

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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NANOSTRING TECHNOLOGIES, INC.  
Petitioner,

v.

PRESIDENT AND FELLOWS OF HARVARD COLLEGE,  
Patent Owner

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U.S. PATENT NO. 11,542,554

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**REPLY DECLARATION OF KIRK CYZMMEK, PH.D. IN SUPPORT OF  
PETITIONER FOR *INTER PARTES* REVIEW**

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## **I. INTRODUCTION AND ENGAGEMENT**

I, Kirk Czymmek, declare as follows:

### **A. Introduction**

1. I am over the age of twenty-one (21) and am competent to make this Declaration. I reside at 603 Martin Pointe Ct, Wildwood, MO 63011.

### **B. Engagement**

2. I have been retained by counsel for NanoString Technologies, Inc. (“NanoString”) in the above-captioned *inter parte* review (IPR) matter as an independent technical expert to review and evaluate specific claims of the U.S. Patent 11,542,554 (“the ’554 Patent”). I have been asked to provide my opinion regarding whether a person of ordinary skill in the art (“POSA”) in the subject matter of the claims would find them anticipated or obvious over certain publications. I previously presented opinions to this effect. *See generally* EX1002.

3. I have further been asked to present opinions in response to those presented by Dr. Hari Shroff in support of the Patent Owner’s response to the Petition. *See generally* EX2024. This declaration sets forth those responsive opinions.

4. I am being compensated on an hourly basis for my work performed in connection with this matter. I have received no additional compensation for my

work in this matter, and my compensation does not depend upon the contents of this report, any testimony I may provide, or the ultimate outcome of the proceeding.

## **II. RESPONSE TO DR. SHROFF'S OPINIONS REGARDING THE LEVEL OF ORDINARY SKILL AND THE STATE OF THE ART**

5. In his declaration, Dr. Shroff states that he reviewed my definition for a POSA but identified no points of disagreement regarding this definition. EX2024

¶ 11. During cross-examination, Dr. Shroff specifically testified that he did not recall any points of disagreement:

Q. Is there anywhere in your declaration, Exhibit 2024, where you identify any points of disagreement with Dr. Czymmek's definition of the level of ordinary skill in the art?

A. Nothing immediately comes to mind, in answer to your question.

EX1082 at 120:17-22.

6. As I explained in my original declaration, a POSA “would have understood the fluorescence imaging technologies themselves, including fluorescence labeling strategies, use microscopes to collect and record and process images and image data to obtain 2D or 3D spatial information relating to the imaged cells or tissues. A POSA would have had experience and/or knowledge of optical systems (such as microscopes), three dimensional and volumetric imaging technologies, including microscopy, microfluidics, and related computational analysis and image-based software programs for acquiring and analyzing 3D images,

including generating 3D spatial information, and processing and quantification relating to signals received from labeled materials.” EX1002 ¶ 69.

7. In my opinion, these basic competencies of the skilled artisan are important. Indeed, both Dr. Shroff and I agree that “three dimensional and volumetric imaging technologies” were basic things that were known and understood by a skilled artisan. Given that this was the case, there cannot be any question that a POSA would have had no trouble extending the Chen MERFISH method to 3D imaging and would have been motivated to do so. This point alone negates Dr. Shroff’s arguments against obviousness.

8. During cross-examination, Dr. Shroff further confirmed several points that a POSA would have understood in the relevant November 2015 time frame. As I explain below, this testimony further confirms obviousness.

9. First, Dr. Shroff confirmed, by reference to his own exhibits, that it was well known the art how to generate a 3D image from a stack of 2D images, testifying that “in general, the idea of taking a stack to acquire a volume was known.” *Id.* at 121:8-13. He confirmed that the tiling of adjacent focal planes to generate a 3D image “was known in 2015.” *Id.* at 227:17-22. Dr. Shroff likewise confirmed that a “POSA would have known that there were certain techniques that would preferentially focus light in one plane relative to others,” including, for instance, light sheet microscopy, total internal reflection fluorescence microscopy, and two-

photon microscopy. *See id.* at 122:14-123:21. This is important because it confirms that as of November 2015 POSAs were well aware of a general approach for generating 3D images based on stacking a series of 2D images. This confirms broadly that 3D imaging was an obvious and natural thing to apply in connection with microscopy techniques that generated 2D images.

10. Second, and consistent with the foregoing, Dr. Shroff confirmed that there were multiple microscopy modalities that could be used for generating 3D images. *See, e.g., id.* at 163:11-19 (A “POSA would have known that, generally speaking, there would be techniques like widefield microscopy and [con]focal microscopy, light sheet microscopy, structured illumination microscopy, a POSA would have known that such 3D imaging techniques existed.”). He confirmed that in November 2015 a POSA would have known that different modalities for 3D microscopy were available, including widefield, [con]focal, and light sheet microscopy. *Id.* at 133:16-21. Dr. Shroff similarly confirmed that, with respect to the various microscopy modalities, a POSA would have understood the “trade-offs for focusing light and taking z-stacks in three dimensions.” *Id.* at 124:8-16; *see also id.* at 138:1-3 (A “POSA would have understood that some techniques are better than others for determining three-dimensional positional information.”). All of this is important because it confirms that not only did POSAs know of many general approaches for generating 3D images, but that these approaches had been

applied to real-world microscopy systems and had been so well characterized that POSAs understood the strengths and benefits of the different approaches and knew how to select the appropriate technique for the appropriate situation. This again undermines any suggestion that it would not have been obvious to adapt MERFISH to 3D volumetric imaging.

11. Third, Dr. Shroff confirmed that a POSA would have known of techniques to improve resolution over what is available compared to a standard point spread function:

A. You're asking if a person would have known that there are methods to improve resolution over a standard point spread function?

Q. Yes.

A. A POSA would have known that there are methods that, in some contexts, can provide better resolution than what is available under a standard point spread function.

*Id.* at 141:2-142:5. He confirmed that as of 2015, there were “a broad class of methods called super-resolution microscopy methods that a POSA would have known existed.” *Id.* at 142:6-12. These methods included SIM, IPALM, PALM, STORM, and 3D SIM. *See id.* at 142:20-143:18. He similarly confirmed that in 2015 POSAs would have been aware of deconvolution methods to increase resolution that relied upon modeling the point spread function. *See id.* at 145:7-22;

167:10-17. In fact, there was publicly available commercial software for this purpose, such as the SVI <sup>TM</sup>Huygens Deconvolution. *See id.* at 168:15-169:16. As yet another example, Dr. Shroff confirmed that expansion microscopy was available to improve resolution. *See id.* at 146:1-7. Although Dr. Shroff purported to be unaware of whether this technique was available in 2015, I can confirm that POSAs were well-aware of expansion microscopy before November 2015. *See* EX1085. These points are important because it shows that to the extent there were issues associated with adapting MERFISH to 3D, if they were even to arise from a particular implementation, there were several well-known techniques for overcoming these issues and that would have been within the basic competencies of the POSA. This again undermines any contention that a POSA would have been dissuaded from seeking to adapt MERFISH to 3D imaging.

12. In opining about the “State of the Art,” Dr. Shroff presents opinions about how prior art systems were “unwieldy” and “home-built” arrangements. *See* EX2024 ¶¶ 20-21. He opines that such prior art systems were “sprawling” and difficult to assemble and maintain. *Id.* ¶ 21. In my opinion, however, these arguments are irrelevant because there is nothing in the claims that excludes from their scope systems that are, per his characterization, “unwieldy” or “home built.” On cross-examination, I understand Dr. Shroff confirmed that he had no opinion that

there was any sort of connection between a system being “homebuilt” and the issue of obviousness:

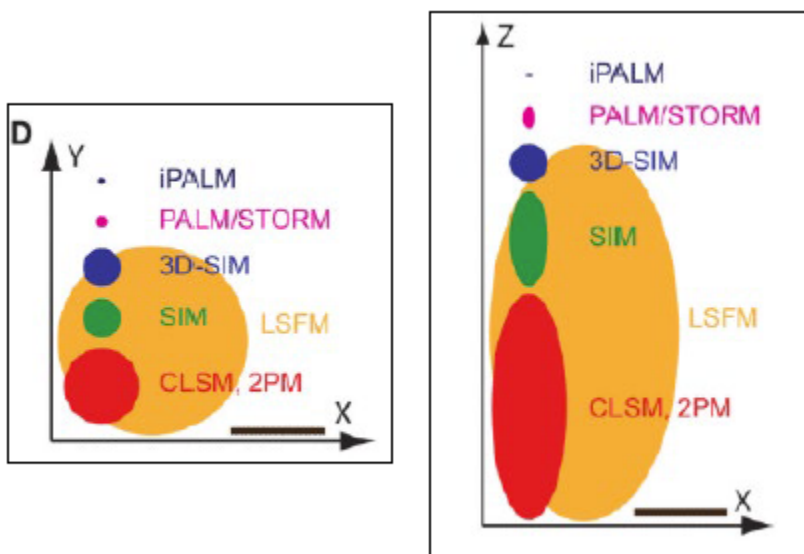
Q. Okay. So homebuilt, to you, weighs against obviousness in this case?

A. Sir, I don't have any expressed opinions about homebuilt and obviousness. Nowhere in this do I say anything about that.

EX1082 at 61:13-17; *see also id.* at 61:18-63:5. Dr. Shroff likewise confirmed that “I wouldn’t say that claim 36 excludes homebuilt systems because nowhere in that - - nowhere in those claims do we have any language about that one way or the other.” *Id.* at 63:22-64:3; *see also id.* at 65:11-14 (“Q. Is it limited, is claim 36 limited to systems that are integrated or monolithic? Do you have an opinion on that? A. Sir, I don't have an opinion on that.”). In my opinion, Dr. Shroff’s opinions regarding the alleged unwieldy nature of homebuilt systems are irrelevant.

13. Dr. Shroff further opines that “axial resolution is more difficult to achieve than lateral resolution.” EX2024 at 21. Dr. Shroff contends that because axial resolution is more challenging than lateral resolution, a POSA would not have attempted to apply MERFISH in a 3D setting. *Id.* ¶¶ 20-32. He opines at length on how the point spread function (“PSF”) is elongated in the z-axis relative to the lateral axes and that this would have impacted resolution. *Id.* Yet, Dr. Shroff’s own figures in ¶ 24 of his declaration confirm that there are multiple microscopy

modalities that have elongated point spread functions in the z-direction. As Dr. Shroff's figure shows, while the LSFM microscopy modality has a relatively extended PSF in the z-direction, there are several modalities that have substantially reduced PSFs in the z-direction, including SIM, 3D-SIM, PALM/STORM, and iPALM:



EX2024 ¶ 24 (citing EX1019, FIG. 1D). These are the same modalities that Dr. 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 this, as I explain above, Dr. Shroff similarly confirmed at his deposition that there were widely available deconvolution methods for improving axial resolution, and that expansion microscopy could also be used to address this problem. See EX1082 at 145:7-22, 146:1-7, 167:10-17, 168:15-169:16. Thus, in my opinion, the

concerns that Dr. Shroff raises about the PSF are overstated and would not have remotely dissuaded a POSA from seeking to adapt MERFISH to 3D. To the extent a POSA was concerned about the PSF or axial resolution, he or she simply would have selected the microscopy modality and/or sample preparation method with the PSF that best-suited their needs.

14. Dr. Shroff further opines that the PSF is “affected by sample thickness,” explaining that with thicker samples it is more difficult to achieve axial resolution. *See* EX2024 ¶ 31. Even to the extent this is true, however, the claims are not limited to any particular sample thickness. As Dr. Shroff confirmed, “I don’t recall seeing any statement about minimal sample thickness.” EX1082 at 220:3-14. In fact, Dr. Shroff confirmed that 3D imaging in a single cell is within the scope of the ’554 patent claims:

Q. You agree that 3D imaging of a single cell, that's within scope of the claims of the '554 patent, right?

A. 3D imaging in a single cell within the scope of the '554 patent?  
Yes, I agree with that.

*Id.* at 180:8-13; *see also id.* at 222:17-22 (“Q. There's no language in the claim that precludes the analysis of a single cell, right? A. As far as I can tell, there's no language in this claim that precludes that.”). Dr. Shroff further confirmed that there “were some researchers in 2015 that, for some samples, were doing 3D microscopy.”

*Id.* at 178:11-16. In fact, a Google Scholar search in the 2000-2015 timeframe reveals roughly 3,000,000 hits related to 3D microscopy. *See* EX1084. As early as 2011, Dr. Shroff himself was performing 3D imaging in samples that had a thickness of roughly 8 microns, which, according to Dr. Shroff, “is about the thickness of a single cell.” EX1082 at 242:5-243:16. Likewise, three years later, in 2014, Dr. Shroff himself was performing 3D spatial imaging and quantification of multiple nucleic acid particles in a single cell. *See id.* at 248:12-251:16. Thus, in November 2015, researchers were interested and motivated to perform 3D imaging of nucleic acids in not just thick samples, but also samples as thin as a single cell. Whatever 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. Any perceived difficulties with 3D imaging of thick samples would not have wholly dissuaded the POSA from attempting to adapt MERFISH to a 3D experiment, but would have simply guided the research to focus on numerous use cases with thinner or smaller samples, such as single cells or thin FFPE prepared tissue slices.

15. Dr. Shroff further opines that there are additional issues related to axial resolution, such as signal-to-noise-ratio and other issues that arise from large numbers of targets. Dr. Shroff opines, for instance, that “increased multiplexing—generating more signal by simultaneously tagging a greater number of nucleic acid

species—can increase the signal density while making it harder to discern signal from individual molecules.” EX2024 ¶ 33. Alleged concerns over signal density are a major theme that runs through Dr. Shroff’s declaration. Briefly, Dr. Shroff’s theory is that the Chen reference is focused on identifying a large number of targets, and this focus would dissuade a POSA from seeking to perform 3D MERFISH because it would have been too difficult to discern large numbers of targets in three dimensions. As Dr. Shroff confirmed, however, the claims require only the analysis of “one or more nucleic acid molecules,” not a large number of targets:

Q. What is the minimum number of molecules encompassed by the scope of claim 36?

A. Well, this particular phrase says determining three-dimensional positional information of said one or more nucleic acid molecules.

Q. So you can satisfy the claims through the analysis of only single molecule, correct?

A. Provided all the elements of the claim are met.

EX1082 at 214:7-17. 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. Tellingly, I understand that Dr. Shroff could not identify any minimum number of targets that could be analyzed with MERFISH. *See id.* at 170:4-173:11. In fact, there is

nothing in the MERFISH method that would preclude the analysis of a small number of targets, nor would a POSA deem it unnecessary or uninteresting to analyze a small number of targets. As I explain above, for instance, in 2014, Dr. Shroff himself was performing 3D imaging of four different nucleic acid targets and their spatial quantification in a single cell and quantification. *See id.* at 248:12-251:16; EX1076 at Fig 2 (describing “spatial location” and “3D rendering” and “proportion of foci containing 4, 3, 2, or 1 distinct vRNA segment”)

### **III. RESPONSE TO DR. SHROFF’S OPINIONS REGARDING CLAIM CONSTRUCTION**

16. I understand that words of the claims of a patent are generally given their plain and ordinary meaning from the perspective of a POSA at the time of the claimed invention. I also understand that the claims of a patent are construed in light of the language of the claims, the patent specification, and the prosecution history of the patent (including prior art references that were cited during the prosecution of the patent). I understand that while the claims are interpreted in view of the specification, it nonetheless inappropriate to limit claims to particular examples or embodiments absent additional reason to do so, such as definition in the specification or a disavowal or disclaimer of claim scope in the specification or prosecution history.

17. In his declaration, Dr. Shroff opines that the term “temperature control” in claim 36 should be interpreted to “mean the ability to actively heat and actively

cool in an apparatus.” EX2024 ¶ 61. I disagree with this opinion for several reasons.

18. Starting with the claim language, in my opinion, there is nothing in the term “temperature control” that would require both active heating and active cooling. 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.

19. Dr. Shroff confirmed at deposition that the plain meaning of “temperature control” is not as narrow as he contends:

Q. Is increasing the temperature of a sample so that you make it a particular temperature, is that a form of temperature control?

A. *It's a form of temperature control*, but not temperature control as defined in the '554 patent, as I describe in paragraph 62 of my declaration.

\* \* \*

Q. Okay. Is cooling a sample so that you bring it down to a particular temperature, is that a form of temperature control?

A. *Cooling is also a form of temperature control*. But the language in the '554 patent is inclusive of heating and cooling, as I describe in my declaration.

EX1082 at 103:7-14, 105:2-8.

20. As Dr. Shroff confirmed on cross-examination, his interpretation of “temperature control” leads to an absurd understanding wherein processes that maintain samples at specific temperatures are inexplicably excluded from the scope of the term “temperature control:”

Q. If you had a system that maintained a temperature at a particular level and did it with only active heating, but didn't have active cooling, would that be a system that provided temperature control?

A. Not in the sense in which I've defined it in my declaration and which the '554 patent defines it.

Q. How about a system that checks to see if the sample is below a desired temperature, and if it is, it heats it up to that temperature and it stops when it reaches that temperature? And then if it starts to drop a bit, a passive cooling requires a bit more to maintain the desired temperature? Would that be temperature controlled?

A. I think the problem is that you're invoking the term “passive cooling,” sir, which doesn't fall within the rubric of the '554 patent.

\* \* \*

Q. I'm trying to describe a system that measures the temperature of

your sample. And if it's below the desired temperature, heats it up to that temperature and then it stops when it reaches that temperature, and then if it starts to drop a bit. From passive cooling, it requires a bit more heat to maintain the desired temperature. Would that be a form of temperature control?

- A. Not in the '554 patent because you have not explicitly given me active cooling.

EX1082 at 112:12-113:10, 114:3-14.

21. Dr. Shroff contends that dependent claim 43, which recites the use of a “heat sink or a Peltier element,” supports his interpretation because a Peltier element is a type of active cooling system. EX2024 ¶ 62. This, however, is only part of a dependent claim. I understand that independent claims must be broader than dependent claims. To the extent a dependent claim is limited to a particular type of active cooling system, I do not see any reason that this requires the term “temperature control” in independent claim 36 to require some form of active cooling.

22. In my opinion, Dr. Shroff does not identify anything in the specification that would support limiting “temperature control” to systems that have both active heating and cooling. Dr. Shroff points to examples in the specification that include both active heating and cooling. *See id.* ¶ 63. But these are only examples, and it is my understanding that such examples do not justify narrowing claim scope.

Moreover, the specification is in fact permissive in its description of “temperature control.” For instance, the 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).” EX1001 at 18:5-7. Stating that the stage “may” include heating “or” cooling, the specification is clear that there is no requirement for *both* heating and cooling. *See also id.* at 30:32-35 (“a heating or cooling apparatus operationally connected to the stage, whereby the heating or cooling apparatus is programmable for time and temperature useful with thermocycling for amplification and sequencing”).

23. Based upon my review, I do not see any definition or disclaimer of claim scope in the specification that would justify limiting the scope of “temperature control.” Consistent with this, Dr. Shroff agreed there was no definition of “temperature control” in the specification:

Q. Is there an explicit definition of the claim term "temperature control apparatus" in the '554 patent?

A. Sir, if you're asking, like, does the patent explicitly define temperature control means X, I don't recall seeing that in the '554 patent.

\* \* \*

Q. Please identify for me where, in the '554 patent, you contend the patent provides an explicit definition of the term temperature

control apparatus?

- A. Sir, I don't contend that the patent, as I just said, explicitly defines temperature control. My contention is 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 at 78:9-14, 79:8-18.

24. When it comes to the question of whether the patentee disavowed or disclaimed claim scope with respect to “temperature control,” Dr. Shroff similarly did not contend that there was any such disavowal or disclaimer. He was not even familiar with the concept:

- Q. Have you ever heard of the concept of a patentee disavowing or disclaiming claim scope?

- A. Sir, I have no idea what you're talking about.

- Q. You don't contend, in your report, that there's any disavowed or disclaimer of claim scope in either the specification or the prosecution history, correct?

- A. My answer's the same. I really don't know what you're referring to.

*Id.* at 81:16-82:6.

25. In sum, I disagree with Dr. Shroff that the term “temperature control” requires the presence of both active heating and active cooling. The term “temperature control” should simply be given its plain and ordinary meaning.

#### **IV. RESPONSE TO DR. SHROFF’S OPINIONS REGARDING THE COMBINATION OF CHEN WITH CAI AND SHEN**

26. In the paragraphs below I respond to Dr. Shroff’s specific opinions regarding the combination of Chen with Cai and Shen

##### **A. The Cited Prior Art Teaches “Temperature Control”**

27. Dr. Shroff first contends that Chen’s FCS2 flow chamber does not have temperature control and similarly did not need temperature control because Chen’s MERFISH experiments used fixed cells, which supposedly do not require temperature control. EX2024 ¶ 70. This is incorrect for several reasons.

28. First, there can be no question that the Biopetechs FCS2 flow cell that was used in the Chen publication included temperature control. As I explained in my original declaration, it was known that the Biopetechs FCS2 flow cell included extensive temperature control systems. *See, e.g.*, EX1002 ¶¶ 142-43, 236. Any suggestion to the contrary is put to rest by a 2020 publication by Su et al., which was published by the same group that published Chen and wherein they provided additional information regarding their MERFISH system. In that publication, they stated that “[a]ll 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 at e8. The publication by Chen that they cite is the Chen publication that I rely upon here, and they make clear that the particular Bioptechs flow cell they used had serial number 060139-2. This serial number corresponds to a Bioptechs flow cell with temperature control capability, as confirmed by the FCS2 instruction manual that I cited in my original declaration. *See* EX1006.006 (referring to serial number BNi060319-2 in lower right-hand corner). Thus, I disagree strongly with Dr. Shroff’s assertion that there is “no evidence” that Chen’s FCS2 flow cell did not have temperature control.

29. Second, any suggestion that the fixed cells in Chen’s MERFISH experiment did not require temperature control is misguided. Chen explains that during the MERFISH imaging process, the “objective was heated to 37°C with a Bioptechs objective heater.” EX1003.011. A POSA would understand from this disclosure that this mean the MERFISH sample was similarly maintained at 37°C with the heating system in the FCS2 flow cell. A POSA would understand that the standard and normal way for an objective heater to be used is in conjunction with the temperature control system in the flow cell to at least ensure thermal stability. By doing this, one would avoid a temperature gradient on going from the sample to the optical system, which would otherwise result in focus drift. Further one would understand that in a system like MERFISH that is based on hybridization chemistry

the standard approach would be to maintain the system at the normal temperature of 37°C to facilitate the chemistry and/or minimize non-specific background.

30. Dr. Shroff further contends that the Bioprotechs FCS2 flow cell does not include “temperature control” because it only includes heating and supposedly lacks active cooling. *See* EX2024 ¶ 71. As I explain above, however, “temperature control” does not require active cooling. *See supra* Part III. As Dr. Shroff acknowledges, the “the ‘FCS2 instruction document’ discloses an FCS2 controller exclusively capable of raising temperature and ***maintaining the temperature.***” EX2024 ¶ 71. I agree that the FCS2 instruction document teaches a system capable of “maintaining the temperature” and, as such, it is my opinion that it teaches “temperature control.”

31. In my original declaration, I further explained that the objective heater by itself was another temperature control system that satisfied the claims. *See* EX1002 ¶ 144. On this point, 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. *See supra* Part III. Dr. Shroff further contends that the objective heater is not “operatively coupled to the sample holder.” EX2024 ¶ 72. Yet, in Chen, the objective is an oil immersion objective.

EX1003.001. An oil immersion is plainly coupled to the sample holder via an oil droplet, as Dr. Shroff himself confirmed on cross-examination:

Q. Are you familiar with oil immersion lenses?

A. Yes, I am, sir.

Q. Is it accurate that in those lenses, an oil droplet is placed in the front of the lens and it's moved to contact the cover slip for the sample?

A. Yeah. That is accurate, sir.

EX1082 at 82:7-14.

**B. The Cited Prior Art Teaches Determination Of Three-Dimensional Positional Information**

32. Dr. Shroff contends that “3D imaging was not a contemplated option for MERFISH.” EX2024 ¶ 88. This, however, is incorrect. As I explained in my original declaration, the Chen MERFISH publication directly contemplates 3D imaging. See EX1002 ¶¶ 128, 183, 189, 204. Most notably, as I explain in my original declaration in connection with claim element 36[d] pertaining to the use of a z-axis motion control system, Chen expressly states that “because a fraction of the cell could be outside the imaging area or focal depth” that “[t]iling images of adjacent areas and adjacent focal planes could be used to improve the counting accuracy.” EX1003.012. By this disclosure, the 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. To remedy this, they expressly state that one can generate 3D data by “tiling” adjacent areas and “adjacent focal planes” (i.e., adjacent 2D images along z-axis). Thus, there can be no question that Chen does indeed expressly contemplate 3D imaging.

33. Indeed, in his own declaration, Dr. Shroff describes exactly this process for generating 3D images in his discussion of optical sectioning. See EX2024 ¶ 36. During cross-examination, Dr. Shroff confirmed that this approach based on tiling adjacent areas and adjacent focal planes that is referred to in Chen could be used to generate 3D images with widefield microscopy and that it was known in 2015:

Q. All right. Let's go to Section 4.1. This is a section entitled "Three-Dimensional Optical Sectioning." This is describing, in this section, how a person of ordinary skill in the art would generate a 3D image from a series of 2D images; is that right?

A. Yeah, lines -- let's see, can be used to collect a series of 2D images in a specimen by moving the focal plane across the 3D specimen. That is what it's saying here, sir.

Q. And that's on page 24, correct?

A. Those sentences are on page 24.

Q. Okay. So let's take a look at Figure 17. Figure 17 is showing those series of focal planes?

A. Yes.

Q. Okay. And each of those focal planes is a 2D image?

A. Each of those focal planes, it refers to a 2D location, 2D plane.

Q. And can you tile those adjacent focal points to make a 3D image?

A. In principle, you can tile those focal planes to make a 3D image. Of course, that's not what was done in the reference, the prior reference image that was suggested by Dr. Czymmek.

EX1082 at 225:15-226:10.

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.

*Id.* at 228:4-14.

Q. And we talked about this earlier, the strategy of tiling adjacent focal planes to generate a 3D image, that was known in November of 2015, right?

A. I think tiling was known in 2015. And, again, I'd like to reiterate that, of course, this method was not applied to do any 3D analysis in Chen 2015

*Id.* at 227:17-228:3

34. Notably, upon reviewing Dr. Shroff's declaration, it appears that he did not address at all my discussion of the disclosure in Chen of tiling images from adjacent areas and adjacent focal planes. This disclosure, however, is important because, as I explain in my original declaration, it is an express disclosure that would guide a POSA to use a z-axis motion control system with Chen to generate 3D images. *See* EX1002 ¶ 189.

35. As Dr. Shroff acknowledges in ¶ 89 of his declaration, Chen further teaches that "Chen further states that "[f]rom an imaging perspective, any optical sectioning technique could be used in MERFISH to allow the imaging of RNAs in thicker cells or tissues." EX1003, 012; EX2024 ¶ 89. I discussed this in my original declaration. *See* EX1002 ¶ 140. Dr. Shroff contends that this disclosure is simply referring to using sectioning to remove background fluorescence. EX2024 ¶ 89. A POSA, however, would not view Chen's discussion of "optical sectioning" in this way.

36. The full passage at issue in Chen is as follows:

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

optically sectioned these cells by collecting stacks of images at different focal depths through the entire depth of the cells. We aligned the images in consecutive focal planes and then computed for each cell the fraction of RNAs that were detected in the three dimensional stack but not in the basal focal plane. We found that only a small fraction,  $15 \pm 1\%$  (mean  $\pm$  SEM across six different RNA species) of RNA molecules were outside the imaging range of a fixed focal plane without z-scanning. These measurements also confirmed that our excitation geometry illuminated the full depth of our 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. This passage was explaining that for the 2D experiments that were performed, the authors of Chen were able to determine that only a few of the targets were outside of the plane they imaged because this particular cell line, while having a volume, was relatively flat. The subject of the passage was thus ensuring that the large majority of targets in the sample were imaged in their chosen test sample. With thicker samples, the authors of Chen explain that one can use any optical sectioning to ensure that all targets are included in the cell/tissue volume. This passage is not about using optical sectioning to remove fluorescence background but is rather proposing to use 3D imaging by optical sectioning to capture the full sample.

37. It is true that Chen contemplates using optical sectioning for the additional purpose of avoiding fluorescence background. But, when Chen contemplates its use for that purpose, it expressly says so. Specifically, Chen states “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.” EX1003.10. However, the passage in which Chen states that “any optical sectioning technique could be used in MERFISH to allow the imaging of RNAs in thicker cells or tissues” does not mention background fluorescence and is instead contemplating the use of adjacent focal planes for 3D imaging of an entire cell to capture RNA species outside the axial range of their oblique incidence illumination geometry.

38. Consistent with this, Dr. Shroff’s own discussion of “optical sectioning” in ¶ 36 of his declaration describes this as a method for generating 3D data. See EX2024 ¶ 36; EX2078 at 25. As I explain above, Dr. Shroff confirmed this again when he was asked about this method on cross-examination:

Q. All right. Let's go to Section 4.1. This is a section entitled "Three-Dimensional Optical Sectioning." This is describing, in this section, how a person of ordinary skill in the art would generate a 3D image from a series of 2D images; is that right?

A. Yeah, lines -- let's see, can be used to collect a series of 2D images in a specimen by moving the focal plane across the 3D specimen. That is what it's saying here, sir.

*See EX1082 at 225:15-226:10.*

39. Beyond the foregoing teaching in Chen, I further rely on the Cai reference, which again teaches 3D imaging. As I explained in my original declaration, Cai teaches the use of three-dimensional scanning along the Z-axis, and that this is to provide "three dimensional spatial information of a target." EX1032.004; EX1004 at 21:50-51; EX1002 ¶ 191. Dr. Shroff tries to discount this disclosure as a "single sentence," EX2024 ¶ 99, but, in fact, Cai includes actual 3D imaging data, as Dr. Shroff confirmed on cross-examination. For instance, Dr. shroff testified as follows with regard to Figure 16:

Q. Now, if you look at the figure caption, it says "3D coronal rendering of the motor cortex." What does that mean to you?

A. It means that on this particular brain sample, somebody associated with Cai took a stack, using some microscope, and then they rendered that stack into the image that we see in Figure 16.

\* \* \*

Q. You agree that Figure 16 on the left is a 3D image?

A. I mean, I agree that it's a rendering of a 3D object.

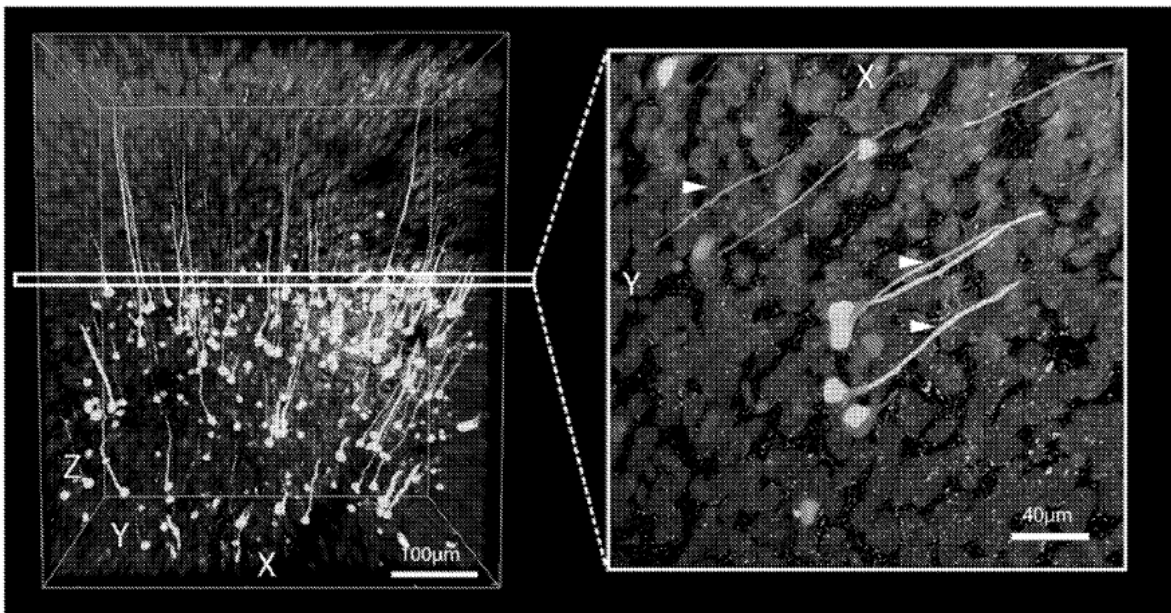
\* \* \*

Q. Okay. It's 3D data, correct?

A. What's shown in Figure 16 is a rendering of some 3D data.

EX1082 at 261:20-262:5, 262:11-14, 263:13-15; *see also id.* at 264:13-22 (Dr. Shroff confirms that Fig. 18 of Cai discloses 3D data). Figure 16 of Cai is shown below, which depicts a 2D slice (right) from a 3D volume (left):

Fig. 16



EX1004.0019 at Fig. 16. Cai's disclosure of 3D imaging is thus extensive.

**C. It Would Have Been Obvious To Adapt Chen To Image Data In Three Dimensions**

40. The Chen publication reports 2D data with MERFISH and 3D validation data using single molecule FISH using the same apparatus as was used for 2D MERFISH, thus it would have been obvious and routine to adapt Chen for generating 3D FISH data. Below, I address Dr. Shroff's arguments to the contrary, all of which I disagree with.

41. Dr. Shroff contends that "Dr. Czymmek does not explain the particular modifications that a POSA would have made to actually convert Chen's 2D MERFISH imaging setup into a volumetric imaging system for determining three-dimensional positional information of nucleic acid molecules per the teachings in Cai." EX2024 ¶ 92. Dr. Shroff goes on to contend that a "POSA would have known that the light sheet microscope imaging system in Cai was completely different from Chen's 2D MERFISH imaging setup and was not designed for use with MERFISH." *Id.* ¶ 94. Dr. Shroff's arguments to this effect, however, are incorrect and miss the point.

42. Most important, my proposed combination of Chen with Cai is not a chimera of the two references wherein the different features of each approach are mindlessly melded together. Nor is it the case that I was not specific as to what features of Cai and Shen would be used in conjunction with Chen. As I explain in my original declaration, "to the extent the Board finds that Zhuang does not

sufficiently disclose a Z-motion control system, it would have been obvious to combine the teachings of a Z-motion control system from Shen and/or Cai.” EX1002 ¶ 192; *see also id.* ¶ 206 (“Cai teaches the claimed Z-axis motion control system to permit three-dimensional interrogation through a stack of focal planes.”); *id.* ¶ 207 (“As Zhuang has the goal of identifying ‘distinct spatial distribution patterns of RNAs,’ it is my opinion that 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.”). 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 on somehow combining the numerous particularized features of Chen with those of Cai and or Shen, or generating some sort of hybrid between the widefield microscope from Chen with the light sheet microscopy of Cai.

43. Accordingly, it is irrelevant that there are differences between, for instance, the light sheet microscope in Cai and the widefield imaging system in Chen. The key point is that the z-axis control system in Cai and Shen can be applied to Chen. Indeed, on both widefield systems (Chen) and light sheet systems (Cai), one can use z-axis control to generate a series of images from different focal planes to generate a 3D image, or with any other optical sectioning approach, for

that matter. Dr. Shroff, for instance, confirmed this for widefield microscopy, as used in Chen:

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.

EX1082 at 228:4-14. Dr. Shroff likewise confirmed that in November 2015, widefield microscopy was used for 3D imaging. *See id.* at 133:16-21 (“Q. What were some of the 3D microscopy modalities that were available in November 2015?

A. One example would be widefield microscopy; another example would have been [con]focal microscopy; another example would have been light sheet microscopy.”); *id.* at 212:17-20 (“Q. Now, with widefield imaging, is that ever used with this concept of making Z stacks? A. Widefield imaging can be used in the context of taking a Z stack.”).

44. Notably, to the extent the claims recite the use of any specific microscopy modality, it is only widefield microscopy in claims 34 and 69. As Dr. Shroff confirmed, there is no disclosure in the ’554 patent of any *new* improvements

to widefield microscopy to make it more suitable for 3D imaging for use with the alleged invention. At most, the '554 patent refers to certain known prior art methods for use with

Q. Sure, let's do it that way. Is there any disclosure in the '554 patent of improvements to widefield microscopy to make it more suitable for 3D?

A. What do you mean by "more suitable for 3D," sir?

Q. Better suited.

A. In what sense?

Q. Any sense. Any sense, whatsoever, in the broadest meaning of the term.

A. Okay. Great. So, as I said, the Broxton light field microscope and the Abrahamsson fast multicolor 3D imaging disclosure, they both are geared towards improving the speed of widefield microscopy.

Q. Okay. That's prior art. Anything else?

A. As I said before, the deconvolution reference, the '554 patent says because optical microscopy text points recognizes a point spread function, deconvolution methods reassign and focus light, which can serve to, effectively, increase resolution. So I'm just reading the method.

So this gets back to our earlier discussion about how, in principle,

deconvolution can get you to the diffraction.

Q. So the Broxton, Wilson, and Abrahamsson articles, that you mentioned in your previous answers, those are all prior art references?

A. These are all prior art references.

Q. Okay. Anything else in the patent that constitutes disclosure of improvements to widefield microscopy to make it better suited for 3D?

A. Not that I'm aware of at this time.

EX1082 at 203:9-205:10. All of this evidence is important because it confirms that it would be no great leap to adapt the 2D MERFISH approach in Chen based on widefield microscopy to a 3D approach. Indeed, it was known that widefield could be used for 3D microscopy (including through the tiling of adjacent areas and adjacent focal planes) and the '554 patent itself does not suggest that any new developments would be required. Just the opposite, the '554 patent assumes in general, widefield microscopy can be adapted from 3D using prior art approaches.

45. A theme that runs through Dr. Shroff's declaration is the idea that Chen, Cai, and Shen are microscopy systems, all with z-control mechanisms, that are supposedly radically different from one another, such that a POSA would not think to combine them or would not have a reasonable expectation of success of combining

them. My understanding of obviousness from counsel, however, is that obviousness does not require proof that references can be combined in a bodily fashion. Rather, my understanding is that a POSA is also a person of ordinary creativity, not an automaton, so the fact that it would take some creativity to combine some prior art references does not defeat a finding of obviousness.

46. In my opinion, Dr. Shroff's declaration does not consider these principles, but instead applies an unduly rigid standard of what a POSA would have found obviousness in view of his or her knowledge, which, as Dr. Shroff does not deny, was extensive. As I detail below, none of the supposed challenges that Dr. Shroff identifies towards combining Chen with Cai or Shen would present significant obstacles for the skilled artisan.

47. Dr. Shroff contends that a POSA would not seek to adapt MERFISH to 3D because of supposed issues with axial resolution, particularly with the widefield microscopy modality used in Chen. *See* EX2024 ¶ 84. Yet, as I detail above, POSAs were aware of microscopy modalities with small PSFs in both the lateral and axial directions including image processing methods such as deconvolution to address both lateral and axial resolution. *See supra* Part II. Likewise, Dr. Shroff confirmed, like Chen reported, and a POSA would know, that widefield microscopy *could* be used for 3D imaging. Further, the supposed issues with axial resolution are only severe with very thick samples, and there is no requirement in the claims

for a minimum sample thickness. As I explain above, the claims cover the analysis of samples as thick as only a single cell (i.e., a few microns in size), and POSAs, including even Dr. Shroff, were highly motivated to carry out such experiments. *See id.* POSAs would have had no trouble addressing axial resolution with such samples.

48. Dr. Shroff further opines that my “argument does not demonstrate what motivation a POSA would have to modify Chen’s 2D MERFISH setup to 3D MERFISH to arrive at the claimed volumetric imaging system.” EX2024 ¶ 103. The motivation, however, is straightforward. As I explained in my original declaration, it “is my opinion that to accomplish this sort of tiling and to understand the spatial landscape of the transcriptome in three dimensions, a POSA would have known that a Z-axis motion control for the focal plane would have been required.” EX1002 ¶ 189. Likewise, as I explained, because “Zhuang has the goal of identifying ‘distinct spatial distribution patterns of RNAs,’ it is my opinion that a POSA would have been motivated to use the MERFISH method for 3D investigation of cells and tissues and would have looked to the relevant teachings in Shen and Cai regarding Z-axis motion control that provides for 3D imaging.” *Id.* ¶ 207. As I explain, such control systems were “standard and well known to a person of ordinary skill.” *Id.* ¶ 190.

49. Dr. Shroff further contends that a POSA would not be motivated to adapt Chen for 3D imaging because this would be contrary to an alleged goal in Chen “to increase the multiplexity of 2D MERFISH imaging.” EX2024 ¶ 104. According to Dr. Shroff, “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.” *Id.* ¶ 105. In Dr. Shroff’s view, a POSA would not have bothered to adapt MERFISH to 3D because he or she would think that doing so would be too hard if there were large numbers of targets to be imaged. I disagree with this for at least two reasons.

50. First, 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. As I note above, Dr. Shroff was unable to identify any minimum number of targets that could be analyzed with MERFISH. *See id.* at 170:4-173:11. In fact, there is nothing in the MERFISH method that would preclude the analysis of a small number of targets, nor would a POSA deem it unnecessary or uninteresting to analyze a small number of targets. As I explain above, for instance, in 2014, Dr. Shroff himself was performing 3D imaging and spatial quantification in a single cell of four different nucleic acid targets. *See id.* at 248:12-251:16; EX1076. A POSA would not be so unreasonable as to believe it is

pointless to adapt MERFISH to 3D because one might not be able to analyze as many targets as is possible when one is performing pure 2D imaging.

51. Second, there is no requirement in the claims for a large number of targets. The claims only require the detection of “one or more” nucleic acid molecules. 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, not a large number of targets.

52. Interestingly, in support of his argument that a POSA would not adapt MERFISH to 3D because of Chen’s focus on high multiplexity, Dr. Shroff relies on patents directed to MERFISH that were applied for by the research group that published Chen. EX2024 ¶ 49. Dr. Shroff explained his opinions as follows:

Q. What I'm asking is, why you believed these patent applications from the Chen group, that are directed to MERFISH, were relevant to your opinions in this case?

A. One of the central arguments in my declaration, sir, is that Dr. Czymmek is claiming that by combining Chen, Shen and Cai, one could get to the claims of the '554 patent. But one of my arguments, sir, is that a person skilled in the art would not have combined these applications. And the reason for that is that the Chen paper, the MERFISH paper, the goal of that paper is to increase the multiplexing, increasing the throughput of MERFISH. It has nothing to do with 3D MERFISH. And so, in

that context, in my declaration, what you're seeing on pages 44, 45, and 46, has to do with the discussion of increasing multiplexity in MERFISH.

EX1082 at 18:19-19:14; *see also id.* at 21:1-4 (“The reason I cited these patent applications, sir, is that I'm trying to make an argument that MERFISH is directed towards multiplexity.”); *id.* at 21:14-19 (“So after 2015, after the date of the '554 patent, the point is that the MERFISH group were, themselves, interested in going after multiplexing. That's the context in which these paragraphs should be read and the context in which I cite these documents.”); *id.* at 22:7-17 (“Q. Okay. Is it fair, then, that you believe these patent applications, that are directed towards MERFISH, reflect evidence of what the goal of MERFISH is? A. Of course. These patent applications, the ones that I am citing here, are examples after 2015. So in that sense, you know, it's happening after the date of the '554 patent, but it's certainly consistent with the idea that this group and the field, in general, was moving towards increased multiplexing.”).

53. Given this testimony, then, it seems reasonable to review the Chen group patent filings to assess the goals of MERFISH. To this end, I've reviewed U.S. Patent 12,209,237, which I understand is one of the patents that issued based on the application that Dr. Shroff discussed in ¶ 49 of his declaration. *See* EX0173 at (21) (referring to application 17/374,400). Importantly, this patent expressly teaches

that MERFISH can be used for 3D imaging, stating “the determination may also be spatial, e.g., the position of the nucleic acid within the cell or other sample may be determined in two or three dimensions.” EX1073 at 14:34-40; *see also id.* at 18:44-58 (“As mentioned, in some cases, the determination may be spatial, e.g., in two or three dimensions.”); *id.* at 22:49-62 (“In some cases, the positions of entities within the sample may be determined in two or even three dimensions.”). This disclosure in the patents directed to MERFISH confirms that MERFISH was intended to be used for 3D imaging.

54. Dr. Shroff further contends that “the signal density of a 3D multiplexed sample to create challenges with computational complexity” EX2024 ¶ 112. Dr. Shroff contends that this would “diminish” the expectation of being able to successfully adapt MERFISH for 3D imaging. *Id.* My understanding from counsel is that obviousness only requires a reasonable expectation of success, not an absolute expectation of success. Dr. Shroff never opines that a POSA would have found it impossible or overwhelming to adapt MERFISH to 3D because of computational challenges. He only says it might have made things more difficult, without ever saying how much more difficult it would be or what the specific challenges would be. I disagree that a POSA would have been dissuaded to adapt MERFISH to 3D based on alleged computational challenges. The Chen paper described results with 2D MERFISH, and extending this to 3D would have only

required a linear increase in the required dataset size for computation due to the need to analyze images in multiple focal planes. In other words, the needed computation power would simply scale linearly with the number of focal planes, and in 2015 there was more than sufficient computational power that was readily available to address this.

**D. Response to Dr. Shroff's Opinions Regarding Secondary Considerations**

55. Dr. Shroff opines that secondary considerations support his opinions of non-obviousness. I disagree that Dr. Shroff has identified any secondary considerations that suggest non-obviousness.

56. As an initial matter, I understand from counsel that to rely upon secondary considerations, one must show a nexus between the particular secondary consideration and the claimed invention. To this end, Dr. Shroff contends that both CosMx and Xenium embody the '554 patent and then argues that these products are commercially successful and that they fulfilled a long-felt need. *See* EX2024 ¶ 118-28. Dr. Shroff, however, has not shown that either CosMx or Xenium use any claim of the '554 patent.

57. As to the CosMx product, Dr. Shroff presented no opinions that it satisfies the “temperature control” apparatus requirement in claim 36. *See* EX2024 ¶ 120-23. Dr. Shroff confirmed this on cross-examination:

Q. So with regard to the CosMx product, and the temperature

control apparatus of claim 36A2, you don't identify anything of the CosMx product that satisfies that, correct?

- A. Yes, in paragraph 121, I go through the temperature control apparatus system of claim 36 and how it's coupled with the sample, and then I explicitly show that in the Xenium 10x device, there is such a temperature control device.

I agree that is not showing for the CosMx.

EX1082 at 276:10-21. Thus, with respect to CosMx, Dr. Shroff has not shown that there is a nexus to the claims.

58. The same is true with respect to the Xenium system. Claim 36 of the '554 patent requires "two or more light sources." As to this requirement, Dr. Shroff opines as follows:

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.

EX2024 ¶ 124. Thus, Dr. Shroff assumes that because there are "multiple channels" there are multiple light sources. This, however, is incorrect. A single light source can be used to generate multiple optical channels. Dr. Shroff confirmed this on cross-examination:

Q. Now in that context, when you have multiple channels, you need to have a separate light source for each channel, right?

A. Well, not necessarily. Some fluorophores have different emission channels but are serviced by the same light source.

Q. You can have multiple channels with only a single light source?

A. Oh, yeah.

Q. How might that work?

A. Well, you could have an emission channel that detects GFP fluorescence, but GFP could be excited by multiple wavelengths of light.

Alternatively, you could have one channel of excitation that excites GFP that could detect to emission in two separate emission channels.

Q. Is it possible to have a single light source that's, like, the broadband light source, where you apply filters to filter out only certain wavelength ranges?

A. It is possible to have such a light source in fluorescence emitting systems.

Q. And how do those work?

A. It's a very general question. I'm not sure what you're getting at. But you'll have a light source and then you put different filters in front of it, just as you said, sir.

Q. And what kind of broadband light sources are there that are available that might be used in that type of setup?

A. You could have arc lamp, you could have LEDs, those would be two examples of light sources.

Q. Any others?

A. You could have a white light laser for example.

Q. Any others?

A. There may be others, but these are the ones that come readily into my mind.

Q. And how long have those kinds of light sources been available for? Were they known to people of skill in the art in 2015? A All of those light sources were around in 2015, sir.

EX1082 117:1-119:1. As Dr. Shroff thus confirms, the mere presence of multiple optical channels does not prove the presence of multiple light sources. Accordingly, it is my opinion that Dr. Shroff has not shown that there is a nexus between Xenium and/or CosMx and the claims of the '554 patent.

59. To the extent Xenium or CosMx are commercially successful, I see no evidence that this is tied to any aspect of the claims. Dr. Shroff, for his part, likewise has no opinion of anything novel in the claims:

Q. What, if anything, is there that you contend is novel or inventive in any of the claims of the '554 patent?

- A. Sir, it's not really what I think is important or inventive, it's really about Dr. Czymmek's erroneous assertion that one can combine these prior art in order to come up with what is described in claim 36.
- Q. Okay. Sitting here today, as 10x's expert on obviousness, if there is anything that you contend is novel or inventive in any of the claims of the '554 patent, please identify that now.
- A. A number of assumptions in your statement there, sir. I wouldn't describe myself as 10x's expert on obviousness. Again, as I just said, you know, my role in this is refuting or rebutting Dr. Czymmek's erroneous assertion that one could achieve what is disclosed in the '554 patent by combining Chen's MERFISH with Shen and Cai.
- Q. Sitting here today, as expert – as 10x's expert on obviousness, do you have any opinion about whether there's anything novel or inventive in any of the claims of the '554 patent?
- A. Sir, my opinion has to do with Dr. Czymmek's opinion and why I think that is erroneous, as I describe in my declaration.
- Q. You know, sitting here today, as 10x's expert on obviousness, can you identify anything that you would contend is novel or inventive in any of the claims of the '554 patent?
- A. My answer is the same, sir. I wouldn't describe myself as 10x's expert on obviousness. My role in this is to refute the erroneous assertion of Dr. Czymmek, that one could arrive at claim 36 by

combining MERFISH with Shen and Cai.

EX1082 at 73:2-74:20. Likewise, when asked if there was any step in the claims that was not in the prior art, Dr. Shroff could not answer:

Q. Okay. If there's any particular step in many of the claims of the '554 patent that you contend is not in the prior art, please identify that now.

A. I'd have to do a thorough analysis on that, sir. I couldn't give you an immediate answer there.

*Id.* at 72:16-73:1. Like Dr. Shroff, I cannot identify anything in the claims of the '554 patent that is not in the prior art or that is novel or inventive. As such, it is difficult to see how there could be any feature of CosMx or Xenium related to the claims that could explain any commercial success that might have or any long-felt need that might have existed for these products.

60. Notably, Dr. Shroff does not know what made either CosMx or Xenium commercially successful:

Q. I was asking something different. Do you know what features of CosMx made CosMx commercially successful?

A. Sir, I wouldn't be able to speculate on what features made it commercially successfully.

Q. Do you know what features made Xenium commercially successfully?

A. Same answer, sir, I don't know what features exactly makes it commercially successful.

*Id.* at 44:15-45:2. In my opinion, however, any commercial success or long-felt need stems not from anything in the claims, but from various features that are not in the claims. This can be seen in the promotional literature that Bruker and 10x supply for their products, CosMx and Xenium products, respectively.

61. Consider, for instance, EX1080, which is promotional literature for the CosMx product. This document promotes features such as being able to analyze a large number of targets, the ability to analyze proteins, the ability to carry out certain specific applications, specific in situ hybridization chemistry, a cloud based computing system, an integrated data viewer, specific gene panels that are available, FFPE and FF compatibility, the ability to perform cell segmentation, easy sample preparation methods, compatibility with different sample types, and a grant support package. *See generally* EX1080. None of these features that Bruker promotes are part of the claims of the '554 patent, a point Dr. Shroff confirmed on cross-examination. *See generally* EX1082 at 279:11-302:13. In my opinion, to the extent CosMx is commercially successful, it is not due to any features in the claims of the '554 patent, which are all part of the prior art, but rather due to the combination of features that are not in the claims and that Bruker promotes to its customers.

62. As another example, consider EX1081, which is promotional literature for the Xenium product. This document promotes features of Xenium such as the DAPI nuclear stain, the ability to analyze a large number of targets, the ability to profile protein expression, compatibility with desktop tools and downstream third-party tools, chemistry that uses padlock probes, short sequencing time, and onboard data processing. *See* EX1081. During his deposition, Dr. Shroff again confirmed that these features were not part of the claims. *See* EX1082 at 302:21-310:15. As with CosMx, to the extent Xenium is commercially successful, it is not due to any features in the claims of the '554 patent, which are all part of the prior art, but rather due to the combination of features that are not in the claims and that 10x promotes to its customers.

63. Finally, Dr. Shroff contends that industry praise for Xenium is a further indicator of non-obviousness. *See* EX2024 ¶ 130. Dr. Shroff's arguments, however, simply prove my point that any praise for Xenium is not connected to the claims. Dr. Shroff opines, for instance, as follows:

Publications utilizing Xenium have praised the platform for the breadth of the system's sequencing ability, the smooth workflow provided by the integrated system, and the ability of the platform to provide high-resolution spatial data for nucleic acid molecules.

*Id.* Here, Dr. Shroff opines that Xenium receive praise for its sequencing ability, smooth workflow, and high resolution. Yet, none of these features are part of the

claims. Dr. Shroff confirmed that there's no minimum resolution requirements. *See* EX1082 at 215:16-216:2. As Dr. Shroff explained, the "claim nowhere says anything about a minimum resolution number. That language is not in the claim." *Id.* at 217:2-4. Likewise, there is no requirement in the claim that the system be "integrated." Dr. Shroff, tellingly had no opinion to this effect. *See id.* at 65:11-14 ("Q. Is it limited, is claim 36 limited to systems that are integrated or monolithic? Do you have an opinion on that? A. Sir, I don't have an opinion on that."). Of course, there is no requirement in the claims for "sequencing" either. Thus, Dr. Shroff identifies no aspect of Xenium that has received praise and that has any connection to the claims.

64. As another example, Dr. Shroff points out that a researcher praised Xenium as follows: "[t]he intuitive design of [Xenium's] bench workflow and post-run analysis software may be the most valuable aspect of this high-throughput in situ technology to accelerate science." EX2024 ¶ 130. Yet, intuitive bench workflow and post-run analysis software, in my opinion, has nothing to do with the claims. Again, Dr. Shroff identifies no aspect of Xenium that has received praise and that has any connection to the claims.

**V. DECLARATION IN LIEU OF OATH**

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1034 of Title 18 of the United States Code.

Dated: 3/17/2025

By: \_\_\_\_\_