

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

CURIO BIOSCIENCE INC.,
Petitioner,

v.

PROGNOSYS BIOSCIENCES INC. AND 10X GENOMICS, INC.,
Patent Owner and Real-Party-in-Interest.

IPR2025-00236, IPR2025-00237
U.S. Patent Nos. 10,662,468, 11,001,879

**DECLARATION OF JOSEPH D. PUGLISI, PH.D. IN SUPPORT OF
PETITION FOR *INTER PARTES* REVIEW**

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TABLE OF CONTENTS

	<u>Page</u>
I. Introduction.....	1
II. Legal Standards	4
A. Claim Construction	5
B. Anticipation and Obviousness.....	6
C. Priority and Written Description	9
III. Overview of the '468 and '879 Patents	10
IV. Level of Ordinary Skill in the Art	12
V. Overview of a Person of Ordinary Skill in the Art's Knowledge	15
A. Background Molecular Biology	15
B. Cells and Tissues	20
C. Spatial Analysis of Nucleic Acids.....	23
D. Background Techniques for Spatial Tissue Analysis	27
1. Detection of Nucleic Acids.....	28
2. Nucleic Acid Arrays.....	30
3. Sequence Barcoding.....	36
E. Overview of Relevant Spatial Analysis Technologies.....	43
1. In Situ Hybridization.....	43
2. 2D-PCR.....	44
3. Array-Based In-Situ Reverse Transcription	47
VI. Prior Art References of the Grounds	61

TABLE OF CONTENTS
Cont'd

	<u>Page</u>
A. Cantor (Grounds 1-2)	61
B. Armani (Ground 2)	62
C. Frisen (Ground 3)	62
VII. Claim Construction	67
A. “a first sequence that specifically binds” / “a first sequence that binds”	68
VIII. Prior Art Anticipates and/or Renders Obvious the Challenged Claims of the '468 and '879 Patents	71
A. Ground 1: Obviousness over Cantor in view of Knowledge of a POSA	76
1. Claim 1	76
2. Claim 2	116
3. Claim 6 ('468 Patent) / Claim 5 ('879 Patent)	117
4. Claim 7 ('468 Patent) / Claim 6 ('879 Patent)	118
5. Claims 12-13 ('468 Patent) / Claims 11-12 ('879 Patent) ...	120
6. Claim 18 ('468 Patent) / Claim 17 ('879 Patent)	120
7. Claim 20 ('468 Patent) / Claim 21 ('879 Patent)	121
8. Claim 21 ('468 Patent) / Claim 23 ('879 Patent)	122
9. Claim 23 ('468 Patent) / Claim 27 ('879 Patent)	124
10. Claims 25-26 ('468 Patent) / Claim 28 ('879 Patent)	124
11. Claim 30 ('468 Patent) / Claim 30 ('879 Patent)	126

TABLE OF CONTENTS

Cont'd

	<u>Page</u>
B. Ground 2: Obviousness over Cantor in view of Armani and Knowledge of a POSA.	129
1. Claim 1 ('468 Patent / '879 Patent)	129
2. Claim 21 ('468 Patent) / Claim 23 ('879 Patent).....	136
3. Claim 23 ('468 Patent) / Claim 27 ('879 Patent).....	137
4. Other Dependent Claims.....	140
C. Ground 3: Anticipation by Frisen.....	140
1. Priority Date	140
2. Claim 1	161
3. Claim 2	169
4. Claim 6 ('468 Patent) / Claim 5 ('879 Patent).....	170
5. Claim 7 ('468 Patent) / Claim 6 ('879 Patent).....	171
6. Claims 12-13 ('468 Patent) / Claims 11-12 ('879 Patent)...	172
7. Claim 18 ('468 Patent) / Claim 17 ('879 Patent).....	174
8. Claim 20 ('468 Patent) / Claim 21 ('879 Patent).....	175
9. Claim 21 ('468 Patent) / Claim 23 ('879 Patent).....	176
10. Claim 23 ('468 Patent) / Claim 27 ('879 Patent).....	178
11. Claims 25-26 ('468 Patent) / Claim 28 ('879 Patent).....	179
12. Claim 30 ('468 Patent) / Claim 30 ('879 Patent).....	180
IX. Conclusion	182

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

Exhibits Cited

EX NO.	<u>PETITIONER’S EXHIBIT LIST</u>
EX1001	U.S. Patent No. 10,480,022 issued to Chee
EX1002	U.S. Patent No. 10,662,468 issued to Chee
EX1003	U.S. Patent No. 11,001,879 issued to Chee
EX1004	U.S. Patent No. 11,549,138 issued to Chee
EX1005	U.S. Patent No. 11,761,030 issued to Chee
EX1006	10x Genomics’ Complaint for Patent Infringement, Case 1:23-cv-01375, Doc. 1, filed December 1, 2023
EX1007	Exemplary Infringement Claim Chart-U.S. Patent No. 10,480,022 Case 1:23-cv-01375, Doc. 1-11, filed December 1, 2023 (EX K)
EX1008	Reserved
EX1009	U.S. Provisional Application No. 61/321,124
EX1010	File History for U.S. Application No. 16/276,235 (2 parts)
EX1011	File History for U.S. Application No. 15/187,661
EX1012	File History for U.S. Application No. 13/080,616
EX1013	Nick J. Proudfoot, <i>et al.</i> , <i>Integrating mRNA Processing with Transcription</i> , Cell, Vol. 108, 501-512, February 22, 2002
EX1014	Uwe Kuhn <i>et al.</i> , <i>Poly(A) Tail Length Is Controlled by the Nuclear Poly(A)-binding Protein Regulating the Interaction between Poly(A) Polymerase and the Cleavage and Polyadenylation Specificity Factor</i> , The Journal of Biologic Chemistry VOL. 284, NO. 34, pp 22803-22814, August 21, 2009

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

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EX1015	James Eberwine <i>et al.</i> , <i>Complementary DNA Synthesis in Situ: Methods and Applications</i> , Methods in Enzymology, VOL. 216, 1992
EX1016	Laurence H. Tecott <i>et al.</i> , <i>In Situ Transcription: Specific Synthesis of Complementary DNA in Fixed Tissue Sections</i> , Science, VOL. 240, pp. 1661-1664, 1988
EX1017	Anna Ligasová and Karel Koberna, <i>In situ reverse transcription: the magic of strength and anonymity</i> , Nucleic Acids Research, 2010, Vol. 38, No. 16
EX1018	Victor Trevino <i>et al.</i> , <i>DNA Microarrays: a Powerful Genomic Tool for Biomedical and Clinical Research</i> , Mol Med 13 (9-10) 527-541, September-Doctor 2007
EX1019	Stahl <i>et al.</i> , "Visualization and analysis of gene expression in tissue selections [sic: sections] by spatial transcriptomics," <i>Science</i> 353(6294):78-82 (July 1, 2016)
EX1020	WO 2012/140224 A1 to Frisen
EX1021	WO 2010/088517 A1 to Emmert-Buck, Armani <i>et al.</i>
EX1022	Michael Armani <i>et al.</i> , <i>2D-PCR: a method of mapping DNA in tissue sections</i> , Lab Chip, 2009, 9(24), 3526-3534; NIH Public Access Author Manuscript
EX1023	Lambda Moses and Lior Pachter, <i>Museum of spatial transcriptomics</i> , Nature Methods, VOL 19, May 2022 [534-546]
EX1024	U.S. Patent No. 11,214,796 issued to Shirai
EX1025	JP2007-14297 to Okano
EX1026	EP 2 130 913 A1 to Okano

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX NO.	<u>PETITIONER'S EXHIBIT LIST</u>
EX1027	Franklin M. Harold, <i>Molecules into Cells: Specifying Spatial Architecture, Microbiology and Molecular Biology Reviews</i> , Dec. 2005, p. 544-564
EX1028	Vanessa M. Brown <i>et al.</i> , <i>Multiplex Three-Dimensional Brain Gene Expression Mapping in a Mouse Model of Parkinson's Disease</i> , <i>Genome Research</i> , 12:868-884, 2002
EX1029	Michael L. Metzker, <i>Sequencing technologies-the next generation</i> , <i>Nature Reviews Genetics</i> , Volume 11, January 2010
EX1030	Poornima Parameswaran, <i>et al.</i> , <i>A pyrosequencing-tailored nucleotide barcode design unveils opportunities for largescale sample multiplexing</i> , <i>Nucleic Acids Research</i> , 2007, Vol. 35, No. 19
EX1031	WO 2009/091934 A1 Cantor
EX1032	U.S. Patent No. 6,620,584 issued to Chee
EX1033	WO 2008/075086 A1 Southern <i>et al.</i>
EX1034	Spellman Declaration EX1002 from IPR2023-01298
EX1035	10x Genomics' Opening Brief in support of Motion to Dismiss, Case 1:18-cv-01800 (D.Del), Doc. 19, filed March 1, 2019
EX1036	Norman P. Gerry, <i>et al.</i> , <i>Universal DNA Microarray Method for Multiplex Detection of Low Abundance Point Mutations</i> , <i>J. Mol. Biol.</i> (1999) 292, 251-262
EX1037	U.S. Patent No. 8,835,358 issued to Fodor
EX1038	U.S. No. 2007/0231824 A1 to Chee
EX1039	Jian-Bing Fan, <i>et al.</i> , <i>Illumina Universal Bead Arrays</i> , <i>Methods in Enzymology</i> , VOL. 410, 2006

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX NO.	<u>PETITIONER’S EXHIBIT LIST</u>
EX1040	10X Genomics’ Petition for IPR Review of Nanostring Technologies' Patent No. 11,377,689 (IPR2023-01298)
EX1041	July 24, 2024 Email from Counsel for Patent Owner to Counsel for Petitioner
EX1042	August 16, 2024 Email from Counsel for Patent Owner to Counsel for Petitioner
EX1043	Scheduling Order, Doc #25, C.A. 23-cv-1375 (D.Del.)
EX1044	Leslie P. Gartner, Ph.D. and James L. Hiatt Ph.D., <i>Color Textbook of Histology</i> , Third Edition, Saunders Elsevier
EX1045	WO 2014/060483 A1 (“Frisen”)
EX1046	Marshall S. Scicchitano <i>et al.</i> , <i>Preliminary Comparison of Quantity, Quality, and Microarray Performance of RNA Extracted From Formalin-fixed, Paraffin-embedded, and Unfixed Frozen Tissue Samples</i> , 2006, J. Histochemistry & Cytochemistry 54(11), 1229-1237.
EX1047	Stephen M. Hewitt, MD <i>et al.</i> , <i>Tissue Handling and Specimen Preparation in Surgical Pathology</i> , Arch. Pathol. Lab. Med. (2008) 132, 1929-1935.
EX1048	US6,133,436 issued to Köster
EX1049-1054	Reserved
EX1055	File History for 16/430,015 (the “’015 Application”)
EX1056	File History for 16/414,213 (the “’213 Application”)
EX1057	File History for 16/402,098 (the “’098 Application”)

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX NO.	<u>PETITIONER’S EXHIBIT LIST</u>
EX1058	Reserved
EX1059	File History for 16/988,284 (the “’284 Application”)
EX1060	Reserved
EX1061	Exemplary Infringement Claim Chart-U.S. Patent No. 11,549,138 Case 1:23-cv-01375, Doc. 1-11, filed December 1, 2023 (EX N)
EX1062	Exemplary Infringement Claim Chart-U.S. Patent No. 11,761,030, Case 1:23-cv-01375, Doc. 1-11, filed December 1, 2023 (EX O)
EX1063	File History for 16/443,771 (the “’771 Application”)
EX1064	File History for 16/437,726 (the “’726 Application”)
EX1065	Exemplary Infringement Claim Chart-U.S. Patent No. 10,662,468, Case 1:23-cv-01375, Doc. 1-12, filed December 1, 2023 (EX L)
EX1066	Exemplary Infringement Claim Chart-U.S. Patent No. 11,001,879, Case 1:23-cv-01375, Doc. 1-13, filed December 1, 2023 (EX M)
EX1067- EX1100	Reserved
EX1101	Curriculum Vitae of Joseph D. Puglisi, Ph.D.
EX1102	Declaration of Joseph D. Puglisi, Ph.D. for IPR2025-00236 & IPR2025-00237

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

I. Introduction

1. My name is Joseph Daniel Puglisi. I am currently the Jauch Professor and Professor of Structural Biology at the Stanford University School of Medicine. I have 40 years of experience in the investigation of nucleic acid and protein chemistry, biophysics, and biology as a professor and as a student.

2. I have been retained by Petitioner Curio Bioscience to provide an independent expert opinion regarding the technology, patents, and relevant prior art at issue in this proceeding. I am being compensated for my time spent consulting on this matter at my normal hourly rate. My compensation does not depend on the outcome of this proceeding.

3. I am an expert in biophysics, biochemistry, molecular biology, cell diagnostics, molecular interactions, DNA and RNA sequencing, genetic testing, nucleic acid preservation, and related fields. My qualifications and credentials are set forth in my *curriculum vitae*, attached as Exhibit 1101. Briefly, I received a B.A. in Chemistry in 1984 from The Johns Hopkins University and a Ph.D. in Biophysical Chemistry in 1989 from the University of California, Berkeley. While pursuing my Ph.D., I received the Bruce Mahan Memorial Teaching Award from the University of California Berkeley's Department of Chemistry.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

4. From 2004 through 2014, I was the Chair of Stanford's Department of Structural Biology. I have been the Director of the Stanford Magnetic Resonance Laboratory since 1997. I have also been the Director of the International School of Biological Magnetic Resonance since 2003. From 1993 through 1997, I was part of the faculty at the University of California, Santa Cruz in the university's Molecular, Cellular, & Developmental Biology Department. From 1991 to 1993, I was a postdoctoral fellow at the Massachusetts Institute of Technology in the institute's Department of Chemistry. From 1989 to 1991, I was a European Molecular Biology Organization (EMBO) postdoctoral fellow at the Institute of Molecular and Cellular Biology at the French National Centre for Scientific Research.

5. I have authored or co-authored over 175 publications in the fields of biophysics, chemistry, molecular and cell biology, including at least five book chapters. I have co-authored a textbook on Physical Chemistry. I have been invited to present on topics in the fields of molecular and cell biology on at least 300 occasions, and presented numerous named Lectureships, including the Linus Pauling Lecture (Stanford), Tom Steitz Lecture (Yale), Ferry Lecture (University of Wisconsin), and O'Keefe Lecture (Arizona State University).

6. I am a member of numerous technical societies, including the National Academy of Sciences since 2014. I have been a member of the Advisory Editorial

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

Board for the EMBO since 2011. I was a council member of the Biophysical Society from 2014–2017. I have been the lead organizer of the past 12 NATO Advanced Study Institute courses on the fields of structural biology and molecular biophysics. In 2007, I was the co-chair for both the Nucleic Acids Gordon Conference hosted by the Salve Regina University, and the American Chemical Society’s RNA Biophysics Symposium.

7. I have been appointed as an editor of a number of scientific publications. I have been a member of the Editorial Board for the Proceedings of the National Academies of Science since 2017. I have been an Editor of the *Biophysics Journal* since 2014. I have been a member of the Advisory Editorial Board for EMBO reports since 2011. I have been an editor of the *Structure* division of the prestigious Cell Press since 2007, and previously served as the Senior Editor from 2003-2007.

8. I received the Merit Award from the National Institute of Health in 2011. I also received the National Institute of Health Director’s Transformative R01 Program Award in 2011. I was an Alfred P. Sloan Research Fellow for the Alfred P. Sloan Research Foundation in 1997. I was also the David and Lucille Packard Fellow for the David and Lucille Packard Fellowship in Science and Engineering from 1994–1999. I thrice received the University of California at Berkeley’s Bruce

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

Mahan Memorial Teaching Award in the university's Department of Chemistry. In 1989, I was inducted into the Sigma Xi Honor Society.

9. My research for the past four decades involved the study of RNA structure and function. In particular, my research includes the investigation of RNA function by studying molecular structure and dynamics using a variety of biophysical and biological tools, such as nuclear magnetic resonance (NMR) spectroscopy, x-ray crystallography, cryogenic electron microscopy, and single-molecule fluorescence methods. I have developed and characterized methods to synthesize, purify, characterize, and stabilize highly pure and structurally stable RNA molecules for these applications, and applied/developed DNA/RNA analysis tools, including sequencing methods. I have also helped develop commercial DNA sequencing and analysis tools, in particular as a longtime advisor to Pacific Biosciences.

II. Legal Standards

10. I have been informed by counsel of general principles concerning patent law and the analysis of whether a patent claim is valid. I have followed these principles in arriving at my conclusions in this declaration. The sections below set forth my understanding of the legal principles that are relevant to my opinions in this declaration.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

A. Claim Construction

11. I understand that the claims of a patent define the scope of the inventions that are protected by the patent. I understand that the language of the claims is construed as it would be understood by a hypothetical person having ordinary skill in the art (a “POSA”) at the time of the invention. Accordingly, where I refer below to a POSA—for example, where I discuss how such a person would have understood the specification or the claims, or what a POSA might have known, among other things—I am referring to such a person working at the time of the invention, even if for the sake of brevity I omit that specific timeframe from my statements.

12. I understand words in a claim will be given their ordinary or customary meaning unless it appears that the inventor used them differently.

13. I also understand that, in construing patent claims, courts seek to determine what a person skilled in the art would have understood the claims to mean primarily in light of the intrinsic evidence of record, which includes the language of the claim itself and other issued claims, the specification, the art cited during prosecution, and the prosecution history.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

B. Anticipation and Obviousness

14. I understand that, for an invention claimed in a patent to be found patentable, or to be valid, it must be, among other things, novel and not obvious in light of what came before it. I understand that patents and publications that were filed and/or published before the filing of a challenged patent are generally referred to as “prior art.”

15. I understand that a patent claim is unpatentable or may be found invalid as anticipated if all claim elements are found in a single prior art reference as arranged in the claim.

16. I understand that a patent claim is unpatentable or may be found invalid as obvious if, at the time when the invention was made, the subject matter of the claim, considered as a whole, would have been obvious to a person having ordinary skill in the field of the technology (the “art”) to which the claimed subject matter belongs.

17. I understand that a claim in a patent is obvious when the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

18. I understand that obviousness may be shown by considering more than one item of prior art, and that the following factors should be considered in analyzing obviousness: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims; and (3) the level of ordinary skill in the pertinent art.

19. I understand that a person of ordinary skill in the art is assumed to have knowledge of all relevant prior art. I understand that one skilled in the art can combine the teachings of various prior art references based on the teachings of the individual prior art references, the general knowledge present in the art, or common sense. I understand that a teaching, motivation or suggestion appearing in the prior art may provide a basis for combining two (or more) prior art references or modifying a given prior art reference. A motivation to combine references may also be implicit in the prior art, and there is no requirement that there be an actual or explicit teaching to combine two references. Thus, one may take into account the inferences and creative steps that a person of ordinary skill in the art would employ to combine the known steps in the prior art in the manner claimed by the patent at issue.

20. I understand that the combination of familiar steps according to known methods is likely to be obvious when it does no more than yield predictable results. I understand that when a work is available in one field of endeavor, design incentives

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

and other market forces can prompt variations of that work, either in the same field or a different one. Likewise, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using that technique to improve the other device in a predictable manner would normally be obvious.

21. I understand that a person of ordinary skill is also a person of ordinary creativity in the field. A person of ordinary skill attempting to solve a problem will not be restricted only to those steps of prior art designed to solve the same problem. Rather, in exercising common sense and ordinary creativity, a person of ordinary skill can draw upon prior art directed to any need or problem known in the field of endeavor at the time of the invention. In other words, the prior art does not need to be directed towards solving the same problem that is addressed in the patent. Further, the individual prior art references themselves need not all be directed towards solving the same problem.

22. I further understand that in many fields, it may be that there is little discussion of obvious techniques or combination, and it often may be the case that market demand, rather than scientific literature or knowledge, will drive design trends. When there is such a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

skill has good reason to pursue the known options within their technical grasp. If this leads to the anticipated success, it is likely the result not of innovation but of ordinary skill and common sense. In that instance, the fact that a combination was obvious to try might show that it was obvious. I understand that the fact that a particular combination of prior art steps was “obvious to try” may indicate that the combination was obvious even if no one had attempted the combination at the time of filing. Likewise, duplicating a known feature from the prior art multiple times in order to take advantage of proven technology typically does not require a leap of inventiveness.

C. Priority and Written Description

23. I understand that during patent prosecution, the applicant may amend the originally filed claims, or submit new claims, between the time an original application is filed and the time at which the patent is issued. I understand that such changes may be made in a continuation application, which is an application that stems from a parent application.

24. I understand that, for a continuation application to be entitled to the filing date of its parent application or parent applications, the claims must find written description support in the earlier application bearing the filing date on which the applicant seeks to rely. If the earlier application on which the applicant seeks to

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

rely is separated from the application for which the benefit is sought by one or more intervening applications, the claims also must find written description support in each intervening application.

25. To evaluate whether claims are supported by a disclosure, such as the patent specification or the specification of a parent filing, I understand that one must consider the written description (including the figures/drawings, text, and original claims) from the perspective of a person of ordinary skill in the art at the time the application was filed. The written description requirement is satisfied if such a person would have recognized that it describes the full scope of the claimed invention as it is finally claimed in the issued patent and that the inventor actually possessed that full scope by the filing date of the original application.

26. I further understand that, while the full scope of a claim or any particular requirement in a claim need not be expressly disclosed in the original patent application, or described in the exact same words used in the claim, a written description that merely renders the claimed subject matter obvious does not satisfy the written description requirement.

III. Overview of the '468 and '879 Patents

27. I understand that in co-pending litigation, EX1006 (District Court Complaint), Patent Owner and its licensee, 10x Genomics, have accused Petitioner

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

of infringing five related patents, including U.S. Patent Nos. 10,662,468 (EX1002) (the “’468 Patent”) and 11,001,879 (EX1003) (the “’879 Patent”) (together, the “Challenged Patents”), addressed here, as well as U.S. Patent Nos. 10,480,022 (EX1001), 11,549,138 (EX1004) and 11,761,030 (EX1005).

28. The ’468 and ’879 Patents are titled “Spatially Encoded Biological Assays,” and name as their inventor Mark S. Chee. EX1002, EX1003. According to their faces, the ’468 and ’879 Patents issued, respectively, from Application No. 16/660,234, which was filed on October 22, 2019, and Application No. 17/144,971, which was filed on January 8, 2021. EX1002 & EX1003, Code (22). The ’468 and ’879 Patents claim priority to “Provisional application No. 61/321,124, filed on Apr. 5, 2010.” EX1002 & EX1003, Code (60).

29. The **ABSTRACTS** of the '468 and '879 Patents state that “[t]he present invention provides assays and assay systems for use in spatially encoded biological assays,” and that the assay system of the invention is “capable of high levels of multiplexing where reagents are provided

to a biological sample in defined spatial patterns; instrumentation capable of controlled delivery of reagents according to the spatial patterns; and a decoding scheme providing a readout that is digital in nature.” EX1002 & EX1003, Abstract. The approach of the patent is illustrated in the flow-diagram provided in Figure 1.

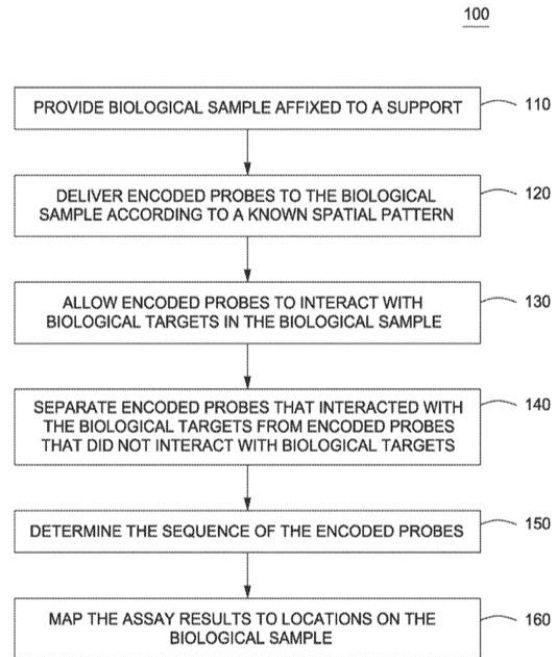


FIG. 1

IV. Level of Ordinary Skill in the Art

30. I have been advised that a “person of ordinary skill in the art,” or “POSA,” is a hypothetical person and that I should consider the following factors in determining the qualifications of a POSA: (1) the educational and experience level of active workers in the field; (2) the sophistication of the technology; (3) the rapidity with which innovations are made; (4) the types of problems encountered in the art;

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

and (5) the prior art solutions to those problems. I understand that not all such factors may be present in every case, and one or more of them may predominate.

31. The relevant technology field for the '468 and '879 Patents is nucleic acid analysis and detection. By virtue of my education, experience, and training, I am familiar with the level of skill in the art of the '468 and '879 Patents before the April 5, 2010 filing date of the '124 Provisional Application.

32. In my opinion, a POSA in the field of the '468 and '879 Patents as of the April 2010 filing date of the '124 Provisional Application would have had a doctorate degree (or equivalent) in bioengineering, genetics, genomics, chemistry, biochemistry, biophysics, molecular imaging or a related discipline with at least 2-4 years of academic, research, or industry experience in genomic technology.¹ In the course of their training and experience, the POSA would have acquired a working knowledge of nucleic acid arrays, combinatorial chemistry techniques for making and using such arrays, nucleic acid sequencing and sample preparation techniques, such as barcoding, imaging methods for signal detection, as well as histopathology

¹ I understand that the POSA is a fictional person. In practice, an individual may have obtained equivalent skill through a combination of less education and greater research and/or industry experience, for example.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

techniques for cell permeabilization and tissue handling. This is confirmed by the

'124 Provisional:

The practice of the techniques described herein may employ, unless otherwise indicated ***conventional techniques*** and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology, ***which are within the skill of those who practice in the art.*** Such conventional techniques include polymer array synthesis, hybridization and ligation of polynucleotides, and detection of hybridization using a label.

EX1009, [00037]; *see also id.*, [0003] (“Conventional methods such as *in situ* hybridization and multiplexed detection of multiple different transcripts have revealed spatial patterns of gene expression and have helped to shed light on the molecular basis of development and disease. Other technologies that have enabled the quantitative analysis of many RNA sequences per sample include microarrays, serial analysis of gene expression (SAGE), and high-throughput implementations of qPCR.”) (citations omitted); *id.*, [00083] (“Conventional sequencing is used as a readout....”); *id.*, [00085] (using “conventional sequencing to determine the structure of the assay products.”).

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

33. By April 5, 2010, I would have had at least the foregoing level of education and experience.

34. In Ground 3, where a priority date of February 14, 2019 applies, a POSA would have at least the foregoing level of skill and education, plus the additional knowledge acquired and documented in the literature in the intervening years.

V. Overview of a Person of Ordinary Skill in the Art's Knowledge

35. In this section, I provide an overview of the various concepts and techniques that a POSA would have known and understood by April 5, 2010.

A. Background Molecular Biology

36. The common specification of the '468 and '879 Patents refers to the analysis and detection of “biological molecules.” These “biological molecules” may be members of several different classes of biological molecules, such as nucleic acids, proteins, lipids, carbohydrates, small-molecule metabolites, or ions, EX1002, 20:48-50, but in this declaration, I focus primarily on nucleic acids.

37. The two main types of nucleic acids relevant to this declaration are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the genetic material found in all living organisms. A fraction of the DNA in an organism encodes genes.

38. DNA is made up of two strands that wind around each other in a shape called a “double helix,” which biologists often depict in schematics that look like a twisting ladder. Each DNA strand is composed of subcomponents called nucleotides consisting of nitrogenous aromatic bases; the nucleotides are linked together to form a continuous strand through phosphodiester linkages. DNA is typically composed of sequences of four such bases, T (thymine), A (adenine), C (cytosine), and G (guanine), which make up the genetic code.

39. Each base on one strand of DNA pairs with a base on the other strand through predictable hydrogen bonding interactions, referred to as “base-pairing.” A preferentially pairs with T and C preferentially pairs with G. This consistent pairing of bases allows DNA to accurately replicate itself, and to be accurately transcribed into a copy of RNA (see below). As discussed in detail below, many methods of analyzing nucleic acids leverage this predictable complementarity to capture, amplify, and detect specific nucleic acids present in a biological system.

40. Each strand is asymmetric and thus has a “directionality,” which scientists refer to by noting the “5’-end” and “3’-end” of a given DNA (or other

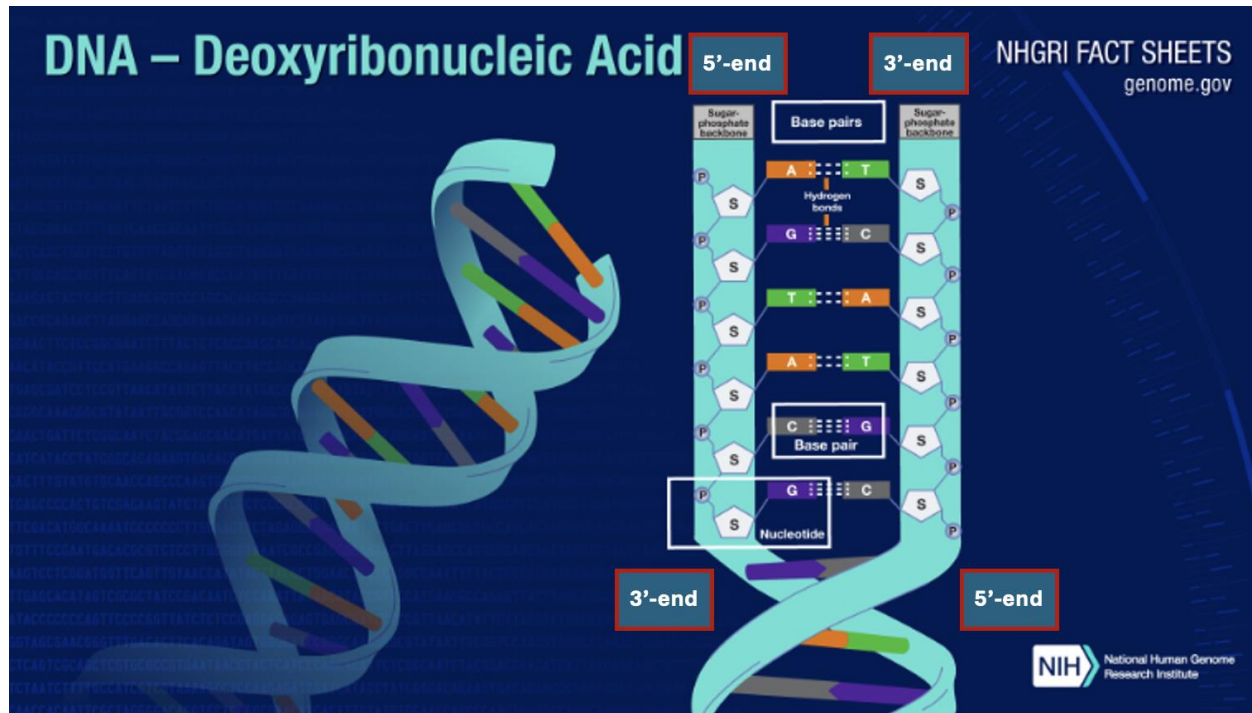
IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

nucleic acid) strand. In double-stranded DNA, one strand runs from 5' to 3', while the other runs from 3' to 5'. I have annotated this concept on the figure below.²



41. The bases are in different orders on different parts of the DNA strand.

DNA is “read” by the order of the bases, that is by the order of the As, Cs, Gs, and Ts. The order of these bases is what is known as the DNA sequence.

² Deoxyribonucleic Acid (DNA) Fact Sheet, Nat’l Hum. Genome Rsch. Inst. (Aug. 24, 2020), <https://www.genome.gov/about-genomics/fact-sheets/Deoxyribonucleic-Acid-Fact-Sheet>.

42. In gene expression, DNA genes are converted into an intermediate called messenger RNA (mRNA) through a process called transcription. During transcription, one strand of DNA holds the information that codes for various genes; this strand is often called the template strand or antisense strand and is copied by a protein machine called RNA polymerase. The other, and complementary, strand is called the coding strand or sense strand (containing codons). Since mRNA is made by copying the template strand, it has the same information as the coding strand. The code for the mRNA will be identical to the coding strand, except in RNA the Thymine (T) bases are replaced with Uridine (U). The illustration below shows the sequence of two complementary strands of DNA, along with the mRNA that would be transcribed from the template strand.

	Directionality	Sequence
DNA Coding Strand	(5'→3')	ACGTTGCATTCCGG
DNA Template Strand	(3'→5')	TGCAACGTAAGGCC
mRNA Transcribed from Template Strand	(5'→3')	ACGUUGCAUUCCGG

43. The transcribed mRNA can then be converted into protein through a process called translation. During translation the sequence information within the RNA, which originally derives from DNA, is “translated” to a sequence of amino acids using 3 base code words (called codons). The flow of genetic information from

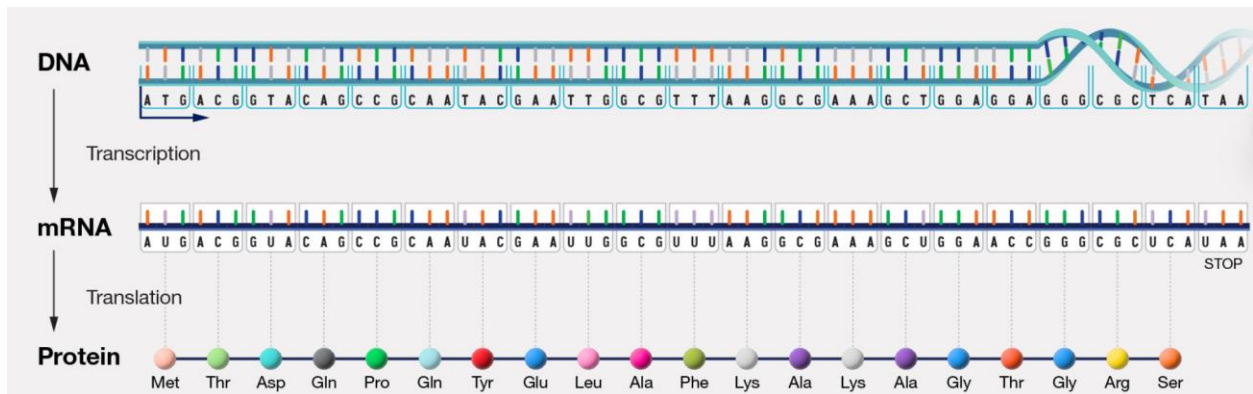
IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

a gene encoded by DNA, into a transcribed mRNA, which in turn is translated to protein is referred to as the central dogma of molecular biology.³ If there is a change to the DNA sequence of a gene (a mutation), that would change the mRNA sequence and could lead to the incorporation of a different amino acid into a protein, possibly disrupting its function. An essential aspect of modern biological research is to understand how this process occurs properly during life and is aberrant in disease.



44. The '468 and '879 Patents' “**BACKGROUND OF THE INVENTION**”

explains that prior art “gene expression analysis and protein analysis have been useful tools in understanding mechanisms of biology,” and that “[c]onventional methods such as in situ hybridization and other multiplexed detection of different transcripts have revealed spatial patterns of gene expression and have helped shed

³ Central Dogma, Nat’l Hum. Genome Rsch. Inst. (Nov. 23, 2024), <https://www.genome.gov/genetics-glossary/Central-Dogma>

light on the molecular basis of development and disease.” EX1002, 1:47-51. The concept of “gene expression” refers to the process by which a gene encoded by DNA gets turned on in a cell to make RNA and proteins. Gene expression reflects the amounts of mRNA or protein, or both, and is analyzed accordingly.

B. Cells and Tissues

45. Cells are the basic building blocks of living things, and can be sorted into two groups: prokaryotes and eukaryotes. Human cells (and other mammalian cells) are eukaryotic cells; such cells are more complex than prokaryotic cells, which can be found in certain unicellular organisms such as bacteria.

46. Eukaryotic cells are highly organized and contain a number of organelles, which are specialized internal components that serve particular functions. These include the cell membrane, which is an outer casing comprised of a double layer of lipid (i.e., fatty acid) molecules, in which a variety of proteins are embedded to serve myriad cellular functions. The nucleus of the cell, also enclosed by a lipid membrane, is where the chromosomes are located, as well as the nucleolus (a membraneless structure), which produces the ribosomes that initiate the processes that produce proteins from the cell using the information encoded by the chromosome.

47. A POSA would have been familiar with a number of well-known techniques for analyzing cells and their components. Light, fluorescence and electron microscopy methods were used traditionally over the prior several decades to visualize cellular architectures and components. Many methods were developed to characterize cells based on proteins expressed on their surfaces. For example, fluorescence activated cell sorting (FACS) uses fluorescent antibodies for cell surface markers to allow individual cells of a given type to be purified. DNA and RNA sequencing methods allow the analysis of the nucleic acid components, and by 2009, could be performed at a single-cell level.

48. Tissues are groups of cells that function together as a unit. For example, tissues can be from different organs (e.g., liver, kidney, heart, muscle, brain, etc.), and often carry cells that are specific to that organ. Tissues can contain multiple cell types, such as muscle and fat cells in skeletal muscle tissues, and neurons and glial cells in brain tissue. Important to many aspects of biological research, tissues can be in a normal, healthy state, or in a disease state (e.g., a cancerous tumor, or degenerated brain tissue).

49. To facilitate research, many techniques for isolating and preparing tissue samples for further investigation have been developed. Once a tissue sample is isolated from a patient or an animal (for example from a surgical resection or

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

biopsy), it must be preserved for further analysis. These techniques are designed “to prepare tissues for study so that they closely resemble their natural, living state.”

EX1044, 1. Steps that may be involved in such processes include:

- **Fixation:** the treatment of tissues with chemical agents to preserve the tissue architecture, such as through dehydration (typically using methanol) or cross-linking methods (typically using formalin, a relative of formaldehyde). This creates a meshwork that maintains the location of biological macromolecules as well as the overall structure of the cell.
- **Freezing:** tissues can be preserved using deep freezing, such as cryogenic preservation in liquid nitrogen.
- **Embedding:** a technique in which tissues are embedded in a material, often paraffin, which hardens to form a block around the tissue, facilitating the slicing of the sample into thin sections.
- **Sectioning:** a technique that involves slicing a tissue into thin slices using a tool called a microtome, which can be performed on embedded or frozen specimens.

See EX1044, 1-2.

50. In the art, there were only a few common techniques for preserving tissues samples, these included (1) freezing the tissues samples and (2) fixing the tissue samples in formalin followed by embedding them in paraffin. Formalin fixation and paraffin embedding (FFPE) was the most common fixation method practiced in the art in 2010 and was known as the “gold standard” for tissue fixation. EX1046, 1229. However, FFPE samples were known to be the inferior choice for nucleic acid analysis because they yielded poor quality RNA in comparison to frozen

or fresh tissue samples, due to chemical reactions of the nucleic acids with the fixation reagents. *Id.*; EX1047, 1933.

51. Once preserved, the tissues can be analyzed using methods to characterize cellular and tissue architectures. Tissues can be stained for traditional microscopy or imaged using fluorescent antibody probes. To explore the interiors of cells, biologists also possess techniques to “permeabilize” the cells to allow large molecules to transit out of the cell’s lipid membrane, for example using alcohols or detergents.

C. Spatial Analysis of Nucleic Acids

52. Biologists can find important information not only in the identity and quantitation of the genes expressed in a biological system, but also in the location at which particular genes are expressed (or not expressed). Not all genes are transcribed at all times. Instead, transcription of each gene in a biological system is highly regulated. Typically, only the genes whose products (i.e. mRNA and protein) are required by a given system at a given time are transcribed.⁴ Not only do these levels

⁴ There are many instances in which transcription is not properly regulated, which results in the over- or under-expression of certain genes. Such issues are implicated in a number of diseases, and are also an important area of investigation.

of transcription differ depending on the requirements of a biological system at a given time, they also may differ depending on the location of given cells in a biological system. For example, levels of transcription of a particular gene may differ in an eye cell as compared to a liver cell. And at an even more localized level, gene expression may differ in different cell types that make up the same tissue. *See, e.g.,* EX1028, 869 (“To explore the relationships between brain regions, we first examined the correlations between each of voxels⁵ in terms of gene expression levels.”). Finally, different transcripts of a gene may have different locations within a cell. For example, a transcript may be localized in the nucleus, in the cytoplasm or

⁵ Voxels are the three-dimensional equivalent of pixels. In this study, the voxels analyzed were segments of mouse brain tissue. *Id.*, 868 (“Here, a method called voxelation is used to investigate the gene expression changes that occur in the mouse brain as a result of a pharmacological model of Parkinson’s disease (PD). The name of the method is derived from the term voxel, which is a cubic 3D image element. Conceptually, voxelation is simple, and uses the direct creation of voxels (cubes) *spatially registered with the brain. RNA extracted from the voxels is analyzed using high-throughput techniques, allowing 3D gene expression patterns to be deduced.*”).

targeted to membranes or organelles. Thus, understanding the spatial aspects of gene expression is central to delineating biological functions and responses.

53. Accordingly, by at least several years before the priority date, scientists had been devising ways to analyze the spatial relationships of biological molecules:

Spatial organization is perhaps the most conspicuous quality of cells and organisms, and also the most elusive. The dissection of life into its molecular constituents—the genes, enzymes, and lipid bilayers—necessarily starts with the destruction of spatial order. Yet we know that most complex functions, such as motility and division, depend on having the right molecules in their proper places. Architecture is what ultimately distinguishes a living cell from a soup of the chemicals of which it is composed. How cells generate, maintain, and reproduce their spatial organization is central to any understanding of the living state.

EX1027, 544 (“Over the past 15 years [before 2005], spatial organization has moved to the forefront of research in cell biology.”). Accordingly, numerous tools and techniques have evolved to answer questions such as “whether there is...a spatial relationship between the position of genes and the location of their products in cell space.” *Id.*, 552.

54. Since “many genes need to be properly regulated in space for the system to function,” many techniques have emerged “[t]o study the spatial patterns of gene expression” by “produc[ing] spatially localized quantification of messenger

IPR2025-00236

IPR2025-00237

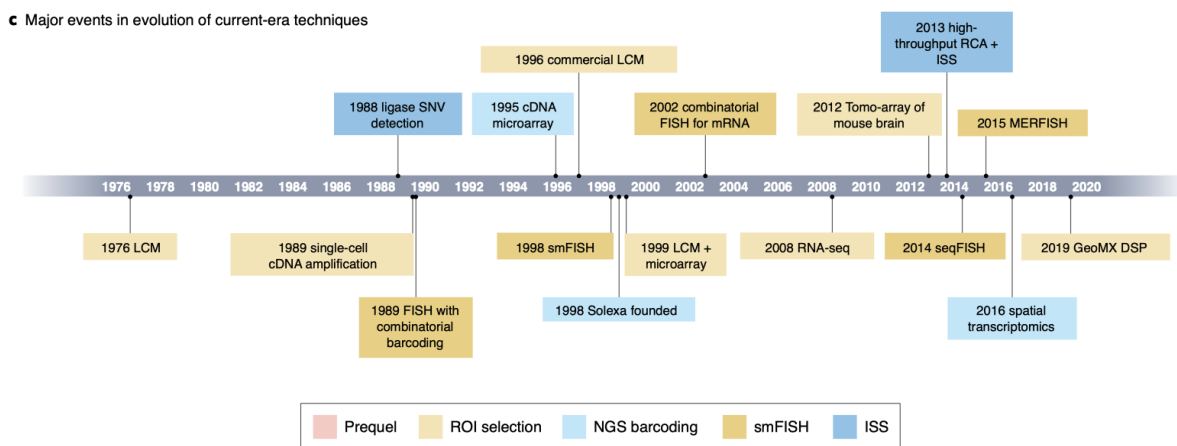
Patent No. 10,662,468

Patent No. 11,001,879

RNA (mRNA) transcripts as proxies for gene expression.” EX1023 (Pachter), 534.

These methods may be referred to as “spatial transcriptomics methods.” *Id.*

55. Pachter’s review article, entitled “Museum of spatial transcriptomics” deeply surveys the literature in this field and “presents a database of literature dating back to 1987 comprehensively documenting the historical evolution and current development in data collection and analysis in spatial transcriptomics.” *Id.* “[I]mportant technologies enabling spatial transcriptomics date back to the 1970s,” and “[t]he foundation for many current-era technologies was built in the decades between the 1970s and 2000s (Fig. 1c).” *Id.*



56. Pachter continues:

For example, ultraviolet (UV) laser was first used to cut tissue in 1976. Popular infrared (IR) and UV LCM systems were first reported in 1996 and were soon commercialized. Some highly multiplexed smFISH

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

technologies, such as sequential FISH (seqFISH), rely on combinatorial barcoding, that is encoding each gene with a combination of colors so transcripts of more genes with easily discernible colors (up to 5) can be quantified simultaneously. Combinatorial barcoding was first reported in immunological DNA FISH in 1989 and was first used for transcripts in 2002. The first unequivocal demonstration of smFISH showing each mRNA molecule as a spot was reported in 1998. Highly multiplexed smFISH would not have been possible without the development of these technologies.

EX1023, 534-536.

57. This historical context documents the existing motivation a POSA would have had as of April 2010 to analyze the spatial distribution and quantification of biological molecules (e.g., nucleic acids, proteins) in biological samples, such as tissues. It also documents the examples of the relevant technologies and background literature that a POSA would have been familiar with at that time.

D. Background Techniques for Spatial Tissue Analysis

58. In this section, I discuss some of the background techniques and technologies with which a POSA would have been familiar, and which are used in the specific implementations of spatial analysis technologies discussed in the following section.

1. Detection of Nucleic Acids

59. Many nucleic acid technologies historically used fluorescence and other labelling and imaging techniques to detect the presence or absence of a target nucleic acid sequence. Fluorescence is a type of emission spectroscopy, in which a molecule absorbs light of a given wavelength, and then releases some of the absorbed energy by re-emitting light of a longer wavelength. For this purpose, many fluorescent dye molecules that absorb and emit light efficiently have been developed. Such molecules can be excited by exposure to light (often using a laser), and because emission occurs at a different wavelength than the exciting light, the exciting light can be effectively filtered out, leading to a high sensitivity of detection of the fluorescent molecule. This high sensitivity is a power of fluorescence-based detection methods.

60. Fluorescence has been particularly useful for the analysis of molecular interactions. For example, nucleic acids are readily labeled with fluorescent dyes to form labelled probes, and when such probes are hybridized with a target, complementary sequence using the principles of base pairing described above, will cause the target-probe complex to be fluorescent upon exposure to an appropriate light source. Thus, the signal is readily detectable with simple imaging or

spectroscopic methods. For example, such techniques were frequently used to detect nucleic acid binding events in microarrays, as discussed further below.

61. Sequencing generally refers to techniques used to determine the order of nucleotide bases present in the DNA or RNA of a biological sample. Around the priority date, next-generation sequencing (NGS) technologies were becoming available. “NGS platforms have dramatically increased throughput and substantially lowered expenditure” as compared to first generation Sanger sequencing, EX1029, 44, “offer[ing] the ability to produce an enormous volume of data cheaply.” *Id.*, 31. Both Sanger sequencing and NGS techniques generate sequencing “reads,” which report the order and identity of nucleotides present in a target nucleic acid of interest.

62. Some applications of NGS techniques included the use of “custom-designed oligonucleotide microarrays...for targeting regions of interest.” *Id.*, 41. Such techniques include “oligonucleotide microarrays [that] have been designed to provide complementary sequences for solid-phase hybridization to enrich” for sequences of interest, before subjecting the captured nucleic acids to NGS. *Id.*, 41 (explaining that “[t]he concept of targeting specific regions of the genome is well established, with PCR being the most widely used method,” and that this may be accomplished “us[ing] NGS platforms to target specific regions of interest.”)

2. Nucleic Acid Arrays

63. Nucleic acid arrays are a class of technologies in which DNA or other nucleic acid sequences are provided on a solid support. Typical solid supports include glass microscope slides and beads. Because “[i]t is well known that complementary single-stranded sequences of nucleic acids form double stranded hybrids,” nucleic acid arrays leverage this property by presenting “thousands of single-stranded sequences that are complementary to target sequences.” EX1018, 528. “[T]he targeted nucleic acid species” can then be “detected by designing several complementary oligonucleotides spanning each entire target sequence” and hybridization events can be read out by a variety of methods, including fluorescence, dyes, and sequencing. *See id.*

64. The illustration below, provided by the National Institutes of Health, provides a general example of one type of implementation of a nucleic acid array:⁶

⁶ DNA Microarray Technology Fact Sheet, Nat’l Hum. Genome Rsch. Inst. (Aug. 15, 2020), <https://www.genome.gov/about-genomics/fact-sheets/DNA-Microarray-Technology>.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

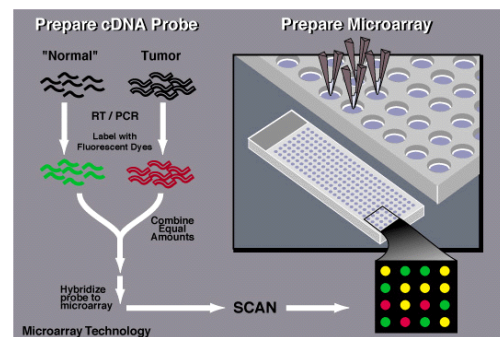
How does a DNA microarray work?

To determine whether an individual possesses a mutation for a particular disease, a scientist first obtains a sample of DNA from the patient's blood as well as a control sample - one that does not contain a mutation in the gene of interest.

The researcher then denatures the DNA in the samples - a process that separates the two complementary strands of DNA into single-stranded molecules. The next step is to cut the long strands of DNA into smaller, more manageable fragments and then to label each fragment by attaching a fluorescent dye (there are other ways to do this, but this is one common method). The individual's DNA is labeled with green dye and the control - or normal - DNA is labeled with red dye. Both sets of labeled DNA are then inserted into the chip and allowed to hybridize - or bind - to the synthetic DNA on the chip.

If the individual does not have a mutation for the gene, both the red and green samples will bind to the sequences on the chip that represent the sequence without the mutation (the "normal" sequence).

If the individual does possess a mutation, the individual's DNA will not bind properly to the DNA sequences on the chip that represent the "normal" sequence but instead will bind to the sequence on the chip that represents the mutated DNA.



65. Around the priority date, there were two main classes of solid supports on which the hybridization probes, sometimes called capture probes, were typically affixed. In one class, “thousands of single-stranded sequences that are complementary to target sequences are bound, synthesized, or spotted to a glass support whose size is similar to a typical microscope slide.” EX1018, 528. A “different technology...uses designed oligonucleotide probes attached to beads that are deposited randomly in a support. *Id.* The position of each bead and hence the sequence it carries is determined by a complex pseudo-sequencing process.” *Id.*

66. Well before 2010, a POSA would have been familiar with a variety of techniques for “decoding” bead arrays to determine the identity and location of each bead in an array. For example, in a 2007 publication, the named inventor, Dr. Chee explained:

U.S. Ser. Nos. 08/818,199 and 09/151,877 describe array compositions that utilize microspheres or beads on a surface of a substrate, for example on a terminal end of a fiber optic bundle, with each individual fiber comprising a bead containing an optical signature. Since the beads go down randomly, a unique optical signature is needed to ‘decode’ the array; i.e., ***after the array is made, a correlation of the location of an individual site in the array with the bead or bioactive agent at that particular site can be made.*** This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the in situ synthesis or spotting techniques of the prior art. Once the array is loaded with beads, the array can be decoded, or can be used, with full or partial decoding occurring after testing, as is more fully outlined below.

EX1038 (Chee-824), [0013]. Other known techniques for spatially addressing bead arrays so that the identity of the probes on a given bead were known included “positional decoding” methods, such as “targeting the placement of the beads” to specified locations, or “selective loading of the sites” of particular locations on an array. *Id.*, [0043].

67. Another decoding method disclosed in Chee-824 (EX1038) involves analyzing “identification sequences” that are also affixed to the beads. EX1038, [0042] (“The present invention is generally based on previous work comprising a bead-based analytic chemistry system in which beads, also termed microspheres, carrying different chemical functionalities are distributed on a substrate comprising a patterned surface of discrete sites that can bind the individual microspheres.”). In a common method of making bead arrays, “the beads may be randomly distributed on the array,” and “can later be ‘decoded’, i.e. after the array is made,” by making “a correlation of the location of an individual site on the array with the bead or candidate agent at that particular site.” *Id*; see also *id.*, [0043] (“[T]he placement of the bioactive agents is generally random, and thus a coding/decoding system is required to identify the bioactive agent at each location in the array.”). “Once the identity of the bioactive agent and its location in the array has been fixed, the array is exposed to samples containing target analytes.” *Id.*, [0044].

68. Chee-824 further discloses decoding that “does not rely on the use of optical signatures, but rather on the use of decoding binding ligands that are added during a decoding step.” *Id.*, [0045]. “The decoding binding ligands will bind either to a distinct identifier binding ligand partner that is placed on the beads, or to the bioactive agent itself, for example when the beads comprise single-stranded nucleic

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

acids as the bioactive agents.” *Id.* “The decoding binding ligands are either directly or indirectly labeled, and thus decoding occurs by detecting the presence of the label.” *Id.* Chee-824 discloses that “[p]referably at least 5000 bead types are simultaneously decoded or decoded in a single decoding experiment.” *Id.*, [0046].

69. Chee-824 discloses methods in which “the bioactive agent is synthesized with encoding sequences that allows decoding to be accomplished independent of the useable portion of the bioactive agent, i.e., nucleic acid sequence.” *Id.*, [0141] (“By ‘useable’ portion is meant the adapter sequence or target sequence.”). For example, “FIG. 12 depicts decoding by sequencing” in which “dye-labeled chain-terminating nucleotides are used to sequence the encoding region of a DNA strand present on a bead.” *Id.*, [0037].

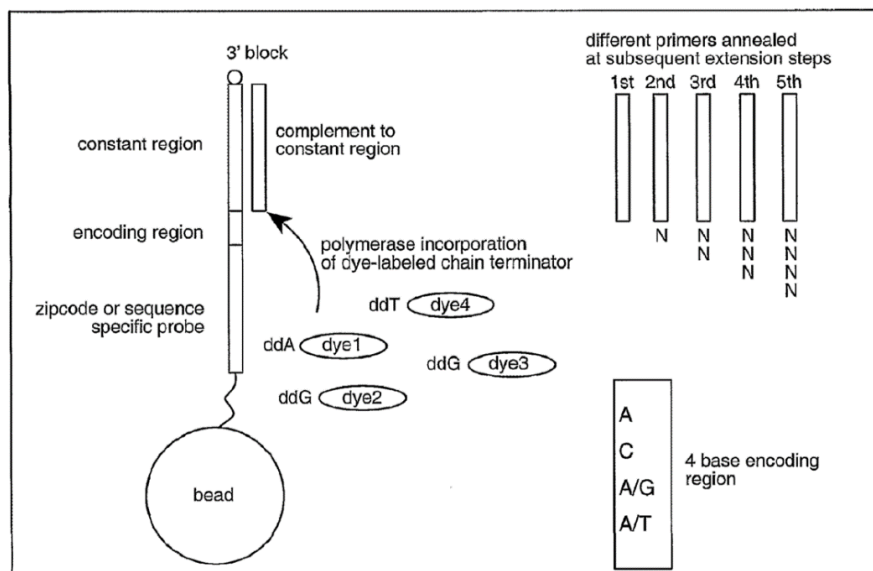


FIG. 12

70. Figure 13 further shows “construction of probes on [a] bead containing encoding sequences, zipcodes, and a gene-specific sequence.” *Id.*, [0038].

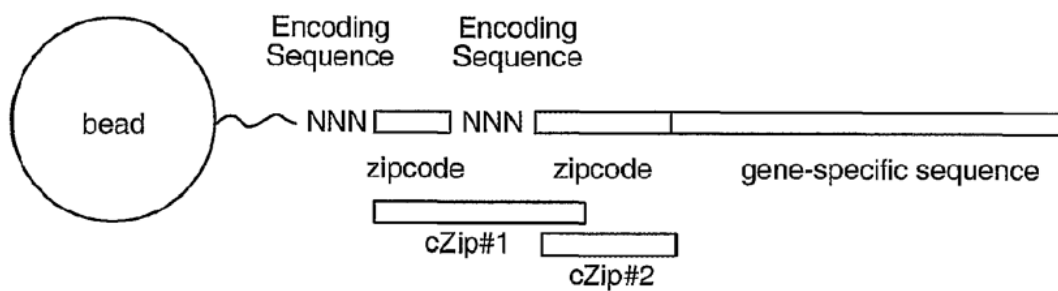


FIG. 13

71. After the encoding sequences are decoded using labels, such as fluorescent labels, the location of the bead with a particular encoding sequence may be recorded, and the decoding reagents may be removed or destroyed so that the array can be used analytically. For example, “following a decoding step where the fluorescent signal of the dyes are detected, the fluorophore is destroyed by an appropriate chemical solution that degrades the fluorescent molecule sufficient[ly] leaving no trace of fluorescence.” *Id.*, [0146]. “In this way, beads are tagged with fluorescent labels, i.e., barcodes of sorts, their location or identity deciphered and recorded.” *Id.* “The beads are then exposed to the ‘fluorescence destroying solution’ and the fluorescence is eliminated, allowing an analytical assay to be conducted.” *Id.*

3. Sequence Barcoding

72. Multiplexing, which allows higher throughput analysis of many samples in parallel, was a key development for nucleic acid sequence analysis before the priority date. As such, a POSA would have been familiar with the concept of barcoding, including using nucleic acid sequences as barcodes to identify and differentiate between different nucleic acid molecules or sets of molecules. *See, e.g.*, EX1037 (Fodor 2009) 3:55-64 (“Methods are disclosed herein for digital counting of individual molecules of one or more targets. In preferred embodiments the targets are nucleic acids.... Targets are labeled so that individual occurrences of the same target are marked by attachment of a different label to different occurrences. The attachment of the label confers a separate, determinable identity to each occurrence of targets that may otherwise be indistinguishable. Preferably the labels are different sequences that tag or mark each target occurrence uniquely.”); *see also* EX1030 (“Multiplexed high-throughput pyrosequencing is currently limited in complexity (number of samples sequenced in parallel), and in capacity (number of sequences obtained per sample).... To overcome these limitations, we have devised a novel barcoding approach to allow for pooling and sequencing of DNA from independent samples, and to facilitate subsequent segregation of sequencing capacity. Forty-eight

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

forward-reverse barcode pairs are described: each forward and each reverse barcode unique with respect to at least 4 nt positions.”).

73. Consistent with this knowledge, I note that in a prior proceeding involving the Fodor patent (EX1037 discussed above), 10x Genomics (Patent Owner’s exclusive licensee and co-plaintiff in this matter) asserted the following with respect to nucleic acid barcoding:

The claims of the Fodor patents are directed to the abstract idea of labeling different objects with different labels. ***This concept is as old as the scientific method and is familiar to every student in any introductory science class*** – when performing an experiment, one needs a control and an experiment and will label them accordingly (whether that means written labels of “control” and “experiment” or “test 1” and “test 2” or “sample A” and “sample B” or some other form of labeling such as placing one in a green cup and one in a blue cup). Even outside the context of scientific experiments, using different labels to differentiate and distinguish between different instances of similar objects is commonplace. For example, different instances of the same model of television set are given different serial numbers for warrantee or recall purposes; people attending a convention are given different name tags; and different books in a library are given different classification numbers (under the Dewey Decimal System) and are assigned different International Standard Book Numbers (ISBNs) (by the Library of Congress). ***The Fodor patents did not invent this idea***

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

they simply take this abstract idea and attempt to limit the use of the idea to the particular technological environment of DNA (and other nucleic acids) by adding “attaching”, “amplifying”, and “detecting” steps and other similarly generic techniques that were well-known and conventional before 2009 (the earliest provisional application).

EX1035, 4-5.

74. Examples of nucleic acid barcoding approaches specifically for correlating particular spatial locations in arrays (such as X,Y coordinates) with particular nucleic acid sequences (barcodes) were disclosed in numerous references before the priority date. For example, Barany 1999 (EX1036) describes a barcoding scheme for microarrays, which is used to direct target nucleic acids “to specific zip-code addresses attached covalently to a three-dimensional gel-matrix array.” EX1036, 251. This barcoding system, which the authors refer to as “zip-codes” defines particular spatial coordinate locations in an array based on unique nucleic acid sequences. For example, by using tetramer (4 nucleotide) bar codes, an array

may be designed that uniquely barcodes up to 256 (4^4) separate locations according to, for example, a grid pattern.⁷

		2nd two bases															
1st two bases		TT	TC	TG	TA	CT	CC	CG	CA	GT	GC	GG	GA	AT	AC	AG	AA
		TT	TC	TG	TA	CT	CC	CG	CA	GT	GC	GG	GA	AT	AC	AG	AA
TT								16'			23'		TTGA 6			TTAG 8	
TC			TCTG 1		30'	TCCC 3			TCGT 5								6'
TG			TGTC 2		36'		TGCG 4							TGAT 7		11'	
TA						18'		TACA 36			33'						
CT	32'		CTTG 9					CTCA 11	CTGT 13								8'
CC					CCTA 33					29'				CCAT 15			
CG	CGTT 10			12'					4'								CGAA 16
CA				34'			25'	CACG 12			CAGC 14		1'			9'	
GT						GTCT 19	24'				GTGC 22			31'			
GC			GCTT 17		14'											22'	GCA 23
GG				20'		GGTA 18	35'										
GA						GATG 34			GACC 20		2'	GAGT 21					
AT								ATCG 28	7'				15'			ATAC 31	
AC							ACCT 27					ACGG 29		5'			13'
AG								AGCC 35									
AA											27'			AGGA 30		19'	AAAG 32

Figure 2. Design of tetramers for use in zip-code arrays. The checkerboard pattern shows all 256 possible tetramers. A given square represents the two bases on the left followed by the two bases on the top of the checkerboard. To be included, each tetramer must differ from all others by at least two bases, and be non-complementary. The chosen tetramers are shown in the white boxes, while their complements are listed as (number)'. Thus, as an example, the complementary sequences GACC (20) and GGTC (20') are mutually exclusive in this scheme. In addition, tetramers that are palindromic, e.g. TCGA (off-diagonal hatched boxes) or repetitive, e.g. CACA (hatched boxes on diagonal from upper left to lower right) have been eliminated. All other sequences which differ from the 36 tetramers by only one base are shaded in light gray. Four potential tetramers were not chosen as they are either all A·T or G·C bases (open boxes).

75. Each additional nucleotide added to a barcode exponentially increases the number of unique available barcodes. For example, using 5-mers as opposed to

⁷ In Barany's method, certain potential barcodes were excluded, such as those having tetramer repeats (e.g., AAAA or TTTT).

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

Barany's tetramers would yield 1,024 (4^5) potential barcodes, and using 10-mers would yield 1,048,576 (4^{10}) potential barcodes. *See also* EX1032, 16:10-17 ("Thus, for an array size of 1000, the decoding sequences must be 5 nucleotides long ($4^5=1024$) to ensure a unique decoding sequence for each candidate agent. In a preferred embodiment the decoding sequence contains from 1 to 1000 nucleotides....").

76. To take another example, Chee-584 (EX1032) describes barcoded bead arrays in which "a spatial or positional coding system is done." EX1032, 28:6-7. These systems allowed for further re-use of barcodes by positioning them on a given array in a manner similar to telephone area codes:

[S]ub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an "area code" that can have the same tags (i.e. telephone numbers) of other subarrays, that are separated by virtue of the location of the subarray. Thus, for example, the same unique tags can be reused from bundle to bundle. Thus, the use of 50 unique tags in combination with 100 different subarrays can form an array of 5000 different bioactive agents. In this embodiment, it becomes important to be able to identify one bundle from another; in general, this is done either manually or through the use of marker beads, i.e. beads containing unique tags for each subarray.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX1032, 28:6-19. *See also* EX1038 (Chee-824), [0146] (“In this way, beads are tagged with fluorescent labels, i.e., barcodes of sorts, their location or identity deciphered and recorded.”).

77. Cantor, which I address further in Grounds 1 and 2 below, disclosed bead arrays in which each bead was labelled or barcoded with a unique “identification sequence” that differed from one bead to another, thereby distinguishing between different beads at different locations in its arrays. A specific example of Cantor’s disclosure “provides a substrate comprising a collection of beads oriented in an array.” EX1031, 4. Each bead “is in association with single-stranded nucleic acid.” *Id.* The bead-associated nucleic acids, which Cantor at times refers to as “the solid phase nucleic acid,” include “a probe sequence,” which “hybridizes to sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid.” *Id.* The bead-associated nucleic acids also include “an identifier sequence.” *Id.* Cantor discloses that in its bead arrays, “each of the solid supports,” i.e., beads, “in the collection share a unique identifier sequence different than the identifier sequence of solid phase nucleic acid of the other solid supports,” i.e., beads. In other words, each bead is identified with unique “identifier sequences.” Since each bead necessarily occupies a distinct physical location, and because each bead possesses an “identifier sequence” unique

IPR2025-00236

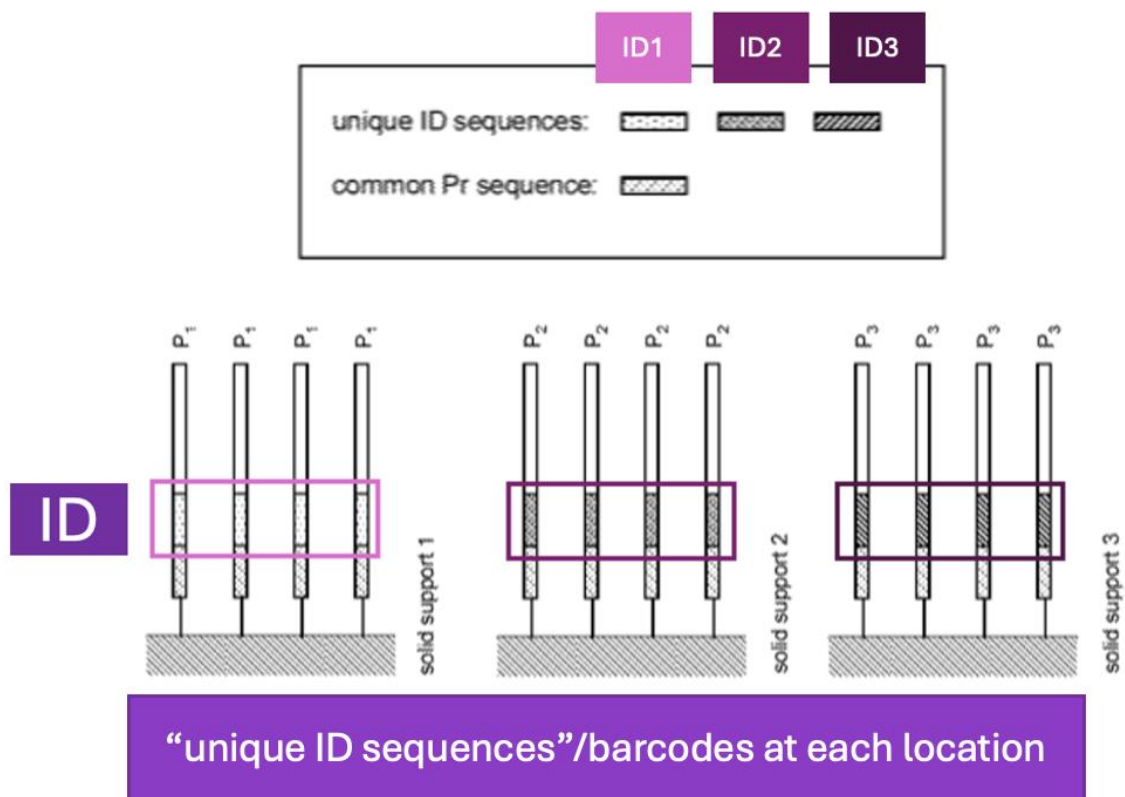
IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

to that bead alone, Cantor's identifier sequences, or barcodes, necessarily correspond with the spatial location of that bead in the array.

78. Cantor's Figure 1 is reproduced below, with annotations showing its use of unique "identification sequences" to identify each unique solid support (i.e., bead) in its arrays, differentiating solid supports at different locations from one another.



E. Overview of Relevant Spatial Analysis Technologies

79. By 2010, a POSA would have been familiar with several methods for analyzing biological molecules in tissue sections, including their spatial arrangement.

1. In Situ Hybridization

80. In situ hybridization (ISH) including fluorescence in situ hybridization (FISH) was a well-established technique by the priority date. The method works by hybridizing a probe molecule that contains a fluorescent tag to nucleic acid targets in fixed, permeabilized cells or tissues. The probe is bound to the target DNA or RNA molecule by targeting and hybridizing to target nucleic acid sequences. Free, unbound probes are then washed away to prevent background signal (e.g., fluorescence from probes that did not bind their target). Originally, this approach used radioactive labeling (simple in situ hybridization). By the late 1980s, the approach had moved to using fluorescently-labeled probes for improved performance by using fluorescence microscopy methods to localize the hybridized probe. By the early 2000s, researchers were using very bright probes with multiple fluorescent dyes, facilitating detection and allowing for single-molecule detection in cells, and also combinations of different fluorophores (similar to barcodes) to allow multiplex detection.

2. 2D-PCR

81. By 2009, a POSA would have been familiar with 2D-PCR techniques in which “[a] 2d map of [a gene] was created from a tissue section and shown to correlate with the spatial area of the sample.” EX1022 (Armani), 3526 (Abstract). These techniques were anticipated to “be easily adapted to assess the status of multiple genes within tissue sections, yielding a molecular map that directly correlates with the histology of the sample,” thus “provid[ing] investigators with a new tool to interrogate the molecular heterogeneity of tissue specimens.” *Id.* This approach merges the power of spatial observation with the nucleic acid analysis methods described above.

82. Armani explained that “[v]ariations in DNA status (mutation, epigenetic modification, insertions, deletions) by location within a tissue section are of interest for elucidating their roles in biology and as clinical markers for diseases.” *Id.*, 3526. Thus, Armani noted a desire and motivation in the art to study nucleic acids in a manner that maintained the spatial organization of the tissue, in contrast to prior methods that “identify genes of interest from tissue specimens by examining cell homogenates (all the cells in the sample mixed together).” *Id.* The use of such cell homogenates “masks a tremendous amount of information” because the intact “tissue comprises a complex milieu of diverse cell populations that define the local

microenvironments.” *Id.* Thus, Armani observed, techniques were necessary to correlate nucleic acid variability within a sample with “the physical location [of nucleic acids] within the tissue section.” *Id.* Armani successfully demonstrated its “method of mapping DNA as a step towards the more challenging task of mapping mRNA.” *Id.*, 3527. Armani subsequently demonstrated the feasibility of its approach with mRNA. EX1021, 69-71 (Example 23) (“This experiment has been performed several times, and shows that it is possible to transfer a tissue vertically into an array of wells, simultaneous[ly] isolating and preserving (without cross-contamination between wells) the positions of the mRNA for subsequent analysis.”).

83. Armani’s approach, which the authors called “2D-PCR, makes use of a miniature array of wells together with protocols that allow tissue lysis, DNA amplification, and target detection within the same well.” EX1022, 3527. The authors demonstrated this technique “via the creation of a 2D detection map, showing the presence/absence of the target DNA.” *Id.* Such methodologies therefore “advance[] studies of gene expression as a function of the type of cell and its position within a tissue.” *Id.*

84. In Armani’s 2D-PCR method, “[a] tissue is sectioned and transferred into a multi-well array device.” *Id.* “The DNA is then extracted from the tissue and subsequently amplified by PCR in the same wells.” *Id.* “The PCR products can be

detected either within the wells or after products are transferred onto one or more detection membranes.” *Id.* This is depicted in Figure 1.

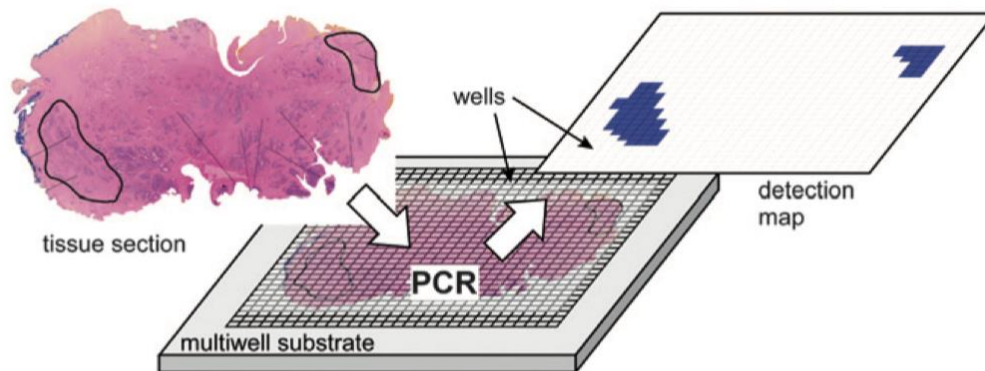


Fig. 1 2D-PCR is achieved by transferring a tissue section vertically into a multi-well array device, isolating tissue subregions. The DNA is extracted and then amplified by the polymerase chain reaction. A detection map is created by imaging the amplified DNA, for example using a DNA-intercalating dye.

85. I note that the Challenged Patents state with respect to Armani that, “[c]ertain PCR assays in a 2D format preserve spatial information [citing EX1022], but these methods have low spatial resolution because they rely on physical transference of tissue into wells, which also prevents random access to tissue samples and high levels of multiplexing.” EX1002, 1:66-2:5; EX1003, 1:64-2:3. The Challenged Patents provide no support for these conclusory assertions, nor do they explain how to solve these purported problems with Armani’s methods. Indeed, the only examples set forth in the Challenged Patents are prophetic examples with no supporting data or results. See EX1002, 30:36-32:37; EX1003, 30:36-32:37.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

Moreover, the Challenged Claims do not preclude methods in which tissue is physically transferred into wells, nor do they recite a particular level of spatial resolution or multiplexing. *See generally*, EX1002-EX1003, claims.

3. Array-Based In-Situ Reverse Transcription

86. A common feature among mature mRNAs that are ultimately translated to protein, especially in eukaryotes, is the presence of a poly(A) tail at the 3' end. EX1013, 501. The poly(A) tail is a series of "A" bases added to the end of an mRNA after it is transcribed. The poly(A) tail is sometimes referred to as polyadenylation. Thus, regardless of the identity of the gene, most mRNAs end with a poly(A) tail, which is typically ~250 "A" nucleotides in mammalian cells. EX1014, 22803.

87. Reverse transcription refers to a biological process by which mRNA is copied into DNA by enzymes called reverse transcriptases. The resulting DNA is referred to as complementary DNA, or cDNA. By the early 1990s, a POSA would have been aware of techniques for "performing cDNA synthesis *in situ*," for example on a tissue sample, "incorporating radioactivity into the cDNA, and examining the autoradiographic distribution of the resultant radiolabeled cDNA." EX1015, 81. This procedure was sometimes referred to as *in situ* transcription (IST), and "was originally used as an mRNA localization technique." *Id.*

88. In short, the IST process involved (1) “annealing a specific oligonucleotide primer to the mRNA in the tissue section” (2) “washing away the unhybridized primer”; and (3) “initiating cDNA synthesis by addition of reverse transcriptase and deoxynucleotide triphosphates.” *Id.* This “allows radiolabeled deoxynucleotides to be incorporated into the growing cDNA molecule in an anatomically defined region,” and subsequently visualized by autoradiographic exposure. *Id.* Those skilled in the art applied this technique for “several applications, including cellular localization of specific mRNAs,...expression profiling of mRNA populations from anatomically defined areas,...and analysis of gene expression in live cells.” *Id.*

89. Early methods of IST were “initiated by in situ hybridization of an oligonucleotide complementary to a specific mRNA, providing a primer that is required for the polymerase activity of reverse transcriptase.” EX1016, 1661. “This priming step allows one to select the individual mRNA to be transcribed,” and “[t]he enzyme then extends the primer with the mRNA acting as a template.” *Id.* This is shown in the figure reproduced below, which shows hybridization of “[a]n oligonucleotide 36 nucleotides (nt) in length, complementary to the sequence encoding amino acids 100 to 111 of rat POMC,” a particular protein encoded by a particular sequence, “as the primer.” *Id.*

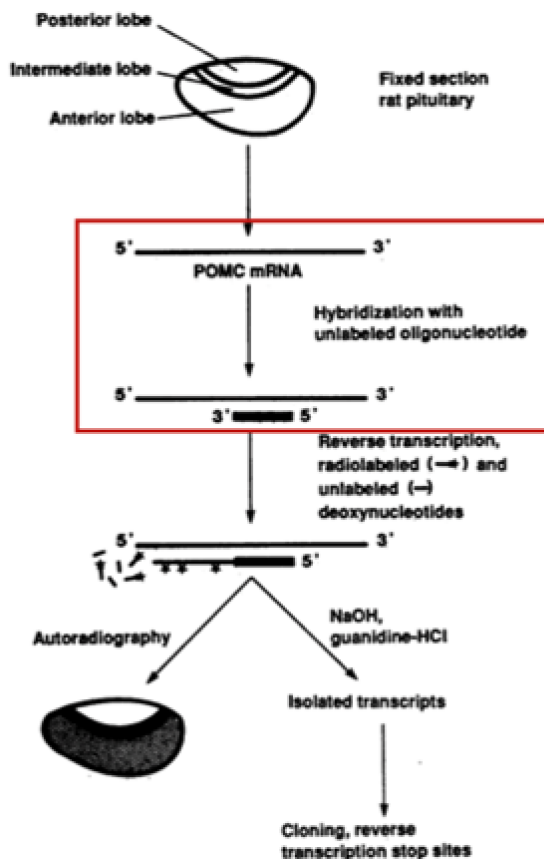


Fig. 1. Schematic outline of the in situ transcription (IST) procedure for the detection of POMC mRNA. An unlabeled oligonucleotide is hybridized to POMC mRNA, followed by reverse transcription within the tissue section in the presence of radiolabeled deoxynucleotides. Autoradiography is then performed for the localization of radiolabeled POMC cDNA. After autoradiography, the transcripts may be denatured and eluted by NaOH or guanidine-HCl treatments, and the transcripts may be either cloned or separated by electrophoresis for subsequent analysis.

90. Subsequently, techniques were developed in which poly-T sequences were used as primer sequences to initiate reverse transcription by hybridizing to the poly-A sequences on any given mRNA. In such techniques, the identity of the gene could then be determined by sequencing the cDNA strand generated by reverse transcription. See EX1017, 3-4 (detecting “polyA RNA sequences in permeabilized HeLa cells” using primers consisting of “oligo dT15,” i.e., TTTTTTTTTTTTTTTT, as well as oligo dT20 and oligo dT25). The use of oligo dT as a generic primer allows the capture and analysis of a generic class of molecules, namely polyadenylated

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

mRNAs. EX1017, 1 (“In this study, we have developed an approach based on a reverse transcription enabling highly specific, effective and fast detection of polyA RNA sequences *in situ*.”).

91. Below I discuss several exemplary references that discuss the use of *in situ* reverse transcription to capture, identify, and analyze the location of nucleic acids in tissue samples. Following reverse transcription of target nucleic acids, these techniques describe detecting and localizing the targets by different techniques, including visualization techniques (e.g., fluorescence) and sequencing techniques.

(a) Detection of Reverse-Transcription Products on Spatial Arrays with Fluorescence

92. Another related set of technologies involved the spatial analysis of gene expression using arrays of capture agents, including poly-T capture agents and gene-specific capture agents, to immobilize mRNA in two-dimensional space and detect its presence using fluorescent or other labeled probes. Representative references demonstrating such techniques include Shirai (EX1024), Okano (EX1026), and Southern (EX1032).

93. Shirai is a United States Patent that was filed on November 29, 2010. EX1024, (63). I understand that even if the challenged claims are determined to be entitled to a priority date of April 5, 2010 (as set forth in VIII.C.1, below, they should

not), Shirai is sufficiently contemporaneous to constitute appropriate evidence of what was known for indicating the level of ordinary skill in the art, the motivations of a POSA, and how a POSA would have understood other prior art disclosures.

94. Shirai discloses methods of creating cDNA libraries from tissue sections using bead arrays that capture target nucleic acids while maintaining information about the spatial locations of those target nucleic acids in the tissue slice. For example, Shirai explains that it “provides a method and/or means for collecting and analyzing an individual cell in a tissue, and at the same time, quantitatively monitoring the expression levels of various genes ***while keeping two-dimensional information in the tissue.***” EX1024, Abstract. Moreover, Shirai “provides a method comprising preparing a cDNA library from mRNA while keeping two-dimensional cellular distribution information and obtaining the gene expression levels at any site or all sites at a level of single cell.” *Id.*

95. In Example 4, Shirai describes providing a plurality of beads: “beads smaller than cells are arranged on a surface.” EX1024, 27:5-6. These beads comprise a plurality of capture agents: “[o]n the beads surface, poly-T probes are immobilized.” *Id.*, 27:6-7. The poly-T probes immobilized on the beads’ surface are configured to interact with at least one mRNA present in a tissue sample: “On the resultant structure,” that is, an array of poly-T coated beads, “a tissue slice is

mounted” and the cells are permeabilized or lysed to release their mRNA, which is then reverse transcribed into complementary cDNA molecules. *Id.*, 27:5-19 (“Cells were lysed in the same manner as above and mRNA molecules were electrophoretically trapped on the surface of beads and reverse-transcribed to obtain a cDNA library sheet.”). Thus, Shirai discloses a plurality of beads comprising capture agents that are configured to interact with nucleic acids (mRNA) present in the tissue slice.

96. The interaction between the poly-T functionalized beads (1901) arrayed on substrate (1903) and mRNA in the tissue (1101) slice is depicted in Figure 19. EX1024, Figure 19; 5:38-39 (“FIG. 19 shows an example of a method for preparing a cDNA library on beads.”); 27:5-32.

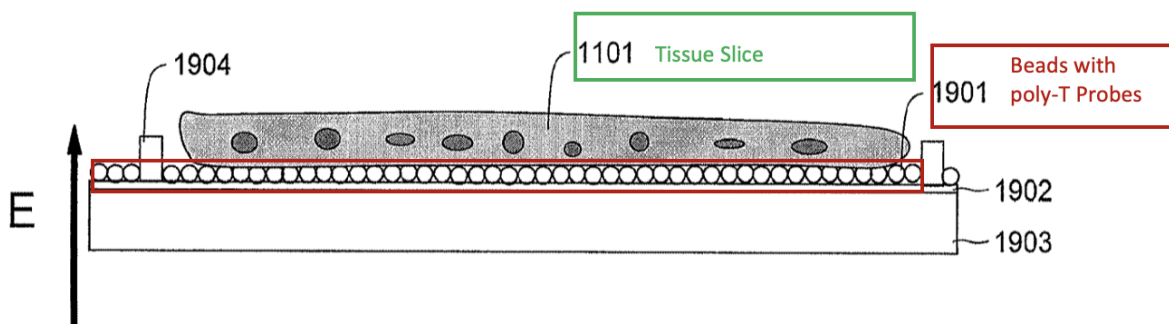


FIG. 19

97. Shirai further discloses a variation of the above method, in which the functionalized beads are arrayed in spatially defined microwells or chambers, each

of which corresponds with a different spatial location of an overlaid tissue sample. EX1024, 27:33-40. Specifically, Figure 20 shows an implementation in which “a number of beads are spread in the bottom of reaction cells in which cells are housed, and the same treatment,” i.e., the mRNA release and capture discussed in connection with Figure 19, “may be performed.”

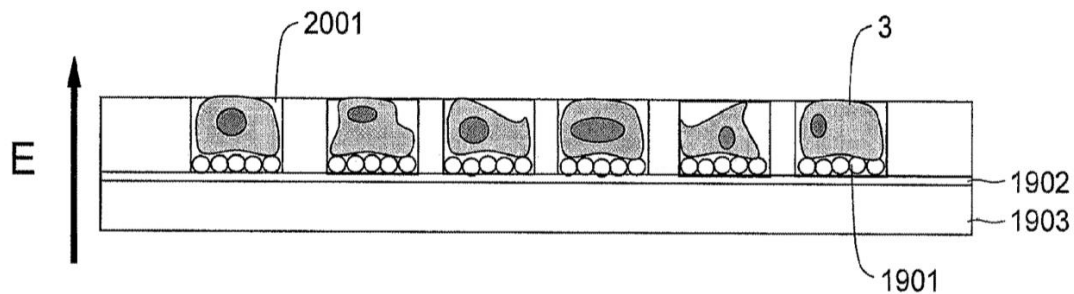


FIG. 20

98. One method that Shirai discloses for preserving spatial/location information regarding the presence or abundance of mRNAs in the tissue sample is by transferring the cDNA library (which contains nucleic acid complements to the mRNA from the tissue) to a sheet or membrane. EX1024, 27:15-25 (“Cells were lysed in the same manner as above and mRNA molecules were electrophoretically trapped on the surface of beads and reverse-transcribed to obtain a cDNA library sheet.”). Gene expression may then be detected based on, for example the use of fluorescent probes, and a spatial map may be generated. *See, e.g., id.*, 10:46-55 (“In

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

the present invention, the results from the detection of gene expression in the two-dimensional cDNA library obtained as mentioned above can be compared to two-dimensional positional information of a sample to obtain correlation data between the specific position in the sample and gene expression. Examples of such two-dimensional positional information of a sample include a microscopic image of a cell sample or a tissue section sample, and a fluorescent image or a chemiluminescent image obtained by other labeling methods.”); Figs. 24-26.

99. The disclosures of Okano (published on December 9, 2009) are similar to those in Shirai, describing “a method, a chip device, and a system for quantitatively measuring intercellularly and intracellularly-localized mRNA and protein ***without loss of local space information.***” EX1026, Abstract. Okano explains that “cell content is trapped atop a substrate or an electrode in the form of a two-dimensional projection of a cell,” and the cellular contents are released such that “mRNA is immobilized to a two-dimensional surface,” where it can be “identified with a labeled probe which hybridizes to the mRNA.”

100. Okano explains that by 2009, POSAs were motivated to identify and detect mRNA and proteins in tissue samples while maintaining the spatial orientation of such molecules. For example, Okano notes that “methods for the identification and detection of mRNA and proteins, which are the respective

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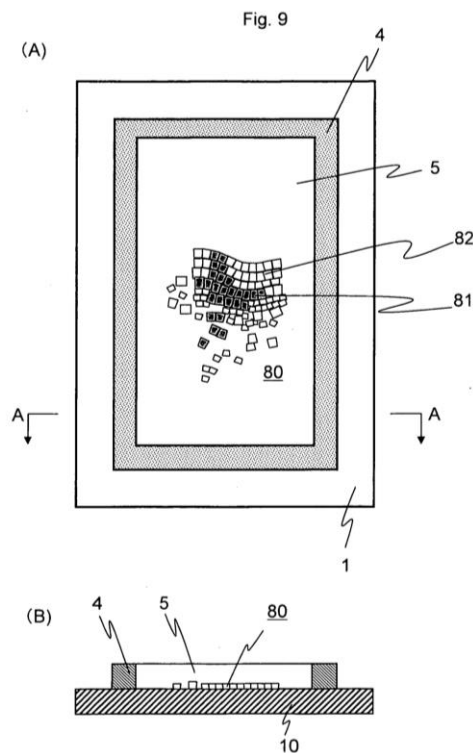
IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

products of transcription and translation performed using a DNA probe chip or protein chip” had been “thoroughly investigated,” and that such methods typically were “performed if a sample in the form of a solution can be prepared.” *Id.*, [0007]. For example, “[i]n mRNA testing, the RNA component is extracted in advance...reverse transcriptase is used to synthesize cDNA by means of a reverse transcription reaction, and amplification is performed by means of PCR amplification and cRNA synthesis.” *Id.* Okano observed that in the case of cell and tissue samples, “there are instances in which it is problematic to convert the cell component being measured into a solution state,” because the process of doing so would “cause[] localized information for each cell to be lost.” *Id.*, [0008]. Since “even within individual cells, mRNA, which is an expressed gene, is localized,” and “change[s] in accordance with a cell’s state of activity,” Okano noted the desirability of detecting such molecules while maintaining information about their spatial context. *Id.* Thus, Okano described and established a POSA’s motivation to perform methods in which “mRNA and proteins localized between or inside cells” can “be quantitatively detected in a state in which local space information is retained,” enabling the generation of “information useful for medically-directed testing drug discovery, and to more accurately describe biological functions.” *Id.*

101. For example, Okano's Figure 9 shows plan and cross-sectional views of "the cell component sorting chip of Example 3," which describes "an instance in which a tissue-like specimen of aggregated cells, for example, a histological section is immobilized to a two-dimensional surface while position information of an intracellular substance is retained." EX1026, [0072].



102. "[A] tissue-like specimen of aggregated cells is the target," and this specimen is applied to an array of "indexing probes in which 10 nm gold particles are bonded to probes for an EpCAM^[8] mRNA sequence." *Id.* These probes

⁸ This refers to the gene for "Epithelial Cellular Adhesion Molecule."

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

“function as a bonding means for trapping a biological substance,” and when “the biological substance is mRNA,” as in Example 3, such substances are those which “have[] a complementary sequence that can hybridize” to the target mRNA. *Id.*, [0014]. “[T]wo-dimensional position information and quantity can be measured at the cellular level of each biological substance by arranging multiple probes to the spots in each section as with a conventional DNA microarray.” *Id.*, [0031]. Thus, “cell position is maintained, and labeling probes are provided with appropriate identifiers that selectively bond to intracellular substances that are, in turn, bonded with affinity to a plurality of probes arranged in cell immobilization sections.” *Id.* The labeling probes can then be detected at their bound position to reveal the spatial locations of EpCAM throughout the tissue sample. *See id.*, [0072].

103. Similarly, Southern discloses the detection of target nucleic acids on arrays so that “[t]he spatial arrangement of the samples is maintained” and the location of “results of the analysis to be matched to individual samples.” EX1033, Abstract. “One analytical procedure that can be used with” Southern’s arrays “involves capture of mRNA by hybridisation to an immobilised capture DNA, followed by reverse transcription of the mRNA using the immobilised DNA as a primer.” *Id.*, 9:17-25. In this procedure, Southern discloses creating a complementary cDNA by extending the probe, using the captured mRNA as a

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

template, using in situ reverse transcription. Southern discloses using optical means, such as fluorescence, as the method for detecting the spatial location of an interaction between its capture agent and a target nucleic acid:

For RNA analytes, detection can be achieved by incorporating fluorescent nucleotides into a complementary strand using an enzyme such as reverse transcriptase (e.g. the avian myeloblastosis virus (AMV) reverse transcriptase). ***For example, cDNA may be made in situ by hybridising mRNA to oligonucleotide probes on a support, and using the immobilised probe as a primer. The reverse transcription reaction preferably incorporates labelled nucleotides into the cDNA in order to facilitate detection of the hybridisation*** [10]. This can be achieved by the use of dNTPs with suitable fluorophores attached. Unlike a sequencing reaction, it is not necessary to use different coloured fluorophores for different nucleotides, because individual nucleotides do not need to be distinguished. Similarly, there is no need to label every nucleotide, and so 1, 2, 3 or 4 of dATP, dCTP, dGTP and dTTP may be labelled, and a mixture of labelled and unlabelled dNTPs can be used. Incorporation of a large number of fluorophores into the cDNA (e.g. in at least 5% of incorporated dNTPs, such as $\geq 10\%$, $> 20\%$, $\geq 30\%$, $\geq 40\%$, $\geq 50\%$, $\geq 75\%$, or more) means that ***the cDNA can readily be detected by any of the familiar means of fluorescence detection, thus revealing a positive signal even for a single hybridisation event. Thus even low-abundance mRNAs can be detected.***

EX1030, 27:12-28. In addition to detection by fluorescence, Southern continues by noting that “[a]fter in situ reverse transcription has been performed, there is initially a RNA/DNA hybrid, wherein the DNA will typically include a label for detection.” *Id.*, 28:8-9. Southern observes that detection can include an “amplification reaction,” *Id.*, 28:19-22, and that “[a]mplification in situ of captured target analytes should enable a wide range of useful applications” including “single molecule sequencing.” *Id.*, 39:25-28.

104. As explained further below, others expressly reported using sequencing as a readout for analyzing nucleic acids on spatially addressable arrays.

(b) Detection of Reverse-Transcription Products on Spatial Arrays with Sequencing

105. Other techniques utilize nucleic acid capture arrays and reverse transcription (or analogous techniques when the targeted nucleic acid is DNA) to analyze nucleic acid samples and use sequencing as the readout. Such techniques include those disclosed by Cantor (EX1031), which is discussed in more detail in the grounds of unpatentability presented below, and Frisen (EX1012).

106. Approaching the priority date, a POSA would have found using sequencing-based techniques as a readout for nucleic acid assays as an alternative for fluorescence-based detection methods to have been an obvious and natural

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

substitution. First, sequencing methods provide a direct readout of the nucleic acid identity, without the use of probe hybridization, imaging, or microscopy to indirectly confirm the identity of a target sequence. Second, using the analogy presented by 10x Genomics regarding the Fodor patent, labelling target nucleic acids with fluorescence would have been akin to a “form of labeling such as placing one in a green cup and one in a blue cup,” whereas nucleic acid barcodes simply apply the concept of “serial numbers” to nucleic acids. EX1035, 4-5. The use of nucleotide “serial numbers” or “barcodes” allows the creation of an exponentially greater number of labels as compared to using colors like green or blue. As noted above, the number of unique barcodes that can be generated by barcodes consisting of only A/C/G/T is 4^n , where n is the number of nucleotides in each barcode sequence string. This allows the creation of large sets of unique barcodes or tags, thereby enabling higher degrees of multiplexing and throughput using the short read-length sequencing technologies available before April 2010. *See e.g.*, EX1030. As Chee-824 explains, “[d]ecoding by sequencing has the advantage that encoding a vast number of distinct codes” may be accomplished “by using only a small number of nucleotides.” EX1038, [0142].

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

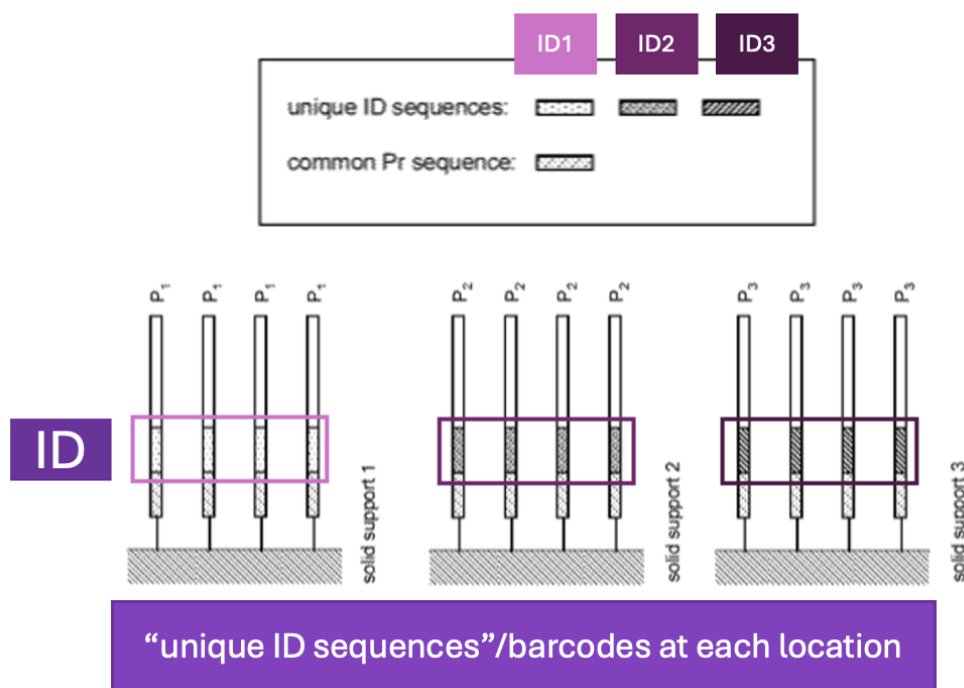
Patent No. 11,001,879

VI. Prior Art References of the Grounds

A. Cantor (Grounds 1-2)

107. Cantor, Patent Cooperation Treaty App. No. WO 2009/091934A1, titled “Single Molecule Nucleic Acid Sequence Analysis Processes and Compositions,” is an international patent application publication that was published on July 23, 2009. EX1031. Accordingly, Cantor is prior art to the '468 and '879 Patents.

108. As explained above, Cantor discloses bead arrays in which each bead is labelled or barcoded with a unique “identification sequence” that differs from one bead to another, thereby differentiating different beads at different locations in its arrays. This is depicted in the annotated version of Figure 1 below.



IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

109. A POSA would have recognized Cantor's "identification sequences" as a type of sample barcoding, as Cantor disclosed associating each solid support, which necessarily occupies a distinct physical location on its arrays, with a unique "identification sequence" that therefore corresponds to a given support's identity and location.

B. Armani (Ground 2)

110. Armani (EX1022) is an article that was published in the journal *Lab on a Chip* on October 12, 2009. EX1022, 3526. Armani is therefore prior art to the '468 and '879 Patents. Armani discloses the 2D-PCR approach discussed in Section V.E.2, above. As discussed in detail below, Armani provides additional evidence of a POSA's motivations to determine the spatial locations of nucleic acids in a tissue sample, and provides tissue preparation workflows that a POSA would have found obvious to combine with the nucleic acid analysis arrays disclosed by Cantor.

C. Frisen (Ground 3)

111. Frisen is an international patent application publication that was filed in 2011 and published in 2012. EX1020, Fields (22) and (43). As explained below, the priority applications leading to the '468 and '879 Patents do not support the issued claims, so they are not entitled to a priority date before February 14, 2019. Therefore, Frisen is prior art to the '468 and '879 Patents.

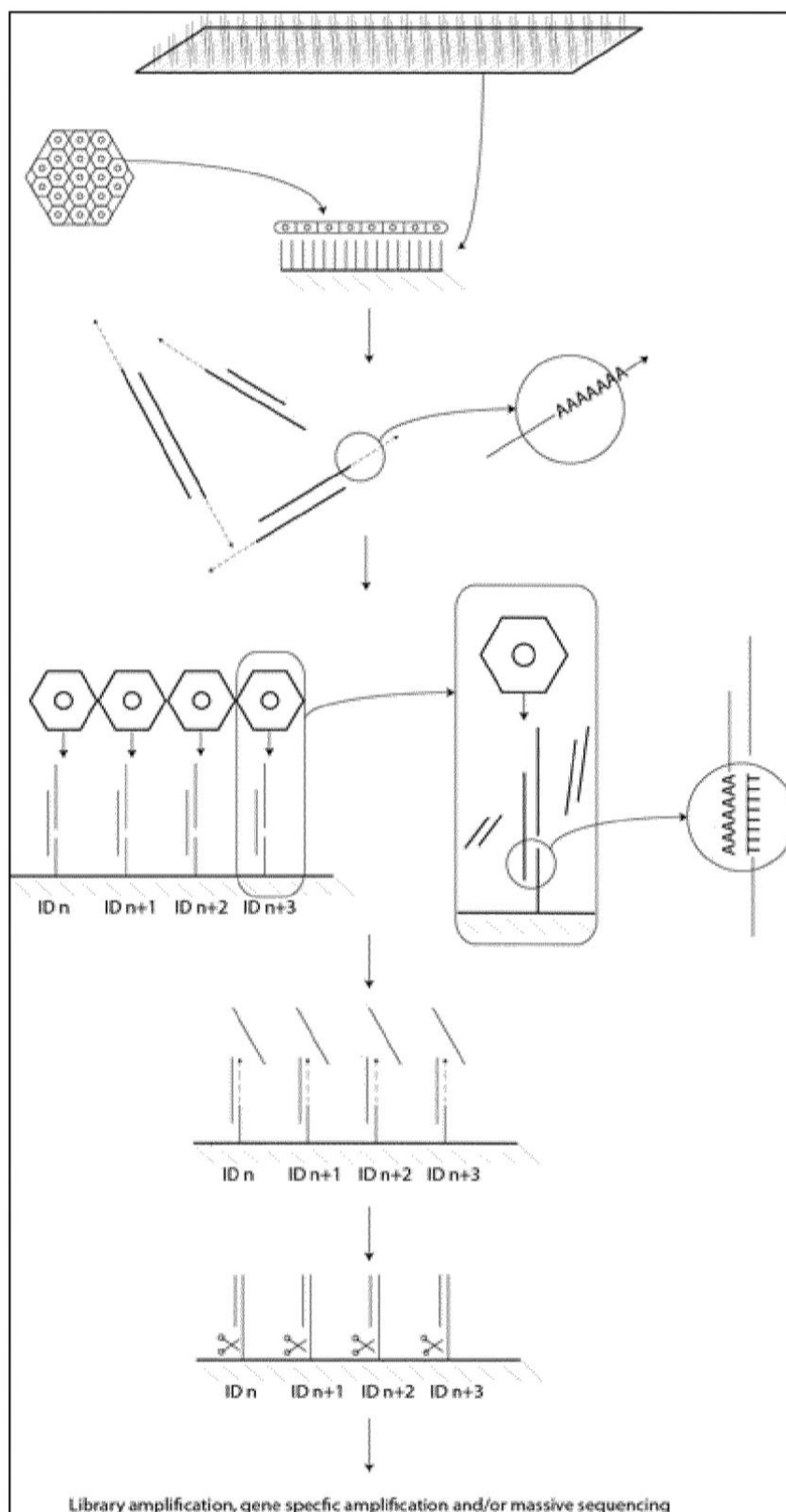
112. Frisen describes “methods and products for the localised or spatial detection of nucleic acid in a tissue sample and in particular [] a method for localized detection of nucleic acid in a tissue sample.” *Id.*, (Abstract). Frisen’s methods involve providing an array of capture probes, where each capture probe comprises “a positional domain that corresponds to the position of the capture probe on the array,” and “a capture domain” that binds to a nucleic acid. *Id.* Frisen then discloses “contacting said array with a tissue sample such that the position of a capture probe on the array may be correlated with a position in the tissue sample,” and then using various techniques to analyze the identity and location of the captured nucleic acids. This is depicted in Figure 16, which “shows a schematic illustration” of the “use of microarrays with immobilized DNA oligos (capture probes) carrying spatial labeling tag sequences (positional domains).” *Id.* Each position has “1) a unique labeling tag (positional domain) and 2) a capture sequence (capture domain).” *Id.*, 68; Figure 16 (depicting different ID (e.g., ID_n, ID_{n+1}, ID_{n+2}, ID_{n+3}) for each spot on the array).

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879



IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

113. I note that in a different case involving 10x Genomics (but not Petitioner or the Chee patents), 10x submitted a declaration from Dr. Paul T. Spellman which stated:

As early as 2011, Stahl *et al.* developed a pioneering spatially barcoded array paradigm for determining the locations of target mRNAs in a biological sample. The scientists disclosed this foundational technology in a patent application filed in 2011 (WO 2012/140224; EX1017 [EX1020 in this proceeding]) and subsequently published it in a 2016 article in Stahl *et al.*, “Visualization and analysis of gene expression in tissue selections [sic: sections] by spatial transcriptomics,” *Science* 353(6294):78-82 (July 1, 2016), and associated supplementary material (“Stahl 2016”; EX1016 [EX1019 in this proceeding]). This technology was also applied in the spatial detection products by Spatial Transcriptomics, Inc. (ST).

EX1034, ¶82.

114. I note that 10x’s characterization of the Stahl/Frisen patent publication as “pioneering” and “foundational” in proceedings not involving the Chee Patents is consistent with my analysis in Ground 3, which shows that the applications leading to the ’468 and ’879 Patents do not provide written description support for the issued claims of the ’468 and ’879 Patents.

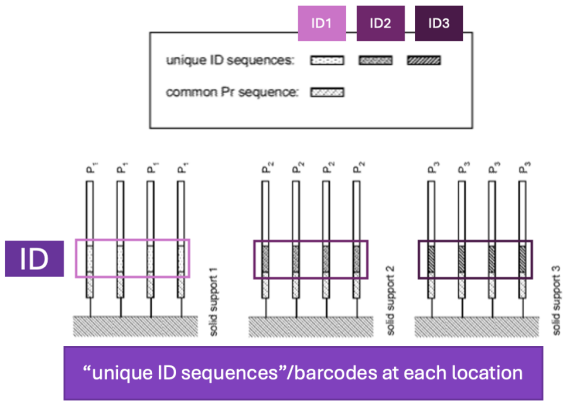
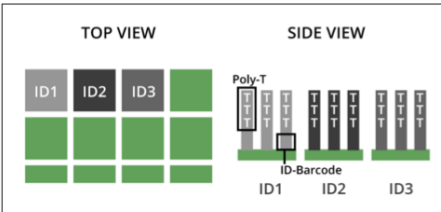
IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

115. Moreover, despite 10x's characterization of the Stahl/Frisen array designs as "pioneering" and "foundational" in other proceedings, it is also important to note that the design of the nucleic acid constructs attached to Cantor's solid supports—which include an identification sequence and a probe sequence—is essentially identical to the ST "array paradigm" described in Stahl. The chart below compares the barcoded arrays disclosed by Cantor in 2009 with those disclosed by Stahl/Frisen in 2011 and 2016.

Cantor 2009 (EX1031) Fig. 1C (Annotated)	“ST Array” (Spellman Declaration, EX1034), ¶¶84-85
 “The solid supports in the collection share a unique identifier sequence different than the identifier sequence of the solid phase nucleic acid of the other solid supports.” EX1031, 2. “A probe [P_N] nucleotide sequence is of a length sufficient to hybridize to a sample nucleic acid nucleotide sequence.” EX1031, 13.	 EX1018, 3. “As depicted above, each spot (identified by a different “ID”) on the solid surface contains a cluster of array probes (vertical rectangular bars in the ST diagram[.]) comprising a unique ‘spatial barcode’ (‘ID’) having a nucleic acid sequence specific to that spot. All the array probes in one spot on the array share the same spatial barcode sequence, which is different from the spatial barcode sequence in the array probes for the other spots.” EX1034, ¶85 (citations omitted). The “Ts” boxed and annotated by Dr. Spellman and captioned “Poly-T” represent poly-T probes that are designed to hybridize to the poly-A tails present on any given mRNA in a sample.

VII. Claim Construction

116. In my analysis throughout this declaration, I have applied the plain and ordinary meaning of the terms of the '468 and '879 Patents, consistent with the way

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

Patent Owner has read these terms in asserting infringement by Petitioner's products in co-pending district court litigation. I understand that, according to Patent Owner, no claim terms require construction.

A. “a first sequence that specifically binds” / “a first sequence that binds”

117. The claims of each patent require a nucleic acid that binds or specifically binds to a nucleic acid. I have reproduced claim 1 (the sole independent claim) of each patent below. I have highlighted in green language that is present in claim 1 of the '468 patent, but not in claim 1 of the '879 patent. I have emphasized and highlighted in yellow pertinent language in the '879 patent relating to the binds/specifically binds claim term.

'468 Patent, Claim 1	'879 Patent, Claim 1
A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising:	A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising:
(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:	(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:
(i) a first sequence that specifically binds the nucleic acid, and	(i) a first sequence that binds the nucleic acid, and

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;	(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;
(b) determining all or a portion of the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid <i>specifically bound</i> to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.	(b) determining the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid <i>specifically bound</i> to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.

118. While claim element 1(a)(i) of the '879 patent appears to be broader than the analogous claim element of the '468 patent, by reciting a capture agent with “a first sequence that binds,” rather than “a first sequence that ***specifically*** binds,” I note that element 1(b) of the '879 patent, like the analogous element of the '468 patent, requires “the nucleic acid specifically bound to the capture agent.” Thus, a POSA would understand that, by operation of element 1(b), the claims of the '879 patent still require the capture probe to “specifically bind” to the target nucleic acid. In any event, because a POSA would understand that a nucleic acid that “specifically binds” necessarily performs the broader function of “bind[ing],” the prior art’s disclosure of probes that “specifically bind” a target nucleic acid, as claimed, satisfy the requirements of both patents.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

119. I understand that in the litigation, the parties dispute whether a poly-T probe constitutes a nucleic acid sequence that “specifically binds” to a target DNA. I understand that Patent Owner/Plaintiffs contend that the binding interaction between poly-T probes and the poly-A tails present on mRNA constitutes “specific binding” in the context of the ’468 and ’879 Patents, which Petitioner disputes. Because the prior art I have identified in this declaration discloses both capture agents that specifically bind a biological molecule of interest, such as gene-specific probes, as well as probes that satisfy the broader implicit interpretation advanced by Patent Owner (such as poly-T probes), the claims are obvious and/or anticipated regardless of which interpretation is applied.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

VIII. Prior Art Anticipates and/or Renders Obvious the Challenged Claims of the '468 and '879 Patents

120. I have determined that the Challenged Claims⁹ are unpatentable on each of the grounds tabulated below:

Ground	Basis	Priority Date Applied
1	Obviousness over Cantor in view of the knowledge of a POSA	April 5, 2010
2	Obviousness over Cantor in view of Armani and the knowledge of a POSA	April 5, 2010
3	Anticipation by Frisen	February 14, 2019

121. The claims of the Challenged Patents recite substantially the same subject matter, with at most minor variations between the related claims from one patent to the other. For example, the sole independent claims of the Challenged Patents are compared side-by-side below. The only difference between the two claims is the presence of certain language in claim 1 of the '468 Patent, highlighted in green, which was omitted from claim 1 of the '879 patent.

⁹ Claims 1, 2, 6, 7, 12, 13, 18, 20, 21, 23, 26, 26, and 30 of the '468 Patent and Claims 1, 2, 5, 6, 11, 12, 17, 21, 23, 27, 28, and 30 of the '879 Patent.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent, Claim 1	'879 Patent, Claim 1
A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising:	A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising:
(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:	(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:
(i) a first sequence that specifically binds the nucleic acid, and	(i) a first sequence that binds the nucleic acid, and
(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;	(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;
(b) determining all or a portion of the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.	(b) determining the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.

122. The minor differences between the two claims are shown below as a “redline” comparison, highlighting the language that Patent Owner deleted from Claim 1 of the '468 Patent to yield Claim 1 of the '879 Patent.

1. → A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising:¶
 - (a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:¶
 - (i) a first sequence that **specifically** binds the nucleic acid, and¶
 - (ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array.¶
 - (b) determining **all or a portion of** the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.¶

123. Because the changes to independent claim 1 of the '879 Patent merely omit certain claim language that was present in independent claim 1 of the '468 Patent, the '879 Patent is rendered obvious and/or anticipated for at least the same reasons as claim 1 of the '468 Patent.

124. The challenged dependent claims also share substantial similarities, as depicted in the table below. The differences are limited to certain additional language present in some claims of the '468 Patent, which is highlighted in green.

'468 Patent, Claim 2	'879 Patent, Claim 2
The method of claim 1, wherein the method further comprises, between steps (a) and (b), a step of removing the tissue section from the array.	The method of claim 1, wherein the method further comprises, between steps (a) and (b), a step of removing the tissue section from the array.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent, Claim 6	'879 Patent, Claim 5
The method of claim 1, wherein the determining step comprises nucleic acid amplification.	The method of claim 1, wherein the determining step comprises nucleic acid amplification.
'468 Patent, Claim 7	'879 Patent, Claim 6
The method of claim 1, wherein the determining step comprises sequencing all or a portion of the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof.	The method of claim 1, wherein the determining step comprises sequencing the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid bound to the capture agent, or a complement thereof.
'468 Patent, Claim 12	'879 Patent, Claim 11
The method of claim 1, wherein the nucleic acid is RNA.	The method of claim 1, wherein the nucleic acid is RNA.
'468 Patent, Claim 13	'879 Patent, Claim 12
The method of claim 12, wherein the RNA is mRNA.	The method of claim 11, wherein the RNA is mRNA.
'468 Patent, Claim 18	'879 Patent, Claim 17
The method of claim 1, wherein the capture agent is immobilized directly or indirectly on a surface of the feature.	The method of claim 1, wherein the capture agent is immobilized directly on a surface of the feature.
'468 Patent, Claim 20	'879 Patent, Claim 21
The method of claim 1, wherein the array comprises a bead array.	The method of claim 1, wherein the array comprises a bead array.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent, Claim 21	'879 Patent, Claim 23
The method of claim 1, wherein the tissue section is a fresh frozen tissue section or a formalin-fixed paraffin-embedded (FFPE) tissue section.	The method of claim 1, wherein the tissue section is a fresh frozen tissue section.
'468 Patent, Claim 23	'879 Patent, Claim 27
The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.	The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.
'468 Patent, Claim 25	
The method of claim 1, wherein the array comprises greater than 500 features.	
'468 Patent, Claim 26	'879 Patent, Claim 28
The method of claim 1, wherein the array comprises greater than 1,000 features.	The method of claim 1, wherein the array comprises greater than 1,000 features.

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

'468 Patent, Claim 30	'879 Patent, Claim 30
The method of claim 1, wherein a feature of the plurality of features comprises greater than 100 capture agents, where each of the greater than 100 capture agents comprises a first sequence that specifically binds to a different nucleic acid and a coding tag that corresponds to a location of the capture agent on the array.	The method of claim 1, wherein a feature of the plurality of features comprises greater than 100 capture agents, where each of the greater than 100 capture agents comprises a first sequence that binds to a different nucleic acid and a coding tag that corresponds to a location of the capture agent on the array.

125. Given this substantial overlap between the two sets of challenged claims, I address these claims together in the Grounds that follow.

A. Ground 1: Obviousness over Cantor in view of Knowledge of a POSA.

1. Claim 1

126. The preambles of claim 1 of each Challenged Patent are identical and recite:

'468 Patent	A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising: [steps (a) and (b)].
'879 Patent	A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising: [steps (a) and (b)].

127. Cantor in view of the knowledge of a POSA renders obvious a “method for determining the presence or abundance of a nucleic acid at a location in a tissue

section” by conducting each of the enumerated steps. Thus, to the extent the preamble is limiting, Cantor in view of the knowledge of a POSA renders it obvious by rendering obvious a method including the enumerated steps, as discussed in detail below.

128. In short, Cantor discloses bead arrays comprising every structural element required by the claim, as depicted in Figure 1. Each bead (solid support) in Cantor’s arrays¹⁰ comprises nucleic acid capture agents comprising (1) a probe sequence (P) that specifically binds a target nucleic acid and (2) an identification sequence (ID) that is unique for each bead, and thus constitutes a coding tag that corresponds to the location of the capture agent on the array. Because each bead necessarily occupies a different spatial address on the array, each ID sequence and

¹⁰ Cantor’s beads constitute the claimed “features.” See ’468 Patent, cl. 17 (“The method of claim 1, **wherein the feature** of the plurality of features **comprises a bead**, well, or channel.”); ’879 Patent, cl. 16 (same). I note that in alleging infringement by Curio’s Seeker product, Patent Owner asserts that a bead necessarily constitutes a “feature.” See also, e.g., EX1065 at 3 (“The tile comprises an array (e.g., a monolayer of beads) comprising a plurality of **features (beads)**, wherein each bead comprises a capture agent....”).

its linked probe is necessarily correlated to a unique location (such as an (X,Y) coordinate) on the array.

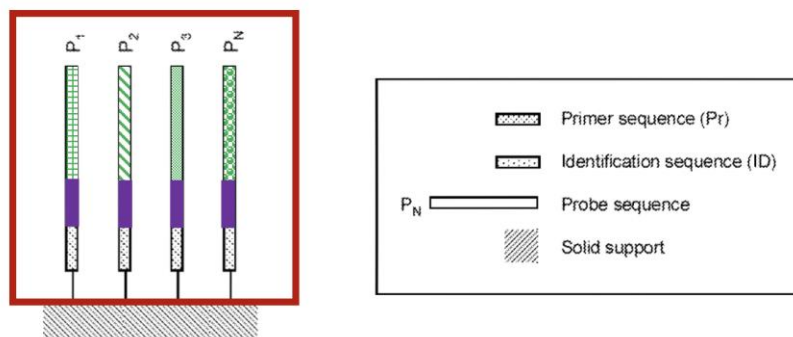
129. Cantor further discloses using such arrays to determine the presence or abundance of nucleic acids at a location in a tissue section. In these methods, target nucleic acids, such as nucleic acids in a tissue sample, are bound to the probe sequences, extended to generate complementary nucleic acids, amplified, and sequenced. EX1031, Fig. 2. Cantor discloses that the nucleic acids produced from its methods include (1) the sequence of the target nucleic acid (or its complement) and (2) the sequence of the ID sequence (or its complement), which corresponds to and thus identifies the bead at which the target nucleic acid was captured. Thus, Cantor's sequencing readouts identify the location at which the capture agents on each bead interacted with a nucleic acid in the tissue section. Moreover, as discussed above in Section V.E, the prior art literature reported many instances of the successful use of similar nucleic acid arrays to determine spatial patterns of nucleic acid expression. This knowledge would have further motivated the use of Cantor's identification sequences to correlate the location of an analyzed nucleic acid with its location in a tissue section, thereby determining the "presence or abundance of [the] nucleic acid at a location."

130. In detail, Cantor discloses “[i]mproved solid supports and methods for analyzing target nucleotide sequences.” EX1031, 1. Cantor expressly discloses that the biological sample analyzed in its methods may be “a solid material such as a tissue [or] cells.” *Id.*, 6.

131. A specific example of Cantor’s disclosure “provides a substrate comprising a collection of beads oriented in an array.” *Id.*, 4. Each bead “is in association with single-stranded nucleic acid.” *Id.* The bead-associated nucleic acids, which Cantor at times refers to as “the solid phase nucleic acid[s],” include “a probe sequence,” which “hybridizes to sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid.” *Id.* The bead-associated nucleic acids also include “an identifier sequence.” *Id.* Cantor discloses that in its bead arrays, “each of the solid supports,” i.e., beads, “in the collection share a unique identifier sequence different than the identifier sequence of solid phase nucleic acid of the other solid supports,” i.e., beads. *Id.*, 2. In other words, each bead is coded with a unique “identifier sequence” that differs from the “identifier sequence” coding each and every other bead.

132. The nucleic acids associated with Cantor’s beads constitute the claimed “**capture agents**” because they are nucleic acid constructs that include a “**probe sequence**” that “[**specifically**] **binds**” with a target nucleic acid, as well as a “**coding**

tag”—Cantor’s “**identification sequence**”—that identifies the specific bead, and thus location, at which a particular binding interaction occurred. Cantor’s Figure 1A shows an example of its solid support, such as a bead, with a plurality of “**capture agents**,” each having the same “**identification sequence (ID)**” and different “**probe sequences**.” I have annotated this figure to show the correspondence between Cantor’s Figure 1 and the requirements of the claimed “capture agents” below. The solid support, which may be a bead, is also shown.



133. Cantor explains that its “[s]olid supports may be provided in an array” including for example “an ‘X by Y array’ for square or rectangular arrays, where the array includes X number of sample locations or solid supports,” such as beads, “in one dimension and Y number of sample locations or solid supports,” such as beads, “in a perpendicular dimension.” EX1031, 17. Thus, a POSA would have understood Cantor to disclose an array of beads at which each bead is necessarily deposited at a particular (X, Y) coordinate location. As noted above, Cantor explains that each bead

IPR2025-00236

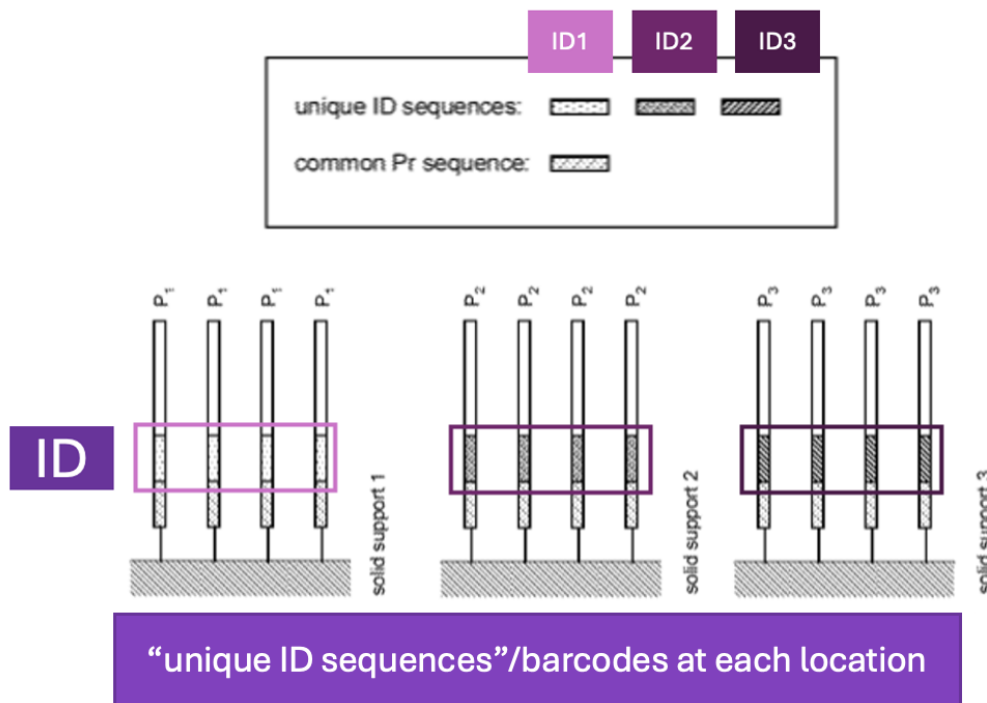
IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

has a unique identification sequence, such that each bead at its unique (X, Y) coordinate location would have a unique identification sequence as part of its capture agent. Such capture agents would also include a “probe” sequence as part of the same single-stranded nucleic acid. Thus, the “identification sequence” corresponds to the location at which the “probe” portion of the capture agent interacts with the tissue section.

134. For example, Cantor’s Figure 1C depicts separate solid supports, such as beads, each having “unique ID sequences” that are correlated to the specific solid supports “solid support [e.g., bead] 1,” “solid support [e.g., bead] 2,” and “solid support [e.g., bead] 3.”



135. Because each unique ID sequence corresponds to a particular bead and thus “barcodes” a given bead (and as a result its location in the array), the existing knowledge of a POSA would have further motivated the use of Cantor’s bead arrays to determine the presence or abundance of a nucleic acid at a location in a tissue section for a variety of reasons. See Sections V.C and V.E, above. For example, Southern, like Cantor, discloses arrays of target-binding nucleic acid probes and expressly instructs the use of such arrays to determine “spatial arrangements” of target nucleic acids. EX1033, Abstract. Southern discloses that the samples suitable for analysis using its methods include “samples containing different types of human cells” such as “tissue samples.” EX1033, 16:28-30. As another example, Armani-517, explains that “[i]n the biological sciences and in clinical medicine there is a need for preserving and mapping the spatial distribution of nucleic acids at the tissue and cellular levels.” EX1021, 1:14-15; *see also* EX1022, 3526 (“[T]o achieve a more complete view, DNA variability within a tissue sample needs to be correlated with the type of cell from which it originates (for example, stroma vs. epithelium), the physical location within the tissue section, and/or histopathological features (for example, a specific disease lesion).”). For example, “in understanding disease it is useful to know which genetic mutations or alterations have occurred and where these changes have occurred within the tissue microenvironment.” EX1021, 1:17-19.

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

Similarly, Okano explains that a POSA would have been motivated to analyze the spatial location of particular nucleic acids in tissue samples because “to more accurately describe biological functions, mRNA...localized between or inside cells must be quantitatively detected in a state in which local space information is retained,” and a POSA would therefore have been motivated to implement techniques that “quantitatively measure the mRNA and proteins localized between or inside cells without losing local space data.” EX1026, [0008]-[0009].

(a) **“contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:”**

136. Step 1(a) of each Challenged Patent is identical and recites:

'468 Patent	(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:
'879 Patent	(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:

137. Cantor discloses each aspect of step 1(a).

138. **First**, Cantor discloses and renders obvious “contacting a tissue section with an array comprising a plurality of features.”

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

139. Cantor discloses nucleic acid binding arrays in which the claimed capture agents are attached to beads, which constitute the claimed “features.” See ’468 Patent, cl. 17 (“The method of claim 1, **wherein the feature** of the plurality of features **comprises a bead**, well, or channel.”); ’879 Patent, cl. 16 (same). I note that in alleging infringement by Curio’s Seeker product, Patent Owner asserts that a bead necessarily constitutes a “feature.” See also, e.g., EX1065, 3 (“The tile comprises an array (e.g., a monolayer of beads) comprising a plurality of **features (beads)**, wherein each bead comprises a capture agent....”); EX1066, 3 (same).

140. Cantor describes its array of features (beads) as follows:

Also, the invention in part provides a **substrate comprising a collection of beads oriented in an array**, where: each bead is in association with single-stranded nucleic acid; **the solid phase nucleic acid comprises an identifier sequence and a probe sequence; the probe sequence hybridizes to sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid**; at least one nucleic acid of the solid phase nucleic acid of each of the solid supports in the collection has a unique probe sequence different than a probe sequence of the solid phase nucleic acid of the other solid supports; **and the solid phase nucleic acid of each of the solid supports in the collection share a unique identifier sequence different than the identifier sequence of solid phase nucleic acid of the other solid supports.**

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX1031, 4; *see also* EX1031, 1 (“Improved solid supports and methods for analyzing target nucleotide sequences are provided herein.”); *id.*, 2 (“In embodiments described herein, a solid support may be in a collection of solid supports, and the invention in part provides collections of solid supports and methods in which a collection of solid supports is contacted with sample nucleic acid. In some embodiments pertaining to solid support collections and methods of use, at least one nucleic acid of the solid phase nucleic acid of each of the solid supports in the collection has a unique probe sequence different than a probe sequence of the solid phase nucleic acid of the other solid supports; and the solid phase nucleic acid of each of the solid supports in the collection share a unique identifier sequence different than the identifier sequence of the solid phase nucleic acid of the other solid supports.”); *id.*, 10 (“Examples of solid supports for use with processes described herein include, without limitation, beads (e.g., microbeads, nanobeads) and particles (e.g., microparticles, nanoparticles).”).

141. Cantor in view of the knowledge of a POSA renders obvious “contacting the tissue section with an array.” Cantor suggests contacting its bead arrays with a tissue section. Specifically, Cantor explains that it discloses “methods in which a collection of solid supports is contacted with sample nucleic acid,”

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX1031, 2, and further explains that its sample may be “a solid material such as a tissue...or a biopsy.” *Id.*, 6.

142. A POSA would have immediately appreciated that a biopsy is a type of tissue section¹¹, and the literature evidences multiple reasons why a POSA would have been motivated to perform array-based nucleic acid analysis on a tissue sample (such as an intact tissue section or a biopsy sample), including because the analysis of such samples would provide information, such as spatial information, that could not be obtained through the analysis of, for example, a tissue homogenate:

Variations in DNA status (mutation, epigenetic modification, insertions, deletions) by location within a tissue section are of interest for elucidating their roles in biology and as clinical markers for diseases. However, to study the molecular basis of normal and pathological conditions, many researchers identify genes of interest from tissue specimens by examining cell homogenates (all the cells in the sample mixed together). Since tissue comprises a complex milieu of diverse cell populations that define the local microenvironments, combining the multiple cell populations into one measurement masks a

¹¹ See, e.g., '879 Patent at 12:9-11 (“Exemplary biological samples include **tissue sections (e.g., including** whole animal sectioning and **tissue biopsies)**, cell populations on slides or culture dishes, and the like.”).

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

tremendous amount of information. Therefore, to achieve a more complete view, DNA variability within a tissue sample needs to be correlated with the type of cell from which it originates (for example, stroma vs. epithelium), the physical location within the tissue section, and/or histopathological features (for example, a specific disease lesion).

EX1022 (Armani), 3526.

When handling cells, which constitute a large percentage of specimens, there are instances in which it is problematic to convert the cell component being measured to a solution state. Since balance of the whole is achieved through mutual interaction between a cell and adjacent cell groups in multi-cellular organisms, pulverizing cell groups will result in a uniform state, causing localized information for each cell to be lost. Further, even within individual cells, mRNA, which is an expressed gene, is localized. Proteins, which are the final product, are also localized within the cell in accordance with their function. These local mRNA and proteins change in accordance with a cell's state of activity, in other words the level of communication between a cell and the adjacent cell group, and the environment in which the cell is placed. Therefore, if an attempt is made to obtain information useful for medically-directed drug discovery, and to describe biological functions more accurately, the mRNA and proteins localized between or inside cells must be quantitatively detected in a state in which local space information is retained.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX1026 (Okano), [0008]. Thus, a POSA would have been motivated to contact Cantor's array with a tissue section, such that the capture agents affixed to its beads interact with nucleic acids in those samples.

143. Specifically, Cantor explains that its capture probes interact with the target nucleic acid by hybridization when contacted with the target nucleic acids. EX1031, 2. (“[T]he solid support and sample nucleic acid are contacted in the mixture under conditions that allow hybridization of the solid phase nucleic acid,” i.e., the probe, “to the sample nucleic acid.”). Thus, Cantor motivates contacting the tissue section with the array to allow for hybridization between the solid phase nucleic acid (capture probes) and the nucleic acids in a tissue sample.

144. Cantor further explains in its Example 1, “Sequence analysis methodology,” that “[s]ample nucleic acid may be prepared by any means commonly known in the art, including but not limited to cell lysis procedures using chemical, physical or electrolytic lysis methods.” *Id.*, 53-54. A POSA would have been familiar with, and would have been motivated to apply such techniques to intact tissue or biopsy samples such that the tissue or biopsy sample disposed on the array is then treated such that the capture probes can hybridize to the nucleic acids in the tissue sample.

145. Indeed, these techniques were frequently applied in the art. For example, Southern discloses the spatial analysis of nucleic acid molecules by contacting tissue-derived nucleic acid molecules with a capture agent affixed to a solid support in methods similar to those disclosed in Cantor. EX1033, 16:28-30; 7:3-7. Southern discloses that, “[i]n some methods, ***samples are applied directly to the support to generate a spatial arrangement of samples***, as illustrated in Fig. 1. Thus, ***when the samples are biological samples comprising cells, the step a) of applying the samples to a support may comprise: (i) applying cells to the support; then (ii) releasing material from the cells.***” *Id.*, 2:17-27. A POSA would recognize that “releasing material from the cells” is synonymous with lysing the cells, as disclosed by Cantor. A POSA would similarly have been familiar with methods for releasing and capturing locally target nucleic acid from cells in a tissue sample disposed on an array, including a bead array, from the background references discussed in Section V.E, above. *See* EX1021-1022 (Armani) (physical pressing and chemical lysis of tissue samples to release target nucleic acid); EX1024 (Shirai) (use of electrophoretic force to lyse cells in tissue sample to locally release target nucleic acid for capture on probes attached to beads); EX1025 (Yasuda) (same); EX1026 (Okano) (same). Thus, a POSA would have been further motivated to dispose the

tissue section on the array disclosed in Cantor and lyse the cells such that the probes can interact with the target nucleic acids within the tissue sample.

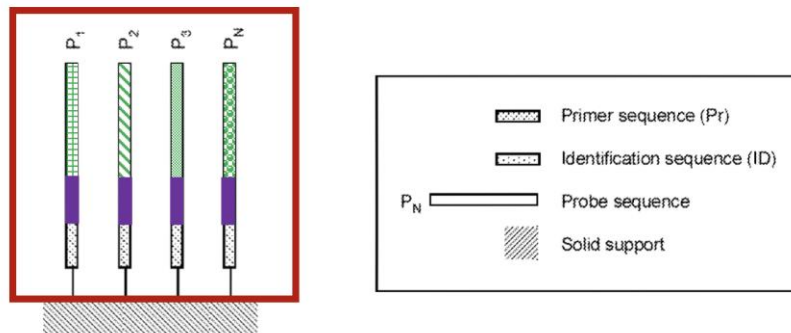
- (i) “[wherein the capture agent comprises:] (i) a first sequence that [specifically] binds the nucleic acid, and”

146. Step 1(a)(i) of each Challenged Patent recites:

'468 Patent	(i) a first sequence that specifically binds the nucleic acid, and
'879 Patent	(i) a first sequence that binds the nucleic acid, and:

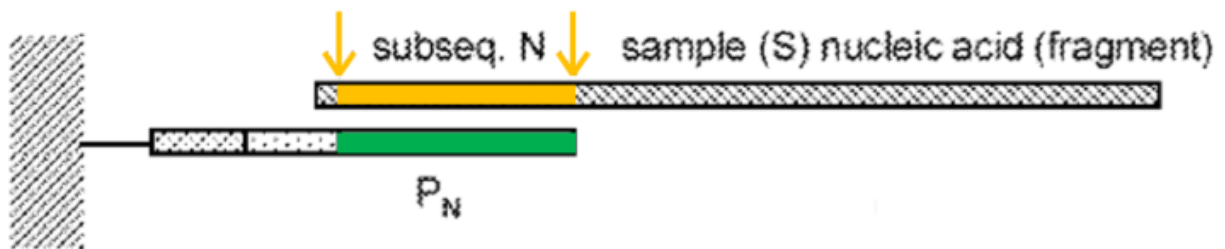
147. To the extent the '879 Patent's claim to a “sequence that binds the nucleic acid” is broader than the '468 Patent's claim to a “sequence that *specifically* binds the nucleic acid,” it is met by Cantor's disclosure of nucleic acids that “specifically bind” a target nucleic acid. *See also* '879, cl. 1(b) (requiring “the sequence of the nucleic acid *specifically* bound to the capture agent”); Section VII.A.

148. Cantor's “capture agents” are configured to “specifically bind” a nucleic acid at a location in a tissue section by hybridization between the target nucleic acid and Cantor's “probe sequences.”



149. For example, Cantor explains that “[s]ample nucleic acid molecule (S) may include a nucleotide sequence (i.e., subseq.N) that hybridizes to a complementary probe sequence (P_N) of the solid phase nucleic acid.” EX1031, 24.¹² This is depicted in Figure 2, excerpted below to show interaction between the “**probe sequence**” of the “**capture agent**” and a portion of a “**biological molecule**,” here a **subsequence (N)** of sample nucleic acid (S).

¹² Cantor discloses both arrays in which the probe sequence is the same at each position, and arrays in which the probe sequence is not the same at each position. See EX1031, Claim 31 (“the solid phase nucleic acid of each solid support share a common probe sequence.”); Claim 32 (“the solid phase nucleic acid of each solid support do not share a common probe sequence.”).



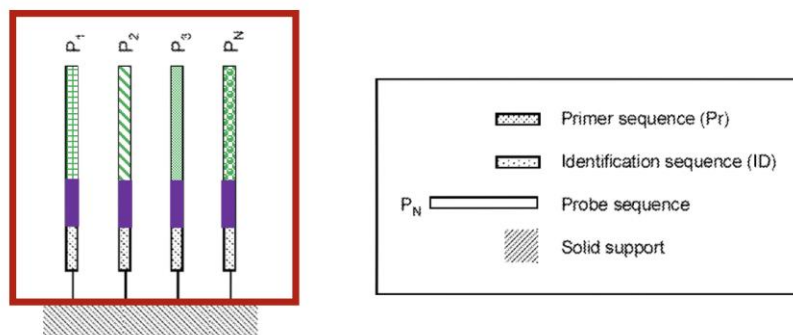
150. Cantor's "probe sequences" specifically bind to target nucleic acids in the tissue section because they are "complementary to subsequences in the sample nucleic acid (e.g., probe sequence species P_1 , P_2 , P_3 ,... P_n are complementary to sample nucleic acid subsequences 1, 2, 3,... N , respectively)." EX1031, 16-17. Cantor explains: "***A probe nucleotide sequence is of a length sufficient to specifically hybridize to a sample nucleic acid nucleotide sequence.*** In certain embodiments a probe sequence is about 5 to about 100 nucleotides in length, and sometimes is about 5 to about 40 nucleotides in length." *Id.*, 14; *see also id.* ("Generally, a shorter probe sequence is selected for applications where the target nucleotide sequence is known or partially known and longer probe sequence are selected for applications in which the target nucleotide sequence or portions thereof are not known. In some embodiments, a probe sequence is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000 or 5000 nucleotides in length.").

(ii) “[wherein the capture agent comprises:] (ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;”

151. Step 1(a)(ii) of each Challenged Patent is identical and recites:

'468 Patent	(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;
'879 Patent	(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;

152. I have reproduced below an annotated version of Figure 1A, which shows an example of Cantor’s **capture agents** affixed to a solid support. Cantor discloses that each “**capture agent**” includes an “**identification or identifier sequence**,” which constitutes the claimed “(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array.”

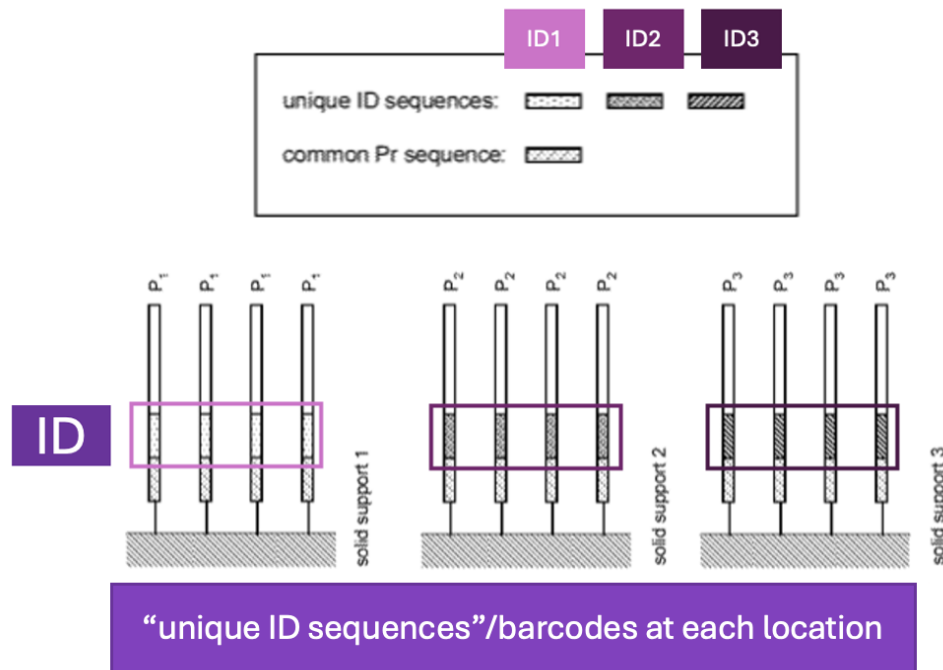


153. For example, Cantor states that “the solid phase nucleic acid of each of the solid supports [e.g., beads], in the collection [e.g., an array] share a unique identifier sequence different than the identifier sequence of the solid phase nucleic acid of the other solid supports [e.g., beads].” EX1031, 4. A POSA would have understood these disclosures to mean that when the solid supports are beads, each capture agent attached to a particular bead included the same “identification sequence,” while the capture agents attached to other beads would have a different “identification sequence.” These differing “identification sequences” would therefore “be useful in part for constructing partial sequence reads into larger sequence constructions,” for example to discern which bead a particular sequence read came from. *Id.*, 15.

154. Cantor explains that “one identification sequence species may differ from a second identification sequence species by one or more nucleotides when the first and second identification sequences are aligned with one another.” *Id.* These identification sequences “may be of any length suitable for analyzing the nucleotide sequence or partial nucleotide sequence of sample nucleic acid,” and “[i]n some embodiments, an identifier sequence is about 5 to about 50 contiguous nucleotides in length, sometimes about 5 to about 20 nucleotides in length and at times about 10 nucleotides in length.” *Id.*

155. Cantor's "identification sequences," which are the claimed "coding tags" also satisfy and render obvious the claim requirement that they "correspond[] to a location of the capture agent on the array." Cantor explains that its "[s]olid supports may be provided in an array" including for example "an 'X by Y array' for square or rectangular arrays, where the array includes X number of sample locations or solid supports," such as beads, "in one dimension and Y number of sample locations or solid supports," such as beads, "in a perpendicular dimension." EX1031, 17. Thus, a POSA would have understood Cantor to disclose an array of beads at which each bead is deposited at a particular (X, Y) coordinate location. Since Cantor's "identification sequence" is "unique" for each bead, each unique bead at a unique (X, Y) coordinate location necessarily possesses a unique identification sequence as part of its capture agent.

156. For example, Cantor's Figure 1C depicts separate solid supports, such as beads, each having "unique ID sequences" that are correlated to the specific solid supports "solid support [e.g., bead] 1," "solid support [e.g., bead] 2," and "solid support [e.g., bead] 3."



157. Moreover, as explained further in connection with step 1(b), Cantor in view of the knowledge of a POSA renders obvious using Cantor's identification sequence to determine the location at which the capture agent interacted with the biological molecule. Specifically, because Cantor's capture agents include a "probe" sequence as part of the same single-stranded nucleic acid as the "identification sequence," Cantor's "identification sequence" necessarily corresponds to the location at which the "probe" portion of the capture agent hybridizes with sample nucleic acid, such as a nucleic acid present in a tissue sample, and thus provides information about the location at which the tissue section interacted with the probe, and thus the location of the captured nucleic acid in the tissue sample. I incorporate

by reference that analysis, which further renders obvious the element of step 1(a)(ii) reciting “the coding tag corresponds to a location of the capture agent on the array.”

- (b) **“determining [all or a portion of] the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.”**

158. Step 1(b) of each Challenged Patent is depicted below:

'468 Patent	(b) [i]determining all or a portion of the sequence of the coding tag, or a complement thereof, [ii] and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, [iii] and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.
'879 Patent	(b) [i] determining the sequence of the coding tag, or a complement thereof, [ii] and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, [iii] and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.

159. Cantor renders obvious claim step 1(b). Specifically, Cantor’s Figure 2 and associated discussion describes how to detect target nucleic acids and determine the identity of both the target nucleic acid sequence and the identification sequence of the bead on which it was captured. As discussed further below, Cantor discloses:

IPR2025-00236

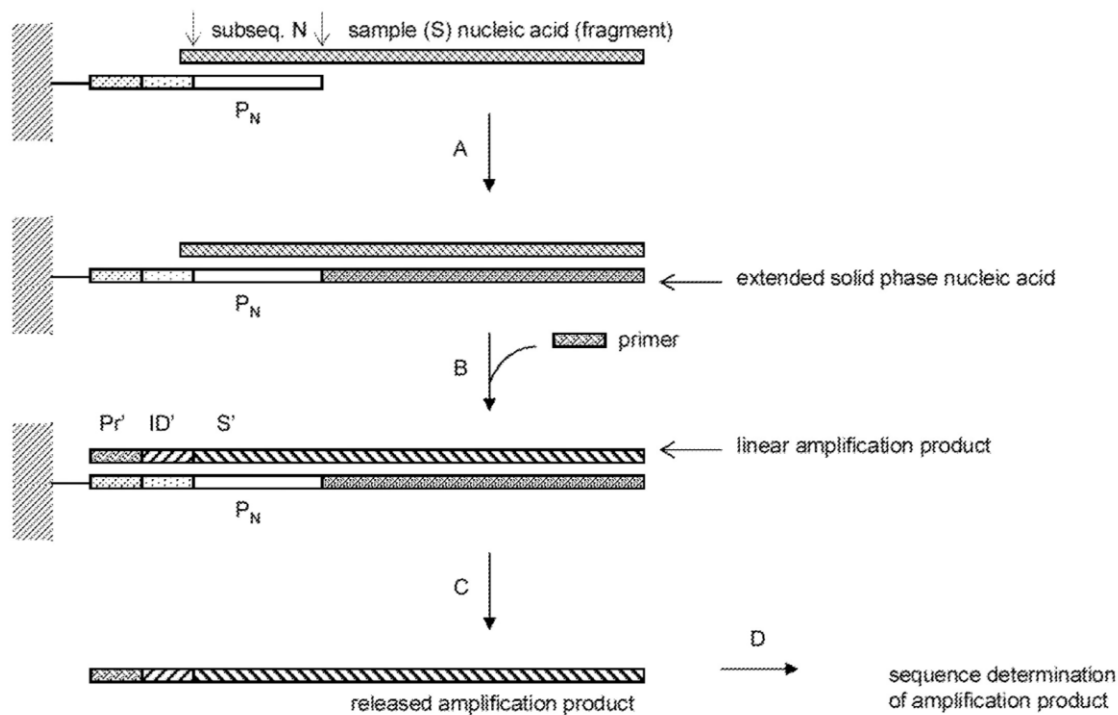
IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

- Each identification sequence corresponds to a unique bead at a unique X,Y position;
- Generating amplified libraries of captured target nucleic acids, which consist of (a) nucleic acids comprising the identification sequence and the complement of the target nucleic acid and (b) nucleic acids comprising the complement of the identification sequence and the target nucleic acid; and
- Sequencing these libraries of amplified nucleic acids.

160. Since Cantor discloses that each identification sequence corresponds to a unique bead at a unique (X,Y) position, when the identification sequence is determined as part of the sequenced amplification product, the presence of a particular identification sequence (or its complement) in a given sequence read identifies the location where the probe portion of the capture agent interacted with the captured sample nucleic acid, and hence the presence of the nucleic acid at a location in the tissue section.



(i) Steps 1(b)(i) and 1(b)(ii)

161. Cantor's extension and amplification workflow determines "[i] [all or a portion of] the sequence of the coding tag, or a complement thereof, [ii] and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof," as claimed.

162. In short, Cantor discloses "sequence determination of [an] amplification product" or its complement. EX1031, Figure 2C-D. Sequencing the "released amplification product" determines the sequence of a complement of the **coding tag (Cantor's identification sequence)** and the sequence of the **nucleic acid**

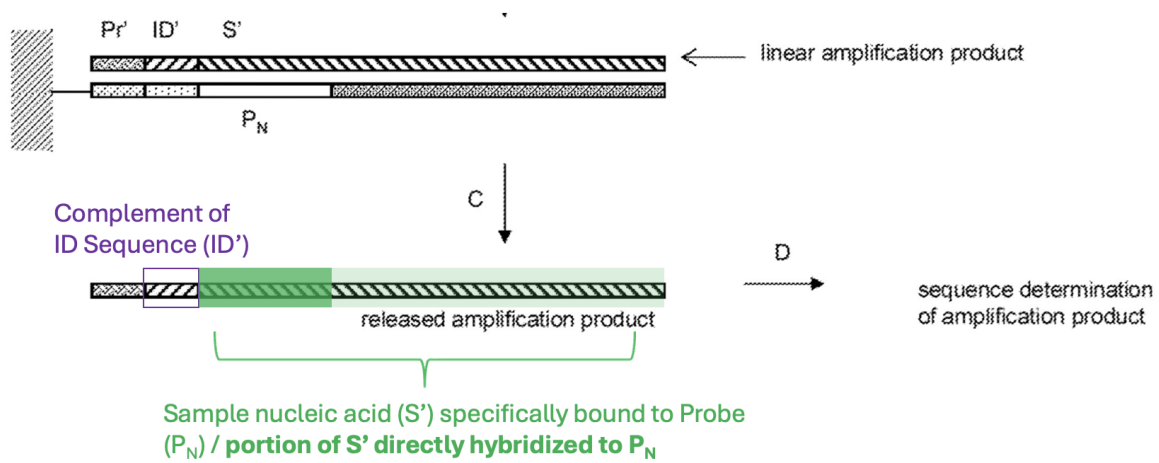
IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

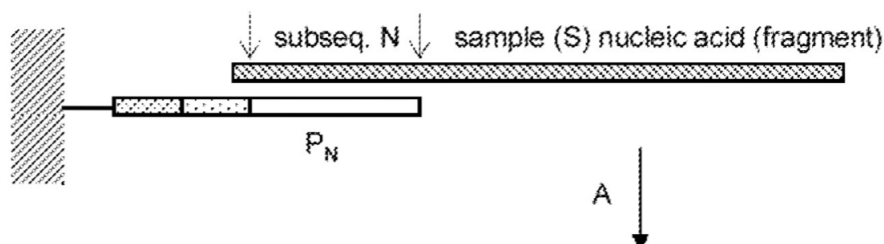
specifically bound to the capture agent, which includes “subseq. N” that is directly hybridized to Cantor’s probe sequence, as well as additional portions of “sample (S) nucleic acid fragment.” *Id.*



Cantor discloses determining “[t]he entire sequence or partial sequence of an amplification product,” called a “read,” and explains that “[a] read may be obtained with or without further amplification.” *Id.*, 25. Accordingly, Cantor discloses determining both the sequence of the strand including the original target nucleic acid molecule, as well as its complement. For example, sequencing the molecule complementary to that shown in Figure 2C-D (as annotated above) would determine the sequence of [i] the identification sequence, and [ii] the complement of the sample nucleic acid specifically bound to probe P_N.

163. In more detail, Cantor discloses methods for extending and amplifying captured target nucleic acids to generate amplified populations of nucleic acids, which contain the target nucleic acid (extended from the probe region which captured it) and the identification sequence, as well as their complements. These populations may then be analyzed by sequencing to determine the identity of the target sequence as well as the identification sequence (i.e., the bead barcode).

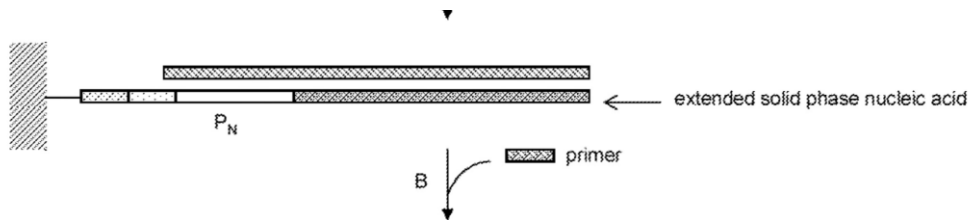
164. The first panel of Figure 2 shows the capture of a target nucleic acid from the biological sample by “specific binding” with the probe region of the capture probe, as discussed above.



165. The next panel shows the “extension” of the capture agent to generate a second strand complementary to the bound target nucleic acid. EX1031, 24 (“Solid phase nucleic acid hybridized to sample nucleic acid (S) can be extended (e.g., illustrated in Figure 2 as A) and “‘extended solid phase nucleic acid’ is generated.”). The nucleic acid depicted to the right of the probe sequence P_N in the figure below shows the extension of the capture agent to include “a nucleotide subsequence

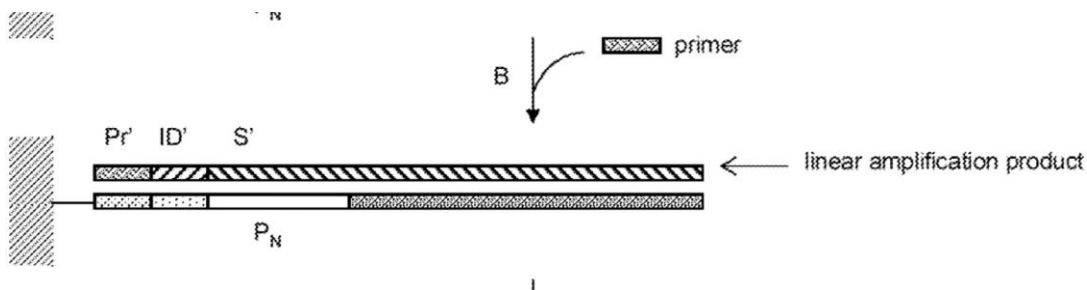
complementary to a target nucleotide sequence in the sample nucleic acid molecule.”

Id.

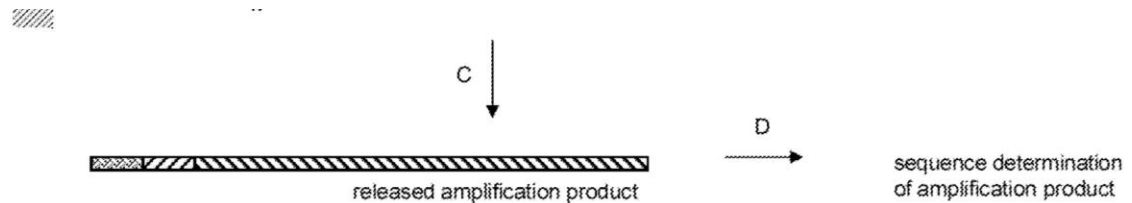


Next, “[e]xtended solid phase nucleic acid may be amplified by hybridizing primer (Pr’), which is complementary to primer sequence (Pr) of the extended solid phase nucleic acid, in the presence of amplification/extension reagents.” *Id.* “The hybridized primer [Pr’] is extended thereby generating an amplification product.”

Id. This amplification product is the top strand shown in the figure panel below, showing the complement to the identification sequence (ID’) added to the top strand via extension from Pr’. “The amplification product contains the primer nucleotide sequence (Pr’), a nucleotide sequence complementary to the identification sequence in solid phase nucleic acid, and a nucleotide sequence identical to or substantially identical to a target nucleotide sequence in the sample nucleic acid molecule.” *Id.*



166. Cantor further discloses that “[t]his linear amplification product can be released from the solid support for sequence analysis (e.g., illustrated in Figure 2 as C).” *Id.*, 24-25.



167. Following release, Cantor discloses that its “[a]mplification products...may be subject to sequence analysis,” that is, the use of sequencing “to determin[e] a nucleotide sequence of an amplification product.” *Id.*, 25. Cantor explains that “[t]he entire sequence or partial sequence of an amplification product can be determined, and the determined nucleotide sequence is referred to...as a ‘read.’” *Id.* “A read may be obtained with or without further amplification,” and accordingly, Cantor discloses determining both the sequence of the strand including the original target nucleic acid molecule, as well as its complement.

168. When the target nucleic acid is mRNA, a POSA would have found it obvious to implement this extension and amplification procedure using reverse transcription techniques. EX1031, 22 (“Conditions under which nucleic acids can be extended and/or amplified by [“extension agents” and “extension reagents”] are

known.”). Cantor explains that “extension agents may include...extension nucleotides, a polymerase and a primer that can hybridize to a primer sequence in solid phase nucleic acid,” such as “polymerases for thermostable amplification (e.g., RNA polymerase for transcription-mediated amplification (TMA),” as well as components “such as reverse transcriptase for TMA reactions, for example.” *Id.*, 22; *see also id.*, 23 (“Thermostable conditions can be maintained and the type and amount of amplification generally is dependent on the extension agents added to the mixture (e.g., primers, RNA polymerase and reverse transcriptase components for TMA (described above)).”).

169. Accordingly, Cantor discloses determining “[i] [all or a portion of] the sequence of the coding tag, or a complement thereof, [ii] and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof.” As discussed below, Cantor renders obvious using this information to determine presence or abundance of the nucleic acid at a location in the tissue section.

(ii) Step 1(b)(iii): “using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section”

170. As explained above, sequencing the released amplification product depicted in Figure 2C-2D determines the sequences of (i) a target nucleic acid and

its complement and (ii) an identification sequence and its complement. Because each identification sequence corresponds to a particular (X,Y) location of a bead in the array, the determined sequences necessarily determine the presence of the nucleic acid at a location (i.e., the location corresponding to the bead bearing the identification sequence). Additionally, the knowledge of a POSA further renders obvious the use of the determined sequences to determine the presence or abundance of a target nucleic acid at a particular location of a tissue section.

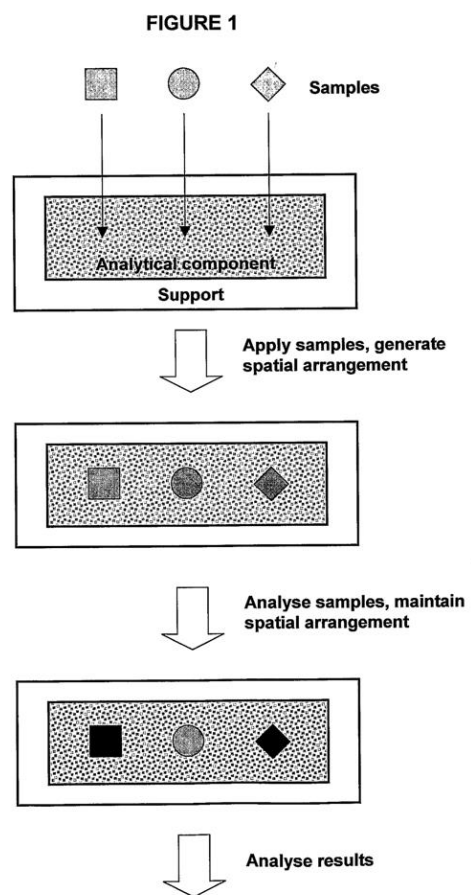
171. A POSA's motivation to correlate Cantor's bead "identification sequences" with the bead's location in array, and thus the location of the "specifically bound" nucleic acid in the tissue section, is confirmed by the knowledge of a POSA. As set forth above, multiple references disclosed the use of nucleic acid arrays, such as bead arrays, to (1) capture or hybridize nucleic acids from a tissue sample through the use of complementary probes; (2) amplify the captured nucleic acid; and (3) determine the identity of the captured nucleic acid and the location at which it interacted with the complementary probe, and thus its location in a tissue sample.

172. This is evidenced, for example, by Southern, which discloses methods of using nucleic acid probe arrays to perform spatial analysis of "samples containing different types of human cells" such as "tissue samples." EX1033, 16:28-30. The

arrays and extension, amplification, and detection procedures disclosed in Southern are analogous to those described in Cantor.

173. Southern explains that “[i]n the methods of the invention, samples are analysed by allowing them to interact with an analytical component on a support. “Target analytes,” such as nucleic acids, “in the samples are detected when the sample interacts with the analytical component.” EX1033, 1:19-21. As an overview:

The invention provides processes for analysing a plurality of different samples. The processes comprise the steps of: a) applying the samples to a support, to which an analytical component is immobilised; and b) allowing the samples to interact with the analytical component, thus permitting analysis of the samples. The samples are applied in step a) to different areas of the support to produce a spatial arrangement of samples on the support. The spatial arrangement of the samples is maintained in step b), thus permitting the results of the analysis to be matched to individual samples. This general approach is illustrated schematically in Fig. 1.



IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

EX1033, 1:22-29.

174. Like Cantor, Southern discloses the detection of target nucleic acid by creating a nucleic acid strand complementary to the target analyte. For example, when the target analyte is an mRNA, Southern discloses creating a complementary cDNA by extending the probe via in situ reverse transcription, using the captured mRNA as a template. Southern discloses using optical means, such as fluorescence, as the method for detecting the spatial location of an interaction between its capture agent and a target nucleic acid:

For RNA analytes, detection can be achieved by incorporating fluorescent nucleotides into a complementary strand using an enzyme such as reverse transcriptase (e.g. the avian myeloblastosis virus (AMV) reverse transcriptase). ***For example, cDNA may be made in situ by hybridising mRNA to oligonucleotide probes on a support, and using the immobilised probe as a primer. The reverse transcription reaction preferably incorporates labelled nucleotides into the cDNA in order to facilitate detection of the hybridisation*** [10]. This can be achieved by the use of dNTPs with suitable fluorophores attached. Unlike a sequencing reaction, it is not necessary to use different coloured fluorophores for different nucleotides, because individual nucleotides do not need to be distinguished. Similarly, there is no need to label every nucleotide, and so 1, 2, 3 or 4 of dATP, dCTP, dGTP and dTTP may be labelled, and a mixture of labelled and unlabelled dNTPs

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

can be used. Incorporation of a large number of fluorophores into the cDNA (e.g. in at least 5% of incorporated dNTPs, such as $\geq 10\%$, $> 20\%$, $\geq 30\%$, $\geq 40\%$, $\geq 50\%$, $\geq 75\%$, or more) means that ***the cDNA can readily be detected by any of the familiar means of fluorescence detection, thus revealing a positive signal even for a single hybridisation event. Thus even low-abundance mRNAs can be detected.***

EX1033, 27:12-28. Southern continues by noting that “[a]fter in situ reverse transcription has been performed, there is initially a RNA/DNA hybrid, wherein the DNA will typically include a label for detection.” *Id.*, 28:8-9. Additionally, Southern observes that detection can include an “amplification reaction,” *Id.*, 28:19-22, and that “[a]mplification in situ of captured target analytes should enable a wide range of useful applications” including “single molecule sequencing.” *Id.*, 39:25-28.

175. Other references within the knowledge of a POSA similarly use and disclose the use of devices and assays similar to Cantor (i.e., array-based capture and amplification of target nucleic acid) to successfully capture nucleic acids, including mRNA, from tissue samples on bead arrays to preserve and determine the spatial location of the captured target nucleic acids in said tissue samples. *See* Section V.E.3, above; EX1024 (Shirai), Abstract (“provid[ing] a method comprising preparing a cDNA library from mRNA while keeping two-dimensional cellular distribution information and obtaining the gene expression levels at any site or all

sites at a level of single cell.”); EX1026 (Okano), [0072] (“[A] tissue-like specimen of aggregated cells, for example, a histological section is immobilized to a two-dimensional surface while position information of an intracellular substance is retained.”). Thus, multiple contemporaneous references confirm why a POSA would have been motivated to correlate Cantor’s bead “identification sequences” with the bead’s location in array, and thus the location of the “specifically bound” nucleic acid in the tissue section.

(iii) A POSA’s knowledge would have further confirmed a POSA’s motivation and reasonable expectation of success in using Cantor’s identification sequences to determine the presence or abundance of a nucleic acid at a location of a tissue section.

176. A POSA’s existing knowledge before the priority date further confirms the motivation and reasonable expectation of success one would have had in using the “identification sequence” present in Cantor’s “capture agents” to determine the location at which the probe of a given capture agent interacted with a target nucleic acid from a tissue sample, and thus the location of the specifically bound nucleic acid in the tissue section.

177. A POSA would have appreciated that Cantor’s disclosure of a unique identification sequence for each bead constituted a unique “barcode” nucleic acid

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

sequence to distinguish one bead from another. *See* Section V.D.3. In addition to Cantor's express disclosures of providing the beads according to an (X, Y) array, a POSA would have found it obvious to determine the identity and location of each bead in the array before using it in Cantor's analytical procedures at least by (i) selective loading of particular beads to particular locations on the array and/or (ii) decoding the array. *See* Section V.D.2, ¶¶65-71.

178. Cantor discloses that its "identifier sequence is about 5[-]50 nucleotides in length." EX1031, 15. In a selective loading procedure, a POSA would have found it obvious to provide beads having, for example, 5 nucleotide identifier sequences that correspond to particular locations in Cantor's (X, Y) array. A POSA would have reasonably expected success in correlating such sequences with particular (X, Y) coordinates given the disclosure of success in using such systems in the prior work of the named inventor and others. *See* EX1032 (Chee-584), 7:30-33 (beads are spatially arranged in "a regular pattern of sites such that the sites may be addressed in the X-Y coordinate plane."); EX1038 (Chee-824), [0044] ("Once the identity of the bioactive agent and its location in the array has been fixed, the array is exposed to samples containing target analytes."); EX1036, 251 (Barany 1999) (describing 4 nucleotide "zip-code" sequences to define spatial locations on an array in a "checkerboard" (X, Y) array pattern).

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

179. For example, the below illustration shows an example of a 16 bead array with unique 5-nucleotide identifier sequences at each (X,Y) location. X coordinates are numbered 1-4, and Y coordinates are lettered A-D. While the illustration below uses 16 total positions, the use of 5 nucleotide barcodes allows uniquely addressing 1,024 unique positions, and each additional nucleotide added to a barcode would have been known to exponentially increase the number of unique available barcodes.

	1	2	3	4
A	AGTGA	TGTGC	TCAAA	AGCTA
B	GTACG	TATCC	GCTTG	CTCGA
C	CCTTC	TTCGT	CTACC	TGGGC
D	TGAAT	CCTTA	CATTG	GATTA

180. For example, in a selective loading technique, to correlate position A1 with the identification sequence AGTGA, a POSA would have found it obvious to deposit a bead (or beads) that was (or were) manufactured to include said sequence according to known methods at the A1 coordinate in the array. *See* EX1038, [0043] (describing “positional decoding” by “targeting the placement of the beads” to predetermined locations on the array). A POSA would have found it obvious to

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

target the placement of the other barcoded beads to the other locations according to similar methods.

181. Alternatively, “the beads may be randomly distributed on the array,” and “can later be ‘decoded’, i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or candidate agent at that particular site can be made.” EX1038, [0042]. A POSA would have been motivated to use such a random loading procedure to simplify and expedite the array generation and fabrication process, and would have found it obvious to decode the array by using any of a number of known decoding procedures. For example, a POSA would have found it obvious to use the decoding by sequencing procedure disclosed in Chee-824, which allows simultaneous decoding of many bead types in a single experiment. *Id.*, [0046]. For example, Chee-824 discloses “decoding by sequencing” of bead arrays comprising probes structured similarly to those used in Cantor’s bead array, comprising an encoding sequence used to identify the particular bead as well as a gene-specific sequence that may be used to assay target nucleic acids. *Id.*, [0037]-[0038], Figs. 12-13. Moreover, the procedures disclosed in Chee-824 allow the array to be used in analytical assays without issue following the decoding procedure. *Id.*, [0146].

182. Thus, assuming that barcoded beads were randomly deposited on a substrate and landed in the positions illustrated in the table above, decoding by sequencing, such as the methods disclosed in Chee-824, would have been an obvious method for obtaining knowledge of the identification sequence at each location before use in subsequent assays. That is, using such methods, a POSA would have been motivated to decode the array to learn that position A1 had the identification sequence AGTGA, position B1 had the identification sequence GTACG, and so on. A POSA would have reasonably expected success in performing such decoding, as doing so would have involved the mere application of well-known techniques, including those described in Chee-824.

183. When the “identification sequence” of a particular bead location is produced as a sequencing readout, a necessary result of Cantor’s amplification and extension procedure, a POSA would then have found it obvious to map the rest of the sequence readout (i.e., the sequence of the target nucleic acid or a complement thereof) to the bead location, thereby determining a spatial location of the biological molecule of the tissue sample. For example, in the example above, a nucleic acid readout from Cantor’s array and methods that included [Target Sequence]-CCTTA would indicate that the nucleic acid that was identified from the tissue sample interacted with the capture agent, and was thus spatially located, at position D2.

184. A POSA would have reasonably expected success in correlating Cantor's "identification sequences" with the location of the beads to which they are bound on an array, at least based on the use of known loading and/or decoding procedures. Indeed, making this correlation would not have required any modifications to Cantor's device, or the process in which it is used to capture, amplify, extend and sequence target nucleic acid sequences.

185. Instead, correlating a particular sequence to a particular location involves (at most) only the mental (or computer implementable) step of associating a particular "identification sequence" with a "particular bead"—features already present in Cantor's device and methods—based on the routine and predictable use of well-known array decoding techniques. And in light of the knowledge in the art motivating a POSA to determine the spatial location and orientation of nucleic acids throughout a tissue sample, it also would have been obvious to document or record the presence of a particular nucleic acid at a particular location in a tissue sample—information learned from sequencing the identification sequence—by mapping it back to the identification sequence's decoded coordinate in the array.

186. Such mapping is illustrated by Armani, which explains that its spatial analysis technique included "the creation of a 2D detection map, showing the

presence/absence of target DNA, on the millimeter scale,” that is, mapping whether target DNA was present or absent at a particular location in the X-Y plane. *Id.*, 3527.

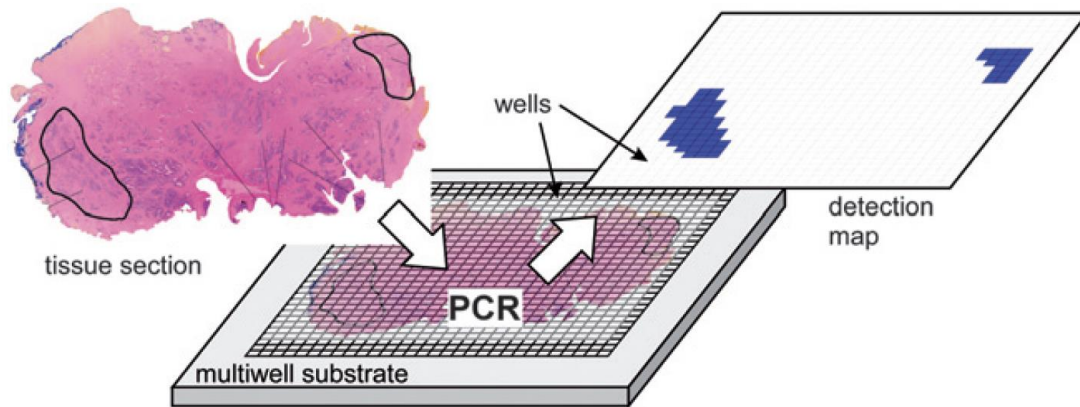


Fig. 1 2D-PCR is achieved by transferring a tissue section vertically into a multi-well array device, isolating tissue subregions. The DNA is extracted and then amplified by the polymerase chain reaction. A detection map is created by imaging the amplified DNA, for example using a DNA-intercalating dye.

The presence of a target nucleic acid at particular locations in the tissue sample is mapped in Armani’s Figure 1, with the blue squares indicating the presence of the target nucleic acid at a particular location. Performing this mapping based on sequencing a particular identification sequence, which correlates to a known location identified through well-known decoding procedures, would have involved routine decoding procedures, and a manual or computer-implemented process for indicating the presence of nucleic acid at a particular location (e.g., through a check mark, or

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

by shading in a box corresponding to the array's grid structure). *See also* Section V.E.3(b) (explaining obviousness of substituting imaging-based readouts with sequencing-based readouts).

(c) Conclusion on Claim 1

187. Accordingly, the combination of Cantor in view of the knowledge of a POSA renders claim 1 obvious.

2. Claim 2

188. Claim 2 of each Challenged Patent is identical, as tabulated below.

'468 Patent	The method of claim 1, wherein the method further comprises, between steps (a) and (b), a step of removing the tissue section from the array.
'879 Patent	The method of claim 1, wherein the method further comprises, between steps (a) and (b), a step of removing the tissue section from the array.

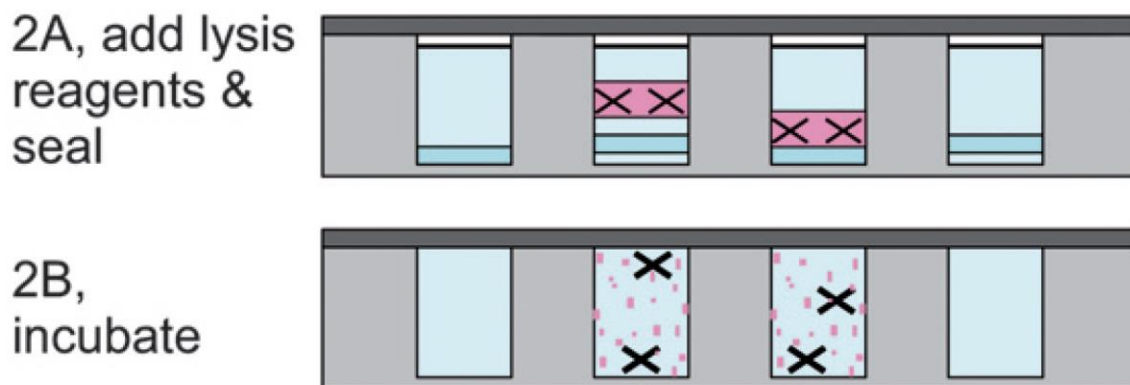
189. I incorporate by reference my analysis of claim 1.

190. Between steps (a) (“contacting...”) and (b) (“determining...”), Cantor renders obvious “a step of removing the tissue section from the array.” Specifically, Cantor discloses that “[s]ample nucleic acid may be prepared by any means commonly known in the art, including but not limited to cell lysis procedures using chemical, physical or electrolytic lysis methods.” EX1031, 54. A POSA would have understood that lysis procedures are techniques that remove a tissue section, because

by lysing open the cells in the tissue section and releasing their contents, the tissue section is destroyed. *See, e.g.*, EX1022 (Armani), 3257 (“the tissue is lysed to release genomic DNA into solution.”).

191. A POSA would have recognized that after the tissue section is contacted with Cantor’s array, as described in Section VIII.A.1.(a) above, the tissue section would need to be lysed so that the capture agents can access the target nucleic acids within the tissue section and then target nucleic acid/capture agent complexes can be extended, amplified, and sequenced as disclosed by Cantor.

192. Armani discloses this very technique in its Figure 2, below. EX1022, 3247 (Fig. 2A-2B (showing removal of tissue section (pink rectangle) following addition of lysis reagents).

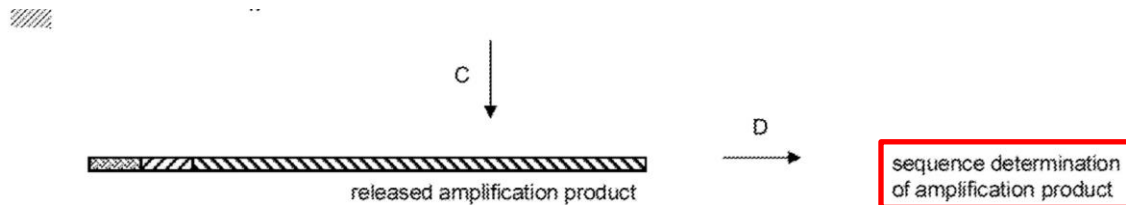


3. Claim 6 ('468 Patent) / Claim 5 ('879 Patent)

193. Claim 6 of the '468 Patent is identical to Claim 5 of the '879 Patent, as tabulated below.

'468 Patent	6. The method of claim 1, wherein the determining step comprises nucleic acid amplification.
'879 Patent	5. The method of claim 1, wherein the determining step comprises nucleic acid amplification.

194. I incorporate by reference my analysis of claim 1, above. As explained in detail with respect to step 1(b), Cantor discloses that “the determining step” comprises nucleic acid amplification, leading to the “sequence determination of [the] **amplification** product,” as excerpted below from Fig. 2C-2D.

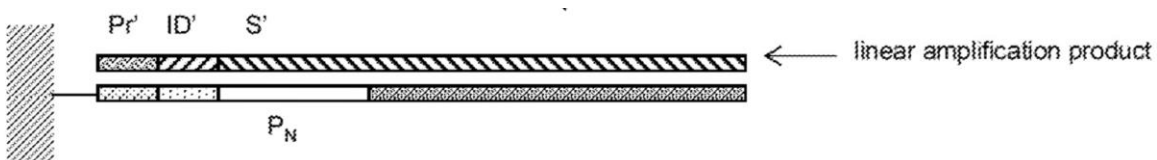


4. Claim 7 ('468 Patent) / Claim 6 ('879 Patent)

195. Claim 7 of the '468 Patent is substantially the same as Claim 6 of the '879 Patent, as tabulated below. The additional language present in claim 6 of the '468 Patent is highlighted in green.

'468 Patent	7. The method of claim 1, wherein the determining step comprises sequencing all or a portion of the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof.
'879 Patent	6. The method of claim 1, wherein the determining step comprises sequencing the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid bound to the capture agent, or a complement thereof.

196. I incorporate by reference my analysis of claim 1, above. Cantor's determining step comprises sequencing the entirety of the "released amplification product," (depicted as the "linear amplification product" in the excerpt of Figure 2 below) as well as sequencing the entirety of the complement of the "released amplification product," (depicted as the bottom strand in the excerpt of Figure 2 below) as discussed with respect to step 1(b), above.



197. Accordingly, Cantor's sequencing in the claimed "determining step" involves sequencing all of the coding tag (i.e., Cantor's identification sequence or its complement), and at least the portion of the target nucleic acid (i.e., at least the portion that was directly hybridized to the capture agent's probe sequence).

5. Claims 12-13 ('468 Patent) / Claims 11-12 ('879 Patent)

198. Claims 12-13 of the '468 Patent are identical to Claims 11-12 of the '879 Patent, as tabulated below.

'468 Patent	12. The method of claim 1, wherein the nucleic acid is RNA. 13. The method of claim 12, wherein the RNA is mRNA.
'879 Patent	11. The method of claim 1, wherein the nucleic acid is RNA. 12. The method of claim 11, wherein the RNA is mRNA.

199. I incorporate by reference my analysis of claim 1, above. Cantor discloses that “[s]ample nucleic acid may comprise or consist essentially of any type of nucleic acid suitable for use with process of the invention, such as sample nucleic acid that can hybridize to solid phase nucleic acid.” EX1031, 6. “A sample nucleic acid in certain embodiments can comprise or consist essentially of...RNA (e.g., **message RNA (mRNA)**, short inhibitory RNA (siRNA), ribosomal RNA (rRNA), tRNA and the like, and/or DNA or RNA analogs....” *Id.*

6. Claim 18 ('468 Patent) / Claim 17 ('879 Patent)

200. Claim 18 of the '468 Patent is substantially the same as claim 17 of the '879 Patent, as tabulated below. The additional language present in claim 18 of the '468 Patent is highlighted in green. Because “wherein the capture agent is

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

immobilized directly” is rendered obvious by the applied prior art, both claims are rendered obvious for the same reasons.

'468 Patent	18. The method of claim 1, wherein the capture agent is immobilized directly or indirectly on a surface of the feature.
'879 Patent	17. The method of claim 1, wherein the capture agent is immobilized directly on a surface of the feature.

201. I incorporate by reference my analysis of claim 1, above. For example, Cantor explains that its capture agents may be affixed to beads by covalent bonding. EX1031, 11-12 (“Solid phase nucleic acid may be in association with a solid support by a covalent linkage....”). Cantor further explains that “[n]ucleic acid may be linked to a solid support without a linker or with a linker [citing references].” *Id.*, 12. A POSA would consider attachment without a linker to be direct immobilization and attachment with a linker to be indirect immobilization. Therefore, Cantor discloses both direct and indirect mobilization of the capture agent onto the surface of a bead of the plurality of beads.

7. Claim 20 ('468 Patent) / Claim 21 ('879 Patent)

202. Claim 20 of the '468 Patent is identical to Claim 21 of the '879 Patent, as tabulated below.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent	20. The method of claim 1, wherein the array comprises a bead array.
'879 Patent	21. The method of claim 1, wherein the array comprises a bead array.

203. I incorporate by reference my analysis of claim 1, above. Cantor discloses bead arrays. *See, e.g.*, EX1031, 4 (“Also, the invention in part provides a substrate comprising a collection of beads oriented in an array....”).

8. Claim 21 ('468 Patent) / Claim 23 ('879 Patent)

204. Claim 21 of the '468 Patent is substantially the same as Claim 23 of the '879 Patent, as tabulated below. The additional language present in claim 21 of the '468 Patent is highlighted in green. Because the use of “a fresh frozen tissue section” is rendered obvious by the applied prior art, both claims are rendered obvious for the same reasons.

'468 Patent	21. The method of claim 1, wherein the tissue section is a fresh frozen tissue section or a formalin-fixed paraffin-embedded (FFPE) tissue section.
'879 Patent	23. The method of claim 1, wherein the tissue section is a fresh frozen tissue section.

205. I incorporate by reference my analysis of claim 1, above.

206. As mentioned previously, Cantor explains that its target/sample nucleic acid may be “a solid material *such as a tissue...or a biopsy.*” *Id.*, 6. Furthermore,

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

Cantor explains in its Example 1, “Sequence analysis methodology,” that the “[s]ample nucleic acid may be prepared by any means commonly known in the art.” *Id.*, 53-54. As described in Section V.B, there were only a handful of methods of preparing a tissue sample known in the art prior to 2010, including freezing a fresh tissue section to create a fresh frozen tissue sample and creating an FFPE tissue sample. Given the limited number of options, it would have been obvious to a POSA to try using a fresh-frozen tissue sample or FFPE tissue sample with the array disclosed in Cantor.

207. Moreover, a POSA would have been motivated to use a fresh-frozen tissue sample with the array disclosed in Cantor. In 2010, fresh-freezing tissue samples was a well-known and common method for preserving tissue samples in a laboratory and clinical setting. Given the prominence of fresh-frozen tissue samples in the field, a POSA would have been motivated use a fresh frozen tissue sample with Cantor’s array, including because of the convenience of using such samples. Additionally, because fresh frozen tissue samples were known to have high RNA/nucleic acid quality, including as compared to formalin-fixed paraffin embedded tissues, a POSA would have been motivated to use fresh-frozen samples in implementations of Cantor’s methods intended to analyze RNA/nucleic acids.

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

9. Claim 23 ('468 Patent) / Claim 27 ('879 Patent)

208. Claim 20 of the '468 Patent is identical to Claim 21 of the '879 Patent, as tabulated below.

'468 Patent	23. The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.
'879 Patent	27. The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.

209. I incorporate by reference my analysis of Claim 1, in particular claim 1(b). As explained in Section VIII.A.1(b)(iii) with respect to claim step 1(b)(iii), generating a map of the presence or abundance of the nucleic acid at a location in the tissue section is an obvious way of documenting the claimed mental step of “using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.”

10. Claims 25-26 ('468 Patent) / Claim 28 ('879 Patent)

210. Claim 26 of the '468 Patent is identical to Claim 28 of the '879 Patent, as tabulated below. Claim 25 of the '468 Patent is broader, and is rendered obvious for the same reasons.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent	25. The method of claim 1, wherein the array comprises greater than 500 features. 26. The method of claim 1, wherein the array comprises 30 greater than 1,000 features.
'879 Patent	28. The method of claim 1, wherein the array comprises greater than 1,000 features.

211. I incorporate by reference my analysis of claim 1, above. Cantor expressly discloses up to at least 10,000 unique beads or solid supports. EX1031, 11 (“In certain embodiments, a solid support collection comprises 2 to 10,000 solid support species, 10 to 1,000 solid support species or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 or 10000 unique solid support species.”). *See also* EX1032 (Chee-584) at 6:6-13 (“Arrays containing from about 2 different bioactive agents (i.e. different beads) to many millions can be made, with very large fiber optic arrays being possible. Generally, the array will comprise from two to as many as a billion or more, depending on the size of the beads and the substrate, as well as the end use of the array. Thus very high density, high density, moderate density, low density and very low density arrays may be made.”); EX1038 (Chee-824), [0047] (“Arrays containing from about 2 different bioactive agents (i.e. different beads) to many millions can be

made....”). As noted above, Cantor’s identification sequences are disclosed to comprise from 5-50 nucleotides in length. Even when the identifications sequences are 5 nucleotides in length, such a configuration allows the assignment of 1,024 (4⁵) unique identification sequences, confirming a POSA’s motivation and expectation of success in using Cantor’s methods to create arrays with 1,000 or more beads.

11. Claim 30 (’468 Patent) / Claim 30 (’879 Patent)

212. Claim 30 of the ’468 Patent is substantially the same as Claim 30 of the ’879 Patent, as tabulated below. The additional language present in claim 30 of the ’468 Patent is highlighted in green.

’468 Patent	30. The method of claim 1, wherein a feature of the plurality of features comprises greater than 100 capture agents, where each of the greater than 100 capture agents comprises a first sequence that specifically binds to a different nucleic acid and a coding tag that corresponds to a location of the capture agent on the array.
’879 Patent	30. The method of claim 1, wherein a feature of the plurality of features comprises greater than 100 capture agents, where each of the greater than 100 capture agents comprises a first sequence that binds to a different nucleic acid and a coding tag that corresponds to a location of the capture agent on the array.

213. I incorporate by reference my analysis of claim 1, above. Cantor discloses each of the plurality of beads (*i.e.*, features) containing at least 100 capture agents (*i.e.*, nucleic acid molecules). For example, Cantor discloses that the “solid

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

phase nucleic acid density per solid support unit [e.g., capture probes per bead] is about 5 nucleic acids to about 10,000 nucleic acids per solid support. The density of the solid phase nucleic acid per unit solid support in some embodiments is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 or 10000 nucleic acids per solid support.” EX1031, 13. As explained above, each capture agent comprises a first sequence that specifically binds to a different nucleic acid (Cantor’s probe sequence) and a coding tag (Cantor’s identification sequence) that corresponds to a location of the capture agent on the array.

214. With respect to the probe sequences, Cantor expressly discloses that a given bead (i.e., one of the claimed features) may contain single stranded nucleic acids with the same bead identifier sequence, but different probe sequences. *See* EX1031, 14 (“In certain embodiments, one solid support comprises about 10 to about 10,000 unique probe sequences (e.g., about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or about 10,000 different probe sequence species); one solid support comprises about 10 to about 1,000 unique probe sequences (e.g., about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or about 1,000 different probe sequence species).”); *see also id.*, cls. 32-35. When each probe

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

sequence on a given bead is different, each necessarily binds to a different nucleic acid, as claimed.

215. In addition, I understand that in the District Court litigation, Patent Owner contends that the limitation “each of the greater than 100 capture agents comprises a first sequence that binds to a different nucleic acid” is satisfied by multiple poly-T tails on the same bead that bind polyA tails on mRNA. To the that extent this argument can be understood, it appears that Patent Owner is interpreting the claimed “first sequence that binds to a different nucleic acid” to be satisfied by a sequence on the capture probe that binds to any molecule present in a sample, as long as another capture probe is not already bound to that molecule, rather than requiring the claimed capture probes to each have a first sequence that binds to/is complementary to a different nucleic acid sequence.

216. Thus, because nucleic acid hybridization interactions generally involve the formation of duplexes through base-pairing, each of the 100 or more probe sequences present on one of Cantor’s beads necessarily binds to a different nucleic acid under Patent Owner’s interpretation of the claims as well.

B. Ground 2: Obviousness over Cantor in view of Armani and Knowledge of a POSA.

217. I incorporate by reference my analysis in Ground 1 above. Here, Armani provides further evidence of a POSA's motivation and reasonable expectation of success in using Cantor's device and methods to determine the spatial location of biological molecules, including mRNA, in a tissue sample. Moreover, Armani provides additional information rendering obvious the step of contacting the array with a plurality of capture agents with a tissue section such that the capture agents interact with nucleic acids of the tissue sample.

1. Claim 1 ('468 Patent / '879 Patent)

218. I have reproduced claim 1 of each patent below for reference:

'468 Patent, Claim 1	'879 Patent, Claim 1
A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising:	A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising:
(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:	(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:
(i) a first sequence that specifically binds the nucleic acid, and	(i) a first sequence that binds the nucleic acid, and

(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;	(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;
(b) determining all or a portion of the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.	(b) determining the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.

219. Cantor in view of Armani and the knowledge of a POSA further renders obvious claim steps 1(a) “contacting the tissue section with an array,” and 1(b) “determin[ing] the presence or abundance of the nucleic acid at a location in the tissue section.”

220. As explained above, Cantor describes a device and methods for detecting nucleic acids using nucleic acid arrays that capture, extend, amplify, and detect target nucleic acids with sequencing. In summary, Cantor discloses providing a plurality of beads with the claimed “capture agents” in the form of nucleic acid constructs that include a “probe sequence” that “specifically binds” with a target nucleic acid, as well as a “coding tag” or “identification sequence” that may be used to identify the specific bead, and thus location, at which a particular binding

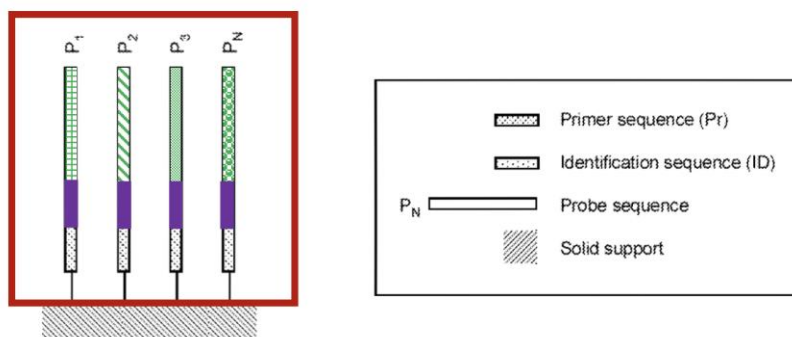
IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

interaction occurred. Cantor's Figure 1A shows an example of its solid support, such as a bead surface, with a plurality of "capture agents," each having the same "identification sequence (ID)" and different "probe sequences." I have annotated this figure to show the correspondence between Cantor's Figure 1 and the requirements of the claimed "capture agents" below.



221. Cantor further explains that its "array can be arranged on any substrate suitable for sequence analysis or manufacture processes described herein," including "substrates having multiple wells or pits," with varying volumes ranging from the microliter to picoliter scale. EX1031, 17-18. Cantor explains that "[w]ells or pits of multiple well and pit substrates may contain one or more solid support units (e.g., each unit being a single bead or particle)." *Id.*, 18; *see also id.*, 19 ("In some embodiments, the microreactor volume is large enough to accommodate one solid support bead in the microreactor and small enough to exclude the presence of two or more beads in the microreactor."). Thus, Cantor discloses providing a plurality of

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

beads containing the claimed capture agents by arranging the beads in a multiple well plate. This arrangement is encompassed by the Challenged Claims. See Dependent Claim 17 ('468 Patent) ("The method of claim 1, wherein the feature comprises a bead, well, or channel."); Dependent Claim 16 ('879 Patent) (same). Cantor's disclosure of arrangements in which beads are placed in wells of a substrate also motivates combining the teachings of Cantor with the teachings of Armani. And, as explained in Ground 1 above, because Cantor further discloses that the biological sample to be analyzed with its methods may be "a solid material such as a tissue [or] cells," EX1031, 6, Cantor expressly discloses all structural elements required for performing the claimed methods.

222. Armani further discloses methods for the analysis of nucleic acids in a tissue section. In Armani's method, (1) "the tissue section is transferred onto the surface of the multi-well array (step 1A) and the pressed onto the surface (1B)," after which "[t]he contents of the well are dehydrated to enable the addition of lysis reagents (1C); (2) "the tissue is lysed to release genomic DNA into solution"; (3) the "DNA is amplified" using PCR; and (4) "the DNA is imaged" using fluorescent detection techniques. EX1022, 3527. This is depicted in Armani's Figure 2.

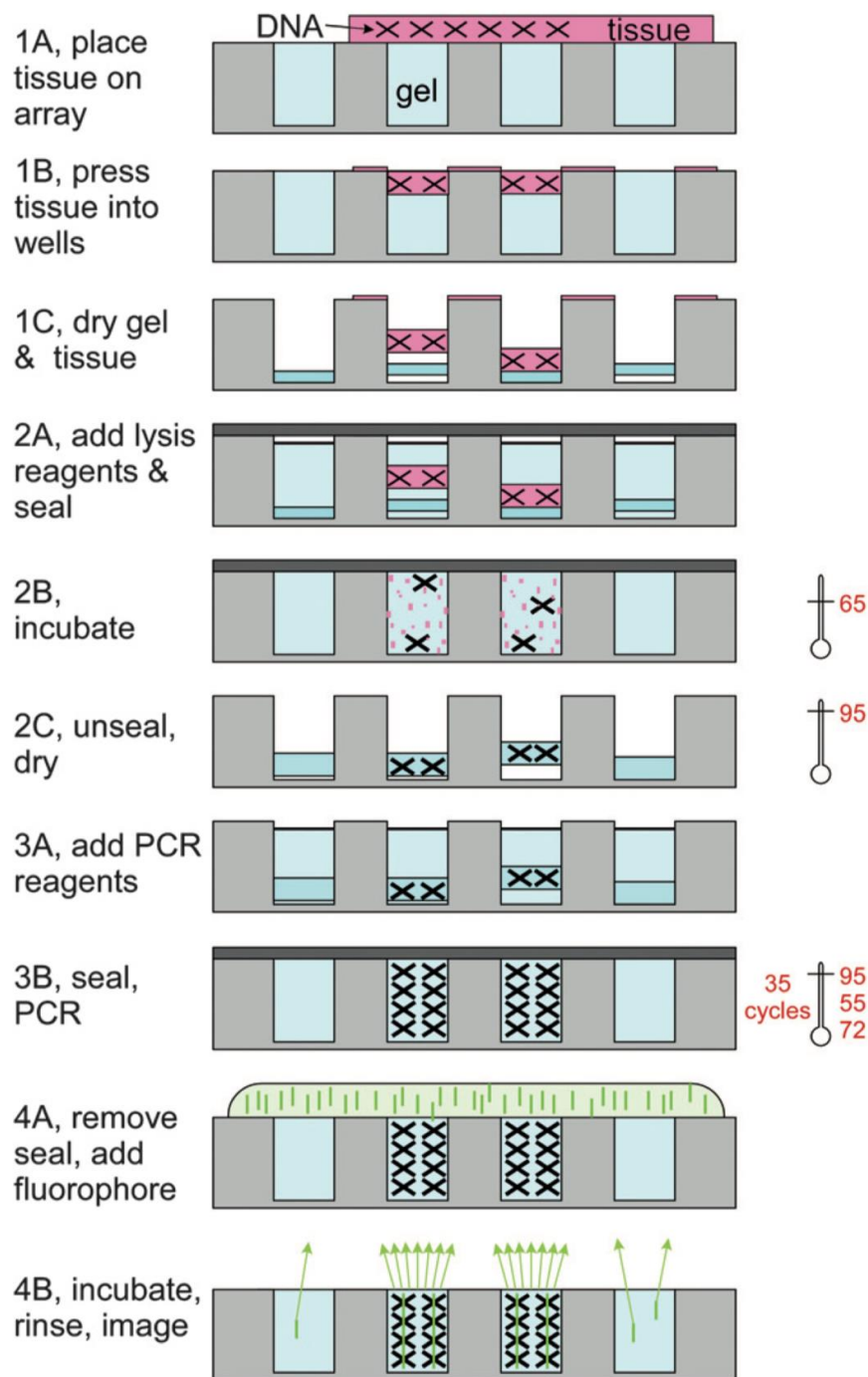


Fig. 2 Experimental protocol used to demonstrate the 2D-PCR concept. Tissue is transferred into wells, isolated, and lysed to extract the genomic DNA (represented by "x"s). PCR is performed to amplify the target DNA, and the targets are visualized with fluorescent dye (indicated by vertical lines "|").

223. A POSA would have found it obvious to combine the teachings of Cantor and Armani by providing Cantor's bead array with the plurality of beads disposed in wells (as noted above, an embodiment expressly contemplated by Cantor), contacting that array with a tissue slice as suggested by Cantor and explicitly disclosed by Armani, and then pressing the tissue sample into the wells and lysing the tissue as described by Armani. EX1022, 3531 (describing "loading the tissue vertically, pressing it directly into the array of wells to isolate tissue subregions while preserving the overall 2D spatial relationship.... This tissue loading technique also has the advantage of beginning with traditional histological sectioning and transfer steps, which are familiar to investigators and clinicians who may wish to adopt this technique for research or diagnosis.").

224. A POSA would then have proceeded with the nucleic acid extension, amplification, and detection by sequencing steps disclosed by Cantor.

225. A POSA reviewing Cantor and Armani would have been motivated to combine their teachings for several reasons. Cantor's protocols facilitate the capture, amplification, and detection of mRNA by sequencing, which Armani indicates was a desirable application for spatial analysis. By using sequencing as a detection technique, rather than visualization (e.g., fluorescence), the combination would allow the detection of even low abundance mRNAs. *See* EX1022, 3532 ("We are

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

currently working on mapping RNA from tissue, since current visualization techniques for RNA cannot reliably detect below a few dozen copies per cell in situ.”). Moreover, the use of Cantor’s methods to generate cDNA libraries of target nucleic acids coded with location information (via Cantors’ identification sequences) would, in some workflows, allow simplification of Armani’s detection procedure by eliminating at least the steps of unsealing the array, adding a fluorophore, incubating, rinsing, and imaging in Armani’s steps 4A-4B. Instead, using sequencing as a detection method would allow a POSA to simply pool the contents of all wells together in a multiplexed sequencing procedure, where the data can then be deconvoluted and spatially mapped based on the read out of location-correlated identification sequences.

226. A POSA would have reasonably expected success in combining the teachings of Cantor and Armani. Indeed, the combination would have involved using Cantor’s device and methods in the manner they are disclosed—to detect nucleic acids from a tissue sample, while specifically using the identification probes to identify the location at which beads were provided. Armani expressly motivates this mental step, and further provides additional specific implementations of how to successfully prepare and process effectively a nucleic acid sample for localized detection.

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

2. Claim 21 ('468 Patent) / Claim 23 ('879 Patent)

227. Claim 21 of the '468 Patent is substantially the same as Claim 23 of the '879 Patent, as tabulated below. The additional language present in claim 21 of the '468 Patent is highlighted in green. Because the use of “a fresh frozen tissue section” is rendered obvious by the applied prior art, both claims are rendered obvious for the same reasons.

'468 Patent	21. The method of claim 1, wherein the tissue section is a fresh frozen tissue section or a formalin-fixed paraffin-embedded (FFPE) tissue section.
'879 Patent	23. The method of claim 1, wherein the tissue section is a fresh frozen tissue section.

228. I incorporate by reference my analysis of claim 1, above.

229. As described in Ground 1, Cantor in view of the knowledge of a POSA renders obvious using a fresh-frozen tissue section with Cantor's array. Armani further renders obvious using fresh-frozen tissue.

230. Armani discloses successfully using fresh-frozen tissue samples with its array. In particular, Armani discloses placing a fresh-frozen tissue section on a multi-well array as shown below in excerpted Figure 2 to detect the presence of a target nucleic acid at various location throughout the tissue sample. EX1022, 3528 (“Two types of tissue were used in these studies: frozen tissue samples from a human

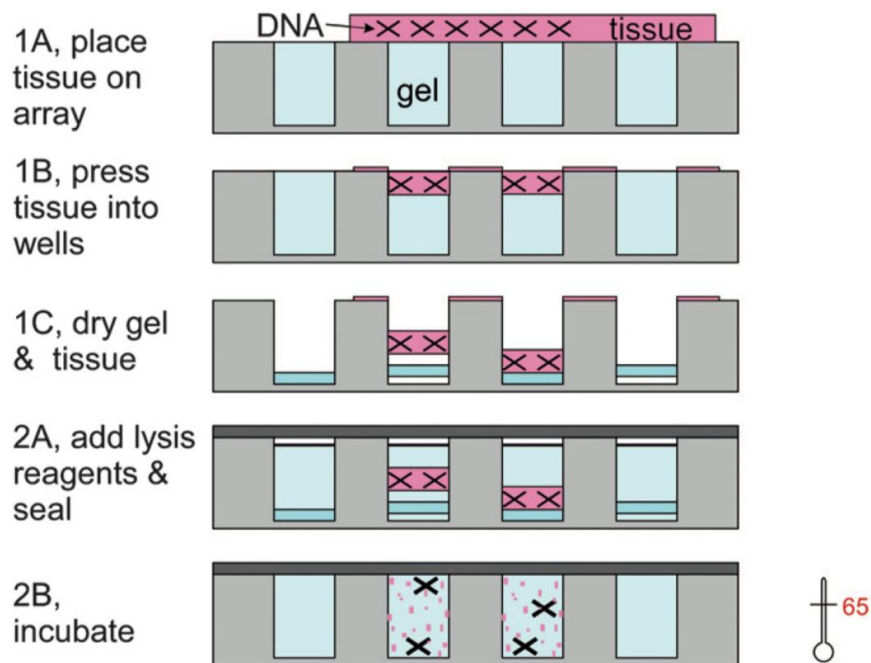
IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

prostate with benign hyperplasia and breast tissue with lobular carcinoma....Tissue specimens were snap frozen and embedded in O.C.T. compound embedding medium (Sakura Fine-Tek, Torrance, CA).”; *id.* (“Tissue sections were transferred to the multi-well device immediately after sectioning while still frozen[.]”).



231. Thus, Armani confirms a POSA’s reasonable expectation of success in using a fresh-frozen tissue section on Cantor’s array.

3. Claim 23 ('468 Patent) / Claim 27 ('879 Patent)

232. Claim 23 of the '468 Patent is identical to Claim 27 of the '879 Patent, as tabulated below.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent	23. The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.
'879 Patent	27. The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.

233. I incorporate by reference my analysis of claim 1, above. The combination of Cantor and Armani renders obvious the step of “generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.”

234. Armani’s Abstract explains that a POSA would have been motivated to map the presence of nucleic acids to their spatial location in a tissue sample, including to “interrogate the molecular heterogeneity of tissue specimens”:

A novel approach was developed for mapping the location of target DNA in tissue sections. The method combines a high-density, multi-well plate with an innovative single-tube procedure to directly extract, amplify, and detect the DNA in parallel while maintaining the two-dimensional (2D) architecture of the tissue. A 2D map of the gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was created from a tissue section and shown to correlate with the spatial area of the sample. It is anticipated that this approach may be easily adapted to assess the status of multiple genes within tissue sections, yielding a molecular map that directly correlates with the histology of the sample.

This will provide investigators with a new tool to interrogate the molecular heterogeneity of tissue specimens.

EX1022, 3526 (Abstract).

235. Armani further explains that its spatial analysis technique included “the creation of a 2D detection map, showing the presence/absence of target DNA, on the millimeter scale,” that is, mapping whether target DNA was present or absent at a particular location in the X-Y plane. *Id.*, 3527. The presence of a target nucleic acid at particular locations in the tissue sample is mapped in Armani’s Figure 1, with the blue squares indicating the presence of the target nucleic acid at a particular location.

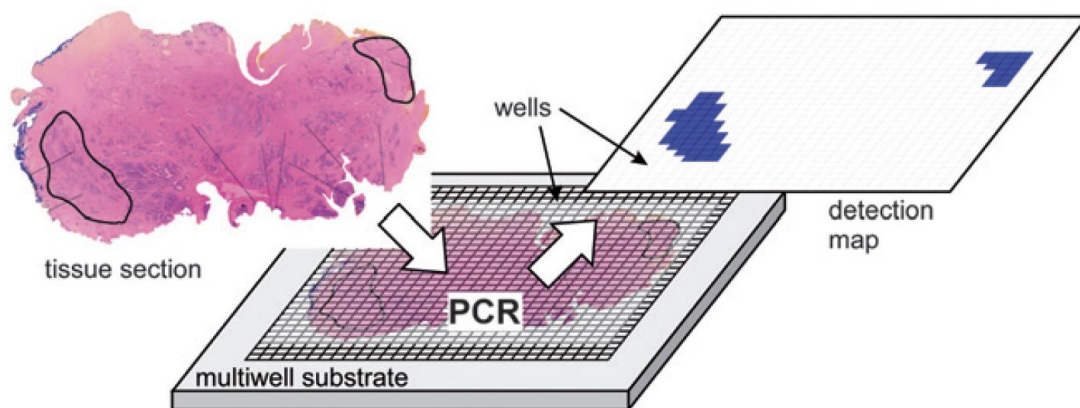


Fig. 1 2D-PCR is achieved by transferring a tissue section vertically into a multi-well array device, isolating tissue subregions. The DNA is extracted and then amplified by the polymerase chain reaction. A detection map is created by imaging the amplified DNA, for example using a DNA-intercalating dye.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

236. Additionally, I note that this mapping step is equally applicable to methods involving the use of Cantor's arrays with the beads arranged on a substrate, as set forth in Ground 1, above. Thus, Cantor in view of Armani and the knowledge of a POSA renders obvious claim 23 of the '468 patent and claim 27 of the '879 patent.

4. Other Dependent Claims

237. The additional features of these claims are disclosed by the combination of Cantor and Armani for the same reasons they are disclosed in Cantor alone. Accordingly, I incorporate by reference my analysis of '468 Patent Claims 2, 6, 7, 12, 13, 18, 20, 23, 25, 26, 30 and '879 Patent Claims 2, 5, 6, 11, 12, 17, 21, 27, 28, and 30, as set forth in Ground 1, above.

C. Ground 3: Anticipation by Frisen

1. Priority Date

238. The '468 Patent issued from Application No. 16/660,234 (the "'234 Application"), which was filed on October 22, 2019. I understand that the '468 Patent/'234 Application purports to claim priority to the following applications:

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

Related U.S. Application Data

- (63) Continuation of application No. 16/443,771, filed on Jun. 17, 2019, which is a continuation of application No. 16/437,726, filed on Jun. 11, 2019, which is a continuation of application No. 16/430,015, filed on Jun. 3, 2019, which is a continuation of application No. 16/414,213, filed on May 16, 2019, which is a continuation of application No. 16/402,098, filed on May 2, 2019, now Pat. No. 10,472,669, which is a continuation of application No. 16/276,235, filed on Feb. 14, 2019, now Pat. No. 10,480,022, which is a continuation of application No. 15/187,661, filed on Jun. 20, 2016, now Pat. No. 10,308,982, which is a continuation of application No. 13/080,616, filed on Apr. 5, 2011, now Pat. No. 9,371,598.
- (60) Provisional application No. 61/321,124, filed on Apr. 5, 2010.

EX1002, Codes (63), (60). These purported priority documents are tabulated below. Because the '661 Application does not provide written description support for the challenged claims, their priority date is no earlier than the February 14, 2019 filing date of the '235 Application. Accordingly, Frisen, which was published on October 18, 2012 is prior art to the '468 Patent. EX1020, Code (43); EX1019.

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

'468 Patent Priority Documents	Filing Date	Exhibit
16/443,771 (the "'771 Application")	June 17, 2019	EX1063
16/437,726 (the "'726 Application")	June 11, 2019	EX1064
16/430,015 (the "'015 Application")	June 3, 2019	EX1055
16/414,213 (the "'213 Application")	May 16, 2019	EX1056
16/402,098 (the "'098 Application")	May 2, 2019	EX1057
16/276,235 (the "'235 Application")	February 14, 2019	EX1010
15/187,661 (the "'661 Application")	June 20, 2016	EX1011
13/080,616 (the "'616 Application")	April 5, 2011	EX1012
61/321,124 (the "'124 Provisional")	April 5, 2010	EX1009

239. The '879 Patent issued from Application No. 17/144,971 (the "'971 Application"), which was filed on January 8, 2021. I understand that the '879 Patent/'234 Application purports to claim priority to the following applications:

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

Related U.S. Application Data

- (63) Continuation of application No. 16/988,284, filed on Aug. 7, 2020, which is a continuation of application No. 16/414,213, filed on May 16, 2019, now Pat. No. 10,787,701, which is a continuation of application No. 16/402,098, filed on May 2, 2019, now Pat. No. 10,472,669, which is a continuation of application No. 16/276,235, filed on Feb. 14, 2019, now Pat. No. 10,480,022, which is a continuation of application No. 15/187,661, filed on Jun. 20, 2016, now Pat. No. 10,308,982, which is a continuation of application No. 13/080,616, filed on Apr. 5, 2011, now Pat. No. 9,371,598.
- (60) Provisional application No. 61/321,124, filed on Apr. 5, 2010.

EX1003, Codes (63), (60). These purported priority documents are tabulated below.

Because the '661 Application does not provide written description support for the challenged claims, their priority date is no earlier than the February 14, 2019 filing date of the '235 Application. Accordingly, Frisen, which was published on October 18, 2012 is prior art to the '879 Patent. EX1020, Code (43); EX1019.

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

'879 Patent Priority Documents	Filing Date	Exhibit
16/988,284 (the "'284 Application")	August 7, 2020	EX1059
16/414,213 (the "'213 Application")	May 16, 2019	EX1056
16/402,098 (the "'098 Application")	May 2, 2019	EX1057
16/276,235 (the "'235 Application")	February 14, 2019	EX1010
15/187,661 (the "'661 Application")	June 20, 2016	EX1011
13/080,616 (the "'616 Application")	April 5, 2011	EX1012
61/321,124 (the "'124 Provisional")	April 5, 2010	EX1009

240. As shown above, each Challenged Patent claims priority through the '661 Application and the '235 Application. Based on my review, and as discussed in detail below, the challenged claims of the '468 and '879 Patents are not entitled to a priority date earlier than February 14, 2019, the filing date of the '235 Application. In my opinion, the challenged claims of the '468 and '879 Patents are not supported by sufficient written description in the Specification of the '661 Application, such that a POSA would have understood the inventor to be in possession of the subject matter recited in the challenged claims. Accordingly, I understand that since the '661 Application does not support the challenged claims, they are not entitled to a priority date earlier than this break in the priority chain, and

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

at the earliest are entitled to the February 14, 2019 filing date of the '235 Application.¹³

241. As I explain below, the '661 Application fails to disclose the subject matter recited in the claims of the '468 and '879 Patents. The preceding '616 Application shares the same specification as the '661 application, and thus fails to provide adequate description of the subject matter recited in the challenged claims for the same reasons. Finally, and as discussed further below, the passages of the '124 Provisional that were omitted from the '661 and '616 Applications also fail to describe the subject matter of the challenged claims.

(a) The '661 Application discloses delivering probes to a fixed tissue sample; not delivering a tissue sample to a previously generated array of substrate-affixed probes.

242. Claim step 1(a) of the Challenged Patents recites, “contacting the tissue section with an array comprising a plurality of features, wherein a feature of the

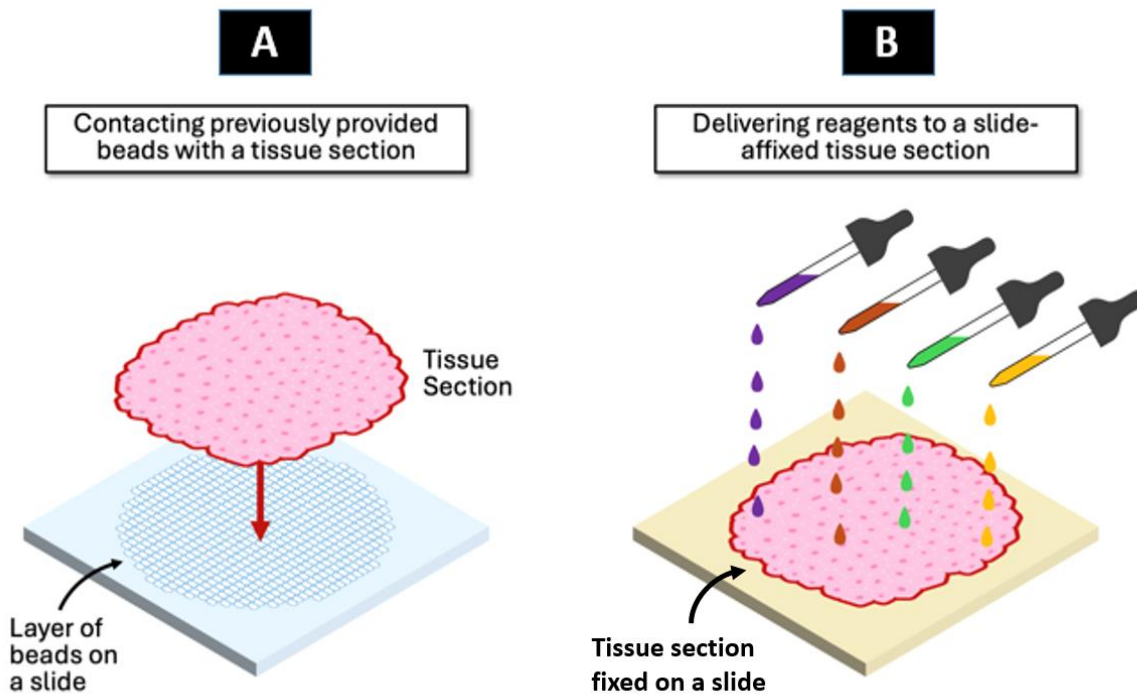
¹³ The '235 Application fails to support the challenged claims for the same reasons discussed herein. However, I understand that for purposes of determining the priority date to which the Challenged Claims are entitled, Frisen is eligible as prior art based on the break in the chain of priority at the '661 Application.

plurality of features comprises a capture agent, wherein the capture agent comprises:

(i) a first sequence that [specifically] binds the nucleic acid.”

243. In my opinion, the '661 Application (including the '124 Provisional) fails to disclose claim step 1(a). The claims as drafted and as read by Patent Owner to assert infringement require first providing an array of features, where each feature comprises a capture agent that binds a target nucleic acid, as well as a coding identifier corresponding to the location of the feature in the array. The claims as read by Patent Owner to assert infringement then recite contacting such an array with a tissue sample by placing the tissue sample on top of the array of features (i.e., beads including capture probes), as depicted in Figure A below. But the '661 Application focuses exclusively on providing a tissue sample affixed to a solid support, and then delivering reagents, such as binding agents,¹⁴ to the top of the support-affixed tissue sample according to a spatial pattern, as depicted in Figure B below.

¹⁴ The priority documents, including the '661 Application, do not refer to “capture agents,” as claimed, instead, they refer to “binding agents.”



In fact, the specification emphasizes that the novelty of the “invention” lies in the delivery instrumentation used in the system of Figure B. “The assay system further provides instrumentation with an ability to deliver reagents in a spatially-defined pattern. This instrumentation, together with software, reagents and protocols, provides a key component of the highly innovative assay system of the invention....” EX1002, 8:64-67; 9:1; EX1003, 8:64-67; 9:1. “In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology.” EX1002, 17:66-67; EX1003, 18:3-4. The claims of the Challenged Patents do not cover the allegedly “highly innovative assay system of the invention” as depicted in Figure B.

244. The differences in these methodologies are not trivial, and the implementation of each requires different sophisticated laboratory instrumentation, techniques, and surface chemistries. For example, in the methods described in the Challenged Patents and their priority applications, probes are delivered to the tissue in solution, as illustrated on the right-hand side. Such techniques require, as discussed below, precise liquid handling and dispensing techniques. And because the probes are provided in solution, the disclosed embodiments repeatedly include a step of “washing” or separating encoded probes that did not bind to a target away from the sample. EX1011, Figs. 1-2; [0028] (“Once encoded probes interact with the biological targets, the encoded probes that interacted with the biological targets must be separated from the encoded probes that did not interact with the biological targets at step 140. In the case where the biological targets are nucleic acids and the encoded probes are oligonucleotides, the separation can be accomplished by, e.g., washing the unhybridized encoded probes from the sample.”). The removal of unhybridized probes is important in the Figure B approach, because if such probes are not washed away, they contribute background “noise” to the readout signal, such as sequencing, making it difficult to detect the interactions of interest. See ¶80, above. By contrast, the claims as read by patent owner to assert infringement against Petitioner purport to encompass probes provided in a solid phase. Such techniques

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

require fabrication technologies and chemistries to create beads or other surfaces with the nucleic acids (such as probes) affixed to the desired locations. A step of separating or washing encoded probes that did not bind to a target would not make sense in the context of these surface chemistries, because each feature would typically consist of multiple probes, some of which would bind a sample nucleic acid and some would not. But because such probes are immobilized on the surface (e.g., covalently bound), it would be extremely difficult and unfeasible to remove unhybridized probes from the array-sample combination without also disrupting the interactions between hybridized surface-affixed probes and the sample.

245. Moreover, the Challenged Patents criticize Armani, which provides a variation of the Figure A approach, stating that its reliance on the transfer of tissue into wells, “prevents random access to tissue samples and prevents high levels of multiplexing.” See EX1002, 1:66-2:5; EX1003, 1:64-2:3. To the extent that the disclosures of the Challenged Patents (but not their claims) purport to address these alleged deficiencies, it is through the delivery of reagents from above the tissue sample. EX1002, 16:15-17 (“Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto* the biological sample.”); EX1003, 16: 19-21 (same).

246. A POSA reviewing the '661 Application (including the '124 Provisional) would not have understood it to demonstrate that the inventor possessed methods of contacting a pre-existing array or arrangement of capture agents, such as probes, with a tissue sample. Instead, a POSA would have understood the disclosure of the '124 Provisional to have been limited to the delivery of reagents, including binding agents and encoded probes, to a tissue sample already affixed to the solid support.

247. For example, the **SUMMARY OF THE INVENTION** explains that “[t]he assay system comprises an assay capable of high levels of multiplexing where encoded probes *are provided to a biological sample* in a defined spatial patterns.” EX1011 ('661 Application), [0007].¹⁵

248. The first “embodiment” disclosed is one in which “the assay system performs the following steps: *providing a sample affixed to a support; delivering encoded probes* for the multiple biological targets to the multiple sites.” *Id.*, [0008]. The next “embodiment” is one in which “there is provided an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid

¹⁵ I note that the '661 Application's paragraph numbering re-starts following the **SUMMARY OF THE INVENTION** section.

targets at multiple sites in a sample, where the assay system performs the following steps: ***providing a sample affixed to a support; delivering oligonucleotide probes*** for multiple nucleic acid targets ***to the multiple sites in the sample*** in a known spatial pattern.” *Id.* [00014]. “Other embodiments of the invention provide an assay system” that “performs the following steps: ***providing a sample affixed to support; delivering encoded probes*** for the multiple protein targets to the multiple sites in the sample....” *Id.*, [00015]. The final “embodiment” described in the **SUMMARY OF THE INVENTION** section similarly involves “***providing a sample affixed to a support; delivering encoded probes*** for the multiple biological targets at multiple sites in a sample in a known spatial pattern.” *Id.*, [00016].

249. Thus, a POSA reviewing the ’661 Application would understand from the inventor’s **SUMMARY OF THE INVENTION** that the alleged invention related to (i) providing a sample affixed to a support and (ii) delivering probes to such a sample. A POSA would not understand these disclosures to demonstrate that the inventor possessed methods of creating arrays of encoded probes on solid supports, such as beads, and subsequently disposing a tissue sample on top of such an array. Such an approach inverts the workflow of the methods disclosed in the ’661 Application’s **SUMMARY OF THE INVENTION**, requires a structure that is not disclosed, and is not mentioned in that section (or elsewhere in the Application).

250. Turning to the '661 Application's figures, "Figure 1 provides a simplified overview of *the assay system of the present invention*." EX1011, [0001].

This overview is explicit that the

"assay system of the present invention" involves first "provid[ing] [a] biological sample affixed to a support" and then "deliver[ing] encoded probes to the biological sample according to a known spatial pattern." EX1011, Fig. 1; see also *id.*, [00025] (describing Fig. 1).

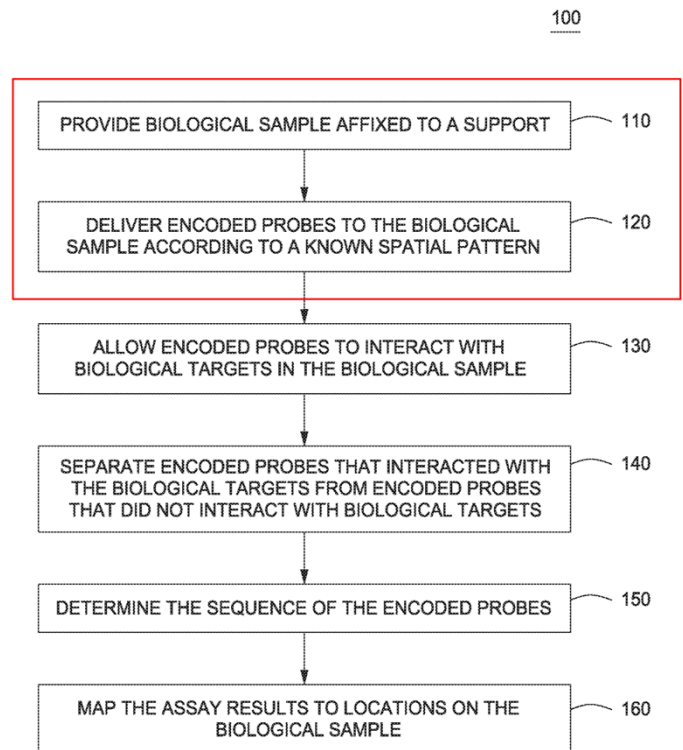


FIG. 1

251. Thus, a POSA reviewing Figure 1 and its caption, which provides an "overview of *the assay system of the present invention*" would understand the inventor considered the methods of the alleged invention to require fixing a sample to a support, followed by delivering encoded probes to the sample affixed to the support.

252. Similarly, Figure 2 “provides a simplified overview of one embodiment of *the assay of the present invention* for detecting nucleic acids.” *Id.*, [0002]. This disclosure again requires “provid[ing] [a] biological sample affixed to a support” and then “deliver[ing] probes” and “deliver[ing] encoding oligonucleotides” to the biological sample.” EX1011, Fig. 2. *see also id.*, [00030], [00031] (describing Fig. 2).

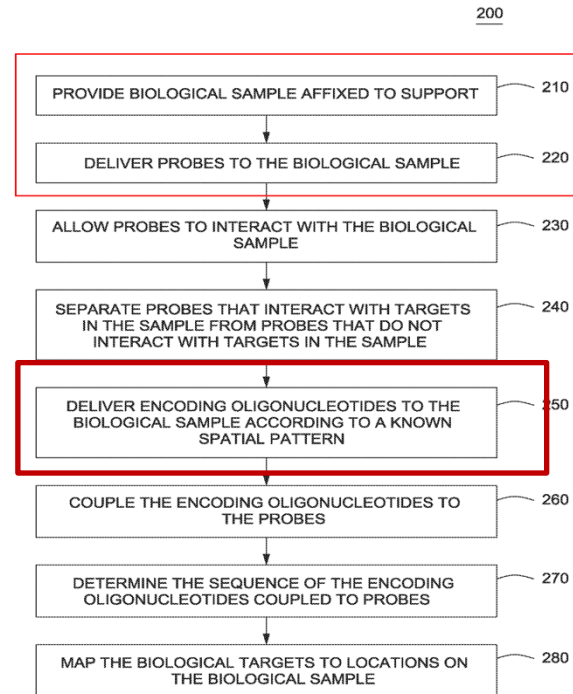


FIG. 2

253. A POSA would not have understood Figures 1-2 to describe providing an array of probes, and then delivering a tissue sample to that array.

254. The remainder of the '661 Application's Specification is replete with additional statements confirming that the “invention” requires (i) providing a sample affixed to a support before (ii) delivering probes to the sample.

255. For example, the **DETAILED DESCRIPTION OF THE INVENTION** section begins with a subsection headed, “**The Invention in General.**” EX1011, [00023]. This section begins:

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

The assay systems of the invention provide spatially-encoded, multiplexed assays comprising 1) an assay capable of high levels of multiplexing with an efficient spatial encoding scheme; 2) ***instrumentation capable of delivering reagents according to a spatial pattern***; and 3) decoding determined by a readout that is digital in nature. The assay systems of the invention detect the presence or absence and relative amount of a biological target or biological activity indicative of a biological target, as well as the location of the biological target or activity in a biological sample, ***e.g., a tissue section or other biological structure disposed upon a support such as a microscope slide or culture dish.***

EX1011, [00023]; *see also id.*, [00024] (“The assay system further provides ***instrumentation with an ability to deliver reagents*** in a spatially-defined pattern. ***This instrumentation***, together with software, reagents and protocols, ***provides a key component*** of the highly innovative assay system ***of the invention....***”).

256. Confirming that the ’661 Application discloses only the delivery of reagents, including probes, to a sample affixed to a solid support, the Specification further notes that the above-referenced “instrumentation capable of delivering reagents according to a spatial pattern” “onto the biological sample” is “***integral to the assay system of the invention.***” EX1011, [00055] (“Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents ***onto the biological sample.***”); *see also, e.g., id.*, [00046] (“Coding tag a1, for example, ***is***

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

deposited on the biological sample in ten discrete areas or spots.... Coding tag a2 ***is deposited on the biological sample*** in ten spots on the second horizontal line.... Coding tag a3 ***is deposited on the biological sample....***”); *id.*, [00048] (“A deposit or dispensing scheme is shown at 530. Like the grid exemplified in Figure 4, encoding oligonucleotides a1 through a4 ***are deposited*** in spots in a pattern.... The grid though shown as a square with spots is actually ***a deposition pattern on a biological sample*** (not shown) such as tissue Section 416 shown in Figure 4.”); *id.*, [00049] (“The target-specific oligonucleotides ***are delivered to the biological sample....***”).

257. Under the subsection headed, “**Assays**” the ’661 Application states that “[t]he assay portion of the assay systems of the present invention comprise the following general steps: ***delivering probes and encoding agents*** where the encoding agents (in some embodiments pre-coupled to the probes) are ***delivered to the sample*** according to a known spatial pattern.” EX1011, [00034]. Further confirming that the invention as disclosed by the priority documents requires delivering probes or reagents to a sample, this section goes on to state that “[t]he samples of the present invention include virtually any biological sample or samples ***that can be affixed to a support*** or provided essentially in a two-dimensional manner.” *Id.*, [00035].

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

258. Because the '661 Application's alleged inventions require the delivery of reagents to samples affixed to a support, the **DETAILED DESCRIPTION OF THE INVENTION** section contains a subsection titled, "**Reagent Delivery Systems.**" EX1011, [00054]. "The reagent delivery system *of the invention* includes instrumentation that allows the delivery of reagents to discrete portions of the biological sample." *Id.* The '661 Application goes on to note that "reagent delivery may be to many different biological samples at one time," although "[a] single tissue section has been exemplified herein." *Id.* The Specification does not, however, contain any disclosure indicating that the system "of the invention" may operate by delivering a tissue section to an array of probes.

259. As noted, the '661 Application provides that "[i]ntegral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto* the biological sample." *Id.*, [00055]. Such techniques are disclosed to include, for example, "ink jet spotting; mechanical spotting...; micro contact printing; photochemical or photolithographic methods; and the like." *Id.*, [00056]; *see also id.*, [00064] ("[T]he reagent delivery system controls the delivery of reagents to specific patterns *on a biological sample surface* using semiconductor techniques such as masking and spraying."); *id.*, [00065] ("In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

technology.”). A POSA would not have understood such disclosures, which necessarily involve delivering reagents on top of a substrate-affixed tissue sample, to demonstrate possession of methods in which a tissue sample is placed on top of an array of capture agents, such as an array of functionalized features, as claimed.

260. The '661 Application itself contains only a handful of references to nucleic acid arrays. These references appear in discussions of the prior art, EX1011, [0004], [0018]; in discussions regarding the use of nucleic acid arrays as potential tools that “can be used to identify the coding tags and probe sequences in the encoded probes of the assay systems of the invention,” but not for use as the inventive assays themselves, *id.*, [00069] (“In yet another alternative, the encoded probes can be amplified and hybridized to a microarray.”); and in discussions involving the use of nucleic acid arrays as “proxies for a tissue section” in the Examples. *Id.*, [00077]-[00078]; [00080]-[00084]. Further, the '124 Provisional’s disclosures regarding “nucleic acid arrays” are in reference to genotyping using prior art techniques. EX1009, [0052] (“Genotyping may be performed using any technique known to those of skill in the art. Preferred techniques permit rapid, accurate determination of multiple variations with a minimum of sample handling. Some examples of suitable techniques involve but are not limited to direct DNA sequencing, capillary electrophoresis, hybridization, allele-specific probes or primers, single-stranded

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

conformation polymorphism analysis, nucleic acid arrays, bead arrays, restriction length polymorphism analysis, random amplified polymorphic DNA, ligase detection reaction, heteroduplex or fragment analysis, differential sequencing with mass spectrometry, atomic force microscopy, pyrosequencing, FRET, and other related techniques. Several methods for DNA sequencing are well known and generally available in the art.”) (internal citations omitted). A POSA would not have understood these references to the use of known and conventional nucleic acid detection techniques for genotyping to disclose that the inventor possessed methods of directly contacting nucleic arrays (or the other listed techniques) with tissue samples to perform the claimed spatial assays. *See, e.g.*, Figs. 1-2.

261. Accordingly, the ’661 Application (including the ’124 Provisional) fails to support the challenged claims of the Challenged Patents. The additional limitations of the challenged dependent claims do not narrow claim 1 in a manner that resolves these deficiencies, and thus all challenged claims are unsupported for the same reasons.

- (b) The '661 Application does not disclose contacting an array with a tissue section such that nucleic acids in the tissue section “bind” or “specifically bind” to the capture probes.**

262. As noted above, the Challenged Patents and their priority applications criticize Armani's tissue preparation procedures:

Certain PCR assays in a 2D format preserve spatial information (see Armani, et al., Lab on a Chip, 9(24): 3526-34 (2009)), but these methods have low spatial resolution because they rely on physical transference of tissue into wells, which also prevents random access to tissue samples and high levels of multiplexing.

EX1011 ('661 Application), [0004]. Yet the subsequent disclosures of the '661 Application contain no teaching on how to prepare the tissue section to allow binding or specific binding when a tissue section is contacted with an array.

263. A POSA would understand, given the '661 Application's criticism of Armani, that the inventor did not envision or possess providing access to the nucleic acids in a tissue sample through physical transference and lysis as in Armani. But the '661 Application does not disclose any alternative techniques for “contacting” an array such that capture probes disposed on the array bind or specifically bind nucleic acids in the tissue section. Thus, a POSA would have understood the '661 Application as having disclosed at most a hope or a wish to obtain access to tissue

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

samples without relying on transference and lysis techniques as disclosed in Armani. But given the failure of the '661 Application to expressly disclose or otherwise point to which techniques to use, a POSA would not have understood the inventor to have possessed such methods.

264. While, as discussed above, techniques for local release of nucleic acids or other biological molecules from tissue samples for capture on probes in bead or other arrays were known, *see, e.g.*, EX1022 (Armani), EX1024 (Shirai); EX1025-1026(Okano); EX1031 (Cantor); EX1033 (Southern), the inventor did not point to such techniques (or in the case of Armani, expressly criticizes and disclaims them). Even though such techniques existed, I understand that pointing to references that render the claimed subject matter obvious is insufficient to establish possession of the claimed invention in the priority documents. Moreso here, given the inventor's criticism and failure to point to such techniques, there is no indication to a POSA in the priority documents that the inventor possessed or even contemplated the use of such techniques.

265. This is an independent reason for which the '661 Application fails to support the challenged claims of the Challenged Patents. The additional limitations of the challenged dependent claims do not narrow claim 1 in a manner that resolves

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

these deficiencies, and thus all challenged claims are unsupported for the same reasons.

(c) Conclusion on Priority Date

266. For the reasons set forth above, the Challenged Claims are not supported by the '661 Application filed on June 20, 2016, and thus are entitled to a priority date no earlier than the February 14, 2019 filing date of the '235 Application. I reserve the right to respond to any arguments advanced by Patent Owner suggesting that the challenged claims are supported by one or more of the '468 and '879 Patents' priority applications.

2. Claim 1

267. The preambles of claim 1 of each Challenged Patent are identical and recite:

'468 Patent	A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising: [steps (a) and (b)].
'879 Patent	A method for determining the presence or abundance of a nucleic acid at a location in a tissue section comprising: [steps (a) and (b)].

268. Frisen anticipates claim 1. Frisen discloses the claimed “method for determining the presence or abundance of a nucleic acid at a location in a tissue section.” For example, Frisen describes its work flow as follows:

[A] method for localised detection of nucleic acid in a tissue sample comprising:

(a) providing an array comprising a substrate on which multiple species of capture probes are directly or indirectly immobilized such that each species occupies a distinct position on the array and is oriented to have a free 3' end to enable said probe to function as a primer for a primer extension or ligation reaction, wherein each species of said capture probe comprises a nucleic acid molecule with 5' to 3': (i) a positional domain that corresponds to the position of the capture probe on the array, and (ii) a capture domain;

(b) contacting said array with a tissue sample such that the position of a capture probe on the array may be correlated with a position in the tissue sample and allowing nucleic acid of the tissue sample to hybridise to the capture domain in said capture probes;

(c) generating DNA molecules from the captured nucleic acid molecules using said capture probes as extension or ligation primers, wherein said extended or ligated DNA molecules are tagged by virtue of the positional domain;

(d) optionally generating a complementary strand of said tagged DNA and/or optionally amplifying said tagged DNA;

(e) releasing at least part of the tagged DNA molecules and/or their complements or amplicons from the surface of the array, wherein said part includes the positional domain or a complement thereof;

(f) directly or indirectly analysing the sequence of the released DNA molecules.

EX1020, 7-8 (“Frisen’s Workflow”); *see also, e.g., id.*, Claims 1, 40, 42.

269. Frisen further explains that its methods involve the implementation “of standard arrays [that] are commercially available and both the number and size of the features may be varied.” EX1020, 15:21-22. Frisen explains that the arrays suitable for its methods include “[c]ommercial arrays...available or known for use in the context of sequence analysis and in particular in the context of NGS technologies. Such arrays may also be used as the array surface in the context of the present invention e.g. an Illumina bead array.” *Id.*, 15:30-34. Thus, Frisen discloses providing its array of capture probes or capture agents by providing a plurality of features, such as beads.

270. As explained further below, Frisen discloses each enumerated element of Claim 1.

- (a) **“contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:”**

271. Step 1(a) of each Challenged Patent is identical and recites:

'468 Patent	(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:
'879 Patent	(a) contacting the tissue section with an array comprising a plurality of features, wherein a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises:

272. Frisen discloses each aspect of step 1(a).

273. **First**, Frisen discloses “contacting a tissue section with an array comprising a plurality of features.”

274. Frisen’s Workflow includes the step of “(b) contacting [an] array with a tissue sample such that the position of a capture probe on the array may be correlated with a position in the tissue sample and allowing nucleic acid of the tissue sample to hybridise to the capture domain in said capture probes.” *Id.*, 7; 25-27; *see also id.*, 13:7-11 (“The distinct position of each nucleic acid (oligonucleotide) spot (each species of oligonucleotide/nucleic acid molecule) is known as a ‘feature’ (and hence in the methods set out above each species of capture probe may be viewed as

a specific feature of the array; each feature occupies a distinct position on the array),....”).

275. **Second**, Frisen’s arrays comprise “a feature of the plurality of features comprises a capture agent, wherein the capture agent comprises” the claimed “first sequence” and “second sequence.”

276. Frisen discloses that its array surface, such as a glass slide or a plurality of beads, comprises a plurality of capture probes with a coding tag. Specifically, Frisen Workflow includes the step of “providing an array comprising a substrate on which multiple species of capture probes are directly or indirectly immobilized such that each species occupies a distinct position on the array...wherein each species of said capture probe comprises a nucleic acid molecule from 5’ to 3’ (i) a positional domain that corresponds to the position of the capture probe on the array.” EX1020, 7:16-21. Frisen also explains that each species of capture probe is a “feature.” *Id.*, 13:7-11 (“The distinct position of each nucleic acid (oligonucleotide) spot (each species of oligonucleotide/nucleic acid molecule) is known as a ‘feature’ (and hence in the methods set out above each species of capture probe may be viewed as a specific feature of the array; each feature occupies a distinct position on the array),....”).

277. Frisen Workflow further discloses that “each species” of capture probe comprises “(ii) a capture domain.” *Id.*, 7:24. Frisen discloses that the “capture domain” may be poly-T, EX1020, 18:24-26, or that “[t]he capture domain may be capable of binding selectively to a desired subtype or subset of nucleic acid,” such as “a particular mRNA species e.g. corresponding to a particular gene or group of genes. Such a capture probe may be selected or designed based on [the] sequence of the RNA it is desired to capture. Thus it may be a sequence-specific capture probe, specific for a particular RNA target or group of targets.” *Id.*, 19:15-24.

(i) “[wherein the capture agent comprises:] (i) a first sequence that [specifically] binds the nucleic acid, and”

278. Step 1(a)(i) of each Challenged Patent recites:

'468 Patent	(i) a first sequence that specifically binds the nucleic acid, and
'879 Patent	(i) a first sequence that binds the nucleic acid, and:

279. To the extent the '879 Patent's claim to a “sequence that binds the nucleic acid” is broader than the '468 Patent's claim to a “sequence that *specifically* binds the nucleic acid,” it is rendered obvious by the disclosure in Frisen of nucleic acids that “specifically bind” a target nucleic acid. *See also* '879, cl. 1(b) (requiring “the sequence of the nucleic acid *specifically* bound to the capture agent”); Section VII.A.

280. Frisen Workflow discloses that “each species” of capture probe comprises “(ii) a capture domain.” *Id.*, 7:24. Frisen discloses that the “capture domain” may be poly-T, *id.*, 18:24-26, or that “[t]he capture domain may be capable of binding selectively to a desired subtype or subset of nucleic acid,” such as “a particular mRNA species e.g. corresponding to a particular gene or group of genes. Such a capture probe may be selected or designed based on [the] sequence of the RNA it is desired to capture. Thus it may be a sequence-specific capture probe, specific for a particular RNA target or group of targets.” *Id.*, 19:15-24.

(ii) “[wherein the capture agent comprises:] (ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;”

281. Step 1(a)(ii) of each Challenged Patent is identical and recites:

'468 Patent	(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;
'879 Patent	(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array;

282. Frisen’s capture agents comprise “(ii) a second sequence comprising a coding tag, wherein a sequence of the coding tag corresponds to a location of the capture agent on the array.” Specifically, Frisen’s Workflow discloses “providing an

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

array comprising a substrate on which multiple species of capture probes are directly or indirectly immobilized such that each species occupies a distinct position on the array...wherein each species of said capture probe comprises a nucleic acid molecule from 5' to 3' (i) a positional domain that corresponds to the position of the capture probe on the array.” EX1020, 7:16-23.

- (b) **“determining [all or a portion of] the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.”**

283. Step 1(b) of each Challenged Patent is depicted below:

'468 Patent	(b) [i]determining all or a portion of the sequence of the coding tag, or a complement thereof, [ii] and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, [iii] and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.
'879 Patent	(b) [i] determining the sequence of the coding tag, or a complement thereof, [ii] and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof, [iii] and using the determined sequences to determine presence or abundance of the nucleic acid at a location in the tissue section.

284. In Frisen’s Workflow, after step (b) (contacting the array), Frisen discloses the steps of “(c) generating DNA molecules from the captured nucleic acid

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

molecules using said capture probes as extension or ligation primers, wherein said extended or ligated DNA molecules are tagged by virtue of their positional domain,” “(e) releasing at least part of the tagged DNA molecules and/or their complements or amplicons from the surface of the array, wherein said part includes the positional domain or a complement thereof;” and “(f) directly or indirectly analyzing the sequence of the released DNA molecules.” *Id.*, 7:29-8:5. For example, when the released DNA is sequenced this “results in a transcriptome with exact positional information” because each positional domain is “correlated with a position in the tissue sample.” *Id.*, 6:2-16; Figure 1. “The sequence data can then be matched to a position in the tissue sample, which enables the visualization, e.g. using a computer, of the sequence data together with the tissue section, for instance to display the expression pattern of any gene of interest across the tissue section (Figure 2).” *Id.*, 6:8-11.

(c) Conclusion on Claim 1

285. Accordingly, Frisen anticipates claim 1.

3. Claim 2

286. Claim 2 of each Challenged Patent is identical, as tabulated below.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent	The method of claim 1, wherein the method further comprises, between steps (a) and (b), a step of removing the tissue section from the array.
'879 Patent	The method of claim 1, wherein the method further comprises, between steps (a) and (b), a step of removing the tissue section from the array.

287. I incorporate by reference my analysis of claim 1. Frisen discloses removing the tissue section from the array between claimed steps (a) and (b):

The tissue sample, i.e. any residual tissue that remains in contact with the array substrate following the reverse transcription step and optionally imaging, if imaging is desired and was not carried out before reverse transcription, ***preferably is removed prior to the step of releasing the cDNA molecules from the array.*** Thus, the methods of the invention may comprise a step of washing the array. Removal of the residual tissue sample may be performed by any suitable means and will be dependent on the tissue sample. In the simplest embodiment, the array may be washed with water. The water may contain various additives, e.g., surfactants (e.g. detergents), enzymes etc to facilitate to [sic] removal of the tissue....

EX1020, 35:34-36:19.

4. Claim 6 ('468 Patent) / Claim 5 ('879 Patent)

288. Claim 6 of the '468 Patent is identical to Claim 5 of the '879 Patent, as tabulated below.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent	6. The method of claim 1, wherein the determining step comprises nucleic acid amplification.
'879 Patent	5. The method of claim 1, wherein the determining step comprises nucleic acid amplification.

289. I incorporate by reference my analysis of claim 1, above. Frisen discloses that “any method of nucleic acid analysis may be used in the analysis step” of Frisen’s Workflow. EX1019, 9:6-13. “Typically this may involve sequencing, but it is not necessary to perform actual sequence determination. For example sequence-specific methods of analysis may be used. For example a sequence-specific amplification reaction may be performed, for example using primers which are specific for the positional domain and/or for a specific target sequence, e.g. a particular target DNA to be detected (i.e. corresponding to a particular cDNA/RNA or gene etc.). An exemplary analysis method is a sequence-specific PCR reaction.”
Id.

5. Claim 7 ('468 Patent) / Claim 6 ('879 Patent)

290. Claim 7 of the '468 Patent is substantially the same as Claim 6 of the '879 Patent, as tabulated below. The additional language present in claim 7 of the '468 Patent is highlighted in green.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

'468 Patent	7. The method of claim 1, wherein the determining step comprises sequencing all or a portion of the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof.
'879 Patent	6. The method of claim 1, wherein the determining step comprises sequencing the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid bound to the capture agent, or a complement thereof.

291. I incorporate by reference my analysis of claim 1, above. As discussed with respect to claim 1, Frisen discloses that the determining step may comprise sequencing all or a portion of the sequence of the coding tag, or a complement thereof, and all or a portion of the sequence of the nucleic acid specifically bound to the capture agent, or a complement thereof. EX1019, 9:6-8 (“[A]ny nucleic acid analysis may be used in the analysis step. Typically this may involve sequencing....”). These claims are unpatentable as anticipated for the same reasons set forth with respect to step 1(b), above.

6. Claims 12-13 ('468 Patent) / Claims 11-12 ('879 Patent)

292. Claims 12-13 of the '468 Patent are identical to Claims 11-12 of the '879 Patent, as tabulated below.

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

'468 Patent	12. The method of claim 1, wherein the nucleic acid is RNA. 13. The method of claim 12, wherein the RNA is mRNA.
'879 Patent	11. The method of claim 1, wherein the nucleic acid is RNA. 12. The method of claim 11, wherein the RNA is mRNA.

293. I incorporate by reference my analysis of claim 1, above. Frisen further discloses:

It will be seen therefore that the array of the present invention may be used to *capture RNA, e.g. mRNA of a tissue sample* that is contacted with said array. The array may also be used for determining and/or analysing a partial or global transcriptome of a tissue sample or for obtaining a spatially defined partial or global transcriptome of a tissue sample. The methods of the invention may thus be considered as methods of quantifying the spatial expression of one or more genes in a tissue sample. Expressed another way, the methods of the present invention may be used to detect the spatial expression of one or more genes in a tissue sample. In yet another way, the methods of the present invention may be used to determine simultaneously the expression of one or more genes at one or more positions within a tissue sample. Still further, the methods may be seen as methods for partial or global transcriptome analysis of a tissue sample with two-dimensional spatial resolution.

EX1020, 11:21-33.

7. Claim 18 ('468 Patent) / Claim 17 ('879 Patent)

294. Claim 18 of the '468 Patent is substantially the same as Claim 17 of the '879 Patent, as tabulated below. The additional language present in claim 18 of the '468 Patent is highlighted in green. Because “wherein the capture agent is immobilized directly” is anticipated by the applied prior art, both claims are anticipated for the same reasons.

'468 Patent	18. The method of claim 1, wherein the capture agent is immobilized directly or indirectly on a surface of the feature.
'879 Patent	17. The method of claim 1, wherein the capture agent is immobilized directly on a surface of the feature.

295. I incorporate by reference my analysis of claim 1, above.

296. Frisen’s Workflow discloses “providing an array comprising a substrate on which multiple species of capture probes are directly or indirectly immobilized such that each species occupies a distinct position on the array...wherein each species of said capture probe comprises a nucleic acid molecule from 5’ to 3’ (i) a positional domain that corresponds to the position of the capture probe on the array.” EX1020, 7:16-21.

297. Frisen also discloses immobilizing the capture agents to beads, for example using “[c]ommercial arrays” such as “an Illumina bead array.” EX1020, 15:30-33. Frisen further explains that the “probes on a microarray may be

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

immobilized, i.e. attached or bound, to the array preferably via the 5' or 3' end, depending on the chemical matrix of the array.” *Id.*, 16:3-5. “The covalent linkage used to couple a nucleic acid probe on an array substrate may be viewed as both a direct and indirect linkage, in that although the probe is attached by a ‘direct’ covalent bond there may be a chemical moiety or linker separating the ‘first’ nucleotide of the nucleic acid probe from the, e.g, glass or silicon, substrate, i.e. an indirect linkage.” *Id.*, 16:10-17.

8. Claim 20 ('468 Patent) / Claim 21 ('879 Patent)

298. Claim 20 of the '468 Patent is identical to Claim 21 of the '879 Patent, as tabulated below.

'468 Patent	20. The method of claim 1, wherein the array comprises a bead array.
'879 Patent	21. The method of claim 1, wherein the array comprises a bead array.

299. I incorporate by reference my analysis of claim 1, above. Frisen further explains that its methods involve the implementation “of standard arrays [that] are commercially available and both the number and size of the features may be varied.” EX1020, 15:21-24. Frisen explains that the arrays suitable for its methods include “[c]ommercial arrays...available or known for use in the context of sequence analysis and in particular in the context of NGS technologies. Such arrays may also

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

be used as the array surface in the context of the present invention e.g. an Illumina bead array.” *Id.*, 15:30-33. Thus, Frisen discloses providing its array of capture probes or capture agents by providing them on a plurality of beads in a bead array.

9. Claim 21 ('468 Patent) / Claim 23 ('879 Patent)

300. Claim 21 of the '468 Patent is substantially the same as Claim 23 of the '879 Patent, as tabulated below. The additional language present in claim 21 of the '468 Patent is highlighted in green. Because the use of “a fresh frozen tissue section” is anticipated by the applied prior art, both claims are anticipated for the same reasons.

'468 Patent	21. The method of claim 1, wherein the tissue section is a fresh frozen tissue section or a formalin-fixed paraffin-embedded (FFPE) tissue section.
'879 Patent	23. The method of claim 1, wherein the tissue section is a fresh frozen tissue section.

301. I incorporate by reference my analysis of claim 1, above. Frisen discloses that “[t]he tissue sample may be prepared in any convenient or desired way and the invention is not restricted to any particular type of tissue preparation. Fresh, frozen, fixed or unfixed tissues may be used. Any desired convenient procedure may be used for fixing or embedding the tissue sample, as described and known in the art.” EX1020, 29:25-29.

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

302. Frisen expressly calls out fresh frozen tissue as an example of such a suitable technique:

As a first representative example of a tissue sample for use in the invention, the tissue may be prepared by deep freezing at a temperature suitable to maintain or preserve the integrity (i.e. the physical characteristics) of the tissue structure, e.g. less than -20°C and preferably less than -25, -30, -40, -50, -60, -70 or -80 °C. The frozen tissue sample may be sectioned, i.e. thinly sliced, onto the array surface by any suitable means. For example, the tissue sample may be prepared using a chilled microtome, a cryostat, set at a temperature suitable to maintain both the structural integrity of the tissue sample and the chemical properties of the nucleic acids in the sample, e.g. to less than -15°C and preferably less than -20 or -25°C. Thus, the sample should be treated so as to minimize the degeneration or degradation of the nucleic acid, e.g. RNA in the tissue. Such conditions are well-established in the art and the extent of any degradation may be monitored through nucleic acid extraction, e.g. total RNA extraction and subsequent quality analysis at various stages of the preparation of the tissue sample.

EX1020, 29:30-30:8.

303. Frisen also expressly calls out FFPE tissue sections as suitable for its methods:

IPR2025-00236
IPR2025-00237
Patent No. 10,662,468
Patent No. 11,001,879

In a second representative example, the tissue may be prepared using standard methods of formalin-fixation and paraffin-embedding (FFPE), which are well-established in the art. Following fixation of the tissue sample and embedding in a paraffin or resin block, the tissue samples may be sectioned, i.e. thinly sliced, onto the array. As noted above, other fixatives and/or embedding materials can be used.

EX1020, 30:9-13.

10. Claim 23 ('468 Patent) / Claim 27 ('879 Patent)

304. Claim 23 of the '468 Patent is identical to Claim 27 of the '879 Patent, as tabulated below.

'468 Patent	23. The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.
'879 Patent	27. The method of claim 1, wherein the method further comprises generating a map of the presence or abundance of the nucleic acid at location(s) in the tissue section.

305. I incorporate by reference my analysis of claim 1. Frisen discloses generating a map of gene expression, i.e., the presence or abundance of mRNAs in the tissue section: "The sequence data can then be matched to a position in the tissue sample, which enables the visualization e.g. using a computer of the sequence data together with the tissue section, for instance to display the expression pattern of any gene of interest across the tissue (Figure 2)." EX1020, 6:8-11.

11. Claims 25-26 ('468 Patent) / Claim 28 ('879 Patent)

306. Claim 26 of the '468 Patent is identical to Claim 28 of the '879 Patent, as tabulated below. Claim 25 of the '468 Patent is broader, and is anticipated for the same reasons.

'468 Patent	25. The method of claim 1, wherein the array comprises greater than 500 features. 26. The method of claim 1, wherein the array comprises 30 greater than 1,000 features.
'879 Patent	28. The method of claim 1, wherein the array comprises greater than 1,000 features.

307. I incorporate by reference my analysis of claim 1, above. Frisen's arrays may contain greater than 1,000 features. EX1020, 28:1-8. ("In representative embodiments of the invention, an array may contain at least 2, 5, 10, 50, 100, 500, 750, 1000, 1500, 3000, 5000, 10000, 20000, 40000, 50000, 75000, 100000, 150000, 200000, 300000, 400000, 500000, 750000, 800000, 1000000, 1200000, 1500000, 1750000, 2000000, 2100000. 3000000, 3500000, 4000000 or 4200000 features. Whilst 4200000 represents the maximum number of features presently available on a commercial array, it is envisaged that arrays with features in excess of this may be prepared and such arrays are of interest in the present invention.").

12. Claim 30 ('468 Patent) / Claim 30 ('879 Patent)

308. Claim 30 of the '468 Patent is substantially the same as Claim 30 of the '879 Patent, as tabulated below. The additional language present in claim 30 of the '468 Patent is highlighted in green.

'468 Patent	30. The method of claim 1, wherein a feature of the plurality of features comprises greater than 100 capture agents, where each of the greater than 100 capture agents comprises a first sequence that specifically binds to a different nucleic acid and a coding tag that corresponds to a location of the capture agent on the array.
'879 Patent	30. The method of claim 1, wherein a feature of the plurality of features comprises greater than 100 capture agents, where each of the greater than 100 capture agents comprises a first sequence that binds to a different nucleic acid and a coding tag that corresponds to a location of the capture agent on the array.

309. I incorporate by reference my analysis of claim 1, above. Each of Frisen's features comprises capture agents comprising a first sequence that binds to a nucleic acid and a coding tag that corresponds to the location of the capture agent on the array, as discussed in relation to claim 1. Moreover, Frisen discloses that each feature can comprises a multiplicity of capture agents, each capture agent with a different capture domain and a positional tag that corresponds to position of the feature/capture agent on the array:

The typical array of, and for use in the methods of, the invention may contain multiple spots, or "features". A feature may be defined as an

area or distinct position on the array substrate at which a single species of capture probe is immobilized. Hence each feature will comprise a multiplicity of probe molecules, of the same species....Each species of capture probe will have the same positional domain (i.e. each member of a species and hence each probe in a feature will be identically "tagged"), but the sequence of each member of the feature (species) may differ, because the sequence of a capture domain may differ. As described above, random or degenerate capture domains may be used. Thus the capture probes within a feature may comprise different random or degenerate sequences.

EX1020, 27:4-16. Frisen further discloses that “the term ‘multiple means two or more, or at least two, e.g., 1000, 2000, 5000, 10,000, or more.” *Id.*, 12:19-25 (“[I]t is envisaged that conventional-type arrays with many hundreds, thousands, tens of thousands, hundreds of thousands or even millions of capture probes may be used.”). A POSA would understand from these disclosures that Frisen envisaged using high-density arrays with greater than 100 capture agents per feature, and that each of the capture probes in Frisen’s features can be designed to bind a different molecule than the other capture probes present at a feature.

310. In addition, I understand that in the District Court litigation, Patent Owner contends that the limitation “each of the greater than 100 capture agents comprises a first sequence that binds to a different nucleic acid” is satisfied by

IPR2025-00236

IPR2025-00237

Patent No. 10,662,468

Patent No. 11,001,879

multiple poly-T tails on the same bead that bind polyA tails on mRNA. To the extent that this argument can be understood, it appears that Patent Owner is interpreting the claimed “first sequence that binds to a different nucleic acid” to be satisfied by a sequence on the capture probe that binds to any molecule present in a sample, as long as another capture probe is not already bound to that molecule, rather than requiring the claimed capture probes to each have a first sequence that binds to/is complementary to a different nucleic acid sequence.

311. Thus, because nucleic acid hybridization interactions generally involve the formation of duplexes through base-pairing, each of the 100 or more probe sequences present on one of Cantor’s beads necessarily binds to a different nucleic acid under Patent Owner’s interpretation of the claims as well.

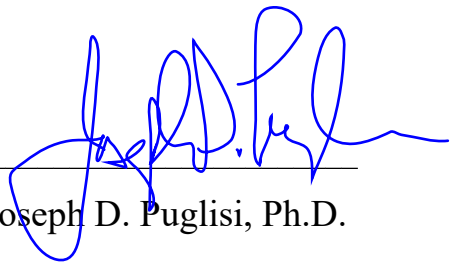
IX. Conclusion

312. For the reasons set forth above, it is my opinion that the challenged claims are not patentable.

IPR2025-00236
IPR2025-00237
U.S. Patent No. 10,662,468
U.S. Patent No. 11,001,879

I hereby declare that all statement 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 1001 of Title 18 of the United States Code.

Dated: November 27, 2024

BY: 
Joseph D. Puglisi, Ph.D.