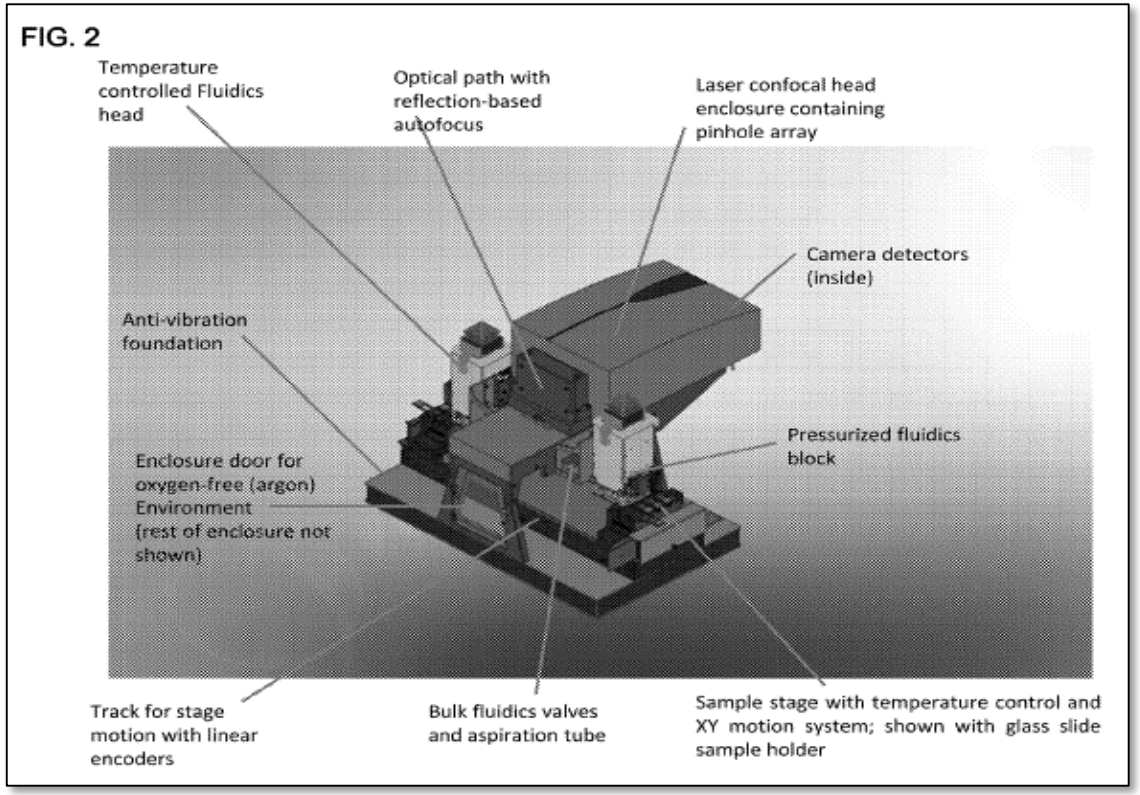
EX1001, face

be optically detected. According to one aspect, 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. The auto-

EX1001, 5:29-33

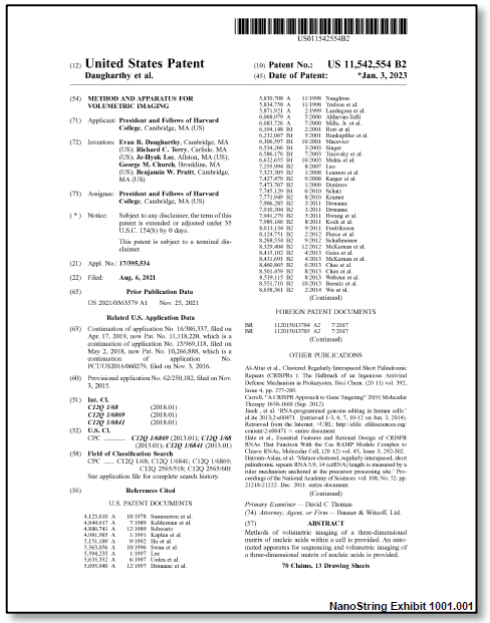
of light signals in three dimensions. The device includes hardware and software functionally assembled to enact a protocol of chemical manipulation (i.e., nucleic acid sequencing) and imaging of the three dimensional nucleic acid containing matrix which is contained within a suitable vessel or stage. The device may be referred to as a fluidic

EX1001, 16:46-51



EX1001, Fig. 2

554 patent: independent Claim 36



US011542554B2

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(60) Provisional application No. 62/250,182, filed on Nov. 3, 2015.

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C12Q 1/6869 (2018.01)
C12Q 1/6841 (2018.01)

(52) **U.S. Cl.**
CPC ***C12Q 1/6869*** (2013.01); ***C12Q 1/68*** (2013.01); ***C12Q 1/6841*** (2013.01)

(58) **Field of Classification Search**
CPC C12Q 1/68, C12Q 1/6841, C12Q 1/6869; C12Q 2565/518; C12Q 2565/601
See application file for complete search history.

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(Continued)

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(57) **ABSTRACT**

Methods of volumetric imaging of a three-dimensional matrix of nucleic acids within a cell is provided. An automated apparatus for sequencing and volumetric imaging of a three-dimensional matrix of nucleic acids is provided.

70 Claims, 13 Drawing Sheets

NanoString Exhibit 1001.001

36. A system comprising:

(a) a stage comprising:

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

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

(iii) an XY motion control system;

(b) a fluidics system comprising:

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

(ii) a fluid dispenser operationally coupled to a pump, which pump is configured to provide said one or more fluid reagents from said one or more reservoirs to said sample holder; and

(c) an optical system comprising:

(i) an objective;

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

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

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

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

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

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

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

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

EX1001, claim 36

3D positional information of a nucleic acid molecule

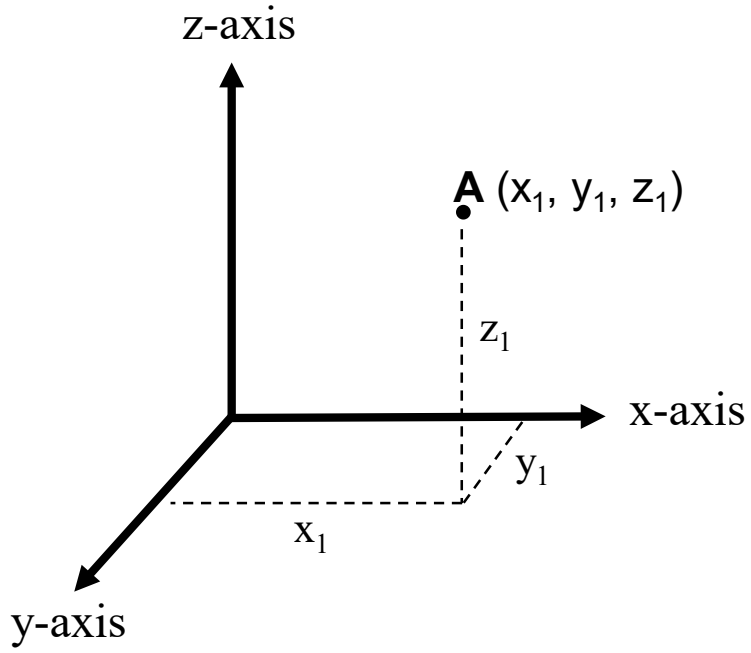
DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

The claims require determining three-dimensional (3D) positional information of one or more nucleic acid molecules

Claim 36:

(e) a computing system comprising . . . **determining three-dimensional positional information of** said one or more **nucleic acid molecules**.

EX1001, claim 36



Determining 3D positional information of a nucleic acid molecule involves resolving positional coordinates of the molecule in x-, y-, and z-axis

dimensional matrix material. In this manner, a three-dimensional matrix of covalently bound nucleic acids of any desired sequence is provided. Each nucleic acid has its own three dimensional coordinates within the matrix material and each nucleic acid represents information. According to one

EX1001, 3:10-14

another. In this context, the nucleic acid molecules are rigidly fixed to the extent that they maintain their coordinate position within the matrix. It is to be understood that even

EX1001, 3:33-35

Resolution is the minimum distance at which two points can be distinguished through a microscope

Toomre:

> **Resolution:** the minimum distance at which objects can be distinguished when imaged with a microscope; often characterized by the full width half maximum of the point-spread function

EX1018, 287

- Lateral resolution is along x- and y-axis
- Axial resolution is along z-axis

Point-spread Function (PSF) determines resolution

Dr. Shroff:

"Resolution can be quantified through the **PSF**, a **measure of the three-dimensional diffraction pattern of a point light source** that effectively describes how blurry a point source appears when viewed under a microscope."

EX2024, ¶22 (citing EX1018, 004; EX1019, 009; EX1027, 023*)*

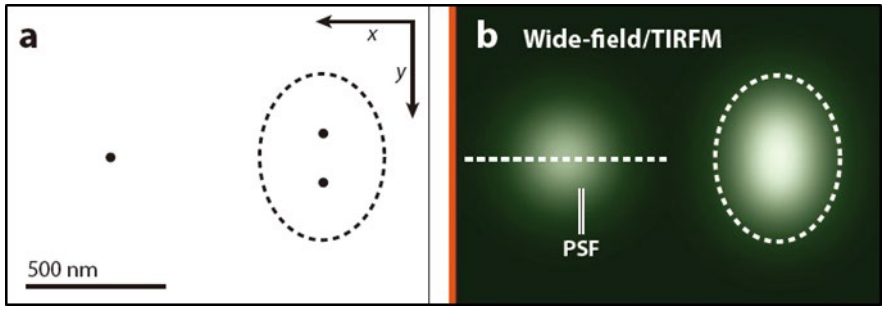
"The **PSF** determines the **ability to resolve two point sources that are in close proximity** to one another—if the **distance between the two points is too small and the PSF is too large**, then the **blur generated** from each point will overlap and the **two points will not be resolvable**. . . . [**A] single point could be localized** by determining the **center of the PSF**, which represents the localized position of the point source."

EX2024, ¶25 (citing EX1018, FIGs. 1A, 1B; EX1027, 023)

Zhang:

The point-spread functions (PSFs) of fluorescence microscopes, e.g., the wide field fluorescence microscope (WFFM), the laser scanning confocal microscope (LSCM), and the disk scanning confocal microscope (DSCM), play a central role in understanding imaging performances, such as the theoretical resolution limit and the optical sectioning capacity. A great amount of research has focused on deriving more and

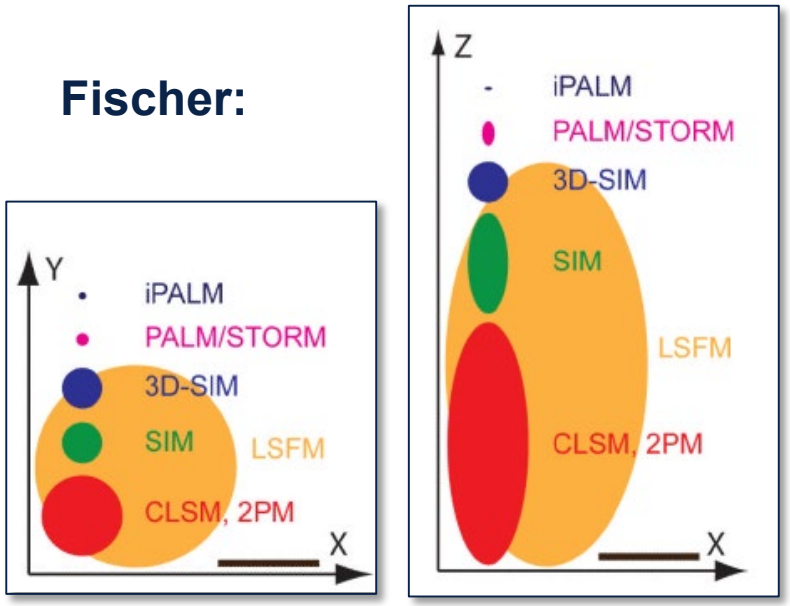
EX2044, 1819



EX1018, Fig. 1a-1b

Axial resolution is more difficult to achieve than lateral resolution

Fischer:



EX1019, Fig. 1

Dr. Shroff:

"As shown in the figure, the effective **PSF in the axial dimension is significantly elongated** compared with the effective PSF in lateral dimensions. EX1019, FIG. 1D. Given this elongation in the axial range, resolving individual point sources of light **along the z-axis** was understood to be **more challenging and complex** than resolving individual point sources of light in the lateral (x-y) range, as the **elongated PSF along the z-axis** contributes to greater **overlap with the diffraction patterns** from other point sources along the z-axis and a concomitant increased **difficulty in resolving individual point sources** along that axis."

EX2024, ¶24

objective lens; see Box 1 and Figure 1). The characteristic anisotropy of the PSF is seen as "Z-stretch" in XZ-projections of reconstructed 3D image sets obtained with uni-axial fluorescence microscopy. This reduced axial resolution and Z-stretch can introduce profound errors in tracking of objects in 4D and in co-localization in multichannel 3D imaging [1].

EX1019, 001

Dr. Czymmek admits that PSF is elongated along the z-axis

Dr. Czymmek:

Q. Okay. Thanks. Do you have an understanding of the term point spread function in the context of imaging?

A. I do.

Q. Would you please tell me what that is?

A. So in reference to a microscope and I'm going to use an example here, if you put a small sub-resolution bead -- and I'm going to, again, give simply an example. Fluorescent bead of around 100 nanometers for a typical microscope.

That bead will have a spread to it that's related to defraction. So this is what's limiting the resolution of the light microscope, and every microscope has an optical transfer function which depends on the objective lens and so forth.

The aberrations as it's looking through the sample. So point spread function basically looks like an hourglass or football shape in the Z-axis, but it also has these defraction patterns which cause an actual round object to look differently. Again, typically an hourglass or football shaped is how it's referred to in three dimensions.

EX2043, 43:17-44:14

Axial resolution was well known to be 2-3 times worse than lateral resolution

Waters:

2006). Resolution in the z-axis (z) is worse than lateral resolution in the light microscope, and is given by

$$z = \frac{2\lambda\eta}{(NA)^2},$$

where η is the refractive index of the specimen. It is important to understand

EX2045, 1141

Toomre:

Resolution can be quantified by analyzing a microscope's point-spread function (PSF), which effectively describes how blurry a point-like object (a single molecule or small fluorescent bead) (**Figure 1***b*) will appear. A cross

high NA. The axial resolution can be approximated by the formula $d_z \approx n\lambda_0/NA^2$, with n being the refractive index, and is typically 2 to 3 times worse than the lateral resolution. λ_0

EX1018, 004

Fischer:

The first and most obvious challenge of 3D imaging is that in conventional, uni-axial microscopy arrangements, the resolution along the Z-axis of the microscope is substantially (~2x) worse than in the lateral dimensions due to the inherent shape of the point spread function (PSF; the three-dimensional diffraction pattern of a point source viewed through an objective lens; see Box 1 and Figure 1). The characteristic anisotropy of the PSF is seen as "Z-stretch" in XZ-projections of reconstructed 3D image sets obtained with uni-axial fluorescence microscopy. This reduced axial resolution and Z-stretch can introduce profound errors in tracking of objects in 4D and in co-localization in multichannel 3D imaging [1].

EX1019, 001

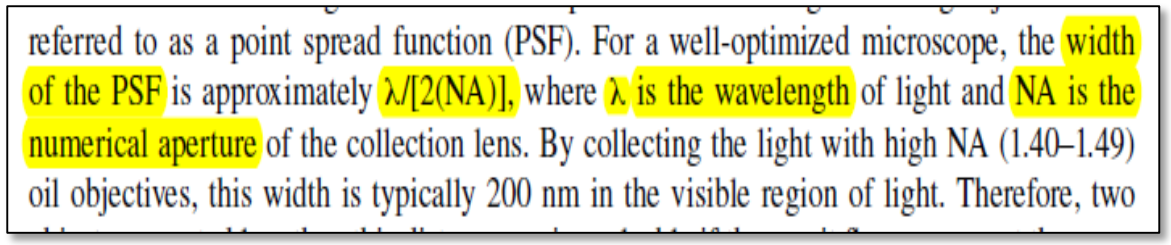
Numerical aperture, emission wavelength, and refractive index impact PSF

Dr. Shroff:

"A POSA would have known that the **PSF is influenced by** several factors, including the **numerical aperture** ("NA") of the microscope's objective lens, the **wavelength of emission light** (e.g., fluorescence) from the sample, and the **refractive index** of the sample, the imaging medium, and the objective lens. EX1027, 023; EX1018, 024."

EX2024, ¶27

Yildiz:



referred to as a point spread function (PSF). For a well-optimized microscope, the width of the PSF is approximately $\lambda/[2(NA)]$, where λ is the wavelength of light and NA is the numerical aperture of the collection lens. By collecting the light with high NA (1.40–1.49) oil objectives, this width is typically 200 nm in the visible region of light. Therefore, two

EX1027, 023

Signal-to-noise (SNR) and signal-to-background (SBR) complicate resolution

Waters:

level (Fig. 2 A). Noise causes imprecision in measurements of pixel intensity values, and therefore a level of uncertainty in the accuracy of the measurements. To detect the presence of a signal, the signal must be significantly higher than the noise level of the digital image

EX2045, 1136

of the digital image. SNR affects spatial measurements as well as intensity measurements; precise determination of the location of a fluorescently labeled object depends on SNR (Fig. 2, D and E)

EX2045, 1136

(Hiraoka et al., 1990). However, illuminating light above and below the image focal plane excites fluorophores above and below the image focal plane. Light emitted from these out-of-focus fluorophores is collected by the objective lens, and appears as out-of-focus background in the in-focus image of the specimen. In

EX2045, 1139

Dr. Shroff:

"The **background fluorescence** caused by out-of-focus fluorophores **impairs the quality of the image** obtained from fluorescence imaging by increasing the amount of noise in the sample, thereby **decreasing the SNR**."

EX2024, ¶34

Signal density complicates resolution

Dr. Shroff:

"Signal density is another factor that a POSA would have understood to influence the resolution of a microscope. Signal density in microscopy is a measure of how many **individual signals are detected and imaged within a single focal plane**. If too many signals are located in close proximity to each other, then **signal density is increased** and **it becomes difficult to discern individual signals**"

EX2024, ¶38

Lubeck:

transcript levels are low. When the density of transcripts is high, diffraction-limited fluorescent spots of the same color will overlap and make barcode readout impossible.

EX2051, 3

Chen:

As 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. On the basis of our imaging

EX1003, 008

Petitioner's obviousness theory requires 3D MERFISH

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petition's obviousness theory requires modifying Chen's 2D MERFISH setup for use in 3D MERFISH

Petition:

"A POSA would have been **motivated to use [Chen's] MERFISH method for 3D investigation** in order to obtain meaningful spatial information in the z dimension, furthering [Chen's] goal of 'identify[ing] distinct spatial distribution 'patterns of RNAs.' EX1003.002; EX1002 ¶ 207."

Pet., 39

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

Pet., 29

Dr. Czymmek:

"The long-known success of these methods would have provided a POSA with a reasonable expectation that the application of Z-axis scanning and imaging to **[Chen's] MERFISH protocol** would have been successful."

EX1002, ¶191

"As [Chen] has the goal of identifying 'distinct spatial distribution patterns of RNAs,' . . . a POSA would have been motivated to use the **MERFISH method for 3D investigation** and would have looked to the relevant teachings in Shen and Cai regarding Z-axis motion control that provides for 3D imaging."

EX1002, ¶207

"[T]o the extent [Chen] is found or argued not to disclose determining 3D positional information, . . . this was disclosed in Cai and it would have been **obvious to modify [Chen]** to permit a POSA **to use MERFISH to determine three-dimensional positional information** about one or more nucleic acid molecules."

EX1002, ¶219; see also id., ¶¶150, 153, 170, 183, 186, 305, 312

POR, 30-32; PO Sur-Reply, 1; EX2024, ¶¶67-68

Petitioner's Reply confirms Petition's theory of modifying Chen's 2D MERFISH setup for use in 3D MERFISH

Petitioner's Reply:

As detailed in the Petition, a POSA would have wanted to **adapt Chen's MERFISH method for 3D** and had no trouble doing so. *See, e.g.*, Petition at 33-34, 39.

Pet. Reply, 10

The foregoing shows that even if there were issues **adapting MERFISH to 3D**, there were well-known techniques for overcoming these issues that would have been within the basic competencies of the POSA.

Pet. Reply, 19

Dr. Czymmek:

This again undermines any suggestion that it would not have been obvious to **adapt MERFISH to 3D volumetric imaging**.

EX1086, ¶10

This again undermines any contention that a POSA would have been dissuaded from seeking to **adapt MERFISH to 3D imaging**.

EX1086, ¶11; see also id., ¶¶ 13, 14

POR, 30-32; POR Sur-Reply, 1; EX2024, ¶¶67-68

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner cannot change its obviousness theory

"**[Petitioner] chose which grounds of invalidity to assert in its petition** and it chose not to assert this new one. Unlike district court litigation — where parties have greater freedom to revise and develop their arguments over time and in response to newly discovered material — the expedited nature of IPRs bring with it an **obligation for petitioners to make their case in their petition to institute**. While the Board's requirements are strict ones, they are **requirements of which petitioners are aware** when they seek to institute an IPR."

Intelligent Bio-Sys. v. Illumina Cambridge, 821 F.3d 1359, 1369 (Fed. Cir. 2016)

"Rather than explaining how its original petition was correct, Continental's subsequent arguments amount to an entirely new theory of *prima facie* obviousness absent from the petition. **Shifting arguments** in this fashion is **foreclosed by statute, our precedent, and Board guidelines**. *See* 35 U.S.C. § 312(a)(3)…."

Wasica Fin. GmbH v. Cont'l Auto. Sys., Inc., 853 F.3d 1272, 1286 (Fed. Cir. 2017)

"[T]he Board did not err in **refusing the reply brief** as improper under 37 C.F.R. § 42.23(b) because **[Petitioner] relied 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

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner is foreclosed from a new theory on motivation to perform single- or low-plex 3D imaging

NEW REPLY THEORY

Petitioner's Reply:

"Because the **claims require detecting only 'one' nucleic acid molecule**, Petitioner's argument that the claims are not obvious because one of Chen's goals is high multiplexicity is irrelevant and entitled to no weight. *See* EX1086 ¶ 51."

Pet. Reply, 14

"[T]here is **nothing about MERFISH** that would preclude **analyzing a small number of targets**, nor would a POSA deem this unnecessary or uninteresting."

Pet. Reply, 14

Dr. Czymmek:

"[T]he **claims require** only the analysis of '**one or more nucleic acid molecules**,' not a large number of targets. . . . Thus, whether a POSA would have viewed it as difficult to perform **3D MERFISH for a large number of targets is not relevant** to the analysis here because the claims require the analysis of only a single target."

EX1086, ¶15

"[T]o 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**."

EX1086, ¶50

Petitioner is foreclosed from a new theory on motivation to select more complex microscope modality

NEW REPLY THEORY

Petition relies on modifying Chen's setup:

As documented above, Zhuang's system is built around an Olympus IX-71 optical microscope. For example, Zhuang discloses that its sample is "imaged on a home-built microscope constructed around an Olympus IX-71 body and a 1.45 NA, 100× oil immersion objective and configured for oblique incidence excitation." EX1003.011. As such, Zhuang discloses an optical system. EX1002 ¶¶ 163-164.

Pet., 28

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

Pet., 26

A POSA would have been motivated to combine the relevant teachings in Shen with Zhuang because Zhuang explicitly requires an autofocusing system for the MERFISH method. EX1003.011; EX1002 ¶ 186. A POSA would have had a reasonable expectation of success, because Shen teachings modifying the Olympus IX-71 microscope, the same one used in Zhuang, in the same way to accomplish the same goal. EX1002 ¶ 187.

Pet., 32

Petitioner's Reply raises a new theory:

known existed." *Id.* at 142:6-12. These methods included SIM, IPALM, PALM, STORM, and 3D SIM, as shown in the image above. *See id.* at 142:20-143:18. If a POSA were concerned about the PSF, he or she could have simply selected a microscopy modality with a sufficiently low PSF. *See* EX1086 ¶ 13

Pet. Reply, 18

Dr. Czymmek raises a new theory:

Shroff identified in his deposition, as I discuss above. Thus, to the extent a POSA was concerned with the PSF in attempting to adapt MERFISH to 3D, he or she could have simply selected a microscopy modality with a sufficiently low PSF. Beyond

EX1086, ¶13

PO Sur-Reply, 22-24

10X GENOMICS

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

No suggestion of a volumetric imaging system for determining 3D positional information of one or more nucleic acid molecules

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

There is no dispute that the goal of Chen's MERFISH is increased multiplexed imaging

Chen (Zhuang):

Single-molecule RNA imaging approaches, such as single-molecule fluorescence in situ hybridization (smFISH), are powerful tools for counting and mapping RNA; however, the number of RNA species that can be simultaneously imaged in individual cells has been limited. This makes it challenging to perform transcriptomic analysis of single cells in a spatially resolved manner. Here, we report multiplexed error-robust FISH (MERFISH), a single-molecule imaging method that allows thousands of RNA species to be imaged in single cells by using combinatorial FISH labeling with encoding schemes capable of detecting and/or correcting errors.

EX1003, 001

Last, we sought to further increase the throughput of our MERFISH measurement by simultaneously imaging ~1000 RNA species. This increase

EX1003, 007

Petitioner:

A POSA would have been motivated to use two or more colors of light as taught by Cai with the method of Zhuang because it would have further increased the multiplexity of barcoding, which would have furthered Zhuang's objective of "rapidly increas[ing] the number of RNA species that can be probed simultaneously." EX1003.002; EX1002 ¶¶ 173-174. A skilled artisan would

Pet., 30

Moffitt (Zhuang):

MERFISH significantly increases the multiplexing of smFISH measurements by utilizing a combinatorial labeling approach to associate unique barcodes with individual RNA species and then by reading out these barcodes through a series of sequential hybridization and imaging measurements. Combinatorial labeling approaches have been utilized previously to

EX1021, 003

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POR, 19-24, 36-38; EX2024, ¶¶39-49, 80-83; PO Sur-Reply, 14-17

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

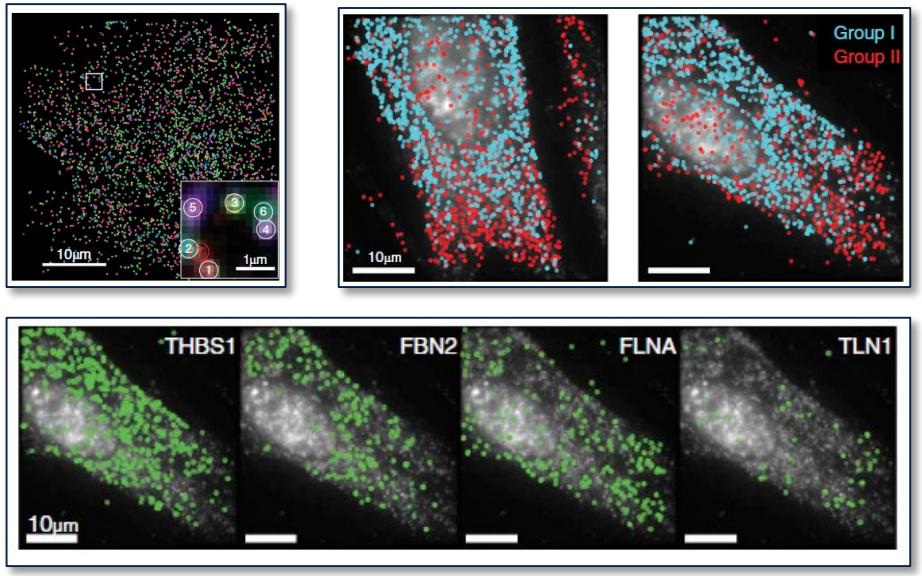
There is no dispute that all of Chen's MERFISH data are 2D data

Dr. Shroff:

"[Chen's] fluorescent images solely depict fluorescence imaging from **Chen's MERFISH setup in a 2D format**."

EX2024, ¶86

Chen:



EX1003, Figs. 2B, 4A, 4C

Petitioner:

"The authors of Chen acknowledged that **their initial experiments involved only 2D data**, which may have caused them to miss some targets."

Pet. Reply, 6

Dr. Czymmek:

"[T]he authors of Chen are stating that **the experiments they performed generated 2D data**, and that this may cause one to miss some of the targets."

EX1086, ¶32

"The Chen publication reports **2D data with MERFISH**…"

EX1086, ¶40

Chen's data analysis confirms the MERFISH experiments were setup for 2D

Chen:

Construction of measured words

Fluorescent spots were identified and localized in each image by using a multi-Gaussian-fitting algorithm (*38*) assuming a Gaussian with a uniform width of 167 nm. This algorithm was used

EX1003, 012

RNA. To determine the copy number per cell, the number of each RNA species was counted in individual cells within each 40- by 40-μm imaging area. This number accounts for the majority but not all RNA molecules within a cell because a fraction of the cell could be outside the imaging area or focal depth. Tiling images of adjacent areas and adjacent focal planes could be used to improve the counting accuracy.

EX1003, 012

Dr. Shroff:

"A POSA would have understood this disclosure to indicate that Chen's setup uses a **Gaussian function that only considers the 'width' parameter ('167 nm')** to localize RNA molecules in each image."

EX2024, ¶87

"A POSA would have understood that the application of a Gaussian function to adjacent 2D image areas (the '**40-by 40-μm imaging areas**' described above) teaches a setup for **determining 2D information** for imaged molecules rather than 3D information, as the **authors only contemplated the use of x- and y-axis information for the molecules** within these imaging areas."

EX2024, ¶87

Chen's disclosure of z-scanning is in reference to *counting* RNA molecules, not determining 3D positional information

Chen:

smFISH. For this purpose, we optically sectioned these cells by collecting stacks of images at different focal depths through the entire depth of the cells. We aligned the images in consecutive focal planes and then computed for each cell the fraction of RNAs that were detected in the three-dimensional stack but not in the basal focal plane. We found that only a small fraction, 15 ± 1% (mean ± SEM across six different RNA species) of RNA molecules were outside the imaging range of a fixed focal plane without *z*-scanning. These

EX1003, 012

Dr. Shroff:

"Chen expressly concludes that 'only a small fraction' of the RNA molecules were 'outside the imaging range of a fixed focal plane' targeted by Chen's MERFISH setup, ultimately **confirming** that—as predicted by the authors—optical sectioning and z-scanning were **unnecessary for Chen's 2D MERFISH approach**."

EX2024, ¶88

Chen's z-scanning quality control experiment

- Not MERFISH
- Purpose was to *count* RNA molecules
- Confirmed that z-scanning was *not necessary*

EX2024, ¶88

POR, 42-43; EX2024, ¶¶88-90; PO Sur-Reply, 8-12

Chen's disclosure of tiling refers to *counting* RNA molecules, not determining 3D positional information

Chen:

RNA. To determine the copy number per cell, the number of each RNA species was counted in individual cells within each 40- by 40-μm imaging area. This number accounts for the majority but not all RNA molecules within a cell because a fraction of the cell could be outside the imaging area or focal depth. Tiling images of adjacent areas and adjacent focal planes could be used to improve the counting accuracy.

EX1003, 012

Dr. Czymmek:

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, 57:13-19

Mere disclosure of z-scanning or tiling is *not* a suggestion of determining 3D positional information of nucleic acid molecules

Chen:

smFISH. For this purpose, we optically sectioned these cells by collecting stacks of images at different focal depths through the entire depth of the cells. We aligned the images in consecutive focal planes and then computed for each cell the fraction of RNAs that were detected in the three-dimensional stack but not in the basal focal plane. We found that only a small fraction, 15 ± 1% (mean ± SEM across six different RNA species) of RNA molecules were outside the imaging range of a fixed focal plane without *z*-scanning. These

EX1003, 012

RNA. To determine the copy number per cell, the number of each RNA species was counted in individual cells within each 40- by 40-μm imaging area. This number accounts for the majority but not all RNA molecules within a cell because a fraction of the cell could be outside the imaging area or focal depth. Tiling images of adjacent areas and adjacent focal planes could be used to improve the counting accuracy.

EX1003, 012

Dr. Shroff:

Q Now, in November 2015, were people of ordinary skill in the art aware of the concept of stacking focal claims 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

POR, 42-43; EX2024, ¶¶88-90; PO Sur-Reply, 8-12

POSAs knew that optical sectioning refers to *removing out-of-focus light* from other planes

Dr. Shroff:

"[A] POSA would have known that **optical sectioning** is a technique that allows for **removal of out-of-focus fluorescence** and helps to enhance contrast and improve image quality."

EX2024, ¶89

Conchello 2005:

CONFOCAL MICROSCOPY

Optical sectioning acquires images of thin slices of a thick specimen by removing the contribution of out-of-focus light in each image plane. This removal of unwanted light

EX2046, 920

Dobrucki 2007:

Consequently, extensive scattering of exciting light as well as emitted fluorescence occurs at the surface and inside live cells. In wide-field fluorescence microscopy scattered exciting light is not detected in the image, but scattered fluorescence is seen as a bright background. This background causes deterioration of image contrast and, thus, degrades image resolution. This

EX2076, 1778

Mere disclosure of optical sectioning is *not* a suggestion of determining 3D positional information of nucleic acid molecules

Dr. Shroff:

Q Did I read that correctly?

A You did. That is what that sentence says.

Q Okay. Let's go on to the next document.

A Of course. In order to use this technique, one would have to have had a two-photon activator dye, would have had to have known how to put these elements together. And it's unclear to me that this method would work, at all, for any of the dyes used in the body of work we consider today.

So, while it's true to say, as you just read, that the conventional temporal focusing microscopy, in principle, can allow optical sectioning of depths greater than a hundred micron, it's unclear and probably not true that this would have applied in the context of the '554 patent at all.

And furthermore, optical sectioning, as I detail in my declaration, is distinct from three-dimensional imaging. They're two separate concepts.

EX1082, 246:17-248:3

POR, 16-17, 42-43; EX2024, ¶¶36, 88-90; PO Sur-Reply, 10-12

Chen discloses optical sectioning for *removing out-of-focus light* from other planes

Chen:

molecules to be identified (*17*, *48*, *49*). In addition, increasing the number of readout sequences per encoding probe or using optical sectioning methods to reduce the fluorescence background may allow further reduction in the number of the encoding probes per RNA.

EX1003, 010

cells. From 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

Dr. Shroff:

"Chen's disclosure here simply **suggests the use of optical sectioning to remove background fluorescence** arising from planes above or below the 2D focal plane of the MERFISH setup in thick samples."

EX2024, ¶89

"A POSA would have understood that **Chen's discussion of fibroblast cells and optical sectioning suggested a 2D application of MERFISH** rather than using MERFISH to determine 3D positional information of individual nucleic acid molecules."

EX2024, ¶89

A POSA would have had no reason to modify Chen's 2D MERFISH setup into a 3D MERFISH imaging system

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

The test for obviousness is not merely what *could have* been done, it concerns what a POSA *would have* been motivated to do

"[The Board's] 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."

Personal Web Technologies, LLC v. Apple, Inc., 848 F.3d 987, 993-994 (Fed. Cir. 2017)

"[O]bviousness concerns whether a skilled artisan not only **could have made** but **would have been motivated to make** the combinations or modifications of prior art to arrive at the claimed invention."

Belden Inc. v. Berk-Tek LLC, 805 F.3d 1064, 1073 (Fed. Cir. 2015)

10X GENOMICS

PO Sur-Reply, 12-14

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner argues what a POSA *could have* done, failing to demonstrate that a POSA *would have* been motivated to do it

Pet. Reply:

approach based on widefield microscopy for 3D. Indeed, it was known that widefield could be used for 3D microscopy (including through the tiling of focal planes by z-axis control) and the '554 patent acknowledges that no new developments would be required. *See* EX1086 ¶¶ 40-47.

Pet. Reply, 13

known existed." *Id.* at 142:6-12. These methods included SIM, IPALM, PALM, STORM, and 3D SIM, as shown in the image above. *See id.* at 142:20-143:18. If a POSA were concerned about the PSF, he or she could have simply selected a microscopy modality with a sufficiently low PSF. *See* EX1086 ¶ 13

Pet. Reply, 18

Q. Now, this tiling method, where you tile the adjacent areas and the adjacent focal points, is that something that can be done using widefield microscopy?

A. Well, the success of that procedure will depend on how much out-of-focus light there is in the sample, sir.

Q. But it could be done depending on the sample type, correct?

A. Depending on the sample type, it could be done.

Pet. Reply, 12 (citing EX1082, 228:4-14)

PO Sur-Reply, 12-14

According to Petitioner, a POSA would modify Chen with Cai's "Z-axis motion control" and "two or more colors of light"

Two or more colors of light:

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

Pet., 29

encoded signals. *Id.* To the extent that Zhuang is deemed to not disclose the claimed method using two or more colors of light, it would have been obvious to modify Zhuang in view of Cai to include such a feature. *See supra* Section X.1.c.ii; EX1002 ¶ 213.

Pet., 41

Z-axis motion control system:

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

Pet., 32

According to Petitioner, Chen's setup already has a Z-axis motion control system

Chen:

Constant focus was maintained throughout the imaging process with a home-built, autofocusing system. Illumination was provided at 641, 561,

EX1003, 007

Petitioner:

Zhuang includes a Z-axis motion control system for moving a focal plane through the volume of the cell. As discussed above, Zhuang's system is built upon

Pet., 31

Dr. Czymmek:

179. It is my opinion that Zhuang teaches a Z-axis motion control system configured for Z-motion of an objective focal plane through said volume.

EX1002, ¶179

Dr. Czymmek:

Q. Yes. That's the sentence. Now, is it your opinion that Chen's auto focusing system includes a Z access motion control system?

A. Well, in order for you to make corrections in auto focus they would have to have the Z position element.

Q. Is that a yes?

A. That's a yes.

EX2082, 61:14-22

POR, 46-49; EX2024, ¶¶99-102; PO Sur-Reply, 17-18

Petitioner never explains *why* a POSA would have allegedly looked to Cai or Shen for a different (and redundant) Z-axis motion control system

Petitioner:

interrogation."). Thus, the theory of the Petition is not that Cai's array of features should be jammed into Chen's MERFISH, but simply that Cai's z-axis motion control should be applied to Chen to enable stacking of focal planes and hence 3D imaging.

Pet. Reply, 12

Dr. Czymmek:

imaging."). Thus, the specific feature I am proposing to use from Shen and/or Cai is a z-axis motion control system so that one can collect adjacent 2D focal planes to generate 3D images of a cell or tissue volume. My obviousness theory is not based

EX1086, ¶42

Dr. Shroff:

"**Chen's setup already had a 'home-built, autofocusing system**," and Dr. **Czymmek does not provide an explanation** as to why a POSA would have modified this setup with a different autofocus arrangement … Dr. Czymmek does not explain **why a POSA would be motivated** to modify the MERFISH setup in Chen to use a **different autofocusing system** when Chen already outlines an autofocusing system."

EX2024, ¶100

POR, 46-49; EX2024, ¶¶99-102; PO Sur-Reply, 17-18

According to Dr. Czymmek, Chen already suggests using two or more colors of light

Dr. Czymmek:

175. In fact, Zhuang teaches precisely this, stating that "[a]lthough an increase in the number of bits by adding more hybridization rounds would increase the data collection time and potentially lead to sample degradation, these problems could be mitigated by utilizing multiple colors to readout multiple bits in each round of hybridization." EX1003.008. Accordingly, it is my further opinion that even if

EX1002, ¶175

Chen suggests using two or more colors of light to *increase throughput* in its 2D imaging setup

Chen:

pability. Although an increase in the number of bits by adding more hybridization rounds would increase the data collection time and potentially lead to sample degradation, these problems could be mitigated by using multiple colors to readout multiple bits in each round of hybridization.

EX1003, 008

Last, we sought to further increase the throughput of our MERFISH measurement by simultaneously imaging ~1000 RNA species. This increase could be achieved with our MHD4 code by increasing the number of bits per code word to 32 while maintaining the number of 1 bits per word at four (Fig. 1B). This could be implemented by either increasing the number of hybridization round to 32 or maintaining 16 rounds of hybridization, but using two-color imaging in each round. We pursued an alternative approach that did not require an increase in the number of hybridizations or color channels by relaxing the error correction requirement but keeping the error-detection capability. For example, by reducing the

EX1003, 007

POR, 46-49; EX2024, ¶¶99-102; PO Sur-Reply, 17-18

Petitioner never explains *why* a POSA would have allegedly looked to Cai for two or more colors of light if Chen already discloses as much

Dr. Czymmek:

175. In fact, Zhuang teaches precisely this, stating that "[a]lthough an increase in the number of bits by adding more hybridization rounds would increase the data collection time and potentially lead to sample degradation, these problems could be mitigated by utilizing multiple colors to readout multiple bits in each round of hybridization." EX1003.008. Accordingly, it is my further opinion that even if

EX1002, ¶175

Dr. Shroff:

"**Chen had already suggested the use of 'two-color imaging in each round**' as an alternative method to 'increase the throughput of [their] MERFISH system.' EX1003, 007. Chen in fact made this alteration one year later when they reported improvements to the throughput of MERFISH. EX2053, 11048 ("to reduce measurement time, we used multicolor imaging."). Therefore, it is my opinion that **a POSA would not have had a reason to consult Cai's disclosures for any teaching of 'two or more colors of light'** to improve the throughput of Chen's 2D MERFISH setup."

EX2024, ¶102

Petitioner never explains *why* a POSA reading Chen would have consulted Cai for disclosures purportedly already taught in Chen

"This testimony … also **fails to 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 v. Verizon Communications, 694 F.3d 1312, 1327-1328 (Fed. Cir. 2012)

"[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)

POR, 46-49; EX2024, ¶¶99-102; PO Sur-Reply, 17-18

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

A POSA interested in Chen's MERFISH would not have looked to Cai because Cai's seqFISH methods are inadequate for multiplexing

Chen disparages the smFISH and seqFISH methods taught in Cai for their "limited" multiplexing capabilities:

Chen:

in different organisms (*14*, *15*). However, application of the smFISH approach to many systems-level questions remains limited by the number of RNA species that can be simultaneously measured in single cells. State-of-the-art efforts by 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 (*16*–*19*), yet many interesting biological questions would benefit from the measurement of hundreds to thousands of RNAs within a single cell. For ex-

EX1003, 002

16. J. M. Levsky, S. M. Shenoy, R. C. Pezo, R. H. Singer, Single-cell gene expression profiling. *Science* **297**, 836–840 (2002). doi: 10.1126/science.1072241; pmid: 12161654
17. E. Lubeck, L. Cai, Single-cell systems biology by super-resolution imaging and combinatorial labeling. *Nat. Methods* **9**, 743–748 (2012). doi: 10.1038/nmeth.2069; pmid: 22660740 — EX2051
18. M. J. Levesque, A. Raj, Single-chromosome transcriptional profiling reveals chromosomal gene expression regulation. *Nat. Methods* **10**, 246–248 (2013). doi: 10.1038/nmeth.2372; pmid: 23416756
19. E. Lubeck, A. F. Coskun, T. Zhiyentayev, M. Ahmad, L. Cai, Single-cell in situ RNA profiling by sequential hybridization. *Nat. Methods* **11**, 360–361 (2014). doi: 10.1038/nmeth.2892; pmid: 24681720 — EX2052

EX1003, 014

POR, 49; EX2024, ¶102

The Petition's relied-upon sentence in Cai for determining 3D positional information of nucleic acid molecules is no different than general knowledge in the art that 3D imaging techniques existed

Cai:

In some embodiments, an imaging step detects a target. In some embodiments, an imaging step localizes a target. In some embodiments, an imaging step provides three-dimensional spatial information of a target. In some embodiments, an imaging step quantifies a target. By using multiple

EX1004, 21:50-51; Pet., 39

Dr. Shroff:

"The **single generic sentence in Cai** would not have given a POSA the motivation or rationale to modify Chen's 2D MERFISH setup to arrive at the claimed system of the 554 patent. Rather, Cai's single sentence is **no different than citing the background knowledge in the art**."

EX2024, ¶103

Dr. Shroff:

"Cai's single sentence does not indicate how **difficult** of a feat if would be to **modify Chen's 2D MERFISH** imaging setup into a volumetric imaging system for 3D imaging in view of Cai due to the **lack of axial resolution,** the problem of **3D photobleaching,** and the **poor SNR and SBR** for 3D applications of **Chen's highly multiplexed MERFISH setup**. Nor does Dr. Czymmek provide any explanation in this regard."

EX2024, ¶103

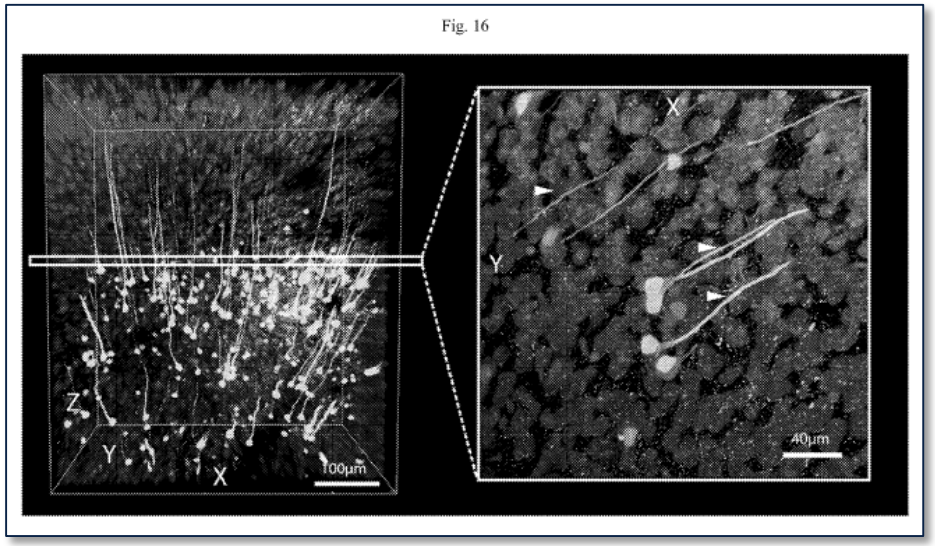
POR, 49; EX2024, ¶102

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner's new evidence in Reply does not provide any motivation to modify Chen's 2D MERFISH setup

NEW REPLY EVIDENCE

Cai Fig. 16:



EX1004, Fig. 16

FIG. **16**. CLARITY with Niss1: 1 mm-thick coronal section (Bregma AP, 2.3-1.3 mm) of a Thy-1-eYFP mouse brain was cleared and stained with NeuroTrace fluorescent Niss1 stain (1:100 dilution, 48 hours, RT). Left, 3d coronal rendering of the motor cortex. Right, 100 μm-thick section of layer V motor cortex. Arrows indicate apical dendrites of the pyramidal neurons (Red-Fluorescent Niss1, Green-eYFP).

EX1004, 11:17-24

eYFP = **fluorescent protein**, not nucleic acid probes

Nissl = fluorescent **stain for neurons**, not nucleic acid probes

EX2082, 82:23-83:8

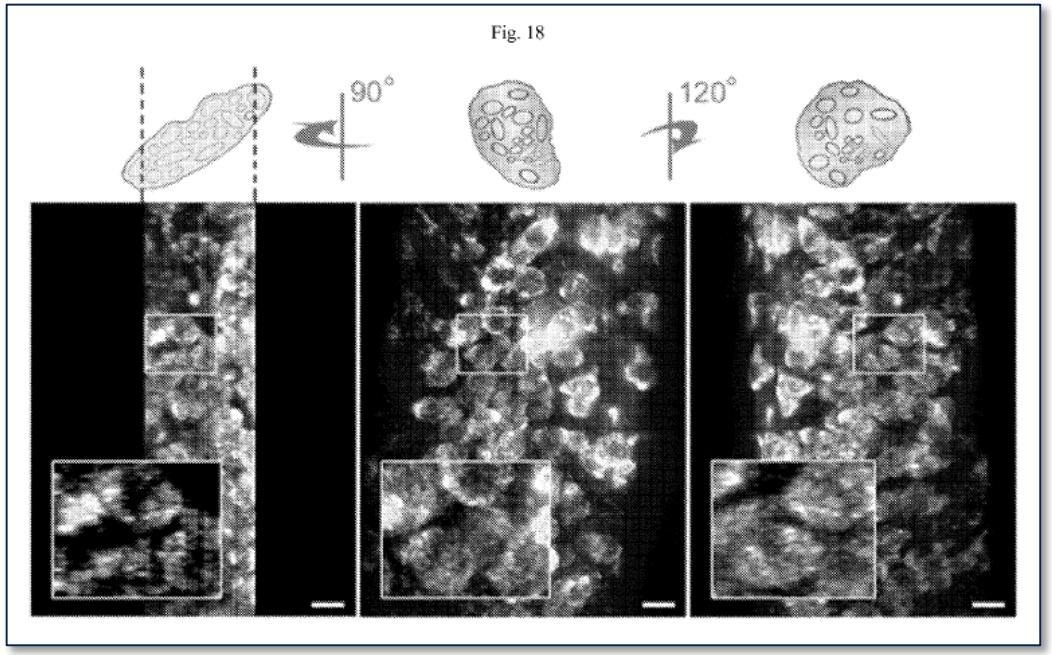
PO Sur-Reply, 18-19; Pet. Reply, 9-10

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner's new evidence in Reply does not provide any motivation to modify Chen's 2D MERFISH setup

NEW REPLY EVIDENCE

Cai Fig. 18:



EX1004, Fig. 18

FIG. **18**. SPIM detects single mRNAs in 100 μm CLARITY brain slices. The slice was scanned over 100 μm. The images were then registered and stitched to a 3D reconstruction. Diffraction limited dots in the image corresponded to single β-Actin mRNAs detected by HCR. Scale bar is 15 μm.

EX1004, 11:27-32

β-actin = single target (**not MERFISH**); single fluorescence output

SPIM = **light sheet** microscope

HCR = requires **amplification**

EX2082, 82:23-83:8

PO Sur-Reply, 18-19; Pet. Reply, 9-10

Petitioner's Reply argument that Cai teaches "extensive actual 3D imaging data" is improper new argument

NEW REPLY EVIDENCE

"**[Petitioner] chose which grounds of invalidity to assert in its petition** and it chose not to assert this new one. Unlike district court litigation — where parties have greater freedom to revise and develop their arguments over time and in response to newly discovered material — the expedited nature of IPRs bring with it an **obligation for petitioners to make their case in their petition to institute**. While the Board's requirements are strict ones, they are **requirements of which petitioners are aware** when they seek to institute an IPR.

Intelligent Bio-Sys. v. Illumina Cambridge, 821 F.3d 1359, 1369 (Fed. Cir. 2016)

[T]he Board did not err in **refusing the reply brief** as improper under 37 C.F.R. § 42.23(b) because **[Petitioner] relied 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

"Rather than explaining how its original petition was correct, Continental's subsequent arguments amount to an entirely new theory of *prima facie* obviousness absent from the petition. **Shifting arguments** in this fashion is **foreclosed by statute, our precedent, and Board guidelines**. *See* 35 U.S.C. § 312(a)(3)…."

Wasica Fin. GmbH v. Cont'l Auto. Sys., Inc., 853 F.3d 1272, 1286 (Fed. Cir. 2017)

PO Sur-Reply, 18-19; Pet. Reply, 9-10

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Cai's light sheet imaging system is a completely different setup compared to Chen's 2D MERFISH setup

Cai:

Light sheet microscopy is applied to directly image CLARITY cleared brains slices. In some embodiments, a mouse brain is mapped in 1 month per machine. In some embodiments, a mouse brain is mapped in one week with 4-5 machines.

Amplification: Amplification of FISH signals allows large FOV imaging of brain slices with 20× low NA objectives. In

EX1004, 44:29-35

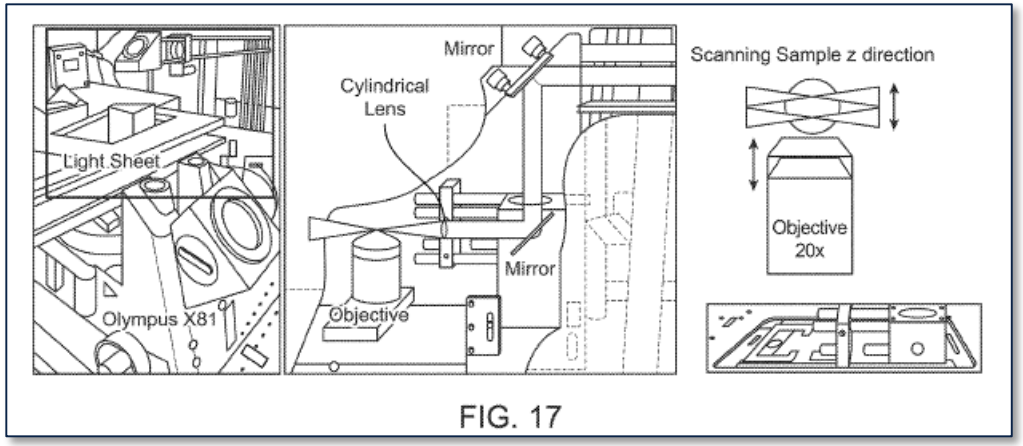


FIG. 17

EX1004, Fig. 17

Chen:

pump (Rainin, Dynamax RP-1). The sample was imaged on a home-built microscope constructed around an Olympus IX-71 body and a 1.45 NA, 100× oil immersion objective and configured for oblique incidence excitation. The objective was

EX1003, 011

Cai's light sheet	Chen's wide-field
Low NA	High NA
20x objective	100x oil immersion objective
Large working distance	Short working distance

POR, 44-46; EX2024, ¶¶93-98; PO Sur-Reply, 18-19

“Bodily incorporation” is not required for obviousness, but Petitioner is still required to provide articulated reasoning with rational underpinning for obviousness

“[R]ejections on **obviousness** grounds **cannot be sustained by mere conclusory statements**; instead, there must be **some articulated reasoning** with **some rational underpinning** to support the legal conclusion of obviousness.”

In re Kahn, 441 F.3d 977, 988 (Fed. Cir. 2006)

POR, 54-56; PO Sur-Reply, 19

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

No motivation to modify Chen's 2D MERFISH setup into a 3D MERFISH system because signal density would limit the ability to multiplex

Chen:

As 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. On the basis of our imaging

EX1003, 008

Lubeck 2012:

Barcoding with conventional fluorescence microscopy is only useful in cases where transcript levels are low. When the density of transcripts is high, diffraction-limited fluorescent spots of the same color will overlap and make barcode readout impossible.

EX2051, 3

Lee 2014:

(*11*). To obtain a spot density that is high enough to yield statistically significant RNA localization, and yet sufficiently low for discerning individual molecules, we developed partition sequencing, in which preextended sequencing primers are used to reduce the number of molecular sequencing reactions through random mismatches at the ligation site (Fig. 2A). Progressively longer sequenc-

EX2050, 1361

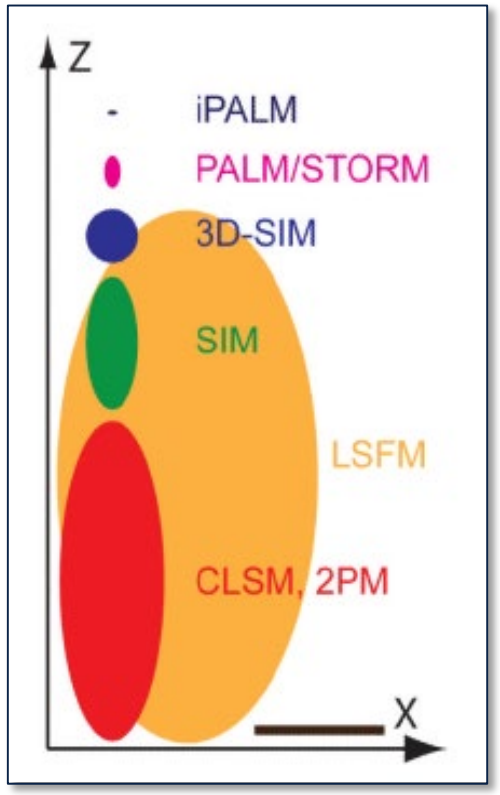
Dr. Shroff:

"A POSA would understand that for any attempts to modify Chen's 2D MERFISH setup into a volumetric imaging system for determining three-dimensional positional information of nucleic acid molecules, a POSA **would have to reduce multiplexity** to improve resolution and limit complexity, thereby **destroying the basic objective of MERFISH**."

EX2024, ¶105

Signal density further complicates poor axial resolution due to z-stretch of the PSF

Fischer:



EX1019, Fig. 1

Dr. Czymmek:

Q. Okay. Thanks. Do you have an understanding of the term point spread function in the context of imaging?

A. I do.

Q. Would you please tell me what that is?

A. So in reference to a microscope and I'm going to use an example here, if you put a small sub-resolution bead -- and I'm going to, again, give simply an example. Fluorescent bead of around 100 nanometers for a typical microscope.

That bead will have a spread to it that's related to defraction. So this is what's limiting the resolution of the light microscope, and every microscope has an optical transfer function which depends on the objective lens and so forth.

The aberrations as it's looking through the sample. So point spread function basically looks like an hourglass or football shape in the Z-axis, but it also has these defraction patterns which cause an actual round object to look differently. Again, typically an hourglass or football shaped is how it's referred to in three dimensions.

EX2043, 43:17-44:14

A POSA would not have had a reasonable expectation of success

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Chen's wide-field imaging setup was known to have poor axial resolution and to illuminate planes above and below the focal plane

Chen:

tification controls (table S1). After each round of hybridization with the fluorescent readout probes, cells were imaged by means of conventional wide-field imaging with an oblique-incidence illumination geometry. Fluorescent spots corresponding

EX1003, 003

Chen:

Because we imaged cells using wide-field imaging with oblique-incidence illumination, without optical sectioning and *z*-scanning, we quantified the fraction of individual RNA species that was outside the axial range of our imaging geometry for six different RNA species using conventional smFISH. For this purpose, we optically sectioned

EX1003, 012

Conchello:

One problem with fluorescence microscopy is that, regardless of where the microscope is focused vertically in a specimen, illumination causes the entire specimen thickness to fluoresce. Thus, it is not unusual that in a given two-dimensional (2D) image more than 90% of fluorescence is out-of-focus light that can completely obscure the in-focus detail and greatly reduce the contrast of what remains. For example, a fluores-

EX2046, 920

North:

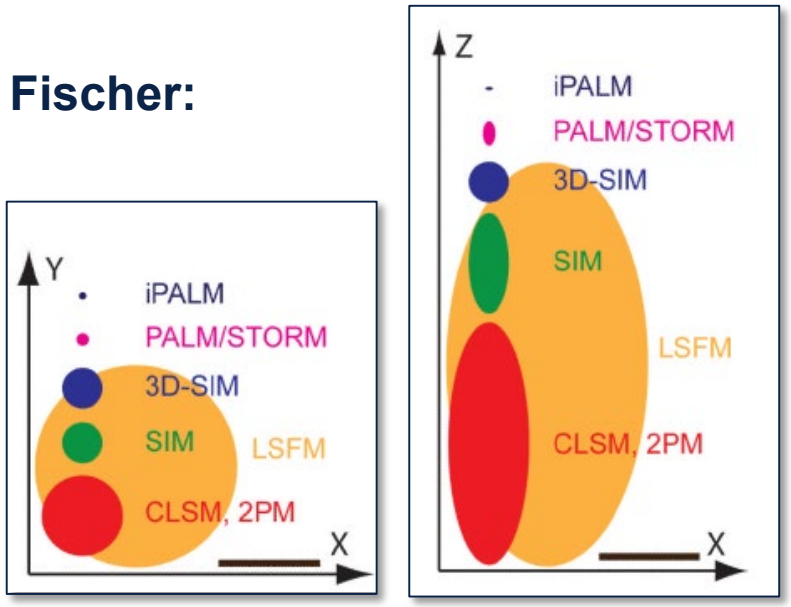
However, wide-field images can provide clear lateral (x- and y-axis) information but only limited axial (z-axis) information. For specimens thicker than a few micrometers, or when precise axial information is required, an instrument that removes out-of-focus blur and permits you to distinguish between the signals in thin "optical" slices may therefore prove superior. Current technologies to

EX1012, 004

POR, 56-58; EX2024, ¶¶110-114; PO Sur-Reply, 19-24

Axial resolution was much more challenging and complex than lateral resolution due to z-stretch of the point spread function

Fischer:



EX1019, Fig. 1

Dr. Shroff:

"[R]resolving individual point sources of light **along the z-axis** was understood to be **more challenging and complex** than resolving individual point sources of light in the lateral (x-y) range, as the **elongated PSF along the z-axis** contributes to greater **overlap with the diffraction patterns** from other point sources along the z-axis and a concomitant increased **difficulty in resolving individual point sources** along that axis."

EX2024, ¶24

objective lens; see Box 1 and Figure 1). The characteristic anisotropy of the PSF is seen as "Z-stretch" in XZ-projections of reconstructed 3D image sets obtained with uni-axial fluorescence microscopy. This reduced axial resolution and Z-stretch can introduce profound errors in tracking of objects in 4D and in co-localization in multichannel 3D imaging [1].

EX1019, 001

POR, 56-58; EX2024, ¶¶110-114; PO Sur-Reply, 19-24

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Axial resolution was well known to be 2-3 times worse than lateral resolution

Waters:

2006). Resolution in the z-axis (z) is worse than lateral resolution in the light microscope, and is given by

$$z = \frac{2\lambda\eta}{(NA)^2},$$

where η is the refractive index of the specimen. It is important to understand

EX2045, 1141

Toomre:

Resolution can be quantified by analyzing a microscope's point-spread function (PSF), which effectively describes how blurry a point-like object (a single molecule or small fluorescent bead) (**Figure 1***b*) will appear. A cross

high NA. The axial resolution can be approximated by the formula $d_z \approx n\lambda_0/NA^2$, with n being the refractive index, and is typically 2 to 3 times worse than the lateral resolution. λ_0

EX1018, 004

Fischer:

The first and most obvious challenge of 3D imaging is that in conventional, uni-axial microscopy arrangements, the resolution along the Z-axis of the microscope is substantially (~2x) worse than in the lateral dimensions due to the inherent shape of the point spread function (PSF; the three-dimensional diffraction pattern of a point source viewed through an objective lens; see Box 1 and Figure 1). The characteristic anisotropy of the PSF is seen as "Z-stretch" in XZ-projections of reconstructed 3D image sets obtained with uni-axial fluorescence microscopy. This reduced axial resolution and Z-stretch can introduce profound errors in tracking of objects in 4D and in co-localization in multichannel 3D imaging [1].

EX1019, 001

Signal-to-noise (SNR) and signal-to-background (SBR) would further complicate single molecule localization in 3D MERFISH

Waters:

level (Fig. 2 A). Noise causes imprecision in measurements of pixel intensity values, and therefore a level of uncertainty in the accuracy of the measurements. To detect the presence of a signal, the signal must be significantly higher than the noise level of the digital image

EX2045, 1136

of the digital image. SNR affects spatial measurements as well as intensity measurements; precise determination of the location of a fluorescently labeled object depends on SNR (Fig. 2, D and E)

EX2045, 1136

(Hiraoka et al., 1990). However, illuminating light above and below the image focal plane excites fluorophores above and below the image focal plane. Light emitted from these out-of-focus fluorophores is collected by the objective lens, and appears as out-of-focus background in the in-focus image of the specimen. In

EX2045, 1139

Dr. Shroff:

"The **background fluorescence** caused by out-of-focus fluorophores **impairs the quality of the image** obtained from fluorescence imaging by increasing the amount of noise in the sample, thereby **decreasing the SNR**."

EX2024, ¶34

No reasonable expectation of successfully revamping Chen's 2D MERFISH setup into a 3D MERFISH system that retains high multiplexed imaging

Single molecule imaging factors: Axial resolution, PSF, SNR, SBR, photobleaching

Chen's 2D MERFISH
- Highly multiplexed single molecule localization in 2D
- No suggestion to perform 3D MERFISH

3D MERFISH
- Highly multiplexed single molecule localization in 3D

Dr. Shroff: "[A] POSA would **not have had a reasonable expectation of success** because of the **lack of axial resolution**, the problem of **3D photobleaching**, and **poor SNR and SBR** for 3D applications in Chen's highly multiplexed MERFISH setup designed for 2D applications in thin cells, all of which would be **exacerbated in an attempt at modification for 3D imaging** in view of Cai."

EX2024, ¶111

POR, 56-58; EX2024, ¶¶110-114; PO Sur-Reply, 19-24

PSF, SBR, and SNR *are* highly relevant when imaging *any* type of sample, be it thick, thin, or small

Petitioner's argument that PSF, SBR, and SNR are only pertinent when imaging thicker samples is incorrect

Issues relevant to PSF when imaging small or thin samples	Issues relevant to SNR when imaging small or thin samples	Issues relevant to SBR when imaging small or thin samples
coverslip distance	aperture size	Autofluorescence
numerical aperture	signal amplification	Non-specific probe binding
refractive index of mounting media	probe brightness	
refractive index of immersion media	exposure time	
scattering properties of the sample	light intensity	
light wavelength	photobleaching	
deconvolution methods		
aperture size		
EX2082, 88:12-92:21	*EX2082, 94:12-100:2*	*EX2082, 100:3-104:16*

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner's Reply belatedly argues that a POSA *could have* selected a different microscopy modality

NEW REPLY THEORY

Petition relies on modifying Chen's setup:

As documented above, Zhuang's system is built around an Olympus IX-71 optical microscope. For example, Zhuang discloses that its sample is "imaged on a home-built microscope constructed around an Olympus IX-71 body and a 1.45 NA, 100× oil immersion objective and configured for oblique incidence excitation." EX1003.011. As such, Zhuang discloses an optical system. EX1002 ¶¶ 163-164.

Pet., 28

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

Pet., 26

A POSA would have been motivated to combine the relevant teachings in Shen with Zhuang because Zhuang explicitly requires an autofocusing system for the MERFISH method. EX1003.011; EX1002 ¶ 186. A POSA would have had a reasonable expectation of success, because Shen teachings modifying the Olympus IX-71 microscope, the same one used in Zhuang, in the same way to accomplish the same goal. EX1002 ¶ 187.

Pet., 32

Petitioner's Reply raises a new theory:

known existed." *Id.* at 142:6-12. These methods included SIM, IPALM, PALM, STORM, and 3D SIM, as shown in the image above. *See id.* at 142:20-143:18. If a POSA were concerned about the PSF, he or she could have simply selected a microscopy modality with a sufficiently low PSF. *See* EX1086 ¶ 13

Pet. Reply, 18

Dr. Cyzmmek raises a new theory:

Shroff identified in his deposition, as I discuss above. Thus, to the extent a POSA was concerned with the PSF in attempting to adapt MERFISH to 3D, he or she could have simply selected a microscopy modality with a sufficiently low PSF. Beyond

EX1086, ¶13

PO Sur-Reply, 22-24

10X GENOMICS

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner's Reply argument that a POSA *could have* selected a different microscopy modality is improper new argument

NEW REPLY THEORY

"**[Petitioner] chose which grounds of invalidity to assert in its petition** and it chose not to assert this new one. Unlike district court litigation — where parties have greater freedom to revise and develop their arguments over time and in response to newly discovered material — the expedited nature of IPRs bring with it an **obligation for petitioners to make their case in their petition to institute**. While the Board's requirements are strict ones, they are **requirements of which petitioners are aware** when they seek to institute an IPR.

Intelligent Bio-Sys. v. Illumina Cambridge, 821 F.3d 1359, 1369 (Fed. Cir. 2016)

"Rather than explaining how its original petition was correct, Continental's subsequent arguments amount to an entirely new theory of *prima facie* obviousness absent from the petition. **Shifting arguments** in this fashion is **foreclosed by statute, our precedent, and Board guidelines**. *See* 35 U.S.C. § 312(a)(3)…."

Wasica Fin. GmbH v. Cont'l Auto. Sys., Inc., 853 F.3d 1272, 1286 (Fed. Cir. 2017)

[T]he Board did not err in **refusing the reply brief** as improper under 37 C.F.R. § 42.23(b) because **[Petitioner] relied 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

10X GENOMICS

PO Sur-Reply, 1-2, 22-24

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner fails to explain *how* a POSA would have modified Chen's imaging setup in view of any of the new microscopy modalities

NEW REPLY THEORY

Fischer:

Mode	Best suited applications	Advantages for 3D Biology	Limitations
		***	
3D SIM*	Macromolecular assemblies and organelles with relatively slow dynamics, e.g.chromatin remodeling. Low scattering samples best.	Resolution ~2x better than diffraction-limited, light efficient	Slow speed, photodamage possible, limited imaging depth, multiple raw images needed per 3D-SIM* image, shot noise not removed, complex optical configuration
3D STORM*/ 3D PALM	Macromolecular assemblies and organelles in fixed difficult to image by diffraction limited imaging., e.g., microbial structure, membranous vesicles.	Resolution ~10x better than diffraction limited, amenable to whole cell imaging, simple optical configuration	Limited imaging depth, slow speed, reducing out-of-focus activation requires either TPM* or light sheet illumination, limited subset of dyes/PA-FPs*, mostly fixed cells
iPALM*	Cell surface-associated macromolecular assemblies and organelles with complex structure in fixed samples: e.g., membrane-bound protein complexes; e.g., adhesion organelles, immune synapses.	Ultra-high (< 20 nm) resolution in 3D, possible to determine protein orientation or conformational change.	Very limited imaging depth, slow speed, limited subset of dyes/PA-FPs*,fixed samples only, complex optical configuration

EX1019, Table 1

Dr. Czymmek admitted that the newly raised microscopy modalities:

- Require specific and **unique optical configurations**
- Require **unique** subsets of **dyes or fluorophores**
- Require **special buffers**
- Use completely **different probes**
- Image proteins (e.g, PALM), **not nucleic acid molecules**
- Are **tedious** and **time-consuming**
- Carry risk of **photobleaching**

EX2082, 116:18-24, 127:7-9, 129:25-130:5, 132:9-133:9, 133:10-21, 136:4-14, 137:8-10, 139:1-12, 141:14-25, 146:20-147:8, 154:18-156:6, 162:7-163:17 .

Petitioner's art combination fails to teach or suggest a temperature control apparatus

"Temperature control apparatus" must have the ability to actively heat and actively cool the sample holder

Claim 36:

(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

EX1001, 33:33-34

Dr. Shroff:

"A POSA would have understood the phrase '**temperature control**' . . . to mean the **ability** to **actively heat and actively cool** an apparatus."

EX2024, ¶61

"A POSA would have recognized that the '**temperature control apparatus**' is operatively coupled to the sample holder . . . and therefore must be **capable** of **actively heating and cooling the temperature of the sample holder**."

EX2024, ¶64

"An apparatus that can **only heat** the sample holder—without the ability to cool the sample holder—**cannot 'control' the sample holder's temperature as described in the 554 patent**, because it is unable to cool the sample holder."

EX2024, ¶64

The intrinsic evidence supports Patent Owner's construction

Claim language:

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

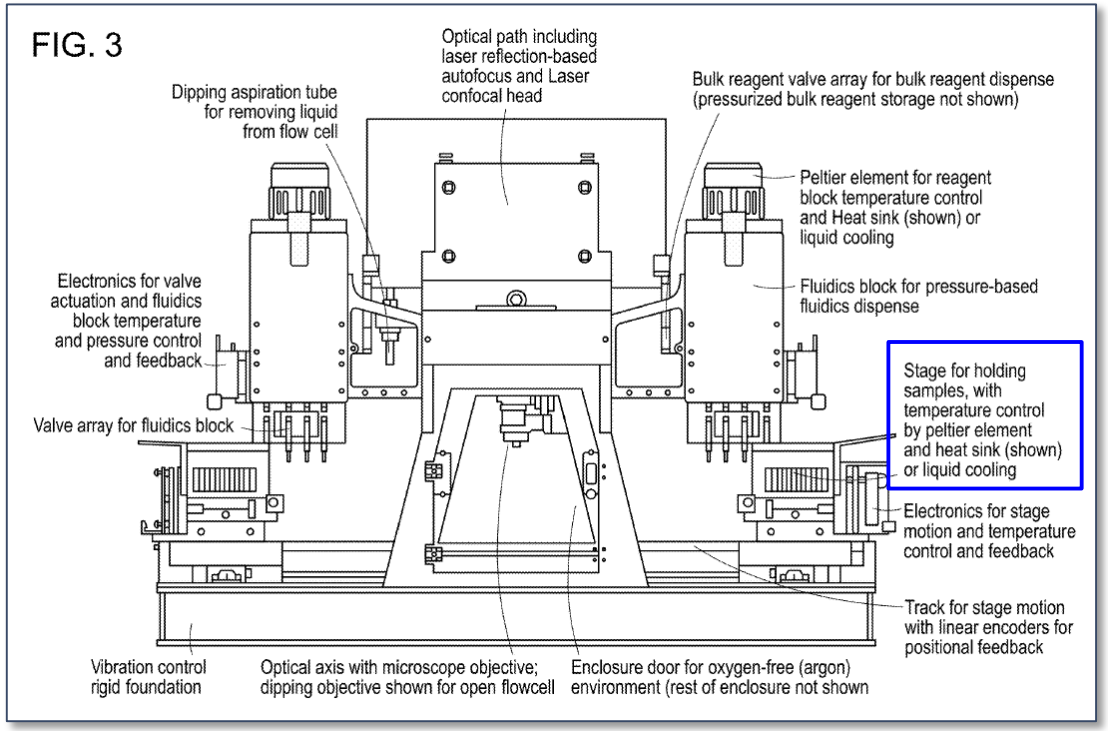
EX1001, 34:31-32

Petitioner:

"**[Heat] sink or a Peltier element** for temperature control . . . These components have **cooling capabilities**."

Pet., 43

Specification:



EX1001, FIG. 3; see also id., 9:34-41, 18:5-10,19:61-62, 26:28-29, 26:36, 27:22-23, 26:35-37. FIG. 5

POR, 27-29; EX2024, ¶¶60-64

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

The 554 specification repeatedly and consistently describes an apparatus having the ability to actively heat and actively cool

"[A POSA] is deemed to read the claim term not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the **specification**."

Phillips v. AWH Corp., 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc)

"[W]hile it is of course improper to limit the claims to the particular preferred embodiments described in the specification, **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)

Petitioner distorts Patent Owner's construction as constantly requiring active heating and active cooling

Dr. Czymmek:

"**[T]he specification states** that the "stage **may** include a heating **or** cooling apparatus where the stage may be heated **or** cooled (such as through thermoelectric cooling using Peltier elements)." . . . Stating that the stage "may" include heating "or" cooling, the specification is clear that **there is no requirement for both heating and cooling**."

EX1086, ¶22; see also Pet. Reply, 3-4

Petitioner:

"**Nothing** in the claim language **requires** "temperature control" to include **both** active heating ***and*** active cooling."

"But just because **part** of a **dependent claim** refers to a particular type of active cooling system **does not mean** that "temperature control" **in independent claim 36 *always* requires active cooling**."

Pet. Reply, 2-3; see also EX1086, ¶21

Patent Owner's construction:

"A POSA would have understood the phrase "temperature control" . . . to mean the **ability** to **actively heat and actively cool** an apparatus."

EX2024, ¶61

Petitioner ignores the word "ability" in Patent Owner's construction

10X GENOMICS

PO Sur-Reply, 5

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner's construction of "temperature control" should be rejected

Dr. Czymmek:

"The concept of **'temperature control'** merely means an **ability to adjust or control temperature**. 'Temperature control' can be achieved many ways, including, for instance, by using **only active heating**: one can heat a sample to a desired temperature, and then **apply additional heat as needed to maintain the temperature** to the extent the sample cools through passive cooling."

EX1086, ¶18; see also Pet. Reply, 2

- Petitioner construction **"is circular"** and "relies on the [claim term control] itself." *Rhode & Schwarz GmBH & Co., KG v. Tektronix, Inc., IPR2018-00643, Paper 41, 14 (P.T.A.B. Sept. 10, 2019)*
- Petitioner's construction ignores the repeated teachings in the 554 specification

Even under Petitioner's construction, Sivaramakrishnan does not show that Chen's FCS2 flow cell has the capability to actively heat

Petition:

"[Chen] discloses that its stage includes a **Bioptechs FCS2 flow chamber**, which is a system that includes temperature control capability. . . . This is taught, for instance, in **Sivaramakrishnan**, published in 2009 and addressing similar types of experiments with the Bioptechs technology. EX1002 ¶ 142; EX1007.004 ('The Bioptechs FCS2 chamber maintains the cells and media at 37°C.')."

Pet., 24

No evidence that Chen necessarily taught or suggested the same FCS2 flow cell as disclosed in Sivaramakrishnan. *Par Pharm., Inc. v. TWI Pharms., Inc., 773 F.3d 1186, 1195-96 (Fed. Cir. 2014)*

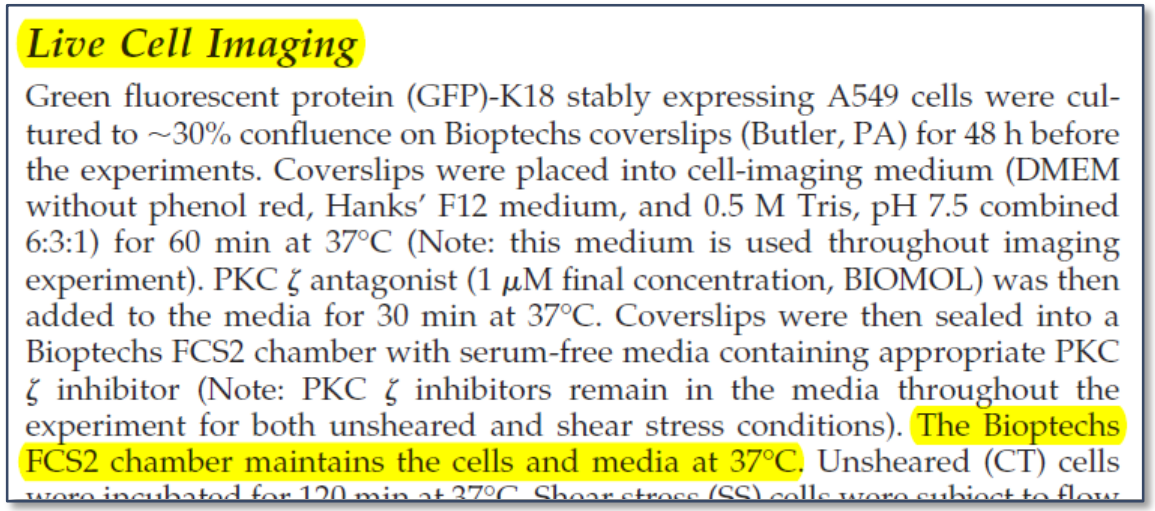
Dr. Shroff:

"Chen does not disclose using any temperature control associated with the FCS2 flow chamber."

EX2024., ¶70

"**Sivaramakrishnan** used an FCS2 flow cell for **imaging live cells**, and thus needed to maintain the FCS2 flow cell at 37°C to keep the cells alive. . . . In contrast, **Chen's 2D MERFISH** experiments **imaged fixed cells**, which do not need to be maintained at 37°C."

Id.



Live Cell Imaging

Green fluorescent protein (GFP)-K18 stably expressing A549 cells were cultured to ~30% confluence on Bioptechs coverslips (Butler, PA) for 48 h before the experiments. Coverslips were placed into cell-imaging medium (DMEM without phenol red, Hanks' F12 medium, and 0.5 M Tris, pH 7.5 combined 6:3:1) for 60 min at 37°C (Note: this medium is used throughout imaging experiment). PKC ζ antagonist (1 μM final concentration, BIOMOL) was then added to the media for 30 min at 37°C. Coverslips were then sealed into a Bioptechs FCS2 chamber with serum-free media containing appropriate PKC ζ inhibitor (Note: PKC ζ inhibitors remain in the media throughout the experiment for both unsheared and shear stress conditions). The Bioptechs FCS2 chamber maintains the cells and media at 37°C. Unsheared (CT) cells

EX1007, 004

POR, 32-33

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Even under Petitioner's construction, Su does not show that Chen's FCS2 flow cell has the capability to actively heat

Petitioner's Reply:

"[A] 2020 publication by **Su et al**.—which was submitted by PO—and **provides the serial number for the Bioptechs FCS2 flow cell system used in Chen**. . . . this serial number corresponds to the very Bioptechs FCS2 system that was relied upon in the Petition and that undeniably **includes temperature control**."

Pet. Reply, 4-5

Petitioner did not argue that Chen's FCS2 flow cell would have been modified to include temperature control

Su:

"All fluid exchanges in this part of the protocol were achieved via the use of a **custom-built fluidics system (Chen et al., 2015)**, with the coverslip mounted in a **FCS2 flow chamber (Bioptechs, 060319-2)**."

EX2009., e7

Chen:

"The sample coverslip was assembled into a **Bioptech's FCS2 flow chamber**, and the flow through this chamber was controlled via a **homebuilt fluidics system composed of three computer-controlled eight-way valves** (Hamilton, MVP and HVXM 8-5) and a **computer-controlled peristaltic pump** (Rainin, Dynamax RP-1)."

EX1003., 011

There is no dispute that Chen's *FCS2 flow cell* lacks the capability to actively cool

Dr. Czymmek:

Q. Let me rephrase the question. How does **Chen's system maintain a temperature** of 37 degrees celsius?

A. It will have a sensor that is reading the current temperature. And if, you know, it's not going to get hotter if the room is not hotter. So it may **turn down the current or turn off the current** to stay within the window of the controlled temperature device and the set point that they maintained.

EX2082, 31:15-25; see also id., 24:8-14, 32:1-7, 33:9-34:1

Q. But for the record, you do agree that that's **passive cooling when it turns off the current**?

A. **Yeah**. If it's not pulling away the heat in an active way using an electronic mechanism, it would be passive, but it's still temperature control.

EX2082, 35:14-20

Dr. Shroff:

"Even if Chen did teach or suggest using an **FCS2 flow chamber** capable of heating and maintaining 37ºC, a POSA would not have understood the system to constitute a "temperature control apparatus," as it would only be capable of raising temperature above ambient temperature, **not actively lowering temperature** below ambient temperatures."

EX2024, ¶71

POR, 33-34; Pet. Reply, 5; PO Sur-Reply, 6-7

There is no dispute Chen's *objective heater* lacks the capability to actively cool

Petition:

"While **PO again** contends that the **objective heater** is not 'temperature control' because it lacks active cooling, see POR at 34, this **relies upon an erroneous construction** of 'temperature control.'"

Pet. Reply, 5

Dr. Czymmek:

"Dr. Shroff again contends that the **objective heater** is not 'temperature control' because it provides only heating and not active cooling. EX2024 ¶ 72. This, however, relies upon the contention that "temperature control" requires active cooling. As I explain above, it is my opinion that this **interpretation of "temperature control" is erroneous**."

EX1086, ¶31

Dr. Shroff:

"[T]he **'objective heater' disclosed in Chen is merely a heating element** rather than a 'temperature control,' as claimed. . . . the instructions for the FCS2 controller indicate that the 'Bioptechs Objective Heater' of the FCS2 comprises a 'heating band and temperature sensor' and includes **no mention of any cooling capacity associated with the objective heater**. EX1006, 006."

EX2024, ¶72

POR, 34; PO Sur-Reply, 6-7

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Chen's objective heater is also not operatively coupled to a sample holder

Petition:

"[Chen] further discloses that its optical system makes use of an oil immersion objective 'heated to 37°C with a Bioptechs objective heater.' . . . **[T]he objective is in thermal contact** with the stage holding the sample, such that the objective heater is an additional temperature control system that becomes coupled to the stage and sample holder."

Pet., 24-25

Dr. Shroff:

"The FCS2 controller instructions teach that [Chen's] **objective heater 'plugs into a separate Temperature Controller** which regulates the objective temperature within 0.1ºC of SETPOINT.' EX1006, 006. This description would teach a POSA that the **objective heater in Chen was operatively coupled to the microscope objective** (the objective being an element recited in **claim 36(c)(i)**) and a separate objective temperature controller, **but not** operatively coupled to the **sample holder as required by claim 36(a)(ii)**."

EX2024, ¶73

Dr. Shroff:

7	Q	Are you familiar with oil immersion
8	lenses?	
9	A	Yes, I am, sir.
10	Q	Is it accurate that in those lenses, an
11	oil droplet is placed in the front of the lens and	
12	it's moved to contact the cover slip for the	
13	sample?	
14	A	Yeah. That is accurate, sir.

EX1082, 82:7-14

Objective Heater Description:

The Bioptechs Objective Heater is an adjustable, thin film, size and shape compliant, heating band and temperature sensor assembly attached to a linear translator. This device attaches to the objective and plugs into a separate Temperature Controller, which regulates the objective temperature within 0.1°C of SETPOINT.

EX1006, 006

Dr. Shroff testified is that Chen's objective lens is in contact with the sample coverslip via an oil droplet

POR, 34; Pet. Reply, 5-6

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner's argument that it would have been obvious to add a Peltier element to Chen's system is conclusory and unsupported

"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)

Petitioner's Reply:

"Finally, even if PO's construction is adopted, it does not help PO because the **Petition established that a Peltier element, which provides active temperature control, is obvious**. See **Petition at 43**; EX1002 ¶ 233; EX1082 at 86:8-12, 97:17-98:3, 99:11-20."

Pet. Reply, 6

Petition at 43:

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

Pet., 43

- Petition did not argue for claim 36 that adding a Peltier element was obvious
- Petitioner offered no explanation why a POSA would have been motivated to modify Chen's 2D MERFISH imaging setup to include Peltier element

PO Sur-Reply, 7-8

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Objective indicia confirm non-obviousness

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Nexus is presumed

"[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)

Dr. Shroff:

"Accordingly, the claims of the 554 patent are embodied in the **Xenium** and CosMx imaging systems, as these systems **embody the claimed features of the volumetric imaging system** of claim 36 and are **coextensive with them**."

EX2024, ¶128

Dr. Shroff, element 36(c):

"Claim 36 also discloses an 'optical system' for the claimed invention, comprising 'two or more light sources' and 'one or more detectors.' EX1001, 33:43-54. These elements are once again reflected in the **User Guide for the Xenium system**. . . . The User Guide also includes extensive instructions for image scanning that repeatedly refer to adjusting or turning multiple channels on or off. EX2060, 73. **The multiple channels of the Xenium system indicate that multiple sources of light are used in the system,** for which at least one detector would be needed to detect said light channels. EX2061, 58."

EX2024, ¶124

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petitioner fails to credibly rebut presumption of nexus

Petitioner's only criticism on Xenium nexus:

"**Dr. Shroff assumes that because there are 'multiple channels' there must be multiple light sources**. This, however, is wrong. **A single light source can be used to generate multiple optical channels**, as Dr. Shroff confirmed. See EX1082 117:1-119:1; EX1086 ¶ 58."

Pet. Reply, 21-22

Patent Owner Sur-Reply:

"**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."

PO Sur-Reply, 24-25

Petitioner fails to rebut Patent Owner's economics expert's testimony on commercial success

Patent Owner's economics expert, Ms. Davis:

"Based on my analysis, I believe **there is evidence of commercial success for the system covered by the 554 patent**. In my opinion, the evidence related to commercial success supports a finding that the 554 patent is nonobvious."

EX2026, ¶30

"In my determination of whether **Xenium has been commercially successful**, I have considered a number of factors that a reasonable businessperson would consider in determining commercial success and I understand are instructive in determining commercial success in an IPR."

EX2026, ¶31

Patent Owner Sur-Reply:

"**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 pinions."

PO Sur-Reply, 25

Q. Do you consider yourself an expert in economics?
A. I do not consider myself an expert in economics.
Q. Do you consider yourself an expert in accounting?
A. I do not consider myself an expert in accounting.
Q. Do you consider yourself an expert in finance?
A. I do not consider myself an expert in finance.

EX2082, 196:3-16

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Thank you

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE