

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BIO-RAD LABORATORIES, INC.,
Petitioner,

v.

CALIFORNIA INSTITUTE OF TECHNOLOGY,
Patent Owner

Case No. IPR2024-01451

U.S. Patent No. 11,827,921 B2

DECLARATION OF KEVIN STRUHL, PH.D.

TABLE OF CONTENTS

	<u>Page</u>
I. INTRODUCTION	1
II. EXPERT QUALIFICATIONS AND CREDENTIALS.....	1
III. BASIS FOR OPINIONS, AND MATERIALS REVIEWED.....	3
IV. UNDERSTANDING OF THE LAW	9
A. A Person Having Ordinary Skill in the Relevant Field During the Relevant Time	9
B. Claim Interpretation in <i>Inter Partes</i> Review.....	11
C. Anticipation	12
D. Obviousness.....	12
E. Priority Date	14
V. BACKGROUND ON RELEVANT TECHNOLOGY.....	15
A. Technology	15
B. The '921 Patent and the Challenged Claims	19
C. The '921 Patent's Prosecution History	26
D. Overview of Larson.....	26
E. Overview of Saxonov	42
F. Saxonov Provisional and Priority Date	52
G. Overview of Silverbrook.....	54
VI. CLAIM CONSTRUCTION	54
VII. GROUNDS OF CHALLENGE.....	55
A. Ground 1 (Claims 1-10 and 13-19): Anticipation by Larson...	55
B. Ground 2 (Claims 2, 4, 6-7, and 14-19): Obviousness in View of Larson.....	62
C. Ground 3 (Claims 1-10 and 12-19): Obviousness In View of Larson and Saxonov	63

D.	Ground 4 (Claim 11): Obviousness In View Of Larson, Saxonov, and Silverbrook	78
VIII.	DECLARATION	79

DECLARATION OF DR. KEVIN STRUHL

I, Kevin Struhl, declare as follows:

I. INTRODUCTION

1. I am making this Declaration at the request of California Institute of Technology (“Patent Owner” or “Caltech”) in the matter of IPR2024-01451, a petition for *Inter Partes* Review of U.S. Patent No. 11,827,921 (“the ’921 Patent”).

2. I am being compensated for my work in these matters at a rate of \$1200 per hour for my professional consulting services. I am also reimbursed for my reasonable expenses incurred in connection with my work in this proceeding. My compensation is not in any way contingent upon the outcome of this *Inter Partes* Review. I have no interest in the outcome of this proceeding or any related litigation.

II. EXPERT QUALIFICATIONS AND CREDENTIALS

3. My qualifications for presenting the opinions in this Declaration are set forth below and in my *curriculum vitae*, a copy of which is provided as EX2002.

4. I earned Bachelor’s and Master’s degrees in biology from the Massachusetts Institute of Technology (MIT) in 1974. I earned a Ph.D. in biochemistry from Stanford University in 1979. I have been a professor of Biological Chemistry and Molecular Pharmacology at Harvard University since

1982.

5. In addition to teaching courses in molecular biology and biochemistry while serving on the faculty at Harvard University for nearly 43 years, I actively researched various topics combining genetic, molecular, biochemical, genomic, and evolutionary approaches to study many aspects of gene regulation in yeast, and the processes of cellular transformation and formation of cancer stem cells. In the course of conducting my research, I have used multiplex polymerase chain reaction (PCR). Indeed, I started using multiplex PCR twenty years ago, which is before real-time PCR machines existed.

6. I have also accrued substantial industry experience. For example, I served as a member of the advisory board for Pharmagenics Inc. from 1991 to 1997. I was also a member of the advisory board for Scriptgen Inc. from 1993 to 2000. I also was a member of the advisory board for Sangamo Inc. from 1999 to 2015. I am currently a member of the advisory board for UTR Therapeutics.

7. I have authored or co-authored over three hundred publications in the fields of biochemistry and molecular biology.

8. Additionally, I was one of the founding editors of *Current Protocols in Molecular Biology*, the manual which publishes the standards for experimental design, methods, and analyses in all areas of molecular biology. I have authored procedures for quantitative PCR for this journal.

9. I have been qualified as a PCR and/or DNA sequencing expert in multiple litigations, including in proceedings before U.S. District Courts, the International Trade Commission, and Patent Trial and Appeal Board.

III. BASIS FOR OPINIONS, AND MATERIALS REVIEWED

10. The opinions set forth in my Declaration are based on my personal knowledge gained from my education, on my personal experience, and on the review of the documents and information described in this Declaration.

11. In preparation of this Declaration, I have considered:

- Exhibit 1001: U.S. Patent No. 11,827,921 “the ’921 Patent”
- Exhibit 1002: Declaration of Carl A. Batt, Ph.D.;
- Exhibit 1003: Joint Claim Construction Statement, filed on June 18, 2024, Dkt No. 65, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23- cv-06360, N.D. Cal.;
- Exhibit 1004: U.S. Patent Application Publication No. US 2013/0040841 A1 “Saxonov”;
- Exhibit 1005: Information Disclosure Statement noting Saxonov from Prosecution File History of U.S. Patent No. 10,068,051 filed on March 23, 2018;
- Exhibit 1006: Notice of Allowance from Prosecution File History of U.S. Patent No. 11,827,921 filed on August 10, 2023;

- Exhibit 1007: U.S. Patent Provisional Application No. 61/510,013
(filed July 20, 2011) (Serge Saxonov, Simant Dube, John F. Regan, applicants) “Saxonov Provisional”;
- Exhibit 1009: U.S. Patent Application Publication No. US
2011/0312704 A1 “Silverbrook”;
- Exhibit 1012: U.S. Patent No. 11,827,921 Infringement Claim
Charts from Infringement Contentions, served on March 21, 2024,
ChromaCode, Inc. et al. v. Bio-Rad Laboratories, Inc., Case No.
5:23-cv-06360, N.D. Cal.;
- Exhibit 1013: Internet Archive WayBackMachine, Feb. 22, 2024,
archived copy of Synbio Technologies, Precision and Accuracy
with TaqMan Probes, <https://synbio-tech.com/precision-and-accuracy-with-taqman-probes/>,
<https://web.archive.org/web/20240519044916/https://synbiotech.com/precision-and-accuracy-with-taqman-probes/>, accessed Sept.
12, 2024;
- Exhibit 1014: Pamela M. Holland, et al., “Detection of specific
polymerase chain reaction product by utilizing the 5’ → 3’
exonuclease activity of *Thermus aquaticus* DNA polymerase,”

Proc. Natl. Acad. Sci. USA, Vol. 88, pp. 7276-7280, August 1991

Biochemistry “TaqMan Paper”;

- Exhibit 1015: Information Disclosure Statement from Prosecution File History of U.S. Patent No. 10,770,170 filed on March 7, 2018;
- Exhibit 1016: Information Disclosure Statement from Prosecution File History of U.S. Patent No. 11,827,921 filed on March 25, 2021;
- Exhibit 1017: U.S. Patent Application Publication No. US 2011/0250597 A1 “Larson”;
- Exhibit 1022: U.S. Patent No. 9,366,632 “Link”;
- Exhibit 1023: Federal Judicial Caseload Statistics (2023), at <https://www.uscourts.gov/statistics-reports/analysis-reports/federaljudicial-caseload-statistics>;
- Exhibit 1024: Civil Minutes - General, filed on December 7, 2023, Dkt No. 34, *ChromaCode, Inc. et al. v. Bio-Rad Laboratories*, Case No. 2:23-cv-08417, C.D. Cal.;
- Exhibit 1025: Patent Owner’s Response to Non-Final Office Action from Prosecution File History of U.S. Patent No. 11,827,921 filed on March 28, 2023;

- Exhibit 1026: Final Rejection from Prosecution File History of U.S. Patent No. 11,827,921 filed on May 10, 2023;
- Exhibit 1027: Joint Stipulation and Order Regarding Filing of Second Amended Complaint and Amended Case Schedule, filed on March 27, 2024, Dkt No. 53, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360, N.D. Cal.;
- Exhibit 1028: Amended Complaint for Patent Infringement of U.S. Patent Nos. 10,068,051 and 10,770,170, filed on October 13, 2023, Dkt No. 12, *ChromaCode, Inc. et al. v. Bio-Rad Laboratories*, Case No. 2:23-cv-08417, C.D. Cal.;
- Exhibit 1029: Patent Owner's Response to Final Office Action from Prosecution File History of U.S. Patent No. 11,827,921 filed on July 10, 2023;
- Exhibit 1030: Non-Final Rejection from Prosecution File History of U.S. Patent No. 11,827,921 filed on December 28, 2022;
- Exhibit 1033: U.S. Patent No. 9,441,266 "Larson patent";
- Exhibit 1036: Nucleic Acids, <https://www.genome.gov/genetics-glossary/Nucleic-Acids>, updated July 7, 2024;

- Exhibit 1037: Introduction of Molecular Beacons,
https://molecularbeacons.org/MB_introduction.html (downloaded July 10, 2024)
- Exhibit 1038: Shivaprasad H. Sathyanarayana and Lauren M. Wainman, “Chapter 2, Laboratory approaches in molecular pathology: the polymerase chain reaction,” *Diagnostic Molecular Pathology*, 2024;
- Exhibit 1039: U.S. Patent No. 10,068,051 Infringement Claim Charts from Infringement Contentions, served on March 21, 2024, *ChromaCode, Inc. et al. v. Bio-Rad Labs.*, Case No. 5:23-cv-06360, N.D. Cal.;
- Exhibit 1040: U.S. Patent No. 10,770,170 Infringement Claim Charts from Infringement Contentions, served on March 21, 2024, *ChromaCode, Inc. et al. v. Bio-Rad Labs.*, Case No. 5:23-cv-06360, N.D. Cal.;
- Exhibit 1041: Order of Consolidation filed August 16, 2024, Dkt. No. 77, *ChromaCode, Inc. v. Bio-Rad Labs., Inc.*, Case No. 5:23-cv-04823, N.D. Cal.;

- Exhibit 1042: Order Reassigning Case filed August 20, 2024, Dkt. No. 78, *In re: ChromaCode Litig.*, Case No. 5:23-cv-04823, N.D. Cal.;
- Exhibit 2002: *Curriculum Vitae* of Kevin Struhl, Ph.D.;
- Exhibit 2003: File History of U.S. Patent No. 11,827,921;
- Exhibit 2004: U.S. Provisional Application No. 61/507,082;
- Exhibit 2005: Patent Scheduling Order dated March 8, 2024, Dkt No. 47, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.);
- Exhibit 2006: Petitioner's Amended Invalidity Contentions, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.)
- Exhibit 2007: Comparison of Saxonov and Saxonov Provisional
- Exhibit 2008: Appendix M to Bio-Rad's Amended Invalidity Contentions;
- Exhibit 2009: Appendix I to Bio-Rad's Amended Invalidity Contentions;
- Exhibit 2010: Docket, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.);

- Exhibit 2011: Plaintiff’s Opening Claim Construction Brief, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.); and
- Exhibit 2012: Reply Claim Construction Brief, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.).

IV. UNDERSTANDING OF THE LAW

12. I am not an attorney. In preparing and expressing my opinions and considering the subject matter of the '921 Patent, I am relying on certain basic legal principles that have been explained to me. These principles are discussed below.

A. A Person Having Ordinary Skill in the Relevant Field During the Relevant Time

13. I understand that the disclosures of patents and prior art references are to be viewed from the perspective of a mythical person of ordinary skill in the relevant field (sometimes referred to by lawyers as “the relevant art”) at the relevant timeframe (a “POSA”). I understand that, for purposes of this IPR proceeding, I should interpret the term POSA as referring to a mythical person to whom an expert in the relevant field could have assigned a routine task with reasonable confidence that the task would be successfully carried out.

14. I have been asked to provide my opinion regarding the “level of ordinary skill in the art” at the time of the invention, which I understand is on or before February 3, 2012.

15. I understand that the hypothetical person of ordinary skill in the art is considered to have the normal skills and knowledge of a person in a certain technical field. I understand that factors that may be considered in determining the level of ordinary skill in the art include: (1) the education level of the inventor; (2) the types of problems encountered in the art; (3) the prior art solutions to those problems; (4) rapidity with which innovations are made; (5) the sophistication of the technology; and (6) the education level of active workers in the field. I also understand that “the person of ordinary skill” is a hypothetical person who is presumed to be aware of the universe of available prior art.

16. I also understand the level of ordinary skill in the art can be evidenced by the prior art. Accordingly, I have also considered the prior art discussed herein in determining the level of ordinary skill in the art.

17. Petitioner asserts that a POSA relevant to the '921 Patent as of February 3, 2012 would have a Ph.D. in molecular biology, genetics, biochemistry, or a related discipline, with at least a year of experience in quantitative and/or digital PCR and fluorescence-based analyte detection. Petition, 9-10.

Alternatively, the Petitioner asserts that a POSA would have been someone with

greater relevant experience sufficient to compensate for having less relevant formal education. *Id.* The Petitioner states that a POSA would have “understood” several technologies and techniques which are enumerated in the Petition. *Id.*

18. I understand that, for purposes of this IPR proceeding, PO does not contest Petitioner’s description of qualifications for a POSA. Therefore, I adopt Petitioner’s definition of a POSA for purposes of this declaration.

19. I would qualify as at least a POSA at all relevant times for purposes of this proceeding, including at least as early as the priority date of the ’921 Patent. I have a Ph.D. in biochemistry with at least a year of experience in quantitative and/or digital PCR and fluorescence-based analyte detection.

B. Claim Interpretation in *Inter Partes* Review

20. I understand that the first step in determining the validity of a claim is for the claim to be properly construed. I understand that “claim construction” is the process of determining a patent claim’s meaning. I also understand that the proper construction of a claim term is the meaning that a POSA would have given to that term during the relevant timeframe.

21. For purposes of *Inter Partes Review*, I understand that the Board applies the *Phillips* standard, under which claims are to be given their ordinary and customary meaning as understood by a POSA at the time the application was filed. I understand that this analysis is performed by primarily looking to the patent’s

claims, specification (including drawings), and prosecution history (the “intrinsic” evidence). I understand that if the claim is unclear in view of the intrinsic evidence, then outside (“extrinsic”) evidence also may be considered.

C. Anticipation

22. I understand that a patent claim is unpatentable as anticipated if a POSA during the relevant timeframe would have understood a single prior art reference to disclose an embodiment that expressly or inherently teaches every limitation contained in the claim. The disclosure in a reference does not have to be in the same words as the claim, but all of the requirements of the claim must be described in enough detail, or necessarily inherent in an embodiment disclosed in the reference, to enable a POSA looking at the reference to practice the claimed invention.

D. Obviousness

23. I understand that a patent claim also, or alternatively, is unpatentable if it would have been obvious to a POSA during the relevant timeframe. I understand that the following factors must be evaluated to determine whether the claimed subject matter was obvious: (1) the scope and content of the prior art; (2) the difference or differences, if any, between the subject matter defined by the claim of the patent under consideration and what is disclosed in the prior art; (3) the level of ordinary skill in the relevant field at the time of the patent’s earliest

priority date or, possibly, at an earlier time; and (4) so-called “objective evidence of non-obviousness.” However, I understand that, for the present, I can ignore both the possibility that obviousness is to be determined at a date earlier than the patent’s earliest priority date and the possibility that Patent Owner will submit objective evidence of non-obviousness.

24. I understand that prior art references can be combined to support a finding that a claim is unpatentable for obviousness when there was an apparent reason for one of ordinary skill in the art, at the time of the invention, to combine the references and that such apparent reasons include: (A) identifying a teaching, suggestion, or motivation to combine prior art references; (B) combining prior art methods according to known methods to yield predictable results; (C) substituting one known element for another to obtain predictable results; (D) using a known technique to improve a similar device in the same way; (E) applying a known technique to a known device ready for improvement to yield predictable results; (F) trying a small number of identified, predictable potential solutions, with a reasonable expectation of success; and (G) identifying that known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the relevant field.

25. Moreover, I understand that so-called “objective indicia of non-obviousness” (also known as “secondary considerations”) like the following can be considered when assessing obviousness: (1) commercial success; (2) long-felt but unresolved needs; (3) copying of the invention by others in the field; (4) initial expressions of disbelief by experts in the field; (5) failure of others to solve the problem that the inventor solved; and (6) unexpected results. I also understand that evidence of objective indicia of non-obviousness must be commensurate in scope with the claimed subject matter.

E. Priority Date

26. I understand that to be entitled a priority date of an earlier-filed application, the earlier-filed application must provide support for the invention claimed in the later-filed application as required by the “written description” requirements of 35 U.S.C. § 112. I understand that an adequate written description requires showing that the inventor was in possession of the invention at the time of the patent application’s filing.

27. I understand that in order to show the possession of the claimed invention, a POSA must discern the claimed invention with all of its limitations based on disclosure of the patent or patent application at issue, including the originally filed claims, specifications, and/or the drawings.

V. BACKGROUND ON RELEVANT TECHNOLOGY

A. Technology

28. A variety of techniques, including polymerase chain reaction (PCR), have been developed to detect a target polynucleotide analyte, such as a particular genetic sequence, within a biological sample. *See* EX1001('921 Patent), 11:12-17, 11:55-12:35; *see also, e.g.*, EX1017(Larson), ¶¶3-6 (“Assays have been developed that rely on analyzing nucleic acid molecules from bodily fluids for the presence of mutations...The advent of PCR and real-time PCR methodologies has greatly improved the analysis of nucleic acids from both throughput and quantitative perspectives.”).

29. Caltech’s innovations described and claimed in the '921 Patent and its priority applications addressed a long-standing problem in the field: namely, as the number of analytes in a biological sample increases, it quickly becomes significantly more complicated and difficult to uniquely identify those analytes. EX1001('921 Patent), 1:35-47. The term “multiplexing” refers to a broad class of techniques for detecting multiple analytes in a biological sample under a single test (also referred to as an “assay”). EX1001('921 Patent), 1:35-47; *see also* EX1017(Larson), ¶45. Early assays used PCR to amplify (create copies of) each of

the target polynucleotide analytes, and often involved gel electrophoresis to separate the various PCR products that distinguished the analytes.

30. A subsequent multiplex PCR approach uses “probes” to bind to target analytes and fluoresce with different colorimetric labels (fluorophores) corresponding to the different analytes, with results being detected with appropriate optical channels. EX1001('921 Patent), 11:18-51, 8:33-62; *see also* EX1017(Larson), ¶¶4-5 (“qPCR reactions are monitored either using a variety of highly sequence specific fluorescent probe technologies[.]”). Optical channels can be configured to detect specific wavelength(s) of light, *i.e.*, colors the probes respectively produce when the probes bind or hybridize to complementary genetic sequences in the target analytes, such that each optical channel corresponds to a probe color (wavelength). EX1001('921 Patent), 28:27-45. For example, a probe for a particular genetic sequence is designed to be complementary to that sequence, and they bind via hybridization. *See* EX1002, ¶¶36-37, 39-40, 42; EX1001('921 Patent), 11:18-51. The fluorophore emits light only if the probe has bound its target within the sample. EX1001('921 Patent), 9:20-32, 11:18-51. A TaqMan probe is a particular type of probe that functions by releasing a fluorescent reporter molecule upon the probe binding to its target DNA sequence and subsequent PCR amplification, wherein the 5' exonuclease activity of the extending DNA polymerase cleaves the probe, separating the fluorescent reporter from a quencher

molecule, resulting in an increase in fluorescence. EX1001('921 Patent), 10:32-34 (“‘TAQMAN probe’ generally refer[s] to hydrolysis probes used to detect the presence of an analyte in a polynucleotide amplification assay.”); EX1017(Larson), ¶47; *see also* EX1002(Batt Decl.), ¶¶36-37, 39-40, 42. Such techniques to detect a target polynucleotide analyte in a biological sample were generally known, and commercially available TAQMAN probes were, and still are, commonly used to detect specific genetic sequences in this manner.

31. For example, a probe with a blue fluorophore binds analyte A and its light is observed in a blue channel; and a probe with a red fluorophore binds analyte B and its light is observed in a red channel. EX1001('921 Patent), 11:55-12:35. Of course, increasing the numbers of probe types (each with distinct fluorophore color) and channels (one per color) increases the number detectable analytes. EX1001('921 Patent), 12:39-45. However, this has the limitation of the number of distinct colorimetric probes and optical channels that could be assayed simultaneously. *Id.*

32. Some multiplexing techniques use probes with colorimetric labels (fluorophores) of the same color. *See* EX1017(Larson), ¶47; EX1001('921 Patent), 8:33-44. Here, color alone does not distinguish the analytes from one another: light in the channel means an analyte is present, but does not identify *which* analyte is present. *Id.* Analytes that are indistinguishable from one another in a

given assay may be referred to as “degenerate.” *See* EX1001(’921 Patent), 16:46-56. Some techniques (*e.g.*, Larson) tried to avoid this problem by (i) highly diluting the sample, (ii) physically dividing the diluted sample into droplets, (iii) observing the intensity of light from each droplet, and (iv) drawing conclusions about the analytes based on that intensity. *See, e.g.*, EX1017(Larson), ¶6. For example, probes with the same color could be designed to fluoresce at different intensities, so as to identify the analyte using the intensity. *See* EX1017(Larson), ¶12.

33. When a dilute sample is divided into droplets, different droplets may contain different numbers of unique analyte molecules. EX1017(Larson), ¶6. The “Poisson distribution” describes the statistics of how many molecules the droplets contain. *See id.* In the “limiting dilution” regime, the majority of droplets contain zero analyte molecules, fewer droplets contain exactly one analyte molecule, even fewer contain exactly two analyte molecules, still fewer contain exactly three analyte molecules, and so on. EX1017(Larson), ¶6. At terminal dilution, the sample is sufficiently diluted that the Poisson distribution basically guarantees that almost all droplets are either empty or contain only a single analyte molecule, with droplets rarely containing multiple analyte molecules. *Id.* At terminal dilution, to best avoid droplets containing multiple analyte molecules, most droplets are empty, which wastes material and time.

34. The '921 Patent refers to analytes that are indistinguishable from one another in a given assay as being “degenerate.” EX1001('921 Patent), 16:47-50. At the time the '921 Patent and its priority applications were filed, a significant need remained for inexpensive, material-efficient, and time-efficient multiplexing that could non-degenerately identify a higher number of targets within a given sample, without the need to add another optical channel corresponding to each additional analyte. EX1001('921 Patent), 1:35-47, 8:45-62.

B. The '921 Patent and the Challenged Claims

35. The '921 Patent inventors conceived of a solution – described and claimed in the '921 Patent and its priority applications – that enables non-degenerate detection of more polynucleotide analytes in a single sample solution volume, in any combination of presence or absence, than the number of wavelength components used to detect them. The application that issued as the '921 Patent was filed on May 1, 2020 as a continuation of application no. 15/914,356, filed March 7, 2018, which was a continuation of application no. 14/451,876, filed August 5, 2014 as a continuation of Application No. 13/756,760, filed February 1, 2013, now U.S. Patent No. 8,838,394. EX1001('921 Patent), §§(22), (63). The '921 Patent claims priority to Provisional Application Nos. 61/594,480, filed February 3, 2012 and 61/703,093, filed September 19, 2012. EX1001('921 Patent), §(60).

36. The '921 Patent inventors recognized that in traditional multi-channel methods such as described above, “the number of detectable analytes is equal to the number of spectrally resolvable fluorophores. Therefore, the number of analytes may only be increased by increasing the number of spectrally resolvable fluorophores.” EX1001('921 Patent), 12:39-45. The inventors developed a technique to non-degenerately identify analytes in a sample, using *fewer* fluorophores than the number of analytes. *Id.*, 8:45-66. Such scheme is distinctive from prior multi-channel techniques or terminal dilution-based techniques described above. *Id.*; see ¶¶28-33 of this Declaration. Instead, “each analyte to be detected is encoded as a value in each of two components of a signal (*e.g.*, intensity and wavelength),” these components are cumulatively measured for the sample, and the “presence or absence of an analyte may then be determined based on the values used to encode the presence of the analyte (*i.e.*, those values in the coding scheme).” *Id.*, 8:66-9:5, 9:41-47.

37. Table 3 of the '921 Patent, reproduced below, describes an example in which seven analytes (A-G), plus control, are encoded using combinations of four fluorophores (fewer fluorophores than analytes):

TABLE 3

Encoding of eight analytes (including control) with four colors and up to four colors per analyte.				
	Blue	Green	Yellow	Red
Ctrl. (p)	1	0	0	0
A	1	0	0	1
B	1	0	1	0
C	1	1	0	0
D	1	0	1	1
E	1	1	0	1

TABLE 3-continued

Encoding of eight analytes (including control) with four colors and up to four colors per analyte.				
	Blue	Green	Yellow	Red
F	1	1	1	0
G	1	1	1	1

EX1001('921 Patent), 13:44-14:9.

38. As the '921 Patent explains,

The presence of the control and analyte A is indicated by a result of 2001. The presence of the control and analyte G is indicated by a result of 2111. These results express the cumulative intensity of the signal in each color. For example, the result 2111 has a 2X signal intensity in the blue channel, while the result 1000 has only a 1 X signal intensity in the blue channel.

Id., 14:14-21.

39. While these example results are for a single analyte, the '921 Patent also explains how the inventive coding method of Table 3 may be used to detect ***combinations*** of different analytes in a single sample volume. For example, the following excerpt from Table 4 demonstrates that the cumulative signal intensity from the combination of analytes A-F in a single sample volume is different, and measurably distinguishable from, that from the combination of analytes A-G in a single sample volume:

7	3	3	3	pABCDEF
8	4	4	4	pABCDEFG

EX1001('921 Patent), 15:60-61.

40. The '921 Patent inventors recognized that in some circumstances, a result may be degenerate “because it can indicate more than one possibility in

terms of the presence or absence of an analyte. For example, with reference to Table 4, result 5233 is degenerate because it can be decoded as either pADFG or pBDEG.” *Id.*, 16:47-52. The inventors solved this problem by reducing or eliminating degeneracy from the coding scheme, “thereby increasing the confidence with which an analyte is detected.” *Id.*, 16:57-59. The ’921 Patent describes different ways to eliminate degeneracy such that every possible combination of analytes has a unique cumulative intensity. *Id.*, 22:11-23:56.

41. One example method of eliminating degeneracy according to the ’921 Patent encodes the individual analyte states using intensity levels that are separated by integer $\log_2$ differences. *Id.* This design is non-degenerate because for N analytes, there are 2^N states, and with each analyte uniquely encoded by a $\log_2$ encoding intensity value (1,2,4,8,16, etc.). The cumulative sums of analyte intensities for any combinatorial state using this $\log_2$ encoding method produce a unique cumulative intensity value for each state. This is because any N digit combinatorial state can be represented as a binary number with N digits. The presence or absence of eight different analytes in an assay can be encoded using a binary representation, such as (00100110). In this system, each analyte is assigned an intensity based on its presence and its binary position. For example, the first position corresponds to $2^0 = 1$, the second is $2^1 = 2$, the third is $2^2 = 4$, and so on. The base-10 equivalent of the binary number represents the cumulative intensity

signal, with each value being unique due to the distinct binary representation of each combinatorial state. This ensures that every possible combination of analytes has a unique cumulative intensity, allowing for precise detection and differentiation. *See id.*, 23:38-56.

42. The '921 Patent claims are directed to the innovations providing assays and kits that can unambiguously detect the presence or absence of each of M analytes, in any combination of presence or absence, in a single sample volume, based on detection of F wavelength components, when M is greater than F. *See, e.g., id.*, 67:9-20. For example, claim 1 of the '921 Patent recites:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,
wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,
wherein said fluorescent signal comprises F wavelength components, and
wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,

wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single sample volume.

EX1001('921 Patent), 67:9-26.

Claim 14 of the '921 Patent recites:

14. A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:

a. each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and

b. each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value;

wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence, and wherein the kit does not require the use

of mass spectrometry or immobilization of said M analytes, and wherein the analytes are simplified in the single sample volume.

EX1001('921 Patent), 68:19-38).

C. The '921 Patent's Prosecution History

43. I understand that the Examiner of the '921 Patent allowed the application on the basis that “[t]he claims recite an empirical assay for detecting the presence and absence of M analytes with F wavelength components wherein M is greater than F without using mass spectrometry or immobilization.” EX2003, 4525-4526. I agree with the Examiner’s assessment, for at least the reasons that I will explain in the sections which follow.

D. Overview of Larson

44. U.S. Patent Application Publication No. US 2011/0250597 to Larson et al. (“Larson”) was filed on February 11, 2011, and issued on October 13, 2011. EX1017(Larson), §§(22), (43).

45. Larson discloses “droplet based digital PCR and methods for analyzing a target nucleic acid using the same.” EX1017(Larson), Abstract.

Larson states that:

Methods of the invention involve novel strategies for performing multiple different amplification reactions on the same sample simultaneously to quantify the abundance of multiple different DNA targets, commonly known to those familiar with the art as “multiplexing”. Methods of the invention for multiplexing dPCR assays promise greater plexity—the number of simultaneous reactions—than possible with existing qPCR or dPCR techniques. EX1017(Larson), ¶45.

Larson states that it is “based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in any one droplet even when multiple primers/probes targeting different alleles are present.” *Id.*, ¶¶45, 98, 99. That is, that the samples are sufficiently diluted that a single target allele (analyte) is located within a droplet.

46. Larson describes terminal dilution as “the vast majority of reactions contain either one or zero target DNA molecules for practical intents and purposes,” and limiting dilution as “some reactions contain zero DNA molecules, some reactions contain one molecule, and frequently some other reactions contain multiple molecules, following the Poisson distribution.” EX1017(Larson), ¶6.

47. Larson asserts various advantages of having one allele per droplet:

Having *only one* haploid genome (i.e., one allele) per droplet gives droplet PCR advantages over standard singleplex or multiplex PCR in tubes or microwells. For example, in traditional PCR, both alleles are present in the reaction mix so if there is a difference in the PCR efficiency between alleles, the allele with the highest efficiency will be over represented. Additionally, there can be variances in the sequence to which the PCR primers hybridize, despite careful primer design. A variance in the primer hybridization sequence can cause that primer to have a lower efficiency for hybridization for the allele that has the variance compared to the allele that has the wild type sequence. This can also cause one allele to be amplified preferentially over the other allele if both alleles are present in the same reaction mix.

These issues are avoided in droplet based PCR because there is only one template molecule per droplet, and thus one allele per droplet. Thus, even if primer variance exists that reduces the PCR efficiency for one allele, there is no competition between alleles because the alleles are separated and thus uniformly amplified.

EX1017(Larson), ¶¶99-100.¹

¹ Emphasis added throughout except where noted.

48. Larson discloses digital PCR (dPCR) assays for detecting different numbers of analytes. For example, FIG. 15 (reproduced below) discloses a “9-plex dPCR assay for spinal muscular atrophy with only two fluorophores, showing the process of optimizing droplet intensities.” EX1017(Larson), ¶35, FIG. 15.

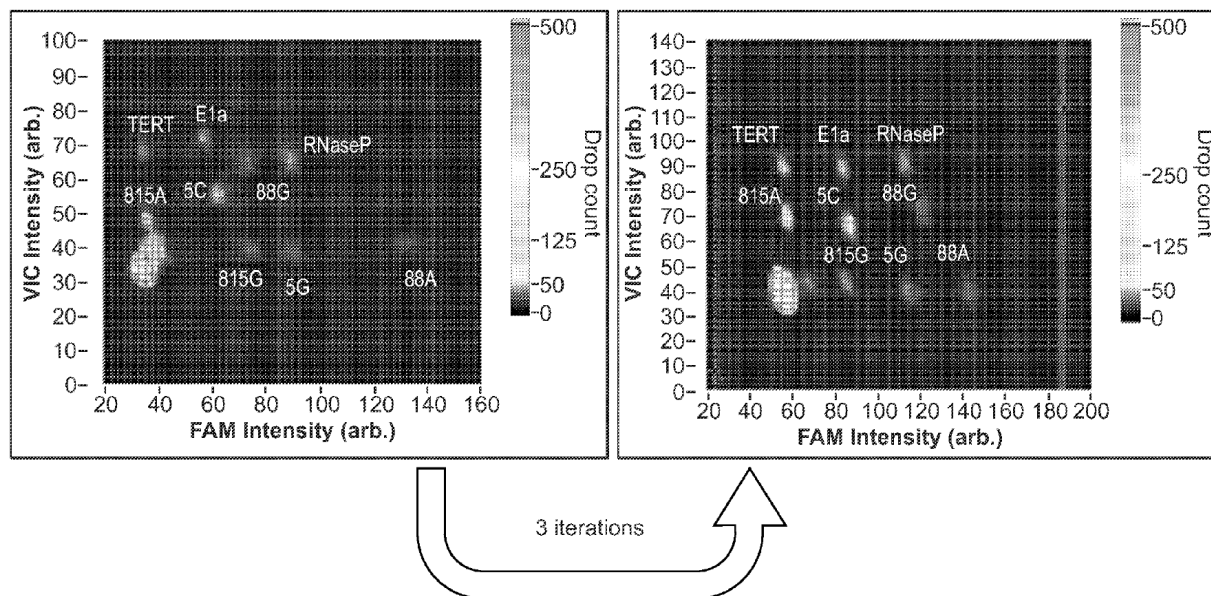


FIG. 15

49. Larson’s FIG. 15 shows only single-analyte clusters (respectively, TERT, 815A, E1a, 5C, 815G, RNaseP, 88G, 5G, 88A). EX1017(Larson), ¶35, FIG. 15. Other graphs in Larson similarly appear to show measurements from only single-analyte droplets, or single-analyte clusters. *See, e.g.*, EX1017(Larson) at FIG. 5B (showing peaks for “one molecule SMN2” and “one molecule E1a” and omitting any double-positive peaks), FIG. 5C (showing clusters for TERT, RnaseP, E1A, SMN1, and SMN2 and omitting any double-positive clusters), FIG. 6B

(showing clusters for TERT, 815A, RNaseP, E1a, and 815G and omitting any double-positive clusters), FIG. 7C (showing peak for “droplet containing a single molecule of target 1” and omitting any double-positive peaks), FIG. 8D (“[h]istogram showing three populations of droplets; with target1, with target2, without target1 or target2” and omitting any clusters containing both target1 and target2), FIG. 9D (“[h]istogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets” and omitting any clusters containing both target1 and target2, any clusters containing both target 1 and target3, any clusters containing both target2 and target3, and any clusters containing all three targets), FIG. 14a (showing clusters for c.815A, BCKDHA, c.815G, SMN1, and SMN2 and omitting any double-positive clusters), FIG. 18A (showing clusters for TERT, SMN1, and SMN2 and omitting any double-positive clusters), FIG. 19A (showing clusters for TERT, 5C, and E1a and omitting any double-positive clusters), FIG. 20A (showing clusters for TERT, 88G, and RNaseP and omitting any double-positive clusters), FIG. 21B (showing clusters for TERT, 88G, and RN5CoP and omitting any double-positive clusters), FIG. 21E (showing clusters for TERT, 6C, and E1b and omitting any double-positive clusters); and FIG. 21H (showing clusters for TERT, SMN1, and SMN2 and omitting any double-positive clusters).

50. Larson teaches “[h]igher degrees of multiplexing would require greater dilution,” because of the possibility for unintended “cross-reactivity” between analytes in a given droplet. EX1017, ¶129. Larson describes the possibility for unintended “cross-reactivity” between analytes in a given droplet as follows:

A multiplexes (*sic*) assay can require a more dilute sample. For instance, at 10% occupancy a duplex reaction would have double occupancy 1% of the time. Hence 1 in 10 PCR+ droplets would be doubles, resulting in a final intensity at least as high and possibly higher than the brighter of the two probes. For a simple duplex system the contribution from each probe could be recovered. In this example the total number of PCR+ droplets for probe 1 would be (Probe1)+(Probe1+Probe2). Higher degrees of multiplexing would require greater dilution. For example, for a 4-plex at 1% occupancy the probability of one probe overlapping any of the other 3 is ~3%, and that error may be too high for some applications. The need for large dilutions strongly favors the large number of dPCR reactions.

EX1017(Larson), ¶129.

51. According to the excerpt above, Larson teaches increasing the sample dilution as the number of analytes increases, in order to reduce the probability that any droplet contains even two analytes, let alone more than two.

52. Larson uses the term “occupancy” to describe the number of analytes in droplets:

In a serial dilution the average number of target DNA molecules per droplet—called the “occupancy” from this point forward—should decrease in direct proportion to the DNA concentration. The occupancy was calculated from Poisson statistics using the following equation well known to those experienced in the art:

$$\text{occupancy} = \ln\left(\frac{P + N}{N}\right),$$

where P and N are the numbers of PCR(+) and PCR(−) droplets respectively.

EX1017(Larson), ¶96.

53. According to Larson’s apparent definition of “occupancy,” the term “10% occupancy” refers to a dilution at which 90% of droplets are empty, 10% of droplets contain analyte, and 10% of the droplets that are not empty (that is, 1% of all the droplets) contain exactly two analytes. *See id.*, ¶129. In this situation, in which the original sample must be very highly diluted, Larson teaches that in a duplex system (an assay for two analytes), the contribution from each probe can be

recovered from a droplet that contains both of the analytes, based on the increased intensity from such a droplet relative to droplets containing only a single analyte. *See id.*, ¶129. And, in any event, the information obtained from the single-analyte droplets is sufficient for Larson’s purposes; indeed, this is why Larson expressly teaches to exclude analyzing multi-analyte droplets, as discussed below.

54. And, for “higher degrees of multiplexing,” that is, more than two analytes, Larson teaches that even “greater dilution” is needed. *Id.*, ¶129. Larson gives the example of a “4-plex” (four analyte assay) at a “1% occupancy,” referring to a dilution at which 99% of droplets are empty and 1% of droplets contain an analyte. *Id.*, ¶129. Even at this high dilution, Larson states that the “probability of one probe overlapping any of the other 3” may be “too high for some applications,” so the “need for large dilutions strongly favors the large number of dPCR reactions.” *Id.*, ¶129.

55. Accordingly, Larson expressly teaches to increase the sample dilution as the number of analytes increases, in order to reduce the probability that one probe overlaps any of the other probes – that is, to reduce the probability that any droplet contains even two analytes, let alone more than two.

56. To the extent that any droplets nonetheless contain more than one analyte, Larson expressly teaches excluding such droplets from analysis. More specifically, Larson states that “[m]ethods of the invention are able to detect which

droplets contain a *heterogeneous population of molecules* and are able to *exclude* those droplets from the analysis.” EX1017(Larson), ¶172. Below, I will address each of the emphasized terms in turn.

57. Larson describes a *heterogeneous population of molecules* as follows: “[d]roplets that produce two signals are classified as droplets that contain a heterogeneous population of molecules.” EX1017(Larson), ¶173. In my view, a POSA considering Larson as a whole would have readily understood that droplets containing a heterogeneous population of molecules produce *two signals* because such droplets contain *two different analytes*, each analyte producing a different signal. Indeed, Larson’s descriptions of “heterogeneous population of molecules” are wholly consistent with my interpretation, especially in the context of the highly diluted regime in which Larson operates.

58. As one example, Larson discloses that “probes that hybridize to the wild-type of the target have a different fluorophore attached than probes that hybridize to a variant of the wild-type of the target.” EX1017(Larson), ¶172. Here, a POSA would have readily understood that the “wild-type of the target” corresponds to a first analyte to which a first probe type hybridizes, while the “variant of the wild-type of the target” corresponds to a second analyte to which a second probe type hybridizes. The POSA therefore would have readily understood that each droplet in this scenario produces *two signals*: one corresponding to the

wild-type target, and one corresponding to the variant of the wild-type target. The POSA therefore would have readily understood each droplet in this scenario contains a *heterogeneous* population of molecules as described by Larson.

59. As another example, Larson discloses that “certain droplets will contain a heterogeneous mixture of both target and target variant due to polymerase errors during the PCR reaction.” EX1017(Larson), ¶169. Here, a POSA would have readily understood that the “target” corresponds to a first analyte to which a first probe type hybridizes, while the “target variant” corresponds to a second analyte to which a second probe type hybridizes. The POSA therefore would have readily understood that each droplet in this scenario produces *two signals*: one corresponding to the target, and one corresponding to the target variant produced through PCR error. The POSA therefore would have readily understood each droplet in this scenario contains a *heterogeneous* population of molecules as described by Larson.

60. In contrast to heterogeneous populations that produce two signals, Larson describes a “homogeneous” population as follows: “[d]roplets that produce only a *single* signal are classified as droplets that contain a homogeneous population of target.” EX1017(Larson), ¶172. In my view, a POSA considering Larson as a whole would have readily understood that droplets containing a homogeneous population of molecules produce *one signal* because they contain

only *a single analyte*, which produces a single signal. Indeed, Larson’s descriptions of “homogeneous population of molecules” are wholly consistent with my interpretation, especially in the context of the highly diluted regime in which Larson operates.

61. Illustratively, continuing with the example of wild-type targets, Larson discloses that “[a]nalyze (*sic*) is then performed on only the droplets that contain a homogeneous population of molecules. The analysis may be based on counting, i.e., determining a number of droplets that contain only wild-type target, and determining a number of droplets that contain only a variant of the target.” EX1017(Larson), ¶174. Here, a POSA would have readily understood that the droplets containing “*only* wild-type target” correspond to a first analyte to which a first probe type hybridizes, while the droplets containing “*only* a variant of the target” correspond to a second analyte to which a second probe type hybridizes. The POSA therefore would have readily understood that each droplet in this scenario produces *only one signal*: either a signal corresponding to the wild-type target, *or* a signal corresponding to the variant of the wild-type target. The POSA therefore would have readily understood each droplet in this scenario contains a *homogeneous* population of molecules as described by Larson.

62. Larson expressly discloses that droplets containing a heterogeneous population of molecules—that is, “[d]roplets that produce two signals” are

“detected and excluded from analysis.” EX1017(Larson), ¶173. I will now address, in turn, Larson’s express teachings regarding *how* and *why* to exclude such droplets from analysis.

63. Regarding *how* to exclude “[d]roplets that produce two signals” from analysis, Larson’s ¶¶178-181 form an entire section entitled “Droplet Sorting” which begins as follows: “Methods of the invention may further include sorting the droplets based upon whether the droplets contain a homogeneous population of molecules or a heterogeneous population of molecules.” EX1017(Larson), ¶178. Larson further discloses in this section that:

A sorting module may be a junction of a channel where the flow of droplets can change direction to enter one or more other channels, *e.g.*, a branch channel, depending on a signal received in connection with a droplet interrogation in the detection module. Typically, a sorting module is monitored and/or under the control of the detection module, and therefore a sorting module may correspond to the detection module. The sorting region is in communication with and is influenced by one or more sorting apparatuses.

EX1017(Larson), ¶178.

Larson’s “Droplet Sorting” section concludes with the statement that: “[b]ased upon the detected signal at the detection module, droplets containing a

heterogeneous population of molecules are sorted away from droplets that contain a homogeneous population of molecules.” EX1017(Larson), ¶181.

64. Recall Larson’s teachings (explained above at ¶¶56-61 of this Declaration) that “[d]roplets that produce *two signals* are classified as droplets that contain a heterogeneous population of molecules” and that “[d]roplets that produce only a *single* signal are classified as droplets that contain a homogeneous population of target.” EX1017(Larson), ¶¶172-173. In my view, based on a fair reading of all of these teachings, a POSA readily would have understood that where Larson’s “Droplet Sorting” section describes sorting droplets “[b]ased upon the detected signal at the detection module,” Larson means sorting droplets based on the *number* of signals that the “detection module” detects from respective droplets. Specifically, *two signals* are detected from droplets containing a *heterogeneous* population of molecules, and *one signal* is detected from droplets containing a *homogeneous* population of molecules. In my view, the only reasonable way to interpret Larson’s teachings of how to *detect* and *sort* droplets containing a heterogeneous population of molecules is based on the number of signals (one signal or two signals) detected from each droplet; the droplets with two signals then are *excluded* from analysis.

65. Larson’s *why* of excluding “[d]roplets that produce two signals” from analysis relates to Larson’s concept of “occupancy” discussed above at ¶¶50-55 of

this Declaration. More specifically, Larson describes “multiple occupancy,” and complexities associated with “multiple occupancy,” as follows:

With multiple occupancy arises the complexity of simultaneous assays competing within the same reaction droplet, and also complexity of assigning the resulting fluorescence intensity that involves a combination of fluorescence from two different reaction products that may or may not be equal to the sum of the two fluorescence intensities of the individual reaction products. *However, methods of the invention can accommodate these complications arising from multiple occupancy.*

EX1017(Larson), ¶141.

66. I believe a POSA, considering the above excerpt from Larson’s ¶141 in view of Larson as a whole, readily would have understood that when Larson refers to “multiple occupancy” droplets, such droplets contain “a heterogeneous population of molecules” as discussed above at ¶¶56-61 of this Declaration. Illustratively, Larson’s statement that droplets with “multiple occupancy” produce a “combination of fluorescence from two different reaction products,” is entirely consistent with Larson’s statement that “[d]roplets that produce two signals are classified as droplets that contain a heterogeneous population of molecules.”

EX1017(Larson), *compare* ¶¶141, 173.

67. I further believe a POSA, considering the above excerpt from Larson's ¶141 in view of Larson as a whole, readily would have understood that Larson's statement "methods of the invention can accommodate these complications arising from multiple occupancy" refers to Larson's strategies discussed at length herein: *first*, to increase the sample dilution as the number of analytes increases, in order to reduce the probability that one probe overlaps any of the other probes – that is, to reduce the probability that any droplet contains even two analytes, let alone more than two, as discussed at ¶¶45-55 of this Declaration; and *second*, to detect, sort away, and exclude droplets containing a heterogeneous population of molecules based on the number of signals (one signal or two signals) that each droplet produces, as discussed at ¶¶56-66 of this Declaration.

68. Larson is replete with examples of how to identify a *single* analyte within a droplet, repeats this as a key inventive concept throughout the reference, and indeed I believe that a POSA reasonably would have considered identifying a single analyte within a droplet to be Larson's principle of operation. *See, e.g.*, EX1017(Larson) at abstract ("single target nucleic acid"), ¶7 ("the invention provides a droplet that contains a single nucleic acid template"), ¶8 ("amplify and detect single nucleic acids in droplets... there is only one template molecule per droplet"), ¶11 ("[o]ne or more droplets are formed, each containing a single nucleic acid template"), ¶13 ("single droplets containing the single nucleic acid

template”), ¶18 (“each droplet contains only one template strand...single droplet that contains a single intact nucleic acid strand”), ¶19 (“compartmentalizing a first fluid into portions, each portion containing a single target nucleic acid”), ¶20 (“forming sample droplets containing a single target nucleic acid derived from a patient sample”), ¶45 (“It is based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in any one droplet even when multiple primers/probes targeting different alleles are present”), ¶46 (“the invention provides droplets containing a single nucleic acid template”), ¶49 (“[m]ethods of the invention involve forming sample droplets containing a single target nucleic acid”), ¶68 (“[i]n certain embodiments, the droplets will include only a single nucleic acid template”), ¶99 (“only one haploid genome (i.e., one allele) per droplet”), ¶100 (“[t]hese issues are avoided in droplet based PCR because there is only one template molecule per droplet, and thus one allele per droplet”), ¶101 (“droplet based digital PCR utilizes only one template molecule per droplet...only one amplicon is being generated per droplet”), ¶107 (“there is still only one template molecule per droplet”). *See also* EX1017(Larson) at claim 1 (“[a] microdroplet comprising a single nucleic acid template”), claim 3 (“the single nucleic acid template is a DNA or an RNA”), claim 10 (“forming one or more microdroplets each comprising a single nucleic acid template”), claim 11 (“merging the first and

second fluids to form a droplet comprising the single nucleic acid template”), and claim 15 (“the single nucleic acid template is a DNA or an RNA”).

E. Overview of Saxonov

69. U.S. Patent Application Publication No. 2013/0040841 to Saxonov et al. (“Saxonov”) was filed on July 12, 2012 and issued on February 14, 2013. EX1004(Saxonov), §§(22), (43).

70. Saxonov is directed to “performing a digital assay with multiplexed detection of two or more distinct targets in the same optical channel.” EX1004(Saxonov), Abstract. Regarding “digital assays,” Saxonov explains:

Digital assays generally rely on the ability to detect the presence or activity of individual copies of an analyte in a sample. In an exemplary digital assay, a sample is separated into a set of partitions, generally of equal volume, with each containing, on average, less than about one copy of the analyte. If the copies of the analyte are distributed randomly among the partitions, some partitions should contain no copies, others only one copy, and, if the number of partitions is large enough, still others should contain two copies, three copies, and even higher numbers of copies. The probability of finding exactly 0, 1, 2, 3, or more copies in a partition, based on a given average concentration of analyte in the partitions, is described by a Poisson distribution.

EX1004(Saxonov), ¶3.

71. This portion of Saxonov teaches that in a standard digital assay, most droplets (partitions) are empty, fewer droplets contain exactly one analyte, even fewer contain exactly two analytes, and even fewer contain exactly three analytes; this is consistent with the limiting dilution regime discussed above at ¶33 of this Declaration. As described previously, in the limiting dilution regime, the sample is sufficiently diluted that the Poisson distribution basically guarantees that the majority of droplets contain zero analyte molecules. A lower number of droplets contain exactly one analyte molecule, an even lower number contain exactly two analyte molecules, an even lower number contain exactly three analyte molecules, and so on.

72. According to Saxonov, “[d]igital assays frequently rely on amplification of a nucleic acid target in partitions to enable detection of a single copy of an analyte,” and such “[a]mplification may be conducted via the polymerase chain reaction (PCR).” EX1004(Saxonov), ¶5. Saxonov states that “[a]mplification of the target can be detected optically from a fluorescent probe included in the reaction,” and that “the probe can include a dye that provides a fluorescence signal indicating whether or not the target has been amplified.” *Id.* So, Saxonov clearly uses the term “nucleic acid target” to refer to a genetic

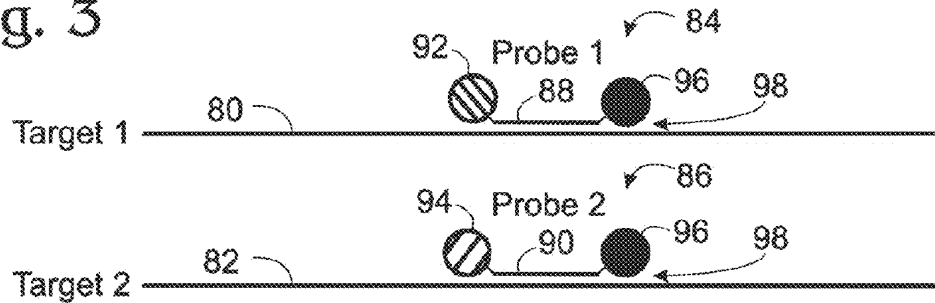
sequence that it is desired to amplify, and then detect using the digital assay using a fluorescent probe, similarly as discussed above in §V.A.

73. Like others, Saxonov tried to multiplex: “[a] new approach is needed to increase the multiplex levels of digital assays.” EX1004(Saxonov), ¶7.

Saxonov identified various shortcomings in then-known multiplexing techniques, such as “instruments with more optical channels, to detect emission from more dyes, are more expensive than those with fewer channels,” and “increasing the number of distinguishable dyes is expensive and becomes impractical beyond a certain number.” *Id.*, ¶6.

74. Saxonov tried solving this problem using a “common or shared channel (‘the same optical channel’)” to detect signals from “reporters for two or more distinct targets.” *Id.*, ¶19. For example, in FIG. 3 (reproduced below), Saxonov illustrates target sequences 80, 82; probes 84, 86 with oligonucleotides 88, 90; and fluorophores 92, 94; when oligonucleotide 88 hybridizes to sequence 80 or oligonucleotide 90 hybridizes to sequence 82, the respective fluorophores separate from quenchers 96 and fluoresce. *Id.*, ¶¶48-52, FIG. 3.

Fig. 3



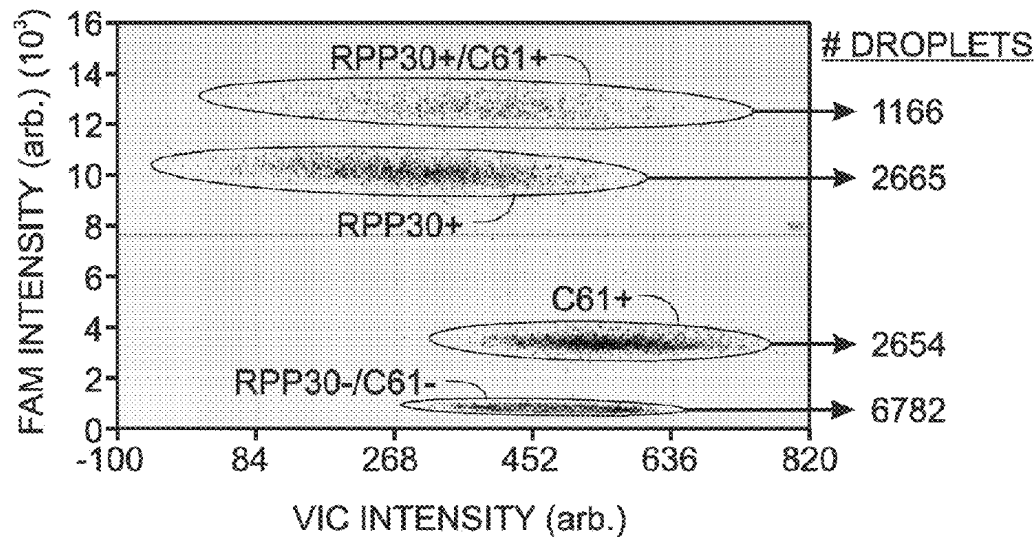
75. Saxonov describes different ways to distinguish the fluorophores' signals based on intensity, including that "one probe may be labeled with a different number of fluorophores than the other probe," or "there may be more or less of one probe than the other in the sample," or "the fluorophores themselves might be different, with one more or less intrinsically fluorescent than the other." EX1004(Saxonov), ¶51. According to Saxonov, the "signal detected from each partition, and the partition itself, may be classified as being positive or negative for each of the reactions/targets contributing to the signal." *Id.*, ¶41.

76. Saxonov states "[t]he data including all permutations of positives will generally fall into 2^N populations or clusters, where N is the number of targets, assuming that each population is distinguishable." EX1004(Saxonov), ¶41.

77. FIG. 5 of Saxonov is part of an example which Saxonov describes as "a first exemplary digital PCR assay, in which multiplexing in a single channel is used to access copy number of the C61 gene... signals from two probes, one for the gene of interest, C61, and one for a reference probe, RPP30, are collected together, as a single signal[.]" EX1004(Saxonov), ¶60. FIG. 5 illustrates clusters

for the C61 and RPP30 genes in a common channel (the FAM channel, where FAM is a type of fluorophore).

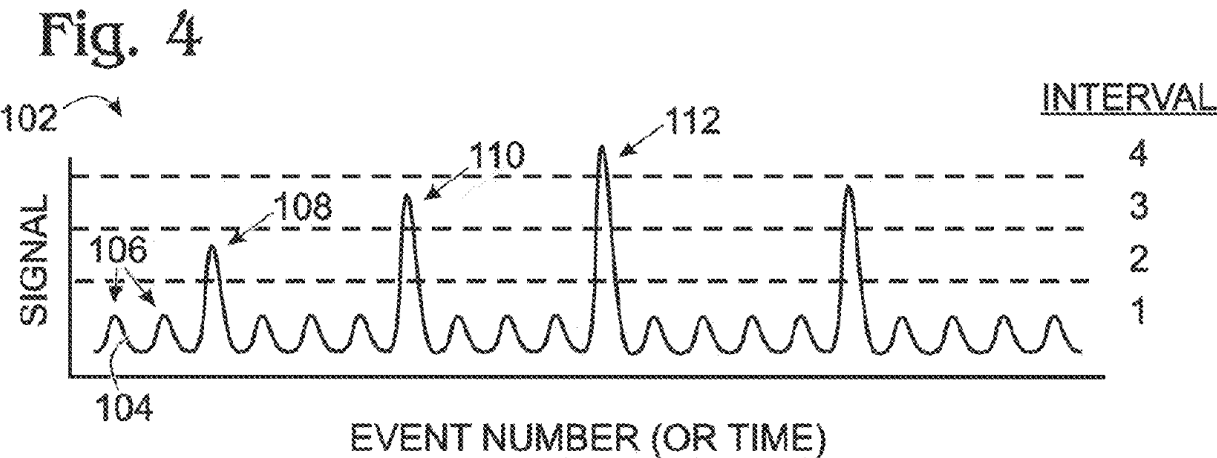
Fig. 5



78. In FIG. 5, the RPP30-/C61- cluster of 6782 droplets “corresponds to droplets that are negative for RPP30 and C61 (i.e., droplets that did not include either gene).” EX1004(Saxonov), ¶64. The C61+ cluster of 2654 droplets “corresponds to droplets that are positive for C61 and negative for RPP30 (i.e., droplets that included the C61 gene but did not include the RPP30 gene).” *Id.*, ¶65. The RPP30+ cluster of 2665 droplets “corresponds to droplets that are positive for RPP30 and negative for C61 (i.e., droplets that included the RPP30 gene but did not include the C61 gene).” *Id.*, ¶66. The RPP30+/C61+ cluster of 1166 droplets “corresponds to droplets that are positive for both RPP30 and C61 (i.e., droplets that included both genes).” *Id.*, ¶67.

79. FIG. 5 of Saxonov is consistent with Saxonov's digital assays description above and with the "limiting dilution" regime (*see* ¶33 of this Declaration): 6782 droplets were empty (the majority); 2654+2665=6485 droplets contained a single analyte (fewer than the number of empty droplets); and 1166 droplets contained both analytes (fewer than the number containing a single analyte).

80. This is also consistent with FIG. 4 of Saxonov, reproduced below, which according to Saxonov shows that peaks 106 "correspond to droplets containing no Target," peaks 108 "correspond to droplets containing Target 1 but not containing Target 2 (T1+/T2-)," peaks 110 "correspond to droplets containing Target 2 but not containing Target 1 (T1-/T2+)," and peaks 112 "correspond to droplets containing both Targets 1 and 2 (T1+/T2+)." EX1004(Saxonov), ¶¶53-57. From FIG. 4, it may be seen that most droplets are empty (peaks 106), with fewer droplets containing one analyte (peaks 108 or 110), and even fewer droplets containing two analytes (peak 112). *Id.*, FIG. 4.



81. Saxonov’s FIG. 5 is fairly simple because there are only two analytes and four populations or clusters. According to Saxonov, as the number of targets (N) increases, the number of clusters increases as 2^N . EX1004(Saxonov), ¶41. Using the tables below, Saxonov shows a single target A (N=1) yields two clusters ($2^1=2$), two targets A and B (N=2) yield four clusters ($2^2=4$), and three targets A, B, and C (N=3) yields eight clusters ($2^3=8$). EX1004(Saxonov), ¶41.

	Target A	Intensity
Population 2	+	Highest
Population 1	–	Lowest

	Target A	Target B	Intensity
Population 4	+	+	Highest
Population 3	+	–	Intermediate
Population 2	–	+	Intermediate
Population 1	–	–	Lowest

	Target A	Target B	Target C	Intensity
Population 8	+	+	+	Highest
Population 7	+	+	–	Intermediate
Population 6	+	–	+	Intermediate
Population 5	–	+	+	Intermediate
Population 4	+	–	–	Intermediate
Population 3	–	+	–	Intermediate
Population 2	–	–	+	Intermediate
Population 1	–	–	–	Lowest

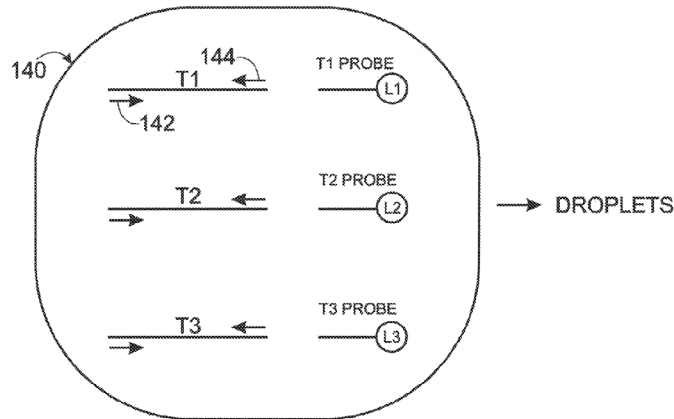
82. As discussed below at ¶¶87-92 of this Declaration, Saxonov’s FIGS. 9 and 10 and ¶¶17, 18, and 76-79 were not carried forward from the Saxonov Provisional and thus it is my opinion, and my analysis assumes, that the subject matter disclosed by FIGS. 9 and 10 and ¶¶17, 18, and 76-79 is not prior art to the ’921 Patent. Nonetheless, even if the Saxonov Provisional is erroneously found to support this subject matter, Saxonov still does not render the ’921 Patent claims obvious when combined with Larson, as discussed below at §§VII.C and VII.D of this Declaration. In any event, Petitioner relies on certain of this subject matter, and therefore, I briefly discuss it below.

83. According to Saxonov, FIG. 9 (reproduced below) is:

a schematic representation of an exemplary bulk phase mixture being separated into droplets during performance of an exemplary digital assay with multiplexed detection

of three or more distinct targets (T1, T2, and T3) using a reporter for each target (a T1 probe, a T2 probe, and a T3 probe) each including a different luminophore (L1, L2, and L3, respectively)[.]

Fig. 9



EX1004(Saxonov), ¶17, FIG. 9.

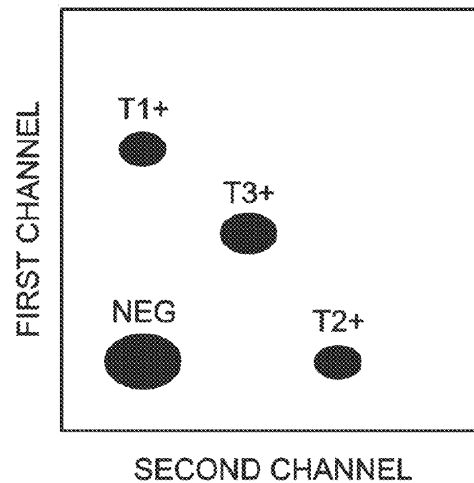
84. FIG. 9 shows that the volume containing the three targets T1, T2, T3 and corresponding probes is divided into droplets.

85. According to Saxonov, FIG. 10 (reproduced below):

is a schematic representation of an exemplary graph showing droplet (or peak) intensities for a T1/T2/T3 droplet assay that may be performed with the droplets of FIG. 9, where data from the T1, T2, and T3 probes are collected in a first optical channel and a second optical channel and plotted as a function of intensity in the first channel (vertical axis) versus intensity in the second channel (horizontal axis), with intensities produced by

exemplary populations or clusters of negative (NEG), T1-positive (T1+), T2-positive (T2+), and T3-positive (T3+) droplets represented by filled ellipses[.]

Fig. 10



EX1004(Saxonov), ¶18, FIG. 10.

86. Note that FIG. 10 does not include double-positive (e.g., T1+/T2+) or triple-positive (T1+/T2+/T3+) clusters, but instead shows just single-analyte clusters (either T1+, or T2+, or T3+). Saxonov states that in FIG. 10, “[t]o simplify the presentation, double-positive populations (T1+/T2+, T1+/T3+, and T2+/T3+) and the triple-positive population are *not shown*,” but alleges that “the populations containing more than one target may be resolved and distinguishable from one another and from the single-target populations and the negative population.” *Id.*, ¶¶78-79.

F. Saxonov Provisional and Priority Date

87. U.S. Patent Application Publication No. 61/510,013 to Saxonov et al. (“Saxonov Provisional”) is a provisional patent application to which Saxonov claims priority. However, the Saxonov Provisional fails to disclose the entire subject matter of Saxonov on which Petitioner relies in Grounds 3 and 4.

88. For example, FIGS. 1-8A and 8B of Saxonov generally correspond to, and are carried over from, FIGS. 1-8 of the Saxonov Provisional, with certain differences. However, FIGS. 9 and 10 of Saxonov are ***absent*** in the Saxonov Provisional. Nor does any disclosure in the Saxonov Provisional, as it would have been understood by a POSA, support or disclose the subject matter relied on by Petitioner from these figures. *See* EX2007, 3, 16-17.

89. As another example, ¶¶2-16, 19-36, 38-53, 45-68, and 72-75 of Saxonov generally correspond to page 1, line 10 through page 20, line 22 of the Saxonov Provisional. *Id.*; *see also* EX2007. However, ¶¶17, 18, 37, 44, 69-71, and 76-80 of Saxonov contain significant new subject matter which is ***not found in, or supported by***, the Saxonov Provisional. *Id.*; *see also* EX2007.

90. Grounds 3 and 4 rely ***heavily*** upon certain of this newly added subject matter. Illustratively, the Petition states that Saxonov’s FIG. 10 “[i]mportantly...teaches an approach to detecting three targets based on just two colors, wherein a first target (T1) is identified by a first color signal, a second

target (T2) is identified by a second color signal, and a third target (T3) is identified by the presence of both color signals.” Petition, 58-59, *reproducing* FIG. 10 and *citing* ¶¶18 and 78-79 of Saxonov. The Petition even goes so far as to present a doctored version of Saxonov’s FIG. 10, which I address further below in ¶¶123-131 of this Declaration. *See* Petition, 60.

91. In short, it is my opinion that Saxonov’s FIGS. 9 and 10 and ¶¶ 17, 18, 37, and 76-80, of which FIG. 10 and ¶¶18, 37 and 78-79² are relied upon in Petitioner’s Grounds 3 and 4, are ***not*** carried over from the Saxonov Provisional and are unsupported by the Saxonov Provisional. I understand that Saxonov’s FIGS. 9 and 10 and ¶¶ 17, 18, 37, and 76-80 therefore are ***not*** prior art to the ’921 Patent, and that these sections are entitled only to Saxonov’s ***non-provisional*** filing date of July 12, 2012, which was ***after*** the ’921 patent’s earliest priority date (February 3, 2012).

92. Saxonov also claims priority to another provisional, U.S. 61/507,082, filed July 12, 2011. EX1004, §(60). However, Petitioner does not indicate any reliance upon the priority date of that provisional. Instead, Petitioner cites only to the Saxonov Provisional, which as explained above does not disclose subject matter upon which Grounds 3 and 4 heavily rely.

² The Petition does not appear to cite FIG. 9 or ¶¶17, 76, or 80 of Saxonov.

G. Overview of Silverbrook

93. U.S. Patent Application Publication No. 2011/0312704 to Silverbrook et al. (“Silverbrook”) was filed on June 1, 2011, and issued on December 22, 2011. EX1009(Silverbrook), §§(22), (43). Silverbrook discloses “a fluorescence-based detection technique to identify target molecules.” *See, e.g., id.*, ¶195. A test module using fluorescence-based detection is controlled by a test module reader, which may be a computer. *Id.*, ¶¶198, 200-201, FIGS. 1, 3, 96, 98. The data generated by the module can also be updated using the test module reader and network. *Id.*

VI. CLAIM CONSTRUCTION

94. For purposes of this declaration, I have reviewed the challenged claims of the '921 Patent in view of their common and ordinary usage and in view of the specification of the '921 Patent.

95. I understand that Patent Owner’s construction of some claim terms differs from that of Petitioner. My analysis below is based on, and consistent with, Patent Owner’s proposed claim constructions set forth at EX1003, EX2011, and EX2012. In particular, I understand that Patent Owner proposes that “degenerate” and “degeneracy” generally describe a situation where a legitimate result is not definitive, because it can indicate more than one possibility in terms of the presence or absence of an analyte. EX1003, 9-11; EX2011, 27-28; EX2012, 18-19.

96. I agree with the Patent Owner's position because this position is consistent with the common and ordinary usage of these terms, and in particular how these terms are used the '921 Patent specification, based on my own understanding as at least a POSA. However, my ultimate opinion that the references cited in the Petition do not teach the challenged claims of the '921 Patent would remain the same even under the Petitioner's proposed constructions.

VII. GROUNDS OF CHALLENGE

97. Petitioner points primarily to Larson as anticipating or rendering obvious the '921 Patent claims, either alone or in view of Saxonov and/or Silverbrook. However, for the reasons set forth below, it is my opinion that none of these references, whether alone or in combination, teach the challenged claims of the '921 Patent.

A. Ground 1 (Claims 1-10 and 13-19): Anticipation by Larson

1. Challenged Claim 1 and Larson Differ in Kind

98. While the '921 Patent and Larson both aim to extend the number of analytes that can be examined simultaneously, they solve these problems in significantly different ways.

99. For example, as noted above at ¶45 of this Declaration, Larson states that it is "based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in

any one droplet even when multiple primers/probes targeting different alleles are present.” EX1017(Larson), ¶¶45; *see also id. at* ¶¶98, 99. That is, in Larson, the samples are sufficiently diluted that a single analyte is located within a droplet.

100. To detect different analytes, Larson extensively dilutes and partitions the sample into numerous droplets such that “some droplets contain zero target nucleic acid molecules, some droplets contain one target nucleic acid molecule, and some droplets may or may not contain multiple nucleic acid molecules (corresponding to limiting or terminal dilution, respectively, as defined above).”

EX1017(Larson), ¶¶6, 52; *see* ¶¶33, 45-55 of this Declaration. Droplets with zero analyte provide no information, but inevitably arise by the procedure.

EX1017(Larson), ¶¶6, 52; *see* ¶33 of this Declaration. This reduces the number of analytes in a droplet, making it easier to identify the analytes, with the easiest case being that of a single analyte per droplet. EX1017(Larson), ¶141 (“the above three methods for higher-plex dPCR are simplest to implement under conditions of terminal dilution, that is when the probability of multiple different target molecules co-occupying the same droplet is very low compared to the probability of any single target occupying a droplet”).

101. To the extent that some droplets may have “multiple occupancy” or contain a “heterogeneous population of molecules,” Larson expressly teaches *excluding* such droplets from analysis, and explains how to do so and the reasons

why to do so. *see, e.g.*, EX1017(Larson), ¶172 (“Methods of the invention are able to detect which droplets contain a *heterogeneous population of molecules* and are able to *exclude* those droplets from analysis”); *see* ¶¶56-68 of this Declaration.

102. Larson not only is replete with examples of how to identify a *single* analyte within a droplet, but Larson repeats this as a key inventive concept, and in view of this teaching I believe that a POSA reasonably would have considered this to be Larson’s principle of operation. *Supra*, §V.D.; EX1017, abstract, ¶¶7, 8, 11, 13, 18, 19, 20, 45, 46, 49, 68, 99, 100, 101, 107, 141, and claims 1, 3, 10, 11, and 15.

103. Larson’s approach has a number of disadvantages. For example, as compared with the ’921 Patent’s approach, Larson’s principle of operation requires partitioning a highly dilute sample into a significant number of droplets, the majority of which provide no information (because the droplets are empty). *See* ¶¶33, 45-55 of this Declaration. Aside from wasting reagents, this is cumbersome to perform and get the optical readouts, because each droplet has to be assessed. As a consequence, the significant number of empty droplets reduces the number of samples that can be analyzed in a given period of time, and the amount of information that can be gathered during that time. Additionally, diluting down to a single analyte is complicated by the possibility that different analytes in the sample may be present at very different concentrations than each other, and this is not

known before performing the dilution, or the assays. Thus, the optimal dilution for analyte A in the sample can be very different from the optimal dilution for analyte B in the same sample. This requires a very broad dilution series, meaning the vast majority of drops are uninformative, *e.g.*, contain either no molecules or too many molecules. Moreover, even different samples containing the same analyte as each other may have different concentrations of that analyte than each other, meaning that the dilution needed to analyze any given one of those samples may be significantly different than the dilution needed to analyze any of the other samples, and none of these dilutions may be knowable in advance. If those samples contain multiple analytes, the problem becomes even more complicated for the reasons stated herein.

104. Excluding any droplets containing multiple analytes, as taught by Larson, adds further complexity and time to the assay, requires additional hardware components to perform the sorting, and is wasteful because droplets containing otherwise useful information about multiple analytes in the sample are simply discarded.

105. The '921 Patent solves the problem of detecting different analytes in a sample by measuring cumulative optical signals using a coding scheme. According to embodiments of the '921 Patent's method, PCR reactions go to completion such that all the original colorimetric probe molecules (whose

fluorophores are quenched) are hybridized, thereby liberating the colorimetric signal. For example, if two probes that are read in the same optical channel have 1000 units of fluorescence for analyte A and 500 units of fluorescence for analyte B, then one can distinguish between 500, 1000, and 1500 units in that channel and hence determine whether the sample contains A, B, or both A and B analytes. Similarly, with appropriate choices of fluorescence units for probes with multiple colors, one can make the same distinction between different analytes. An advantage of PCR reactions going to completion is that the starting concentrations of the analytes does not matter, in stark comparison to the importance of concentration to Larson's method as discussed at paragraph ¶103 of this Declaration.

106. An important feature taught by the '921 Patent is how to choose probe colors and intensities that uniquely determine a given analyte, even if that analyte is mixed with other analytes at the same time. This avoids the degeneracy problem, which arises when the same intensity values are consistent for two different combinations of analytes. For example, if intensity values 1, 2, 4, 8, 16, 32 are chosen for probes read in the same optical channel, every combination of present/absent analytes is unique. This method of decoding cumulative signals is different in kind (not just degree) from Larson, and makes it much easier to analyze multiplexed samples.

107. Claim 1 of the '921 Patent, reproduced above in ¶42 of this Declaration, recites an assay that is “capable of *unambiguously* detecting the presence or absence of each of said at least M analytes, *in any combination of presence or absence, in a single sample volume*, based on detection of said F wavelength components, when M is greater than F.” EX1001('921 Patent), 67:16-20. Claim 14 of the '921 Patent, also reproduced above in ¶42 of this Declaration, recites a kit that likewise is “capable of *unambiguously* detecting the presence or absence of each of analyte of M analytes in a single sample volume, *in any combination of presence or absence*.” EX1001('921 Patent), 68:31-34. While the single sample volume may not necessarily contain *all* analytes at the same time, the power of the '921 Patent lies in its flexibility to detect *any possible combination* of the analytes within the single sample volume, in a manner that each analyte may be uniquely distinguished from each other analyte. See ¶¶35-43 of this Declaration. At the time of the '921 Patent and its priority applications, no such solution was available to solve the problem of how to increase the number of analytes which could be non-degenerately detected in a single sample volume. See ¶¶35-42 of this Declaration; *See also* EX1001('921 Patent), 1:46-47 (“There is a need for multiplexed reactions that can be carried out *in a single solution*.”)

2. Larson Does Not Teach How to Unambiguously Identify Three or More Analytes Within a Single Sample Volume

108. As described above at ¶¶45-68 of this Declaration, Larson teaches various complexities that could arise if two or more analytes are in a droplet, and expressly teaches how to avoid that situation: *diluting* and partitioning the sample into numerous droplets to reduce the likelihood of any droplets having “multiple occupancy,” or containing a “heterogeneous population of molecules,” and *excluding* such droplets.

109. Moreover, Larson is *silent* as to how to analyze droplets having three or more analytes. This is because Larson had no need to try to solve that problem: the single-analyte droplets yielded the information that Larson apparently sought—an identification of whether an analyte was present in the sample—and without having to resolve any of the complications caused by “multiple occupancy.” Larson did not contemplate the solution provided by the ’921 Patent, because there was no need in Larson to solve the problem of how to *unambiguously detect* three or more analytes. Larson’s limiting dilution and sorting/exclusion entirely avoided this issue. Indeed, the Petition itself states that “under *these* conditions”—that is, under the conditions of “terminal dilution, where the probability of a single target molecule in droplets is much greater than, and overwhelms that of multiple targets”—the detectable signal generated for each potential analyte in Larson is “distinct and readily distinguishable, providing ‘unambiguous’ detection of the analytes.” Petition, 29.

110. The Petition appears to argue that the '921 Patent claims are invalid because they are being asserted against assays and kits that purportedly resemble those described in ¶140 of Larson. *See, e.g.*, Petition at 27-32. However, for at least the reasons given at paragraphs ¶¶98-109 of this Declaration, claims 1 and 14 of the '921 Patent, and claims respectively depending therefrom, differ from Larson in several material aspects which go to the very core of Larson's express teachings, inventive concepts, and principle of operation.

B. Ground 2 (Claims 2, 4, 6-7, and 14-19): Obviousness in View of Larson

111. The Petition identifies dependent claims 2, 4, 6-7, and 14-19 as allegedly being obvious over Larson. Petition at 44-51. However, my analysis and views set forth above in ¶¶98-110 of this Declaration regarding Ground 1, describing the many material differences between Larson and claim 1, apply equally (if not more) to claims 2, 4, 6-7 which depend from claim 1, to independent claim 14 which recites similar limitations as discussed above for claim 1, and to claims 15-19 which depend from claim 14.

112. In any event, the Petitioner's remarks regarding claim 14 apparently amount to an argument that the word "kit" is not limiting, and that "it would have been obvious to combine the components disclosed in Larson together in a 'kit.'" Petition, 50-51. I believe that such limited argumentation does not adequately

explain why a POSA would have found it obvious to attempt to obtain the kit which is recited in claim 14, and in claims depending therefrom, based on the teachings of Larson considered *as a whole* (that is, including Larson’s express teachings regarding diluting and partitioning the sample and excluding droplets containing more than one analyte), for at least the reasons set forth above in ¶¶98-110 of this Declaration.

C. Ground 3 (Claims 1-10 and 12-19): Obviousness In View of Larson and Saxonov

113. Whereas Petitioner’s arguments in Grounds 1 and 2 appear to be based on Patent Owner’s positions purportedly taken in the parallel district court litigation (*see* ¶¶98-112 of this Declaration), Petitioner’s arguments in Ground 3 instead appear to be based on the allegation that “Larson, alone or in combination with Saxonov, at a minimum renders obvious the ‘unambiguous’ detection of the ‘presence or absence’ of multiple analytes in such individual subvolumes.”

Petition, 51. However, Petitioner’s errors in Ground 3 are even more amplified than in Grounds 1 and 2, because *both* Larson and Saxonov teach something very different from the language recited in the ’921 Patent claims. I will first touch on the purported teachings of Larson (I have already discussed Larson’s actual teachings at length in other parts of this Declaration); then I will discuss Saxonov’s teachings; and then I will explain why combining Larson with Saxonov falls far

short what is recited in the '921 Patent's claims 1 and 14, and claims respectively depending therefrom.

1. Larson Eliminates the Very “Multiple Occupancy” Situations to Which Petitioner Points

114. In Ground 3, the Petition cites Larson as allegedly teaching “that there may be multiple targets in a single individual droplet and that its methods accommodate such ‘multiple occupancy’ situations” and states “Larson’s assays use conditions and parameters that do precisely this.” Petition, 52, citing EX1017(Larson) ¶¶129, 141 and EX1002(Batt) ¶321. But Larson’s *actual* accommodation of “‘multiple occupancy’ situations” is *incompatible* with an assay or kit that is capable of unambiguously detecting the presence or absence of each of said at least M analytes, *in any combination of presence or absence, in a single sample volume*. See EX1001('921 Patent), 67:16-20, 68:31-34.

115. For example, as discussed above at ¶¶65-67 of this Declaration, a POSA considering Larson’s ¶141 (cited in the Petition), in view of Larson as a whole, readily would have understood that Larson’s statement “methods of the invention can accommodate these complications arising from multiple occupancy” refers to the strategies discussed at length herein: *first*, to increase the sample dilution as the number of analytes increases, in order to reduce the probability that one probe overlaps any of the other probes – that is, to reduce the probability that

any droplet contains even two analytes, let alone more than two, as discussed at ¶¶45-55 of this Declaration and described in Larson’s ¶129 (cited in the Petition); and **second**, to detect, sort away, and exclude droplets containing a heterogeneous population of molecules based on the number of signals (one signal or two signals) that each droplet produces, as discussed at ¶¶56-68 of this Declaration.

116. For at least the reasons described in the preceding paragraph ¶115 and elsewhere in this Declaration, it is my view that if a POSA wished to “accommodate” multiple occupancy situations, the full teachings of Larson would, at most, have motivated that POSA to try to **eliminate** multiple occupancy situations by **diluting** the sample to reduce the probability that any droplet contains two or more analytes, and **excluding** droplets containing two or more analytes from analysis. In my view, these teachings of Larson do not render it at all obvious to instead ***try to obtain, and analyze, droplets that may obtain all possible different combinations of analytes.***

117. The Declaration of Dr. Carl Batt (EX1002), Petitioner’s proffered expert, does not change my view on this subject. Indeed, ¶321 of the Batt Declaration, to which the Petitioner points as allegedly supporting the position that Larson’s “methods accommodate... ‘multiple occupancy’ situations,” merely points to Larson’s ¶¶129, 141 and rehashes Petitioner’s conclusion regarding obviousness.

2. Larson Does Not Teach or Suggest Analyzing Multiple Targets in a Single Droplet

118. Other portions of Ground 3 similarly try to contort Larson’s express disclosure into something Larson clearly teaches away from. For example, Petitioner points to Larson’s FIG. 9 as allegedly showing an “assay involving three targets and two colors, wherein one color is used to identify two of the targets and a second color identifies the third target,” and alleges this “coding scheme allows for determination of precisely which targets are present in a droplet *even when multiple targets are present in a droplet.*” Petition, 55-56. But again, as this Declaration amply establishes, Larson expressly teaches *reducing and excluding* multiple-analyte droplets from analysis in order to *avoid* “complications” that arise in such droplets—not merely in a few selected examples, but as a core inventive concept and principle of operation. *See, e.g.,* ¶¶45-68 of this Declaration.

119. The Petitioner further alleges that from Larson’s FIG. 9C, a POSA: would have understood that the various combinations of analytes would result in distinguishable signals for the three analytes because the signals from P_1 , P_2 , P_3 , P_1+P_2 , P_1+P_3 , P_2+P_3 , $P_1+P_2+P_3$, and so on have different intensities as the combinations are additive and would each sum to unique values in each of the two colors used.

Petition, 57, citing EX1002(Batt), ¶336; EX1017(Larson), FIG. 9C.³

Here, the Petitioner and Dr. Batt hypothesize what scenarios “would result in distinguishable signals” from droplets containing different combinations of three analytes, and allege that the “foregoing coding scheme” is “[c]ritically, consistent with Larson’s teaching that its assays can accommodate ‘multiple occupancy.’”

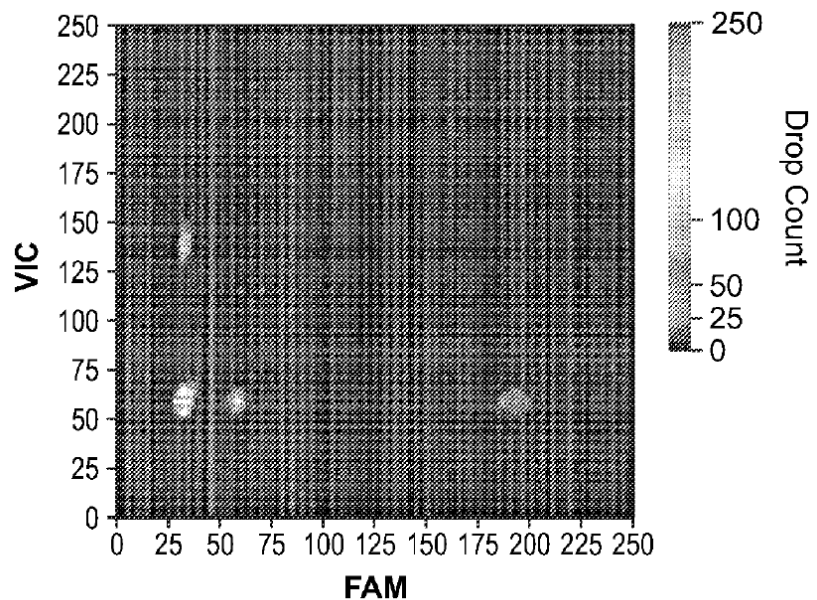
Petition, ¶56, citing EX1002(Batt), ¶¶335-337. But contrary to what is alleged, the scheme is not at all “consistent” with Larson’s teachings regarding “multiple occupancy,” and indeed the Petitioner and Dr. Batt conveniently ignore the fact that Larson expressly *excludes* analyzing signals from droplets that contain combinations of analytes precisely *because* those droplets produce two signals (among other complications that Larson teaches arise within multiple-analyte droplets). *See, e.g.*, ¶¶45-68 of this Declaration.

120. And, even while Petitioner and Dr. Batt point to Larson’s FIG. 9C, they conveniently ignore the related figure FIG. 9D, reproduced below:

³ The “and so on” in this quote is nonsensical because there are only three analytes, and all possible combinations of presence or absence of those three analytes are already enumerated.

FIG. 9D

Histogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets.



EX1017, FIG. 9D.

According to Larson, FIG. 9D illustrates a “[h]istogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets.” EX1017(Larson), ¶121. FIG. 9D does not disclose, and indeed clearly *omits*, any clusters containing *both* target1 and target2, any clusters containing *both* target 1 and target3, any clusters containing *both* target2 and target3, and any clusters containing all *three* targets. Accordingly, what ostensibly is *actual* data from Larson is entirely consistent with my interpretation of the reference as set forth above at ¶¶45-68 of this Declaration, and not at all consistent with Petitioner’s conjectures about what “would result” *if* Larson’s teachings were *not actually followed*. I do not believe that anything in Larson would have led a

POSA to ignore Larson’s express teachings—and indeed, to modify Larson’s principle of operation—to try to obtain the scheme Petitioner proposes.

3. Saxonov Does Not Teach or Suggest Unambiguously
Analyzing Three or More Targets in a Single Droplet

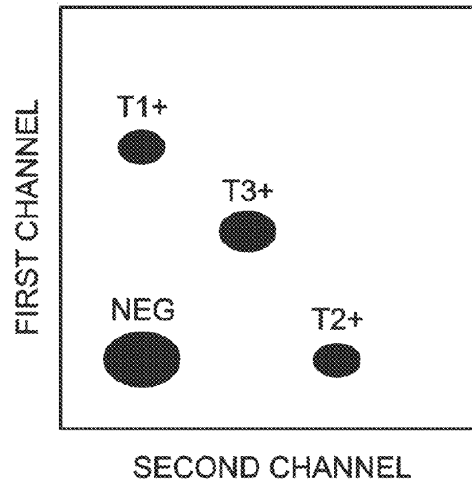
121. Similar to Larson, Saxonov tried to extend the number of analytes that can be simultaneously identified in an assay. For example, Saxonov tried to use the “same optical channel” to detect different analytes, ostensibly to reduce the cost of additional optical channels and optical dyes. *Supra* §V.E. And, like Larson, to detect different analytes, Saxonov extensively diluted and partitioned the sample into numerous droplets such that most of the droplets are empty (contain zero analyte molecules), and the next largest population of droplets contains a single analyte. *Supra* §V.E. Saxonov also is replete with examples of how to identify a *single* analyte molecule within a droplet. *Supra* §V.E. Indeed, for detecting multiple analytes in the same optical channel, droplets with a single analyte molecule also are key to Saxonov’s method. *Supra* §V.E.

122. More specifically, because of the Poisson distribution, it is inevitable that some droplets will contain two analyte molecules, or even more than two (with frequency dropping quickly as the dilution increases). *Supra* ¶33, §V.E. But such droplets are unnecessary in Saxonov’s method for obtaining the data Larson desires. *Supra* §V.E.

123. Although it is my understanding that Saxonov's FIG. 10 and ¶¶78-79 are not prior art to the '921 Patent (*see* §V.F.). Petitioner relies heavily upon these portions (*see* §V.F.). At the same time, these very portions highlight Saxonov's deficient teachings in how to obtain meaningful information, even from double-positive and triple-positive droplets.

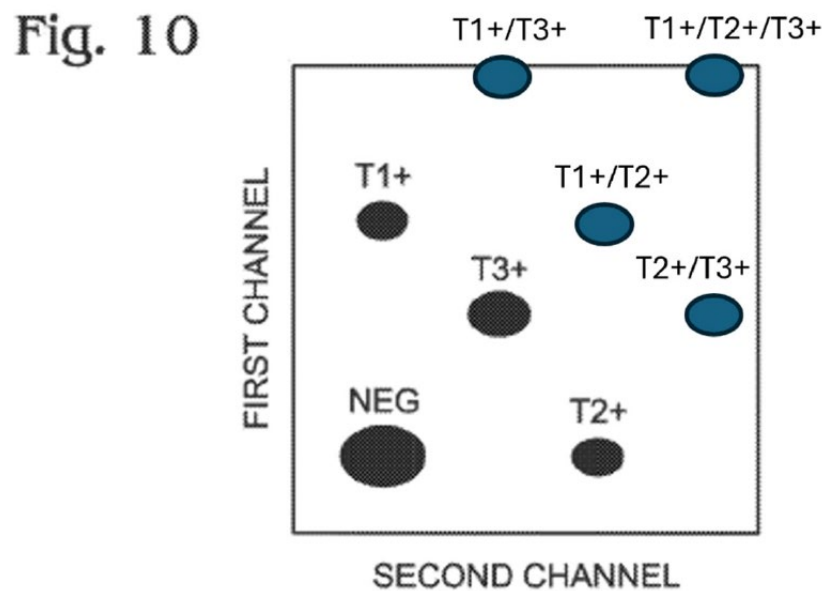
124. For example, Saxonov states that double-positive populations (T1+/T2+, T1+/T3+, and T2+/T3+) and the triple-positive population (T1+/T2+/T3+) in FIG. 10 are not shown “[t]o simplify the presentation” but “may be resolved and distinguishable from one another and from the single-target populations and the negative population.” EX1004(Saxonov), ¶¶78-79. Yet, Saxonov does not actually explain *how* such populations of droplets that contain three or more analytes might actually be “resolved and distinguishable from one another”—let alone in the specific manner recited in the '921 Patent claims. EX1004, ¶¶78-79.

Fig. 10



125. Saxonov’s ¶20 further confirms that Saxonov did not really know, or teach, how to distinguish clusters of multi-analyte droplets—whether in one channel or multiple channels: “[t]hree targets, two in a first channel and one in a second channel, would again generate eight clusters or populations of data, but they would be separated in a two-dimensional intensity space *and so in principle more easily resolvable*.” EX1004(Saxonov), ¶20. This falls far short of teaching a POSA that the clusters are *actually* resolvable, let alone how to design the probes such that the clusters are *resolvable and unambiguously* detected.

126. Moreover, Petitioner proffers the following fabricated version of Saxonov's FIG. 10, which includes the double-positive and triple-positive clusters that Saxonov did not actually illustrate (*i.e.*, Petitioner's figure did not actually appear in Saxonov):

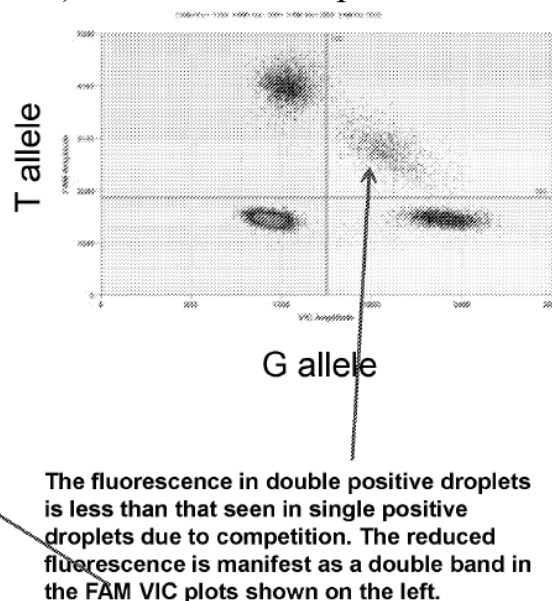


Petition, 60.

127. More specifically, in its own version of Saxonov's ostensible teachings, Petitioner adds double-positive clusters T1+/T3+, T1+/T2+, T2+/T3+, and triple-positive cluster T1+/T2+/T3+ in blue, to Saxonov's FIG. 10. Petition, 60. Petitioner states that "[a]s this modified figure shows, any combination of three targets may be uniquely identified." Petition, 60. Without explaining any basis for alleging the particular cluster locations added to FIG. 10, Petitioner's

proffered expert concludes that “this representation shows how easily each of three targets, however they may be distributed in droplets, are to identify.” EX1002, ¶343.

128. In my view, Petitioner’s version of FIG. 10 is a misleading fabrication. For example, the Saxonov Provisional actually shows a double-positive result in a *different location* than Petitioner’s FIG. 10 asserts. More specifically, the Saxonov provisional includes a plot of two measured analytes (here, the T and G alleles) in two channels, plus an arrow and annotation:



EX1007(Saxonov Provisional), 58.

129. Consistent with FIGS. 5 and 10 of Saxonov, I understand (and a POSA would have understood) that the lower right cluster shows “single positive” for the G allele, and the upper left cluster to be “single positive” for the T allele.

130. The Saxonov Provisional refers to the middle cluster as “[t]he fluorescence in *double positive* droplets,” and states that the droplets’ fluorescence “is less than that seen in single positive droplets due to competition.” *Id.* But the location of this double-positive spot—which appears to be *actual data*—is totally different than that of the double-positive spot in Petitioner’s manufactured version of Saxonov’s FIG. 10. In particular, the measured double-positive spot does *not* occur at a location corresponding to the neat and simple sum of the T+ and G+ single positive clusters, as Petitioner’s fabricated FIG. 10 illustrates. Instead, the actual double-positive spot reported in the Saxonov provisional is *shifted to another location* because of reduced fluorescence “*due to competition.*” EX1007(Saxonov Provisional), 58. Accordingly, a POSA would have expected double-positive and the triple-positive clusters (if any) to occur in *different locations* than those shown in Petitioner’s fictional version of Saxonov’s FIG. 10 asserts.

131. For this reason, in addition to the reasons set forth further above regarding Saxonov’s many deficiencies, it is my opinion that Saxonov fails to teach an assay that is “capable of *unambiguously* detecting the presence or absence of each of said at least M analytes, *in any combination of presence or absence, in a single sample volume*, or a kit that likewise is “capable of *unambiguously* detecting the presence or absence of each of analyte of M analytes

in a single sample volume, *in any combination of presence or absence.*”

EX1001('921 Patent), 67:16-20, 68:31-34.

4. Combining Larson with Saxonov Falls Short of the
Claimed Subject Matter

132. For the reasons set forth above, Saxonov and Larson are both materially deficient in teaching everything recited in the '921 Patent's claims 1 and 14. *Supra*, §§VII.A.-C.3. Nonetheless, Petitioner and its proffered expert try to rationalize why the claimed subject matter would have been obvious over Larson combined with Saxonov. Petition, 58-61; *citing* EX1002(Batt), ¶¶339-348. I disagree with their explanations why, and believe that even if Larson were to be combined with Saxonov for any reason (whether for Petitioner's proffered reason or otherwise), the resulting combination would fall far short of what is claimed in the '921 Patent.

133. For example, Petitioner tries to use the “multiple occupancy” problem, identified by Larson, as a reason to justify combining Larson with Saxonov:

Incorporating Saxonov's teachings that droplets with different combinations of targets can be “separated by intensity” would have been a no-brainer and something a POSA would have been highly motivated to extract the

most information possible from the experiment and address “multiple occupancy” situations.

Petition, 61, citing EX1002(Batt), ¶¶344-346.

The cited section of Dr. Batt’s Declaration elaborates somewhat on
Petitioner’s position:

A POSA would have been highly motivated to do this as well, as discussed, to acquire the maximum amount of information from this kind of costly equipment...but also to adequately deal with the “multiple occupancy” situations that Larson discusses arise when loading targets into droplets with a Poisson distribution. See, e.g., EX1017¶¶ 129, 140-141.

EX1002(Batt), ¶346.

134. However, to emphasize yet again, Larson teaches addressing the “multiple occupancy” problem as follows: *diluting* and partitioning the sample into numerous droplets to reduce the likelihood of any droplets ever containing two or more analytes, and using sorting to *exclude* any droplets with “multiple occupancy” or containing a “heterogeneous population of molecules.” *Supra* §V.D.

135. Accordingly, in my view, if a POSA wished to address the “multiple occupancy” problem, the teachings of *both* Larson *and* Saxonov would have led

them to attempt to *dilute* the sample further to reduce the occurrence of “multiple occupancy.” *Supra* §§V.D., V.E. And, to the extent that there were still droplets with “multiple occupancy,” the teachings of Larson would have led the POSA to use sorting to *exclude* any such droplets. *Supra* §VII.C.1. In my view, nothing in Larson or Saxonov would have rationally motivated a POSA to try to modify Larson to *increase* the number of “multiple occupancy” droplets, e.g., by *increasing* concentration, by *not* excluding “multiple occupancy” droplets, and by *intentionally analyzing* “multiple occupancy droplets,” which seem to be the only way any “multiple occupancy” droplets could be obtained by modifying Larson’s methods. I do not think a POSA would have found it at all obvious to change Larson’s entire principle of operation in such a manner, and Petitioner fails to explain why a POSA would have tried to do so.

136. Moreover, it is my opinion that a POSA would not even have been motivated to combine Larson and Saxonov, because Saxonov does not add any meaningful teachings to Larson. Petitioner admits that Saxonov, like Larson, provides the teaching of loading droplets with targets according to Poisson distribution. Petition, 58. Petitioner further argues that a POSA would have been motivated to combine teachings because “Larson already teaches the same approach utilized in Figure 10 of Saxonov[.]” Petition 60-61. Without pointing out any additional teaching provided by Saxonov, Petitioner argues that it would

have been obvious to combine teachings of Larson and Saxonov. Petition 58-61.

For the reasons discussed above, it is my opinion that Saxonov provides no additional meaningful teachings that cure Larson's deficiencies. *See* ¶¶132-135 of this Declaration. Furthermore, a POSA would not have been motivated to combine the teachings of Larson and Saxonov because combining Saxonov would not have added anything meaningful and thus the combination would have been pointless.

137. Therefore, in my view, claims 1 and 14 of the '921 Patent, and claims depending therefrom, are not obvious over Larson in view of Saxonov.

D. Ground 4 (Claim 11): Obviousness In View Of Larson, Saxonov, and Silverbrook

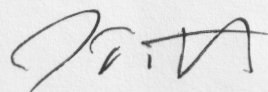
138. The Petition alleges that claim 11 of the '921 Patent is obvious in view of Larson and Saxonov in view of Silverbrook. Petition at 66-67. Claim 11 depends on and adds limitations to claim 1. The Petitioner argues that Silverbrook teaches "PCR and hybridization to identify which analytes are present in a sample and transmitting results of the testing through a computer network." Petition at 66. Even assuming that Silverbrook teaches that for which it is cited, such features do not cure the many deficiencies of Larson and Saxonov as detailed elsewhere herein. *See supra* §§VII.A.-C.

VIII. DECLARATION

139. This Declaration and my opinions herein are made to the best of my knowledge and understanding, and based on the material available to me, at the time of signing this Declaration. I declare under penalty of perjury under the laws of the United States that all statements made herein on 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 § 1001 or Title 18 of the United States Code. I executed this Declaration at Weston, Massachusetts.

Dated: December 30, 2024

By



KEVIN STRUHL