



Bruker v. Harvard, IP2024-00526 **Petitioner's Oral Hearing Presentation**

May 16, 2025

'554 Patent: Claim 36

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

(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, US 11,542,554 at Claims 36, 43
(Petition at 10, 13; Reply at 14)

Dr. Shroff: Cannot Say What Is Novel Or Inventive



Hari Shroff, Ph.D.

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

6 A Sir, it's not really what I think is
7 important or inventive, it's really about
8 Dr. Czymmek's erroneous assertion that one can
9 combine these prior art in order to come up with
10 what is described in claim 36.

EX1082, 2/4/25 Shroff Dep. at 73:2-10 (objections omitted) (Reply at 1)

Dr. Shroff: Cannot Say What Is Novel Or Inventive



Hari Shroff, Ph.D.

3 Q Sitting here today, as expert -- as
4 10x's expert on obviousness, do you have any
5 opinion about whether there's anything novel or
6 inventive in any of the claims of the '554 patent?
8 A Sir, my opinion has to do with
9 Dr. Czymmek's opinion and why I think that is
10 erroneous, as I describe in my declaration.

EX1082, 2/4/25 Shroff Dep. at 74:3-10 (objections omitted) (Reply at 1)

Dr. Shroff: Cannot Say What Is Novel Or Inventive



Hari Shroff, Ph.D.

11 Q Okay. Sitting here today, as 10x's
12 expert on obviousness, if there is anything that
13 you contend is novel or inventive in any of the
14 claims of the '554 patent, please identify that
15 now.

17 A A number of assumptions in your
18 statement there, sir. I wouldn't describe myself
19 as 10x's expert on obviousness. Again, as I just
20 said, you know, my role in this is refuting or
21 rebutting Dr. Czymmek's erroneous assertion that
22 one could achieve what is disclosed in the
1 '554 patent by combining Chen's MERFISH with Shen
2 and Cai.

EX1082, 2/4/25 Shroff Dep. at 73:11-74:2 (objections omitted) (Reply at 1)

Dr. Shroff: Cannot Say What Is Novel Or Inventive



Hari Shroff, Ph.D.

11 Q You know, sitting here today, as 10x's
12 expert on obviousness, can you identify anything
13 that you would contend is novel or inventive in
14 any of the claims of the '554 patent?

16 A My answer is the same, sir. I wouldn't
17 describe myself as 10x's expert on obviousness.
18 My role in this is to refute the erroneous
19 assertion of Dr. Czymmek, that one could arrive at
20 claim 36 by combining MERFISH with Shen and Cai.

EX1082, 2/4/25 Shroff Dep. at 74:11-20 (objections omitted) (Reply at 1)

Dr. Shroff: Cannot Identify Any New Claim Steps



Hari Shroff, Ph.D.

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

20 MR. HOLMAN: Objection. Form.

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

EX1082, 2/4/25 Shroff Dep. at 72:16-73:1 (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

22 Q Do you know if common sense can be a
1 sufficient rationale to combine two concepts that
2 are known to prior art?

3 A Sir, I don't know. Common sense seems
4 like a very vague and nullified term to me. I'm
5 not sure what you're getting at there.

EX1082, 2/4/25 Shroff Dep. at 55:22-56:5 (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

6 Q Do you know if it can be a sufficient
7 justification to prove something obvious as to
8 argue that applying one concept to another concept
9 would have been applying a known problem -- a
10 known solution to a known problem? Would that
11 kind of rationale justify defining obviousness?
12 Do you know?

13 MR. HOLMAN: Objection. Form.

14 A Yeah, again, I'm not sure what you're
15 getting at here, sir.

EX1082, 2/4/25 Shroff Dep. at 56:6-15 (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

7 Q I'm asking something different. Could
8 it, in your view, be sufficient rationale to show
9 obviousness to show that a POSA would have merely
10 been combining prior art elements according to the
11 known methods to get a predictable result?

12 Would that kind of rationale support
13 obviousness?

15 A I'm not sure about that, sir, again.

EX1082, 2/4/25 Shroff Dep. at 57:7-15 (objections omitted) (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

16 Q Would -- could it potentially justify
17 obviousness to show that a prior art combination
18 of two things represents a simple substitution of
19 one known element for another to obtain
20 predictable results?

21 Would that work to show obviousness?
22 Do you know?

2 A Sorry, I'm not sure.

EX1082, 2/4/25 Shroff Dep. at 57:16-58:2 (objections omitted) (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

11 Q Okay. I'm just asking you, could it
12 ever be a sufficient rationale to justify
13 obviousness to argue that applying a known
14 technique to a known device is something that
15 could justify obviousness? Do you know?
17 A My answer is the same, sir.
18 Q Which is what? You don't know?
19 A Just that I'm not sure what you're
20 getting at and I'm not sure I understand the
21 relevance of that to this discussion.

EX1082, 2/4/25 Shroff Dep. at 58:11-21 (objections omitted) (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

22 Q Okay. Could, you know, the argument
1 that it would be obvious to try a certain approach
2 because there's a limited number of identified
3 predictable solutions, possibly justified,
4 defining of obviousness? Do you know?

6 A My answer is the same. I don't
7 understand what you're getting at. It seems like
8 you're discussing very general phenomenon that I'm
9 not sure how it's relevant to this case.

EX1082, 2/4/25 Shroff Dep. at 58:22-59:9 (objections omitted) (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

21 Q If there was an expressed teaching in
22 the prior art to combine these two concepts, could
1 that be a good reason to justify obviousness? Do
2 you know?

4 A Without understanding what you mean by
5 "expressed teaching" and how it relates to this
6 case, I can't really comment on that, sir.

EX1082, 2/4/25 Shroff Dep. at 59:21-60:6 (objections omitted) (Reply at 2)

Dr. Shroff: Does Not Know Obviousness Rationales



Hari Shroff, Ph.D.

7 Q Okay. Would it be a sufficient
8 justification to show obviousness to argue that
9 one is simply applying work in one field of
10 endeavor that was known and applying either the
11 same field or a different one based on design
12 incentives or market forces?

13 Could that justify the finding of
14 obviousness? Do you know?

16 A My answer is the same. I don't see how
17 this relates to our discussion here.

EX1082, 2/4/25 Shroff Dep. at 60:7-17 (objections omitted) (Reply at 2)

Competencies Of The POSA



Kirk Czymmek, Ph.D.

69. A POSA would have understood the fluorescence imaging technologies themselves, including fluorescent label technology, the use of microscopes to collect and record images, and the processing of 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. A POSA also would have had experience and/or knowledge of detectable labels, including fluorescent labels, used for labeling nucleic acids and the use of such labeled nucleic acids to identify and characterize other nucleic acids within a sample based on the *in situ* hybridization of the labeled nucleic acid to nucleic acids of interest having complementary sequence, e.g., sequence to which the labeled nucleic acids will bind through hybridization based on complementary sequences.

EX1002, Declaration Of Kirk Czymmek at ¶169 (Reply at 10)

Competencies Of The POSA



Kirk Czymmek, Ph.D.

70. A POSA in the field of fluorescence imaging would also have had some familiarity with state-of-the-art gene amplification and sequencing technologies. In particular, a POSA would have had some familiarity with these technologies due to the overlap in the underlying fundamentals involving hybridization of nucleic acids and fluorescent labels (and the detection thereof), obtaining image data of fluorescent labeled material, and analysis of data obtained from detection and/or imaging of fluorescent labeled material. Further, to the extent such related technologies were not considered to be within the same field, a POSA would have been aware of these technologies, and recognized that the methods and techniques used in these related technologies would have been useful to address the problems that arise in using fluorescently-labeled nucleic acid probes with *in situ* hybridization, imaging using such, and the processing of resulting images and image data to obtain spatial information relating to the imaged cells or tissues.

EX1002, Declaration Of Kirk Czymmek at ¶170 (Reply at 10)

Dr. Shroff: No Disagreement with Dr. Czymmek



Hari Shroff, Ph.D.

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

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

EX1082, 2/4/25 Shroff Dep. at 120:17-22 (Reply at 10)

Claim Construction

Disputed Claim Term: Temperature Control Apparatus

Claim Term	Petitioner's Construction	PO's Construction
"temperature control"	Plain and ordinary meaning.	The ability to actively heat <i>and</i> actively cool.

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;

EX1001, US 11,542,554 at Claim 36
(Petition at 10)

Dr. Shroff: Does Not Know What Evidence Is Most Important



Hari Shroff, Ph.D.

22 Q Do you know if the claim language is
1 more important than the specification and
2 prosecution when it comes to interpreting claims?
3 Do you know?
5 A Sir, I'm not a lawyer. I don't know
6 which of these things you're saying is more
7 important in the patent.

EX1082, 2/4/25 Shroff Dep. at 76:22-77:7 (objections omitted) (Reply at 2)

Dr. Shroff: Claim Language Is Broader Than PO's Construction



Hari Shroff, Ph.D.

7 Q Is increasing the temperature of a
8 sample so that you make it a particular
9 temperature, is that a form of temperature
10 control?

11 A It's a form of temperature control, but
12 not temperature control as defined in the
13 '554 patent, as I describe in paragraph 62 of my
14 declaration.

EX1082, 2/4/25 Shroff Dep. at 103:7-14 (Reply at 3)

Dr. Shroff: Claim Language Is Broader Than PO's Construction



Hari Shroff, Ph.D.

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

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

EX1082, 2/4/25 Shroff Dep. at 105:2-8 (Reply at 3)

Dr. Shroff: PO's Construction Yields Illogical Results



Hari Shroff, Ph.D.

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

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

EX1082, 2/4/25 Shroff Dep. at 112:12-19 (Sur-Reply at 10)

Dr. Shroff: PO's Construction Yields Illogical Results



Hari Shroff, Ph.D.

3 I'm trying to describe a system that measures the
4 temperature of your sample. And if it's below the
5 desired temperature, heats it up to that
6 temperature and then it stops when it reaches that
7 temperature, and then if it starts to drop a bit.
8 From passive cooling, it requires a bit more heat
9 to maintain the desired temperature.

10 Would that be a form of temperature
11 control?

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

EX1082, 2/4/25 Shroff Dep. at 114:3-14 (objections omitted) (Reply at 3)

Dr. Shroff: PO's Construction Yields Illogical Results



Hari Shroff, Ph.D.

1 Now, you know, we're lucky, we don't
2 even to have air-conditioning in our home. I have
3 a heater in my house that heats our house up to
4 72. Cools a little bit, it falls below 72, it
5 gives a bit more heat. But we never have -- we
6 don't have an air conditioner on our home.

7 Would that be a temperature control
8 system?

10 Foundation. Relevance.

11 A From what you're describing, sir, it
12 sounds a lot like the objective heater in Chen,
13 which I describe, in paragraph 72 of my
14 declaration, doesn't meet the definition of a
15 '554 patent.

EX1082, 2/4/25 Shroff Dep. at 115:1-15 (objections omitted) (Reply at 3)

Dr. Shroff: PO's Construction Yields Illogical Results



Hari Shroff, Ph.D.

4 Q Okay. So a refrigerator is a system
5 that maintains a temperature, a particular
6 temperature that's cold, but you would not call it
7 a temperature control apparatus in the context of
8 the '554 patent because it doesn't include an
9 active heating component; is that right?

11 A Again, the '554 patent, the meaning of
12 temperature control is one which encompasses
13 heating and cooling. The example you just gave of
14 a refrigerator merely maintains temperature.

EX1082, 2/4/25 Shroff Dep. at 108:4-14 (objection omitted) (Reply at 3)

Dr. Shroff: Understood Lexicography Principal (But Only This Rule)



Hari Shroff, Ph.D.

8 Q At the bottom of paragraph 60, as you
9 pointed out, you state that when a patentee
10 explicitly defines a claim term in the
11 specification, definition controls.

12 Do you see that?

13 A I do.

14 Q Are there any other principles related
15 to claim construction that you were informed of in
16 drafting your report?

17 A Nothing comes to mind right now, sir.
18 But, again, I'm not a lawyer. I'm not sure what
19 principles you're referring to.

EX1082, 2/4/25 Shroff Dep. at 77:8-19 (Reply at 2)

Dr. Shroff: No Definition In Specification



Hari Shroff, Ph.D.

9 Q Is there an explicit definition of the
10 claim term "temperature control apparatus" in the
11 '554 patent?

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

EX1082, 2/4/25 Shroff Dep. at 78:9-14 (Reply at 3)

Dr. Shroff: No Definition In Specification



Hari Shroff, Ph.D.

8 Q Please identify for me where, in the
9 '554 patent, you contend the patent provides an
10 explicit definition of the term temperature
11 control apparatus?

12 A Sir, I don't contend that the patent,
13 as I just said, explicitly defines temperature
14 control. My contention is that a POSA would have
15 understood the meaning of temperature control in
16 light of the language of the patent specification
17 and the claims. And I give -- I give examples and
18 I'm happy to read in paragraph 62.

EX1082, 2/4/25 Shroff Dep. at 79:8-18 (Reply at 3)

Dr. Shroff: Unaware Of Whether Claims Can Be Limited To Examples



Hari Shroff, Ph.D.

20 Do you know, one way or the other,
21 whether there are any rules related to whether
22 it's appropriate to limit claims in a patent to
1 examples or embodiments in the spec?

2 Do you know?

4 A Again, sir, my answer has to be the
5 same. I'm not a lawyer, so I'm not sure exactly
6 what you're referring to here.

EX1082, 2/4/25 Shroff Dep. at 80:20-81:6 (objections omitted) (Reply at 2)

Dr. Shroff: Unaware Of Whether Claims Can Be Limited To Examples



Hari Shroff, Ph.D.

8 Let's say that in a patent, there's
9 only a single embodiment. Do you know, one way or
10 the other, whether, in those circumstances, it
11 would be appropriate to construe the claims as
12 being limited to only that embodiment?

14 A Sorry, sir, I don't have the knowledge
15 to answer your question there.

EX1082, 2/4/25 Shroff Dep. at 81:8-15 (objections omitted) (Reply at 2)

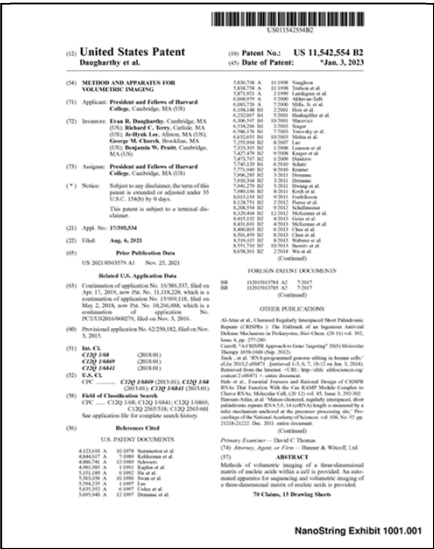
Specification: Both Heating And Cooling Not Required

	
United States Patent Dushkanyuk et al.	
(2)	(19) Patent No. US 11,542,584 B2 July 3, 2023
(3) WITHDRAWN APPLICATION FOR VOLUNTARY PENDING	
(4) Application Product and Methods of Harvest of Cells , Canadian No. 30,143,176	
(5) Inventors Erin B. Dushkanyuk , Cambridge, MA (57a) Michael E. Berry , Cambridge, MA (57b) Jeffrey Lee Wilson , Worcester, MA (57c) George W. Church , Worcester, MA (57d) Benjamin W. Frenkel , Cambridge, MA	
(6) Assignee Product and Methods of Harvest of Cells , Canadian No. 30,143,176	
(7) Notice Subject to any disclaimer, the contents of this paper are intended to inform the public of the status of the application for a patent in the United States. This paper is subject to any and all disclaimer that may appear in this journal.	
(8) App. No. 11,542,584	
(9) Filed Aug. 6, 2021	
(10) US 2021/0005747 A1 Nov. 25, 2021	
Related U.S. Application Data	
(11) Continuation of application No. 15,960,126, filed on Sep. 11, 2017, which is a continuation of application No. 14,578,119, filed on Dec. 11, 2014, which is a continuation of application No. 13,759,418, filed on Nov. 12, 2013, which is a continuation of application No. 13,029,182, filed on Dec. 11, 2010, which is a continuation of application No. 12,929,182, filed on Nov. 28, 2009. 	
(12) Int. Cl. C12N 1/00	
(13) CPC C12N 1/00	
(14) U.S. Class. C12N 1/00	
(15) Field of Classification Search C12N 1/00	
(16) Field of Classification Search C12N 1/00	
(17) References Cited	
(18) U.S. PATENT DOCUMENTS	
(19) Foreign Patent Documents	
(20) OTHER PUBLICATIONS	
(21) Abstract	
(22) Claims	
(23) Drawings	
(24) References	
(25) References	
(26) References	
(27) References	
(28) References	
(29) References	
(30) References	
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(73) References	
(74) References	

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), such as according to a programmed time and temperature, such as is useful with thermo-cycling for applications such as nucleic acid hybridization, amplification and sequencing. The heat-

EX1001, '554 Patent At 18:5-7 (Reply at 4)

Specification: Both Heating And Cooling Not Required



Aspects of the present disclosure are directed to an automated sequencing and volumetric imaging device including a multi axis stage or positioning system including a sample holder for a three dimensional nucleic acid containing matrix, a heating or cooling apparatus operationally connected to the stage, whereby the heating or cooling apparatus is programmable for time and temperature useful with thermo-cycling for amplification and sequencing, a

EX1001, '554 Patent At 28:32-35 (Reply at 4)

Dr. Shroff: No Awareness Of Concepts Of Disavowal Or Disclaimer



Hari Shroff, Ph.D.

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

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

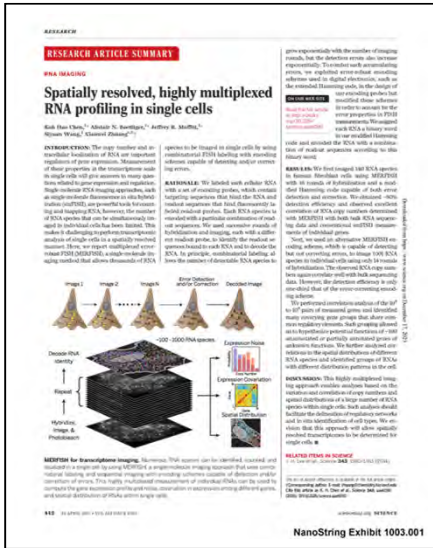
22 Q You don't contend, in your report, that
1 there's any disavowed or disclaimer of claim scope
2 in either the specification or the prosecution
3 history, correct?

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

EX1082, 2/4/25 Shroff Dep. at 81:16-82:6 (objection omitted) (Reply at 3)

**“Temperature Control” Is Anticipated Or
Obvious In View Of Chen**

Chen: Discloses Use Of Bioptechs FCS2



MERFISH imaging

The sample coverslip was assembled into a Bioptech's FCS2 flow chamber, and the flow through this chamber was controlled via a home-built 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). The sample was

EX1003.011 (Petition at 23)

FCS2 Has Temperature Control

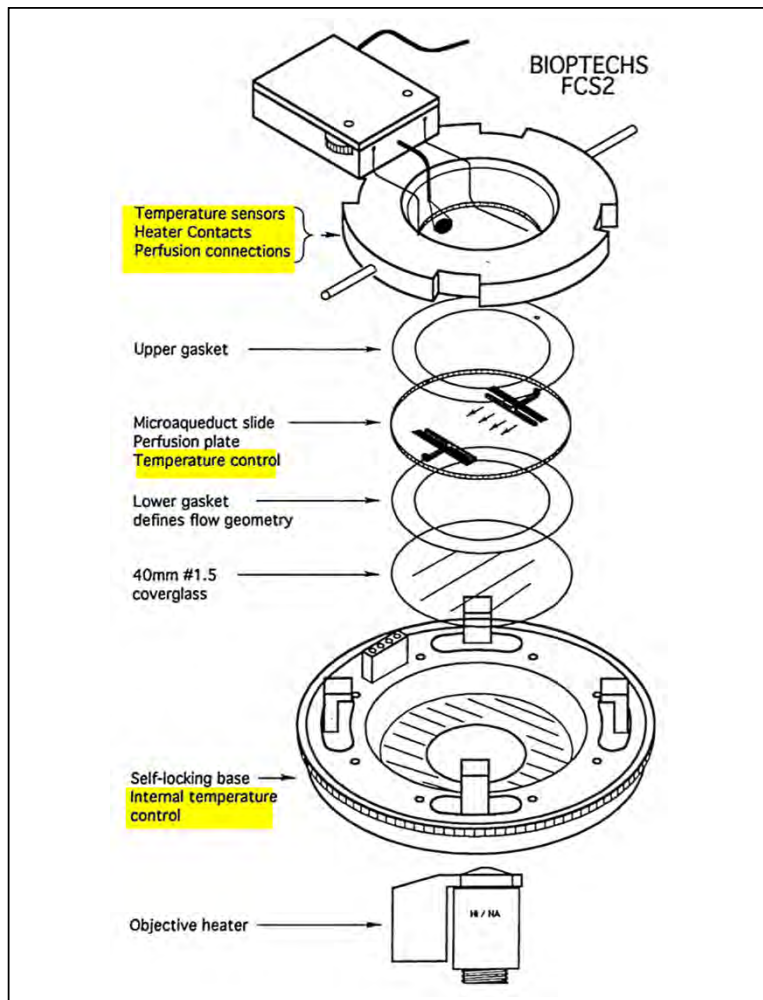


FIGURE 75.4

Microaqueduct slide technique. This system is characterized by sophisticated temperature control, high-volume perfusion rates, and compatibility with standard microscopy techniques.

EX1015, Spector – Volume 2: Light Microscopy and Cell Structure at 1015.017 (Petition at 48)

FCS2 Has Temperature Control



Dr. Czymmek's Declaration

143. Further evidence supports that it was well-known to a POSA that the Bioptechnics FCS2 flow cell includes temperature control. An expanded view of the FCS2 system with the conductive temperature sensors shown at the top is depicted in the figure below from a book chapter regarding the Bioptechnics FCS2 flow cell: (EX1015.017). As the image shows, the microaqueduct slide includes a “[t]emperature control” as does the self-locking base, which includes “[i]nternal temperature control.” *Id.*

EX1002 ¶143 (Petition at 24)

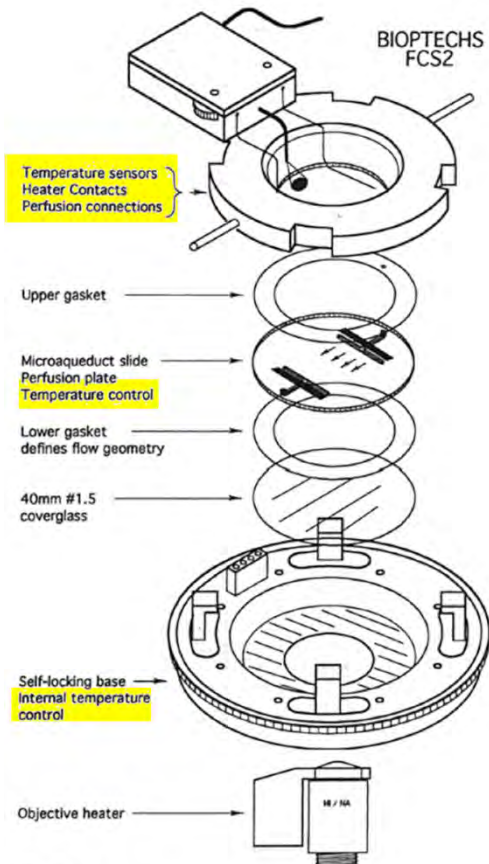
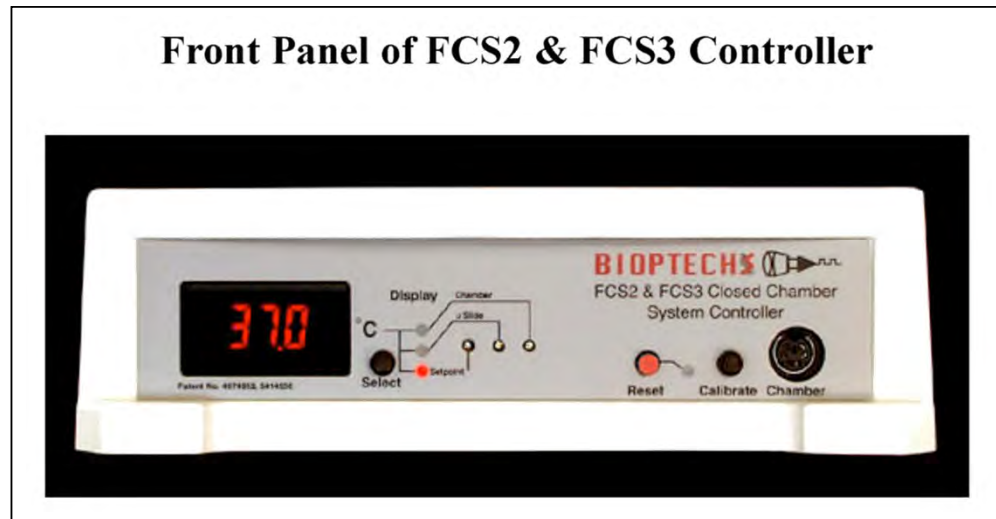


FIGURE 75.4
Microaqueduct slide technique. This system is characterized by sophisticated temperature control, high-volume perfusion rates, and compatibility with standard microscopy techniques.

FCS2 Has Temperature Control



EX1006.Fig.1 (Petition at 44)

Contemporaneous Literature: FCS2 Has Temperature Control

Shear Stress Induced Reorganization of the Keratin Intermediate Filament Network Requires Phosphorylation by Protein Kinase C ζ

Sivara J. Sivaramakrishnan,¹ Jaime L. Schneider,² Albert Sitkov,³
Robert D. Goldman,² and Karen M. Ridge¹

Submitted October 14, 2009; Revised March 2, 2010; Accepted March 30, 2010

[illegible]

INTRODUCTION

neuroanatomical filament (20). The comparison of the cytoskeleton of cells in culture and intracellular cytoskeletons of cells in the brain has revealed several differences, which include: (1) no different genes (Owens et al., 2000); (2) different proteins (Owens et al., 2000); (3) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (4) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (5) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (6) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (7) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (8) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (9) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (10) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (11) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (12) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (13) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (14) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (15) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (16) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (17) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (18) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (19) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000); (20) different organization of the cytoskeleton in neurons of the brain (Owens et al., 2000).

epithelial cells used in this study contain polymers of keratin 1 and 18 (K1/K18; Ridge et al., 2005; Jorjick et al., 2008; Sureshkumar et al., 2009). K1 polymers are important for the mechanical properties of epithelial cells (Ridge et al., 2005; Sureshkumar et al., 2009; Hestermann and Aebi, 2004; Jorjick et al., 2008). The mechanical properties of the integrity of the epithelium, resulting in a wide variety of diseases including cancer, hepatitis, skin blunting disorders, and skin aging (Ridge et al., 2005; Jorjick et al., 2008; Sureshkumar et al., 2009).

Intermediate filaments are dynamic structures that undergo a cycle of assembly and disassembly, and are involved in structural reorganization of the network in response to mechanical forces (Helmke et al., 2003; Yuen et al., 2001; Weis et al., 2003; Sureshkumar et al., 2009). The molecular structure of the K12 network and the local mechanical properties in primary abraded epithelial cells and ABR cells were studied using atomic force microscopy (AFM) and local remodeling of K12 network and a substantial increase in the stiffness of the network were observed (Sureshkumar et al., 2008). The mechanoprotection that regulates the shear stress-mediated structural reorganization of the K12 network is mediated by the K12 network itself.

The assembly state of K12s is regulated primarily by post-translational modifications, such as phosphorylation and ubiquitination of specific residues in the head and tail domains of the proteins (Cheney et al., 1990). Five phosphorylation sites (Ser19, Ser20, Ser21, Ser22, and Ser23) in the head domain of K12s (K12pSer19, K12pSer20, K12pSer21, K12pSer22, and K12pSer23) (Kim and Yoon, 2004) are known to be involved in the regulation of these sites, increase during different cellular processes; for instance, K12pSer23 is highly phosphorylated during mitosis and is important for the regulation of the cell cycle.

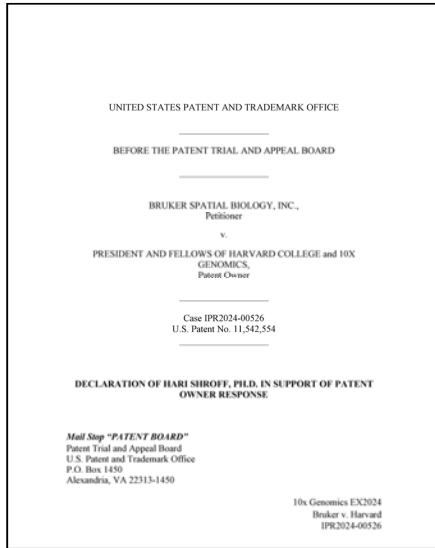
This article was published online ahead of print in *BMJ* in *Press* (<http://www.bmjjournal.org/cgi/doi/10.1136/bmj.e10-e10>) on April 8, 2009.

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 μ M 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 were incubated for 120 min at 37°C. Shear stress (SS) cells were subject to flow through the chamber to yield an average laminar shear stress of 15 dyn/cm² for 120 min at 37°C. Finally, the Bioptechs chamber was mounted onto the stage of an LSM 510Meta confocal microscope (Carl Zeiss) whose stage and objective preequilibrated at 37°C by an air stream stage incubator (model ASI 400; Nevtek, Burnsville, VA). For SS cells flow was continued through the chamber for the entire duration of the experiment. No flow was passed through the chamber for the CT cells. To ensure the health of the cells for the duration of the experiment, samples of media were taken from the Bioptechs chamber at the start and end of the experiment to measure pH, pCO₂ and pO₂ using Novamed Blood Gas Analyzer. No significant changes in pH, pCO₂ or pO₂ were observed in the cell imaging medium from the start to the end of experiment.

EX1007.004 (Petition at 24)

Dr. Shroff: Relies On MERFISH Publications

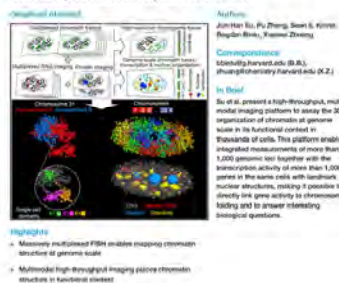


49. The objective of increasing multiplexity in MERFISH is further demonstrated by the research group's later publication and patent applications directed to MERFISH. For example, in 2016, the group reported "an improved MERFISH method that drastically *increases the throughput*" of the procedure. EX2053, 11047. I understand that the same research group has also applied for several patents directed to MERFISH. I understand from reviewing portions of the file histories of U.S. Patent Application Nos. 17/374,000 and 18/184,967 that the research group acknowledged the limitations in multiplexity of the smFISH and seqFISH methods that MERFISH was intended to address. For example, in

EX2024, Declaration Of Hari Shroff at ¶49 (Reply at 9)

MERFISH Publication: Identifies Specific FCS2 That Was Used

Cell Genome-Scale Imaging of the 3D Organization and Transcriptional Activity of Chromatin



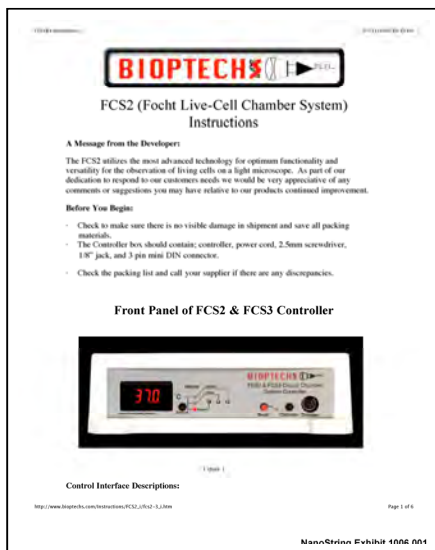
Sequential hybridization of readout probes for sequential or combinatorial FISH imaging

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 (Bioprocess, 060319-2). The setup of this system is described in detail in the “Fluidics system configuration” section. Briefly, the fluidics system consisted of 3-5 computer-controlled eight-way valves (Hamilton, MVP and HVXM 8-5) and a computer-controlled peristaltic pump (Gilson, MINIPLUS 3). Put together, these components allow control of both the rate of fluid flow and of the type of fluid flowing at any given time.

Each round of hybridization consisted of the following general steps:

EX2009 at pg. e7 (Response at 4)

Chen's FCS2 Included Temperature Control



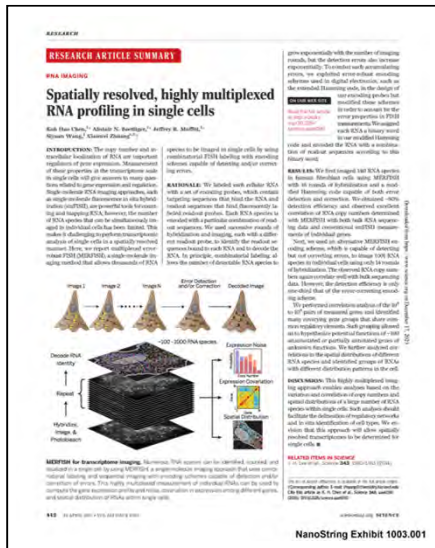
Continual Perfusion with the Micro-Perfusion Pump:

Bioptechs recommends the FCS2 Micro-Perfusion Pump due to its smooth flow profile in slow, flow applications. If dual flow is needed there is also a PC interface board and program that enables alternate control of two pumps to regulate the acceleration and run time of each. The Micro-Perfusion Pump Controller (PC interface) is ideal for changing concentrations of media.

©Bioptechs, Inc., 4/19/2001
BNi060319-2

EX1006 at 1006.006 (Petition at 44, 49; Response at 46)

Chen: Also Uses An Objective Heater



MERFISH imaging

The sample coverslip was assembled into a Bioptech's FCS2 flow chamber, and the flow through this chamber was controlled via a home-built 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). The sample was controlled eight-way valves (Hamilton, MVP and HVXM 8-5) and a computer-controlled peristaltic 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 $\times$ oil immersion objective and configured for oblique incidence excitation. The objective was heated to 37°C with a Bioptechs objective heater.

EX1003.011 (Petition at 24)

Dr. Czymmek: POSA Would Understand FCS2 Has Temperature Control



Kirk Czymmek, Ph.D.

Dr. Czymmek's Declaration

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.

EX 1086 Czymmek Reply Dec. ¶ 29 (Reply at 4; Sur-Reply at 7)

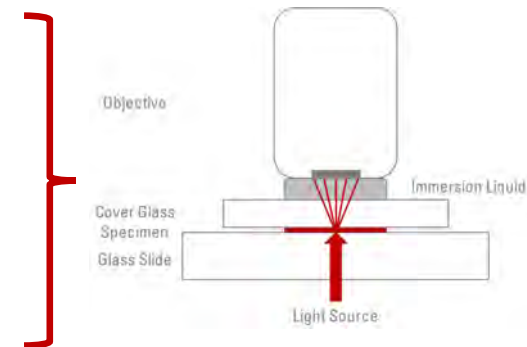
Dr. Czymmek: Objective Heater Provides Temperature Control



Kirk Czymmek, Ph.D.

Dr. Czymmek's Declaration

144. Zhuang also discloses that the “objective was heated to 37°C with a Bioptechs objective heater.” EX1003.011. This is yet an additional temperature control. In my opinion, a POSA would have understood that this objective heater is operatively coupled to the sample holder as a POSA would have appreciated that it was the cells and perfused solutions that required the temperature to be maintained at 37°C. In particular, during an experiment using an oil immersion lens, the objective lens with the oil immersion medium droplet placed on the front lens element is moved to contact the coverslip with the attached sample on the opposite side of the coverslip. Thus, the heated objective is in thermal contact with the sample holder, i.e., the objective is transferring heat through the oil as a medium. The objective heater is therefore an additional temperature control system, additionally minimizing thermal gradients that would cause drift while imaging, and in the process of use becomes coupled to the stage and sample holder.



EX 1002 Czymmek Reply Dec. ¶ 144 (Reply at 4)

Dr. Shroff: Objective Heater Is Coupled To Sample Holder



Hari Shroff, Ph.D.

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, 2/4/25 Shroff Dep. at 82:7-14 (Reply at 6)

Claim 43: Recites “Peltier Element”

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

EX1001, US 11,542,554 at Claims 36, 43 (Petition at 13; Reply at 14)

Dr. Shroff: Peltier Element Is Active Cooling



Hari Shroff, Ph.D.

8 A The Peltier element is an active
9 cooling and heating system in the sense that one
10 has to put energy into the system. Heat sink
11 could be passive or it could be active, depending
12 on the context.

EX1082, 2/4/25 Shroff Dep. at 86:8-12 (Reply at 6)

Dr. Shroff: Peltier Element Is Active Cooling



Hari Shroff, Ph.D.

11 Q And that, in your view, the use of a
12 heat sink or Peltier element satisfies the cooling
13 aspect of your construction of temperature control
14 apparatus, right?

15 A It satisfies cooling. But, again, as I
16 just said, a Peltier element could be used for
17 heating, and a heat sink could be used to remove
18 heat generated as part of the Peltier element's
19 heating operation. This is also something that a
20 POSA would have known in the art.

EX1082, 2/4/25 Shroff Dep. at 99:11-20 (Reply at 6)

Dr. Shroff: Peltier And Heat Sink Are Active Cooling



Hari Shroff, Ph.D.

17 You agree that a heat sink and a
18 Peltier element, those are active cooling
19 elements, right?

20 A Heat sink is an active cooling element
21 insofar as the language of the '554 patent.
22 Peltier elements would be considered to be active
1 heating and cooling elements in the sense that you
2 put in energy and then the element can either heat
3 or cool.

EX1082, 2/4/25 Shroff Dep. at 97:17-98:3 (Reply at 6)

Dr. Shroff: Heat Sink and Peltier Are Active Cooling



Hari Shroff, Ph.D.

5 Q Okay. A person of skill in the art
6 would have recognized that the heat sink or
7 Peltier element in claim 43 would have been
8 recognized as being used for cooling parts of the
9 system, correct?

10 A They would have recognized that, yes.

EX1082, 2/4/25 Shroff Dep. at 99:5-10 (Reply at 6)

Dr. Czymmek: Peltier Element Was Obvious



Kirk Czymmek, Ph.D.

Dr. Czymmek's Declaration

233. A POSA would also have understood that heat sinks and Peltier elements were well known and that both could be used to achieve temperature control. A POSA thus would have understood from Zhuang's disclosures relating to controlling temperature that a heat sink and/or Peltier element could be used to control temperature. As explained, a POSA would have been wholly familiar with heat sinks and Peltier elements and their use because they were commonly used before the date of the alleged invention. It is my further opinion that it would have been obvious to a POSA to include heat sinks or Peltier elements as a part of the temperature control apparatus for the benefit of improved temperature control and cooling capabilities.

EX 1002 Declaration of Kirk Czymmek ¶ 233 (Reply at 6)

Dr. Shroff: POSA Would Have Used Heat Sink Or Peltier



Hari Shroff, Ph.D.

11 Q Why, in 2015, would a person of skill
12 in the art have used heat sink or a Peltier
13 element?

14 A A person skilled in the art would have
15 known, in 2015, that a heat sink or Peltier
16 element could be an element of temperature
17 control.

EX1082, 2/4/25 Shroff Dep. at 96:11-17 (Reply at 6)

Dr. Shroff: No Rebuttal To Dr. Czymmek



Hari Shroff, Ph.D.

16 Q If there is any place in your
17 declaration where you believe you respond to
18 Dr. Czymmek's opinions on claim 43, please,
19 identify where that is now.

20 A I'm not arguing that Dr. Czymmek -- I'm
21 not arguing with Dr. Czymmek that a POSA would
22 have known that heat sinks and Peltier elements
1 could be used to control temperature. Rather,
2 what I'm saying is that the specification of the
3 '554 patent, including claim 43, with respect to,
4 additionally, the independent claim 36, is not the
5 same as what is described in Chen, Cai, and Shen,
6 as Dr. Czymmek asserts.

EX1082, 2/4/25 Shroff Dep. at 90:16-91:6 (Reply at 6)

Dr. Shroff: No Rebuttal To Dr. Czymmek



Hari Shroff, Ph.D.

19 Is there any place where you present
20 opinions that a person of ordinary skill in the
21 art wouldn't have found it obvious to use a heat
22 sink or Peltier element with Chen, Cai, or Shen?

2 A Again, a person of skill in the art
3 would know what heat sinks and Peltier elements
4 are, in the context of temperature controls, but
5 the point that I'm making in my declaration is
6 that the meaning of temperature control vis- -vis
7 these element, in the context of the '554 patent,
8 is distinct from what Dr. Czymmek is asserting how
9 temperature control is used in Chen, Shen, and
10 Cai.

EX1082, 2/4/25 Shroff Dep. at 94:19-95:10 (objections omitted) (Reply at 6)

Dr. Shroff: No Rebuttal To Dr. Czymmek



Hari Shroff, Ph.D.

13 Q Okay. Tell me again where you present
14 the rebuttal on to Dr. Czymmek's opinion that it
15 would have been obvious to use a heat sink or a
16 Peltier for the benefit of improved temperature
17 control and cooling and heating capabilities.
18 Tell me again where that is in your declaration.
19 A Sir, I never asserted that heat sinks
20 or Peltier elements wouldn't have been known to a
21 POSA, that it isn't obvious what a heat sink or
22 Peltier element does. Rather, as I've said
1 before, the material in my declaration, including
2 the paragraphs that I previously stated and the
3 stenographer has on record, have to do with the
4 particular language and meaning of temperature
5 control apparatus in the context of the
6 '554 patent's claim 43 and independent claim 36.
7 Which is distinct from the meaning of temperature
8 control in Czymmek, as referenced in Cai, Chen,
9 and Shen.

EX1082, 2/4/25 Shroff Dep. at 100:13-101:9 (Reply at 6)

Adapting Chen For 3D Imaging Would Have Been Obvious

Claim 36: “Three-Dimensional Positional Information”

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

(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, US 11,542,554 at Claim 36
(Petition at 10)

3D Imaging By Optical Sectioning



4.1 Three-dimensional optical sectioning

It is a matter of fact that the possibility of a 3D reconstruction of an object starting from the acquisition of two-dimensional, 2-D, datasets made by consecutive optical slices, is challenging and powerful towards morphological analysis and volume rendering. The gentle procedure of optical sectioning to get information from different planes of the specimen without being invasive allows preserving structures and functions. A wide-field microscope, Fig. 16 can be used to collect a series of 2D images of the specimen by moving the focal plane across the 3D specimen. A set of 2D images is acquired at various focus positions along the z-axis, Fig. 17. The result of such collection can be arranged in separate views or in a single view that considers all the information collected in the 3D volume, Fig. 18.

EX2078 at 24 (Reply at 7)

3D Imaging By Optical Sectioning

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BRUKER SPATIAL BIOLOGY, INC.,
Petitioner

v.

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

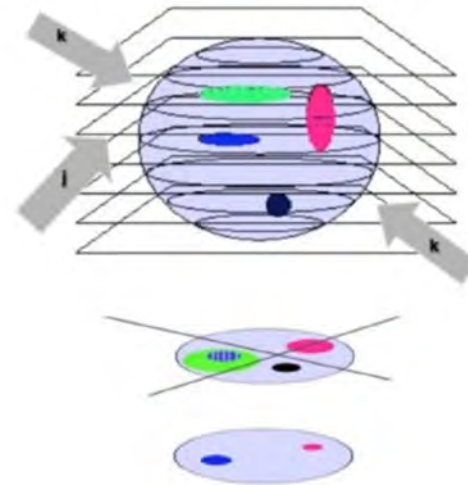
Case IPR2024-00526
U.S. Patent No. 11,542,554

DECLARATION OF HARI SHROFF, PH.D. IN SUPPORT OF PATENT
OWNER RESPONSE

Mail Stop "PATENT BOARD"
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

10x Genomics EX2024
Bruker v. Harvard
IPR2024-00526

Fig. 17 Optical sectioning is referred to a starting in-focus image plane, *j*, within a set of out-of-focus planes, *k*. When the focus of the lens is placed in the plane *j*, blurred information can come from adjacent planes. Such a contamination can be removed by image processing, confocal imaging or two-photon excitation microscopy



EX2024 - Shroff Declaration (PUBLIC - REDACTED) ¶ 36 (Reply at 7)

Dr. Shroff: Stacking Focal Planes Is For 3D Imaging



Hari Shroff, Ph.D.

8 Q Now, in November 2015, were people of
9 ordinary skill in the art aware of the concept of
10 stacking focal claims to do 3D microscopy?

11 A What I would say is that, in general,
12 the idea of taking a stack to acquire a volume was
13 known. But of course in particular, and with
14 reference to our discussion here, what was not
15 known is the know-how to any multiplexed RNA
16 environment obtained axial coordinates, as I
17 discussed at length in my declaration.

EX1082, 2/4/25 Shroff Dep. at 121:8-17 (Reply at 8)

Dr. Shroff: Stacking Focal Planes



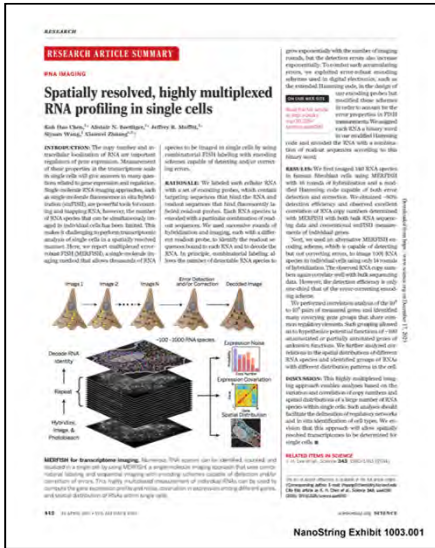
Hari Shroff, Ph.D.

17 Q And we talked about this earlier, the
18 strategy of tiling adjacent focal planes to
19 generate a 3D image, that was known in November of
20 2015, right?

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

EX1082, 2/4/25 Shroff Dep. at 227:17-228:3 (objections omitted) (Reply at 8)

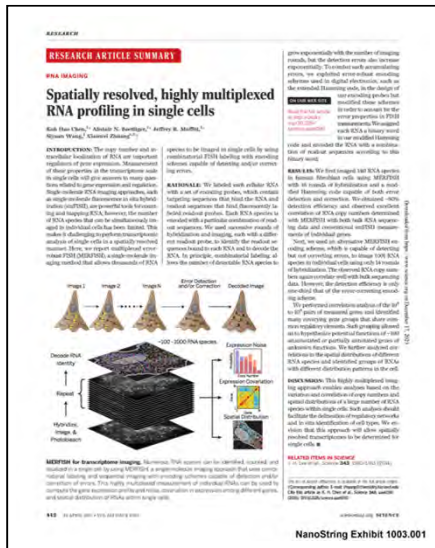
Chen: Teaches Optical Sectioning



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 (Petition at 38)

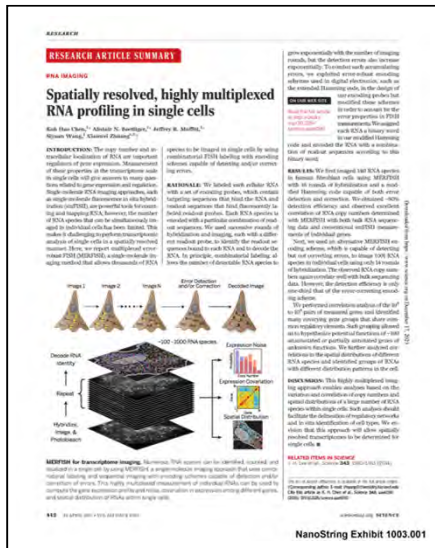
Chen: Teaches 3D Spatial Information



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 (Reply at 8)

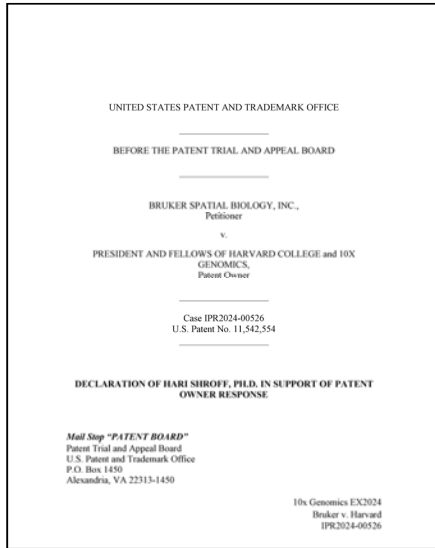
Chen: Teaches 3D Spatial Information



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 (Petition at 34)

Dr. Shroff: Relies On MERFISH Patents



49. The objective of increasing multiplexity in MERFISH is further demonstrated by the research group's later publication and patent applications directed to MERFISH. For example, in 2016, the group reported "an improved MERFISH method that drastically *increases the throughput*" of the procedure. EX2053, 11047. I understand that the same research group has also applied for several patents directed to MERFISH. I understand from reviewing portions of the file histories of U.S. Patent Application Nos. 17/374,000 and 18/184,967 that the research group acknowledged the limitations in multiplexity of the smFISH and seqFISH methods that MERFISH was intended to address. For example, in

EX2024, Declaration Of Hari Shroff ¶ 49 (Response at 19, 22-24, 38; Reply at 9)

Dr. Shroff: MERFISH Patents Show Its Intended Use



Hari Shroff, Ph.D.

1 A The reason I cited these patent
2 applications, sir, is that I'm trying to make an
3 argument that MERFISH is directed towards
4 multiplexity. That's the sense in which I'm using
5 these additional documents, as I describe in my
6 declaration.

EX1082, 2/4/25 Shroff Dep. at 21:1-4 (Reply at 9)

Dr. Shroff: MERFISH Patents Show Its Intended Use



Hari Shroff, Ph.D.

14 So after 2015, after the date of the
15 '554 patent, the point is that the MERFISH group
16 were, themselves, interested in going after
17 multiplexing. That's the context in which these
18 paragraphs should be read and the context in which
19 I cite these documents.

EX1082, 2/4/25 Shroff Dep. at 21:14-19 (Reply at 9)

Dr. Shroff: MERFISH Patents Show Its Intended Use



Hari Shroff, Ph.D.

7 Q Okay. Is it fair, then, that you
8 believe these patent applications, that are
9 directed towards MERFISH, reflect evidence of what
10 the goal of MERFISH is?

11 A Of course. These patent applications,
12 the ones that I am citing here, are examples after
13 2015. So in that sense, you know, it's happening
14 after the date of the '554 patent, but it's
15 certainly consistent with the idea that this group
16 and the field, in general, was moving towards
17 increased multiplexing.

EX1082, 2/4/25 Shroff Dep. at 22:7-17 (Reply at 9)

Chen's MERFISH Patent: Teaches 3D



In addition, 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. In some embodiments, the positions, number, and/or concentrations of nucleic acids within the cell (or other sample) may be determined.

EX 1073 327 Patent at 14:34-40 (Reply at 9)

Chen's MERFISH Patent: Teaches 3D



After introduction of the nucleic acid probes into a cell or other sample, the nucleic acid probes may be directly determined by determining signaling entities (if present), and/or the nucleic acid probes may be determined by using one or more secondary nucleic acid probes, in accordance with certain aspects of the invention. As mentioned, in some cases, the determination may be spatial, e.g., in two or three dimensions. In addition, in some cases, the determination may be quantitative, e.g., the amount or concentration of a primary nucleic acid probe (and of a target nucleic acid) may be determined. Additionally, the secondary probes may comprise any of a variety of entities able to hybridize a nucleic acid, e.g., DNA, RNA, LNA, and/or PNA, etc., depending on the application. Signaling entities are discussed in more detail below.

EX 1073 at 18:44-58 (Reply at 9)

Chen's MERFISH Patent: Teaches 3D

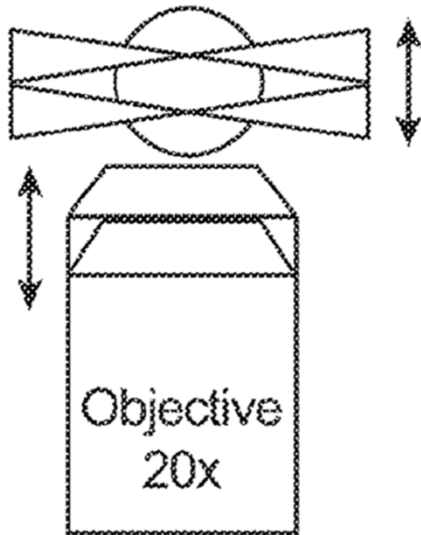


As discussed above, in certain aspects, signaling entities are determined, e.g., to determine nucleic acid probes and/or to create codewords. In some cases, signaling entities within a sample may be determined, e.g., spatially, using a variety of techniques. In some embodiments, the signaling entities may be fluorescent, and techniques for determining fluorescence within a sample, such as fluorescence microscopy or confocal microscopy, may be used to spatially identify the positions of signaling entities within a cell. In some cases, the positions of entities within the sample may be determined in two or even three dimensions. In addition, in some embodiments, more than one signaling entity may be determined at a time (e.g., signaling entities with different colors or emissions), and/or sequentially.

EX 1073 at 22:49-62 (Reply at 9)

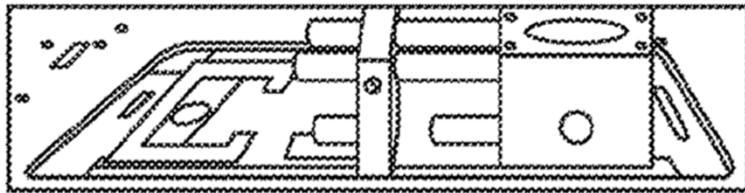
Cai: Teaches Optical Sectioning

Scanning Sample z direction



directly onto the same base of objective. With this configuration, the objective is moving synchronically with the illumination sheet, allowing scanning the sample along z-axis (right, top). The right (bottom) figure also shows that.

EX1004.47:43-45 (Petition at 33)



EX1004.Fig.17
(right-hand portion)
(Petition at 33)

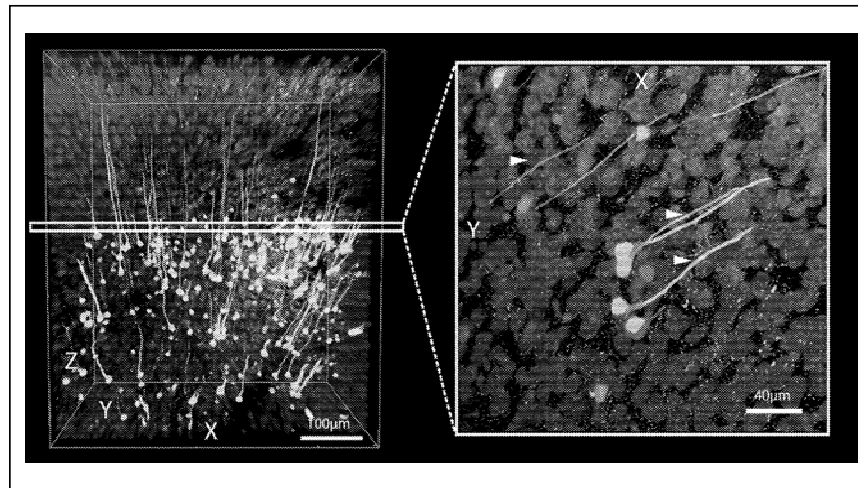
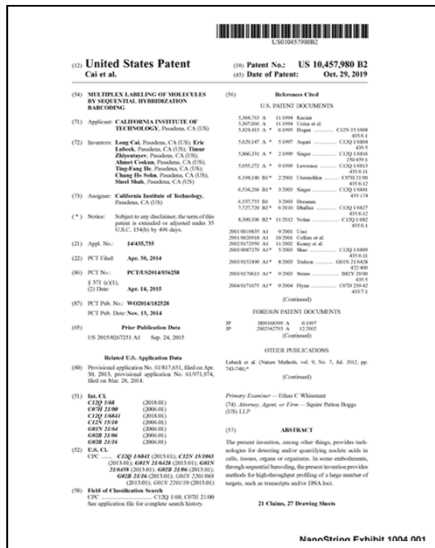
Cai: Teaches 3D Spatial Information

[illegible]

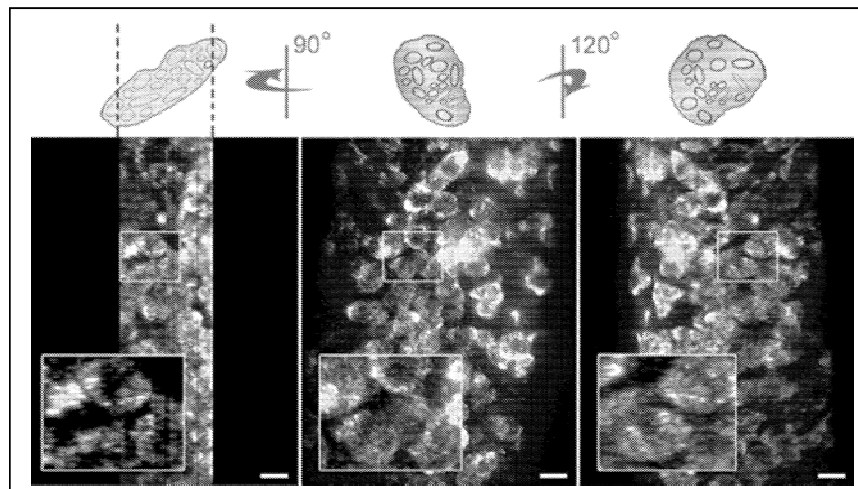
In some embodiments, an imaging step detects a target. In some embodiments, an imaging step localizes a target. In some embodiments, an imaging step provides three-dimensional spatial information of a target. In some embodiments,

EX1004.21:50-51 (Petition at 34)

Cai: Teaches 3D Spatial Information



EX1004 at Fig. 16 (Reply at 10)



EX1004 at Fig. 18 (Reply at 10)

Dr. Shroff: Cai Figure 16 Is 3D Imaging



Hari Shroff, Ph.D.

20 Q Now, if you look at the figure caption,
21 it says "3D coronal rendering of the motor
22 cortex."

1 What does that mean to you?

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

EX1082, 2/4/25 Shroff Dep. at 261:20-262:5 (Reply at 10)

Dr. Shroff: Cai Figure 16 Is 3D Imaging



Hari Shroff, Ph.D.

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

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

EX1082, 2/4/25 Shroff Dep. at 262:11-14 (Reply at 10)

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

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

EX1082, 2/4/25 Shroff Dep. at 263:13-15 (Reply at 10)

Dr. Shroff: Cai Figure 18 Is 3D Imaging



Hari Shroff, Ph.D.

13 Q Now, let's go to Figure 18.

14 A Yes, Figure 18.

15 Q What is being shown in Figure 18?

16 A Again, looking at the caption in
17 Figure 11 -- in column 11 of this document, what's
18 rendered here appears to be beta actin mRNA in a
19 3D reconstruction, where the beta mRNA has been
20 amplified by a technique called HCR.

21 Q So this is 3D data in Figure 18?

22 A It's a rendering of 3D data, sir, yes.

EX1082, 2/4/25 Shroff Dep. at 264:13-22 (Reply at 10)

PO's Argument: Chen Is A "Homebuilt" System

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BRUKER SPATIAL BIOLOGY, INC.,
Petitioner

v.

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

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

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

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Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

III. THE 554 PATENT

A. The 554 patent's claimed system

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

PTAB-IPR2024-00526-20 POR, Patent Owner Response at 24

Dr. Shroff: Homebuilt Is Not In The Claims



Hari Shroff, Ph.D.

22 A Sir, I wouldn't say that claim 36
1 excludes homebuilt systems because nowhere in
2 that -- nowhere in those claims do we have any
3 language about that one way or the other. I'm

EX1082, 2/4/25 Shroff Dep. at 63:22-64:3 (Reply at 21)

Dr. Shroff: No Opinions On “Homebuilt And Obviousness”



Hari Shroff, Ph.D.

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

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

EX1082, 2/4/25 Shroff Dep. at 61:13-17 (Reply at 20)

Dr. Shroff: Claims Not Limited to “Integrated Or Monolithic” Systems

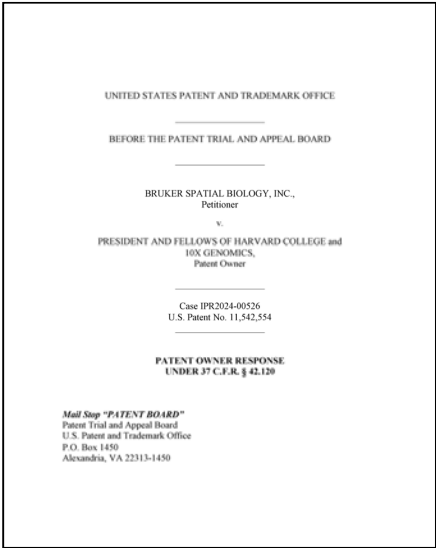


Hari Shroff, Ph.D.

11 Q Is it limited, is claim 36 limited to
12 systems that are integrated or monolithic? Do you
13 have an opinion on that?
14 A Sir, I don't have an opinion on that.

EX1082, 2/4/25 Shroff Dep. at 65:11-14 (Reply at 21)

PO's Argument: Chen Is Focused On High Multiplexing



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

PTAB-IPR2024-00526-20 POR, Patent Owner Response at Pg. 5

Claim Language: Only Requires One Target

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

- (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, US 11,542,554 at Claims 36 (Petition at 10, 13; Reply at 14)

'554 Claim Language: No Minimum Sample Thickness



Hari Shroff, Ph.D.

3 Q Okay. Do the claims require any
4 minimum sample thickness?

5 A I don't recall seeing any statement
6 about minimal sample thickness. Although, as we
7 discussed, and as is in my declaration, the sample
8 thickness certainly has a bearing on the ability
9 to determine accurate three-dimensional positional
10 information, particularly --

11 Q The claim --

12 A -- in the context of MERFISH, in which
13 there are many nucleic acid molecules that are
14 stained.

EX1082, 2/4/25 Shroff Dep. at 220:3-14 (Reply at 16)

'554 Claim Language: Encompasses Analysis Of A Single Cell



Hari Shroff, Ph.D.

8 Q You agree that 3D imaging of a single
9 cell, that's within scope of the claims of the
10 '554 patent, right?

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

EX1082, 2/4/25 Shroff Dep. at 180:8-13 (objection omitted) (Reply at 16)

'554 Claim Language: Encompasses Analysis Of A Single Cell



Hari Shroff, Ph.D.

16 Q That precludes the analysis of the
17 single cell, right? There's no language in the
18 claim that precludes the analysis of a single
19 cell, right?

21 A As far as I can tell, there's no
22 language in this claim that precludes that.

EX1082, 2/4/25 Shroff Dep. at 222:17-22 (objection omitted) (Reply at 16)

'554 Claim Language: No Minimum Resolution Requirement



Hari Shroff, Ph.D.

2 A The claim nowhere says anything about a
3 minimum resolution number. That language is not
4 in the claim. And, yet, as I've explained in my

EX1082, 2/4/25 Shroff Dep. at , 217:2-4 (Reply at 25)

Dr. Czymmek: POSA Would Have Used MERFISH Appropriately



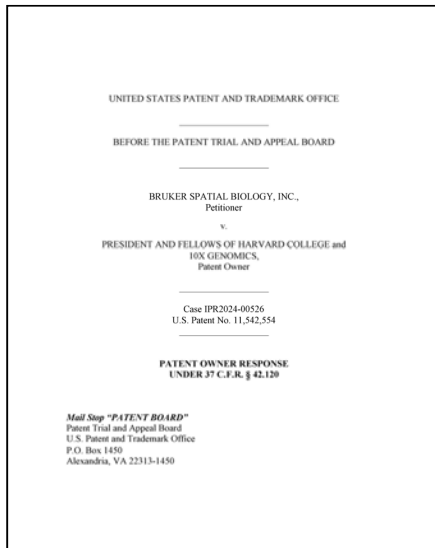
Kirk Czymmek, Ph.D.

Dr. Czymmek's Declaration

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.

EX 1086 ¶ 50 (Reply at 15)

PO's Argument: Chen And Cai Are Different



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

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

Petitioner's reliance on Cai's light sheet microscope system with Z-axis motion control fails to consider the viewpoint of a POSA. **A POSA would have understood that Cai's light sheet microscope is completely different from Chen's 2D MERFISH imaging setup** and was not designed for MERFISH imaging.

PTAB-IPR2024-00526-20 POR, Patent Owner Response at Pg. 44 (Reply at 11)

Law: Bodily Incorporation Not Required



The “test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference.”

In re Keller, 642 F.2d 413, 425 (C.C.P.A. 1981) (Reply at 11)

Law: POSA Is Not An Automaton



A “person of ordinary skill is also a person of ordinary creativity, not an automaton.”

KSR Int'l Co. v. Teleflex Inc., 550 U.S. 398, 421 (2007) (Reply at 11)

Law: Expectation Of Success Need Only Be Reasonable



The law is clear, however, that for obviousness, the expectation of success in modifying the prior art “need only be reasonable, not absolute.”

Pfizer, Inc. v. Apotex, Inc., 480 F.3d 1348, 1364 (Fed. Cir. 2007) (Reply at 15)

'544 Patent: Claims Wide-Field Microscopy

34. The system of claim 1, wherein said optical system is configured for wide-field microscopy.

EX1001, US 11,542,554 at Claim 34 (Reply at 13)

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

EX1001, US 11,542,554 at Claim 69 (Reply at 13)

Dr. Shroff: Optical Stacking May Be Used With Wide-Field



Hari Shroff, Ph.D.

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

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

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

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

EX1082, 2/4/25 Shroff Dep. at 228:4-14 (Reply at 12)

Dr. Shroff: Optical Stacking May Be Used With Wide-Field



Hari Shroff, Ph.D.

17 Q Now, with widefield imaging, is that
18 ever used with this concept of making Z stacks?

19 A Widefield imaging can be used in the
20 context of taking a Z stack.

EX1082, 2/4/25 Shroff Dep. at 212:17-20 (Reply at 12)

PO's Argument: Point Spread Function Makes 3D More Difficult

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BRUKER SPATIAL BIOLOGY, INC.,
Petitioner

v.

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

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

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

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Alexandria, VA 22313-1450

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

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

PTAB-IPR2024-00526-20 POR, Patent Owner Response at Pg. 10 (Reply at 10, 17)

Dr. Shroff: Methods For Improving Resolution Were Known



Hari Shroff, Ph.D.

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

1 Q Yes.

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

EX1082, 2/4/25 Shroff Dep. at 141:20-142:5 (Reply at 18)

Dr. Shroff: Methods For Improving Resolution Were Known



Hari Shroff, Ph.D.

6 Q And what were the methods of -- what
7 methods were available for doing that in
8 November 2015, that a person of ordinary skill in
9 the art would have known of?

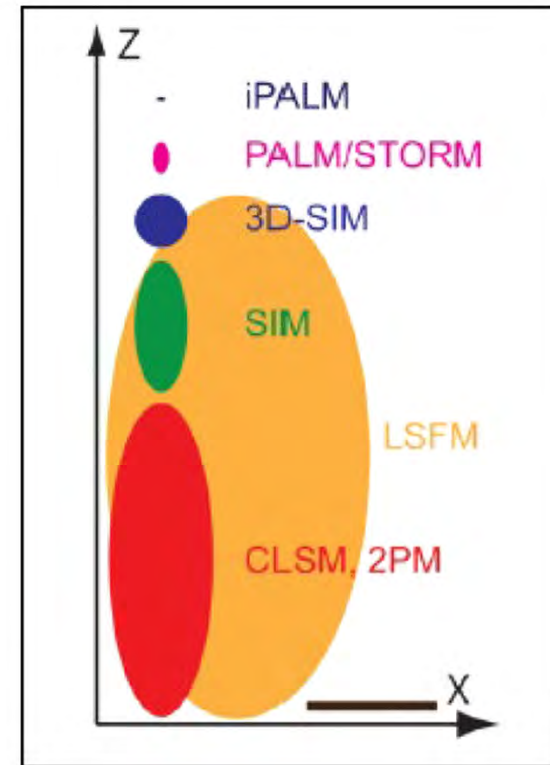
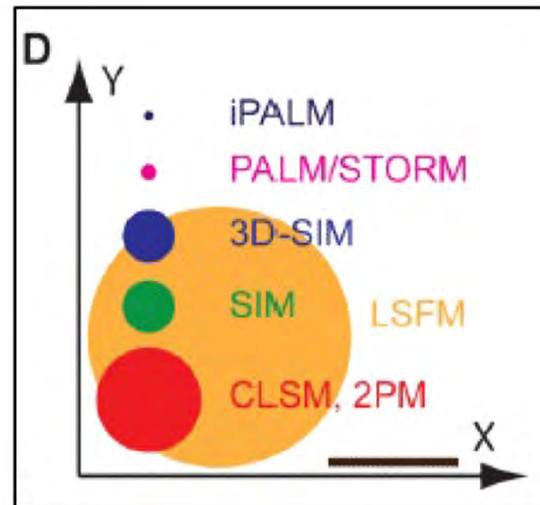
10 A Well, in general, there's a broad class
11 of methods called super-resolution microscopy
12 methods that a POSA would have known existed.

EX1082, 2/4/25 Shroff Dep. at 142:6-12 (Reply at 18)

Microscopy With Narrow PSF In Z-Direction Was Known



Hari Shroff, Ph.D.



EX1019, FIG. 1; EX2024, ¶24.

POR at 12 (Reply at 18)

Dr. Shroff: Super-Resolution Methods Known In 2015



Hari Shroff, Ph.D.

12 What are some of the super-resolution
13 methods that were available?

14 A For example, PALM, for example, STORM.

15 Q These were all available in
16 November 2015?

17 A IPALM, PALM, STORM, 3D SIM were all
18 available in November 2015.

EX1082, 2/4/25 Shroff Dep. at 143:12-18 (Reply at 18)

Dr. Shroff: Expansion Microscopy Could Improve Resolution



Hari Shroff, Ph.D.

1 Q Now, how about expansion microscopy?
2 That's a technique for improving resolution as
3 well, right?

4 A Expansion microscopy is a technique for
5 improving resolution. Regarding whether that was
6 available in 2015, you would have to check the
7 reference.

EX1082, 2/4/25 Shroff Dep. at 146:1-7 (Reply at 18)

Dr. Shroff: Deconvolution Methods Improve Resolution



Hari Shroff, Ph.D.

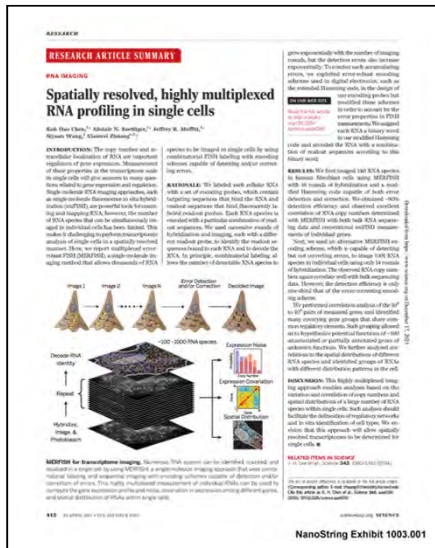
10 Q Now, with regard to axial microscopy, a
11 person of ordinary skill in the art would have
12 known about deconvolution, right?

13 A As I think I said before, a person
14 skilled in the art would have known that
15 deconvolution microscopy could be used to get you
16 to the resolution but not exceed the resolution
17 limit.

EX1082, 2/4/25 Shroff Dep. at 167:10-17 (Reply at 18)

Multiple Light Sources

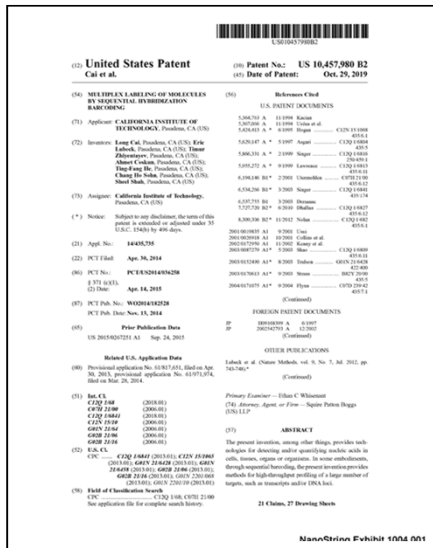
Chen: Teaches Multiple Light Sources



Constant focus was maintained throughout the imaging process with a home-built, autofocus system. Illumination was provided at 641, 561, and 405 nm by using solid-state lasers (MPB communications, VFL-P500-642; Coherent, 561-200CWCDRH; and Coherent, 1069413/AT) for excitation of our Cy5-labeled readout probes, the fiducial beads, and nuclear counterstains, respectively. These lines were combined with a

EX1003.011 (Petition at 29)

Cai: Teaches Multiple Light Sources



to different fluorophores. (b) Composite four-color FISH Data from 3 rounds of hybridizations on multiple yeast cells. Twelve genes were encoded by 2 rounds of hybridization, with the third hybridization using the same probes as hybridization 1. The boxed regions were magnified in the bottom

EX1004.9:34-38 (Petition at 30)

scripts in single cells. (c) Exemplary barcodes. mRNA 1: Yellow-Blue-Yellow; mRNA 2: Green-Purple-Green; mRNA 3: Purple-Blue-Purple; and mRNA 4: Blue-Purple-Blue.

EX1004.9:45-48 (Petition at 30)

Dr. Czymmek: Obvious To Use Cai's Multiple Light



Kirk Czymmek, Ph.D.

Dr. Czymmek's Declaration

172. In my opinion, the detection of different fluorophores as described in Cai would have required using different colors of excitation. This is particularly the case when laser light sources are used, as taught by Cai's listing of laser-excitabile dyes, because lasers emit monochromatic light. Accordingly, a POSA would have understood Cai to teach using two or more light sources and, to the extent it might be deemed not to, a POSA would have found it obvious to include two or more light sources, such as lasers, to provide two or more colors of light for the benefit of greater diversity of signals for generating distinct barcodes given that its use was standard prior to 2015.

EX1002 ¶172 (Petition at 30)

Secondary Indicia Do Not Overcome Bruker's Prima Facie Showing

Dr. Shroff: No Nexus For CosMx



Hari Shroff, Ph.D.

10 Q So with regard to the CosMx product,
11 and the temperature control apparatus of
12 claim 36A2, you don't identify anything of the
13 CosMx product that satisfies that, correct?

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

20 I agree that is not showing for the
21 CosMx.

EX1082, 2/4/25 Shroff Dep. at 276:10-21 (objection omitted) (Reply at 21)

Dr. Shroff: No Nexus For Xenium



Hari Shroff, Ph.D.

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

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

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

9 A Oh, yeah.

EX1082, 2/4/25 Shroff Dep. at 117:1-9 (Reply at 22)

Dr. Shroff: No Understanding Of Basis For Commercial Success



Hari Shroff, Ph.D.

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

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

20 Q Do you know what features made Xenium
21 commercially successfully?

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

EX1082, 2/4/25 Shroff Dep. at 44:15-45:2 (objection omitted) (Reply at 22)

Ms. Davis: Commercial Success Opinions Are Meaningless



Julie L. Davis
Economics Expert

4 Q Did you perform any comparison on the
5 sales and sales growth and profitability of Xenium
6 with any competitor products?

7 A No. There is no data in the record of
8 this proceeding that would allow me to do so.

9 Q So even without a comparison of sales,
10 sales growth and profitability of Xenium with a
11 competitor product, you conclude that Xenium is
12 commercially successful; is that right?

13 A I do.

EX1083, 2/26/25 Davis Dep. at 31:4-13 (objections omitted) (Reply at 22)

Ms. Davis: Relies On Dr. Shroff For Commercial Success



Julie L. Davis
Economics Expert

5 Q Are you aware of what features of Xenium
6 led to its commercial success?

7 A Not specifically. I would have to defer
8 to Dr. Shroff.

9 Q So to clarify, you do not have any
10 understanding of what specific features of Xenium
11 led to its commercial success; is that right?

12 A I have not tried to attribute commercial
13 success to any specific feature in the Xenium
14 products.

EX1083, 2/26/25 Davis Dep. at 43:5-14 (Reply at 22)

PO's Argument: Relies Upon Need For Non-Homebuilt System

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Alexandria, VA 22313-1450

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

The rapid adoption of the Xenium and CosMx systems once they became commercially available demonstrates a long-felt need in the art for a system for volumetric imaging. EX2026, ¶¶@@; EX2040, 5, 8. As Dr. Shroff explains, prior to the commercial availability of these products, few, if any labs had a complete system for volumetric imaging. EX2024, ¶129. At best, artisans had to rely on homebuilt arrangements made from piecemeal compilations of components, which were laborious to construct and not achievable outside of specialized labs.

EX2024, ¶¶20-21, 129. That changed rapidly once CosMx and Xenium became commercially available in late 2022. EX2024, ¶129. Researchers around the world rapidly purchased these volumetric imaging systems, demonstrating that the field had longed for such a system until the 554 patent's system became commercially available. EX2024, ¶129.

PTAB-IPR2024-00526-20 POR, Patent Owner Response at Pg. 65-66 (Reply at 24)

Dr. Czymmek: PO Relies On Praise For Irrelevant Features



Kirk Czymmek, Ph.D.

Dr. Czymmek's Declaration

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.

EX 1086 Czymmek Reply Dec ¶63 (Reply at 24-25)

Dr. Shroff: Praiseworthy Features Are Unclaimed



Hari Shroff, Ph.D.

11 Q Is it limited, is claim 36 limited to
12 systems that are integrated or monolithic? Do you
13 have an opinion on that?

14 A Sir, I don't have an opinion on that.

EX1082, 2/4/25 Shroff Dep. at 65:11-14 (Reply at 25)

16 Q Okay. There's no requirement in the
17 claims that the system has to meet a certain
18 minimum resolution, right?

20 A There's no explicit reference to
21 resolution in claim 36. Although a POSA would
22 have understood the resolution would come to bear
1 determining three-dimensional positional
2 information.

EX1082, 2/4/25 Shroff Dep. at 215:16-216:2 (Reply at 25)

Thank You