



UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

**Dropworks, Inc.
Petitioner,**

v.

**Lawrence Livermore National Security, LLC.
Patent Owner.**

**IPR2021-00302
U.S. Patent No. RE43,365**

(Exhibit 2023)

**DECLARATION OF CARL BATT, PH.D., IN SUPPORT OF
PATENT OWNER'S RESPONSE TO PETITION
FOR *INTER PARTES* REVIEW**

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I. INTRODUCTION

1. I, Carl Batt, Ph.D., have been retained by Weil, Gotshal & Manges LLP, counsel for Patent Owner Lawrence Livermore National Security, LLC (“Lawrence Livermore” or “Patent Owner”). I understand that Petitioner Dropworks has filed an inter partes review Petition against U.S. Patent No. RE43,365 (the ‘365 Patent). I understand that the Patent Trial and Appeal Board (PTAB) Instituted this proceeding based on Dropworks’ Petition. The following discussion and analysis provides my opinions in support of Patent Owner’s Response to the Petition and in response to the opinions provided by Petitioner’s expert, Dr. Meinhart.

A. Qualifications And Experience

2. I am presently the Liberty Hyde Bailey Professor of Biology at Cornell University in the Department of Food Science, and the Director of the Cornell University/Ludwig Institute for Cancer Research Partnership. I am also a founder of Main Street Science and the founder of Nanooze, an on-line science magazine for kids. I am also a co-founder and former co-director of the Nanobiotechnology Center, a National Science Foundation supported Science and Technology Center. I am also appointed as an Adjunct Senior Scientist at the MOTE Marine Laboratory in Sarasota, Florida. My research interests are a fusion of biology and nanotechnology focusing on important biomedical problems.

3. I received my Bachelor's of Science degree in Microbiology from Kansas State University, and Master's of Science and Ph.D. degrees from Rutgers University, Department of Food Science. From 1981 to 1985, I did postdoctoral work at the Massachusetts Institute of Technology. From 2002 to 2012, in partnership with the Ludwig Institute for Cancer Research, I established a Good Manufacturing Practices Bioproduction facility in Stocking Hall. My full curriculum vitae is attached to my report as Appendix A.

4. Over the years, I have published over 270 peer-reviewed articles, book chapters, and reviews. In addition, from 1987-2000 I served as an editor for Food Microbiology, a peer-reviewed journal, and editor for the Encyclopedia of Food Microbiology that was published in 2000. In 2010, I undertook a revision of this publication that was released in 2012. In 2015-2016, I was subject editor for the Elsevier, Food Science Reference module

5. I have made practical use of polymerase chain reaction (PCR) and real-time PCR in my research and teaching since their discovery. PCR and real-time PCR are still integral tools in my research labs, in both public and private practice. In particular, I and others in my laboratory working at my direction, built an instrument in my laboratory for performing real-time PCR for research purposes. In addition, my group has made use of microfluidic devices for a variety of applications

including real-time molecular analyses of various organisms based upon target nucleic acid sequences.

6. In preparing this declaration, I have considered the claims, specification, and prosecution history of the '365 Patent from the vantage point of one of ordinary skill in the art. I have also reviewed the briefing in this inter partes review proceeding, and the accompanying Exhibits, including the declaration of Petitioner's Expert Dr. Meinhart. A list of the materials that I reviewed is included as Appendix B to this report. I am also relying on my expert experience, education, and qualifications as a basis for my analysis and opinions.

7. I reserve the right to modify or supplement my opinions or the bases for my opinions in light of any additional documents, depositions and other testimony, or other evidence that may become available the proceeding progresses. I may supplement Appendix B to incorporate such additional materials.

B. Compensation

8. I am being compensated at my standard billing rate of \$375/hr. My compensation is not contingent on reaching any particular findings or conclusions, or on any outcome in the case. The opinions contained in this report are mine and are based on my own personal knowledge, experience, training, and study of the materials discussed below in this report.

II. UNDERSTANDING OF THE LAW

9. In this section I describe my understanding of certain legal standards. I have been informed of these legal standards by Lawrence Livermore's attorneys. I am not an attorney, and I am relying only on instructions from Lawrence Livermore's attorneys for these legal standards. I understand that there are a limited number of grounds under which a party may contend that a patent claim is invalid in an inter partes review proceeding, including at least: (1) anticipation (under 35 U.S.C. § 102) and (2) obviousness (under 35 U.S.C. § 103).

10. It is my understanding that when interpreting the claims of the '365 Patent, I must do so from the perspective of one of ordinary skill in the art at the relevant priority date. The earliest application to which the 365 Patent claims priority was filed on March 14, 2003. However, as discussed below, the invention of the '365 Patent was conceived of in 2001. My opinion of the person of ordinary skill in the art at the time of the invention in the '365 Patent is set forth in Section III.B., below.

11. To be anticipatory, I understand that each and every element of a claim must be found, either expressly or inherently, within the single prior art reference in as complete detail as recited in the claim. I understand that a reference does not anticipate a claim unless all elements of the claim are disclosed within the four

corners of the reference in the same arrangement as in the claim. I understand that a reference does not anticipate a claim if it is necessary to select and combine different features from different protocols within the reference to meet the various claimed features.

12. I understand that a claimed invention is only unpatentable under 35 U.S.C. § 103 (“obvious”) if the differences between the invention and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains.

13. I understand that in assessing whether a claimed invention would have been obvious at the time the invention was made to a person having ordinary skill in the art, the following factors are considered: (1) the scope and content of the prior art; (2) the differences between the art and the claims at issue; (3) the level of ordinary skill in the art; and (4) objective evidence of non-obviousness. I understand that a claim of obviousness may be based on one or more references, taken in combination.

14. I understand that a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was known in the prior art. It has been explained to me that three requirements must be satisfied for a

combination of prior art references to render obvious a claimed invention. First, the cited art must teach or suggest all the limitations of the invention as recited in the claims. Second, the cited art, coupled with the knowledge generally available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated the ordinary skilled artisan to modify a reference or to combine references. Third, the proposed modification of the cited art must have had a reasonable expectation of success, determined from the vantage point of the ordinary skilled artisan at the time the invention was made.

15. I understand that it is impermissible to use hindsight to arrive at the claimed invention. My understanding is that to preclude hindsight in the obviousness analysis, which I understand to be improper, there must be evidence at the time of the invention of some reason to make the variation or combination. Furthermore, I understand that, to render a claim obvious, the prior art cannot be vague, but rather, must collectively guide a person of ordinary skill in the art towards the particular solution claimed.

16. I understand that obviousness does not exist where the prior art discourages or teaches away from the claimed invention. Furthermore, I understand that “teaching away” does not require that the prior art foresaw the specific invention claimed in the patent and warned against taking that path; rather, the prior art design

itself may teach away from the claimed invention. I understand that in an obviousness analysis, only analogous art may be considered.

17. Art is considered analogous if it is from the same field of endeavor as the patent. If it is not from the same field of endeavor, it may be considered analogous if it is reasonably pertinent to the particular problem with which the patent is involved. A reference is reasonably pertinent if, though in a different field, it would have commended itself to an inventor's attention in considering her problem.

18. I understand that in evaluating whether patent claims are obvious, objective indicia of non-obviousness can be considered, such as commercial success, long-felt but unsolved needs, copying, praise, unexpected results, industry acceptance, failure of others, and skepticism by experts.

III. BACKGROUND

A. The '365 Patent

19. The '365 Patent represents a critical advance in technology that enables analysis of biological materials. The inventors of the '365 Patent recognized that rapid detection of biological materials such as those used in biological warfare could push defense mechanisms from "detect to treat" to "detect to protect", "having a serious impact on the number of casualties from a massive bioagent release." Ex.

1001 at 4:22-29. The inventors discovered that this analysis could be accomplished by using micro-volumes of sample fluid to enable rapid reactions and detection.

20. To accomplish their goal of performing ultra-fast reactions on and analysis of biological material, the inventors used their combined experience with microfluidics, chemistry, and military defense to devise novel devices and methods for conducting PCR reactions in microfluidic devices. Microfluidic devices could be used to manipulate small volumes of reagent fluid to partition sample material such that a single DNA molecule was isolated in an individual partition. By applying heating and cooling elements to the microfluidic devices, PCR could be performed on these encapsulated single-molecule templates to create a sufficient number of copies to form a detectable reaction product.

21. This invention has numerous benefits, which the inventors recognized were applicable “wherever current PCR-type systems exist, including medical, drug-discovery, bio-warfare detection, and other related fields.” *Id.* at 6:37-39. In particular, the '365 Patent explains that the advantages of its system include: “(1) much faster detection time through a reduction in the total number of temperature cycles required, (2) a reduction in the time for each cycle, and (3) removing interference from competing DNA templates.” *Id.* at 4:46-49.

22. An exemplary depiction of one system described in the '365 Patent is shown below in Figure 3.

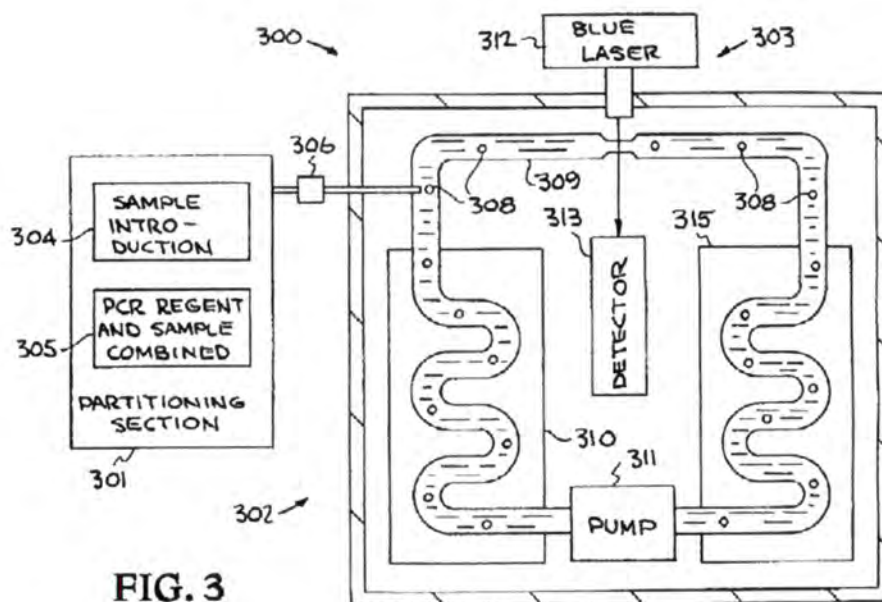


FIG. 3

Id. at Fig. 3.

23. The schematic depicted in Figure 3 includes a partitioning section (301) where sample and PCR reagents are combined, and the resulting sample fluid is injected through orifice (306) to produce microdroplets (308) suspended in an immiscible carrier fluid. *Id.* at 6:59-63. Using this process, “each partitioned DNA-containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction.” *Id.* at 3:56-59. In the embodiment depicted in Figure 3, the droplets are

pumped through a tube (309) and cycled through heating (310) and cooling (315) regions to conduct PCR reactions on the genetic material inside of the droplets. *Id.* at 7:1-4. Specifically, the PCR reaction amplifies target sequences of DNA encapsulated in droplets using PCR primers that only bind to complementary target sequences of DNA. After PCR, the droplets flow past the detection and analysis section (303), which includes a laser (312) and a detector (313). *Id.* at 7:5-8. The laser excites fluorophores released during amplification of target molecules and the detector measures the resulting fluorescence intensity.

24. By partitioning individual molecules into droplets, the systems described in the '365 Patent "reduc[e] the duration of each temperature cycle by effectively increasing the concentration of reactants by enclosing them in picoliter type volumes." *Id.* at 5:18-20. The '365 Patent is distinguished from prior-art test-tube PCR reactions not only by its faster reaction rates, but its ability to partition and analyze single DNA molecules, which allows for ultra-sensitive and fast analysis on a molecular level.

B. State Of The Art

1. Level of Ordinary Skill In The Art

25. I understand that Dr. Meinhart alleges that "a person of skill in the art would have been a researcher interested in microfluidics with at least a bachelor's

degree in a science such as bioengineering, chemical engineering, chemistry, physics, mechanical engineering, or biochemistry; or a bachelor's or master's degree in a relevant discipline and three to five years of relevant experience.” Meinhart Decl. ¶ 51. While I generally agree with this description of a POSITA, in my opinion, Dr. Meinhart's description of what a person of ordinary skill in the art would have been familiar with at the time of the invention leaves out important context that is useful to understand when evaluating the level of knowledge and skill of a POSITA at the time of the '365 Patent.

26. I have reviewed the Preliminary Patent Owner response, and agree that in the 2002 time frame, a skilled artisan would have been a researcher interested in microfluidics with at least a bachelor's degree in a hard science that involves both chemical and physical phenomena, such as chemistry, physics, chemical engineering, bioengineering, or mechanical engineering. However, I also agree that additional information is useful to aid in the understanding of the relevant knowledge of the skilled artisan at the time of the claimed invention.

27. At the time of the claimed invention, microfluidic and droplet-based methods of analysis and detection were in the very early stages of development. For example, even as late as 2006, researchers characterized the field of microfluidics as in its “infancy.” *See e.g.*, Ex. 2001 at 372 (“So, what next for microfluidics? It is

both a science and a technology....It is also in its infancy, and a great deal of work needs to be done before it can be claimed to be more than an active field of academic research.”). Accordingly, at the time of the ’365 Patent, a skilled artisan would have viewed microfluidics as a novel departure from the prior art and would have understood that researchers at the time had only made simple proof-of-concept demonstrations of microfluidic techniques related to the manipulation of fluid reagents in a confined space. *Id.* at 369 (describing the present state of microfluidics in 2006). Such techniques were not yet commercially successful, and would not have been routine to the skilled artisan. *Id.* at 371 (“Above all, [microfluidics] must become successful commercially, rather than remain a field based on proof-of-concept demonstrations and academic papers.”). Accordingly, the application of microfluidic techniques, particularly as applied to the manipulation of fluid reagents, would not have been top of mind to researchers in the field of the claimed invention, and would not have been straightforward to implement or adopt.

28. Further, at the time of the invention, a skilled artisan would have had little if any experience with droplet-based technology. A person of skill in the art at this time would have understood microfluidics to be largely limited to continuous-flow, and would not have been familiar with the use of droplet-based microfluidics, especially for performing reactions. Techniques of creating droplets and

encapsulating particles and reagents in droplets was barely in the proof-of-concept stage as of 2002. Thus, in 2002, the skilled artisan would have had little to no experience conducting reactions in droplets because the field was still considered nascent as of 2008. *See, e.g.*, Ex. 2002 at 216 (“This paper provides a comprehensive review of the field of ‘droplet’ or ‘digital’ microfluidics and its applications. This nascent field has attracted a diverse group of researchers to study the fundamentals of two-phase dynamics in microchannels and also to develop novel solutions for biological and chemical applications that are superior to conventional techniques.”).

29. In support of my opinion, I note that several of the references Petitioner cites, such as Kopp, Curcio, and Schneegaß, are papers focused on describing rudimentary proof-of-concept experiments related to flow-through thermocycling and droplet-based reactions. These papers are exemplary of the type of work that was being done in microfluidics around the time of the invention. For example, Kopp describes the design of a flow-through thermocycling device that performed 20 PCR cycles to amplify a 176-base pair fragment to assess how flow rate impacted the reaction. This demonstrates that researchers at this time were interested in evaluating the basic aspects of flow-through thermocycling in a continuous-flow configuration. A few years later, Curcio used a 15 meter length of tubing and manually injected alternating segments of sample fluid and oil to create a

rudimentary segmented-flow system to amplify DNA. Ex. 1005. Curcio documented several issues with its system and areas for further optimization, concluding that continuous segmented-flow PCR could provide a basis for miniaturized DNA amplification. *Id.* at 7. Notably, Curcio was published in January of 2003, which is after the conception date of the '365 Patent. *Id.* This demonstrates that even in 2003, only a rudimentary apparatus was being used to perform basic proof of concept experiments to explore PCR in segmented-flow systems (which are not droplet-based).

30. Accordingly, it is my opinion that a skilled artisan interested in analyzing biological material and chemical samples would have most likely attempted to follow prior art techniques, such as continuous-flow through thermal cycling, or cell sorting (as discussed in greater detail below). As I understand, even advanced researchers who attempted to perform even simple reactions in droplets struggled to achieve desirable results. *See e.g.*, Ex. 2015 (Thorsen Thesis).

C. “Prior Art” Cited In the Petition

31. Below, I provide a summary of the alleged prior art relied on by Petitioner. As I detail later in my report, I do not agree that the references relied on by Petitioner are prior art to the '365 Patent.

1. Ismagilov

32. Ismagilov describes methods and microfluidic systems for generating plugs in a microfluidic device, and conducting autocatalytic reactions within the plugs. Ismagilov explains that plugs can be created by flowing an aqueous fluid through an inlet port at a junction between the inlet port and a channel containing an immiscible fluid such as oil. The plugs can contain reagents for a reaction. Ex. 1003 at 2:58-60. Plugs are formed at the junction of the inlet port and the channel, when the stream of aqueous fluid contacts the carrier fluid (e.g., oil in the channel. Ex. 1003 at 9:61-67. Ismagilov teaches that surfactants “designed to assemble at the interface between the plugs and the immiscible fluid that surrounds them” may be used to control the surface chemistry of the disclosed devices. *Id.* at 12:9-17. The use of surfactants “may facilitate the control and optimization of plug size, flow, and uniformity” for example, by reducing “the shear force needed to extrude or inject plugs into an intersecting channel.” *Id.* at 31-44. 20:31-65. As explained in greater detail below, Ismagilov does not constitute prior art to the '365 Patent.

2. Curcio

33. Curcio describes a “continuous segmented-flow method” for DNA amplification. Ex. 1005 at 1. In contrast to microfluidic devices that create droplets in an immiscible fluid such as oil, segmented flow reactors such as those described

in Curcio use alternating segments of sample fluid and a spacer fluid to separate the sample fluid into discrete segments. *Id.* at 3. Curcio proposes an experimental apparatus that introduces alternating segments of sample fluid and immiscible fluid into a tube using pressurized helium to create aqueous compartments of sample fluid. *Id.* The system in Curcio is not a microfluidic device. Rather, Curcio describes a rudimentary system that includes a 15 meter length of tubing that transits through three temperature zones for a total of 30 times to circulate sample fluid segments. *Id.* Reactions were performed in the sample fluid as it moved through the tubing. *Id.* at 3-4. However, Curcio notes significant problems with cross-contamination. *Id.* at 4-5 (explaining that “a substantial carryover occurs” between consecutive samples, and that “cross-contamination could also be visually observed in a model experiment, where an aqueous plug was loaded with Bromophenol blue as a front sample”).

3. Quake

34. Quake describes an apparatus to sort particles (e.g. virions) by directing their flow and sorting them. I understand Quake to be primarily directed to sorting particles, such as viral particles, not to performing PCR in droplets. *See, e.g.,* Ex. 1006 at 5:1-3 (“The invention addresses the above-discussed and other problems in the art and provides new devices and methods for sorting viruses and other particles

by flow cytometry.”); *id.* at 2:6-9 (“In a preferred embodiment, the devices and methods of the invention are used to sort or evaluate virions or virus particles. Other preferred embodiments are used to sort or evaluate molecules, such as nucleic acids or proteins, or cells, such as bacteria or pathogens.”). It is my understanding that Quake does not contain any disclosure of performing PCR reactions in droplets, and in fact teaches away from performing PCR in droplets, as discussed in greater detail below.

35. Quake purports to address problems associated with conventional sorting devices such as fluorescence-activated cell sorting (“FACS”) and flow cytometry by creating microfluidic devices allegedly capable of detecting smaller particles, such as viruses as an alternative to PCR. *See id.* at 3:8-10 (“Conventional flow sorters, such as FACS have numerous problems that render them impractical for analyzing and sorting viruses and other similarly sized particles.”); *see generally id.* at 3-4. Quake explains that its methods and devices are “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil” so that the droplets (e.g., containing viruses) can be optically analyzed and sorted based on their contents. *Id.* at 1:16-18. For example, Quake indicates that droplets containing a viral particle with a particular marker can be detected by linking a reporter molecule (such as an optical label) to a characteristic

of the viral particle, and sorted based on this label by controlling valves and/or fluid flow within the device. *Id.* at 32:26-31.

36. I do not understand Quake to teach methods for carrying out PCR, in microfluidic droplets. Rather, I understand Quake to indicate problems with PCR, and recommend the direct optical detection of sorted viral particles as an alternative to PCR detection of viral particles. PCR is mentioned only twice in the entirety of Quake's disclosure. First, in the background section, Quake explains that PCR as an existing method for detecting viruses is defective, and states its intention to offer alternatives to PCR by using a sorting system. *Id.* at 2:25-3:6 ("Molecular techniques such as DNA probes or the polymerase chain reaction (PCR) are used for the detection of viruses where cell culture or serological methods are difficult, expensive or unavailable."). In particular, Quake explains that although PCR is traditionally the method of choice to detect viral DNA or RNA in clinical specimens, and has the advantage of sensitivity, it also comes with a significant risk of cross-contamination from sample to sample that "can outweigh the benefits of detecting small quantities of a target viral nucleic acid." Quake at 2:31-3:6. Thus, Quake presents the use of direct optical detection of viral particles in microdroplets as a preferred alternative to direct PCR detection of DNA or RNA in clinical specimens.

37. The second reference to PCR in Quake describes PCR in the context of other applications of microfabrication technology. *Id.* at 23:26-29 (“*Microfabrication permits other technologies to be integrated or combined with flow cytometry* on a single chip, such as PCR (13), moving cells using optical tweezer/cell trapping (14-16), transformation of cells by electroporation (17), μ TAS (18), and DNA hybridization (5).” (emphasis added). This disclosure is not an explanation of how to perform PCR in the droplets generated by Quake’s system, but simply acknowledges that microfabrication can be used to integrate other techniques into a chip in addition to Quake’s microfluidic sorter.

38. Unlike the ’365Patent, Quake does not rely on droplets as microreactors to perform PCR reactions. Rather, Quake primarily uses droplets to encapsulate viral particles so that they can be sorted and detected. Quake’s method of detecting viral particles does not require any amplification, as Quake claims that optical markers can be used to directly detect the targeted viral particles. The few references Quake does make to performing reactions in droplets are either bare references to performing reactions with no detail on how to implement the reaction, or they are descriptions of reactions that are distinct from PCR and do not require any of the elements critical to performing PCR in droplets. This is consistent, in my opinion,

with Quake's focus on direct detection primarily using optically-labeled surface markers.

4. Holliger

39. Holliger describes methods of “in vitro evolution of molecular libraries” which involves selecting certain enzymes which evolve to have the desired characteristics. Ex. 1007 at 1:3-6. Specifically, Holliger proposes a method to improve the properties of polymerases (e.g., nucleic acid-processing enzymes) by compartmentalizing nucleic acids and nucleic acid-processing (NAP) enzymes in the same compartment. The NAP enzymes can “process” or amplify genetic material. Thus, the activity of the NAP enzyme can be measured based on the quantity of the amplification product produced by the NAP enzyme. *See* Ex. 1007 at 3:14-20; see also *id.* at 5:24-26 (“[T]he post amplification copy number of the nucleic acid member is substantially proportional to the activity of the replicase, the activity of a requisite agent, [or] binding affinity of the first and second polypeptides.”). Unlike the methods described in the '365Patent, which are directed to identifying a target sequence of a nucleic acid (e.g., to detect a particular pathogen), Holliger is directed to the selection of a particular enzyme variant based on its activity level.

40. In Holliger, the nucleic acids which encode the NAP are compartmentalized into vesicles and the NAP is therefore produced within the vesicle. According to Holliger, NAP enzymes replicate nucleic acids. *Id.* at 5:16-18. Thus, by compartmentalizing the nucleic acid sequence which encodes the NAP enzyme, sequences that encode for NAP with enhanced replication activity can be identified. For detection, Holliger describes assaying the contents of the droplets to determine which had greater amounts of nucleic acids resulting from NAP with enhanced replication activity. For example, Holliger describes the use of gel electrophoresis and sequencing to determine the quantity of nucleic acid to perform this assessment. The droplet with the greater amount of nucleic acid resulted from a NAP in that droplet which had a higher replication activity.

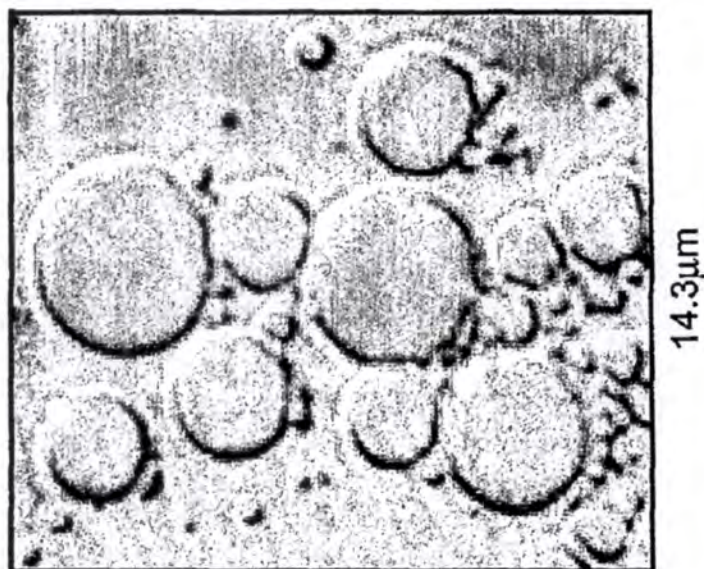
41. Rather than teaching microfluidics, Holliger uses bulk emulsions, formed by adding aqueous solution manually (“dropwise”) to a container of oil in conjunction with stirring, *id.* at 52:7-18, or via mechanical agitation of the water/oil mixture using something like a paddle or whisk:

Creation of an emulsion generally requires the application of mechanical energy to force the phases together. There are a variety of ways of doing this which utilise a variety of mechanical devices, including stirrers (such as magnetic stir-bars, propeller and turbine stirrers, paddle devices and whisks),

homogenisers (including rotor-stator homogenisers, high-pressure valve homogenisers and jet homogenisers), colloid mills, ultrasound and ‘membrane emulsification’ devices.

Id. at 48:14-19. These types of emulsions are formed spontaneously based upon the surface tension between the aqueous sample fluid and the immiscible fluid (oil) when mixed together.

42. Figure 2 of Holliger provides an example of the polydisperse water vesicles formed using Holliger’s process which vary dramatically in size:



Id. at Fig. 2.

43. Figure 2 shows that Holliger’s mechanical agitation methods produce vesicles with wildly varying sizes, rather than the uniform sizes that can be created in a microfluidics device. This is consistent with Holliger’s description that vesicles

are created by first adding an aqueous solution dropwise to a container of oil, and then further dispersing those vesicles by non-specific mechanical disruption. This type of technique provides very little control over the size and the relative contents of the droplets.

44. Holliger purports to describe experiments involving PCR in the mess of random vesicles that are dispersed throughout the emulsion. To generate the vesicles for this experiment, Holliger introduces 200 μL of PCR mixture into a mineral oil, allegedly creating vesicles that are on average 15 μm in size. *Id.* at 59:10-15. This leads to roughly 200 million vesicles,¹ far more than the few thousand droplets that are created in the microfluidic droplet system in Quake. Elsewhere, Holliger teaches generating more than a billion water vesicles per milliliter. *See id.* at 12:21-23 (“With $> 10^9$ microcompartments/ml, emulsion PCR (ePCR) offers the possibility of parallel PCR multiplexing on a unprecedented scale.”)

45. Holliger then transfers the millions vesicles in bulk to a standard PCR tube and then subjects the emulsion to thermal cycling in a standard thermal cycler.

¹ This can be computed based Holliger’s suggestion of using 0.2mL (Ex. 1007 at 59:10-15) and creating emulsions with 10^9 microcompartments per milliliter (Ex. 1007 at 12:21-23).

Id. at 59:16-17. Following thermal cycling, ether is added to break the emulsion and the aqueous portion of the emulsion is recovered in bulk. *Id.* at 59:18-23. Holliger then analyzes this fraction using gel electrophoresis to assess whether any PCR products were formed. *Id.* As I describe in greater detail below, gel electrophoresis involves drawing the sample material using an electrical field through a porous gel to separate the material into different size bands according to size. Thus, different molecules are grouped together based on its size, and the mass of molecules in each band can be measured using the fluorescence of the molecules and a fluorescent dye.

46. Holliger, however, includes nothing to establish what fraction of the millions of vesicles actually contained a PCR product, or whether the PCR products can be attributed to vesicles of a particular size that might be formed in a microfluidic device. Further, because the individual vesicles are destroyed before detection, Holliger's method is not capable of identifying whether any particular size of vesicle contains a PCR product. Holliger therefore does not establish any clear linkage between the vesicle and its ability to support a PCR reaction.

47. Unlike Holliger, the '365Patent uses microfluidic devices to generate droplets, and optical analysis methods to detect PCR products inside the droplets without breaking them. In contrast to Holliger, I understand that the goal of the '365Patent is to detect the presence or absence of a PCR product using optical

detection in droplets as a means to detect the nucleic acid target in the sample. The '365 Patent has no need to determine copy number or otherwise characterize the contents of partitions—it relies on a binary determination of droplet contents. The inventors of the '365 patent discovered that microfluidics could be used to create droplets on a microfluidic chip, perform reactions inside the droplets on the chip, and detect the reaction products inside the droplets by optical analysis creating a seamless method of rapid detection of target nucleic acids. Holliger's goal, however, was to evolve molecular libraries and select improved nucleic acid polymerases. This relies on the quantification of the reaction product inside the droplet, and accordingly requires an analysis method that goes beyond binary detection. This level of analysis is not required nor informs the '365 patent.

5. Schneegaß

48. Schneegaß is a review article primarily focused on describing the state of the art of flow-through thermocyclers for performing on-chip PCR as of 2001. Specifically, Schneegaß reviews the history and designs of various flow-through reactors to compare features of different devices. Ex. 1008 at 101. Flow through thermocyclers perform PCR reactions in sample fluid by flowing it through narrow tubing. Ex. 1008 at 107. Flow-through thermocycling pumps a continuous stream

and does not use droplets to encapsulate reaction components. Therefore, it cannot be used to analyze single-target nucleic acid molecules.

49. Schneegaß also explains the possibility of generating a series of sample plugs, created by pulsing an aqueous liquid plug fluid with another an immiscible liquid medium. *Id.* at 108 (“One of the most attractive features of flow reactors is the possibility to generate a serial sequence of sample plugs, which are separated by small zones of a medium to enhance the sample number capacity of the device.”). This method, which is discussed in theoretical terms without much detail on implementation, is distinct from droplet-based art such as Quake in that it describes “segmented flow” reactions. As described above, segmented flow of a sample is created by alternately injecting an aqueous sample fluid and an immiscible spacer fluid to create discrete segments of sample. The ’365 patent, by contrast, relies on the surface tension between the sample fluid and the immiscible carrier fluid as sample fluid is continuously injected into an immiscible carrier fluid to create uniform droplets in the microfluidic device.

50. The limited discussion Schneegaß provides regarding systems that use aqueous droplets in oil is mainly centered around prior art, such as Litborn and Katsura, which do not disclose performing PCR reactions in droplets in a microfluidic device. Ex. 1008 at 109. Schneegaß makes a transient reference to

performing reactions in droplets but does not provide sufficient details on the specifics or results of this experiment to inform a POSITA.

IV. CLAIM CONSTRUCTION

51. I understand that Petitioner and Dr. Meinhart have advanced the following claim constructions:

- “orifice”/“injection orifice”: Petitioner contends that the terms “orifice” / “injection orifice” should be construed as a “hole to permit fluid flow” / “a hole to permit fluid flow by force.”
- “microfluidic device” / “microdroplet”: Petitioner contends that the term “microfluidic device” should be construed as “a device for manipulating fluid samples and reagents, wherein the fluid flows through a structure having a cross-section of micrometer dimensions.” And that “microdroplet” should be construed as “a droplet having a cross-section of micrometer dimensions.”
- “heater” / “cooler”: Petitioner contends that “heater”/“cooler” should be construed as “a region in which the temperature of a sample partition is raised/lowered.”
- “channel”: Petitioner contends that “channel” should be construed as “a groove in a substrate.”
- “tube”: Petitioner contends that “tube” should be construed as “a long hollow cylinder.”

Petition at 26-28. I apply these constructions herein where they are relevant, but

note that they do not substantively affect my analysis.²

V. THE '365 PATENT IS NOT INVALID

A. Ismagilov Is Not Prior Art

52. I disagree with Dr. Meinhart's and Petitioner's assertion that Ismagilov is prior art to the '365 Patent. Accordingly, it is my opinion that Ismagilov, and Ismagilov in combination with Curcio do not render the '365 Patent invalid.

1. Ismagilov Did Not Disclose PCR Prior To The Effective Filing Date Of The '365 Patent

53. I understand that Petitioner has asserted that Ismagilov has a priority date of July 8, 2002 based on its priority claim to provisional Patent Application No. 60/394,544 (the '544 Application). I do not agree that the '544 Application provides a disclosure that supports the Ismagilov Patent's description of performing PCR in droplets. The earliest disclosure of PCR in Ismagilov is in patent application 10/434,970, which was filed on May 9, 2003—after the March 14, 2003 filing date of the '365 Patent. Therefore, it is my opinion that Ismagilov is not prior art to the

² I also understand that the parties have agreed to certain constructions of these terms in litigation in District Court that are different than the constructions proposed by Petition and Dr. Meinhart in this proceeding. These differences do not affect my opinion.

'365 Patent. Below, I explain the basis for my opinion that the '544 Application does not describe performing PCR in droplets.

54. The '544 Application, entitled "Device and Method For Pressure-Driven Plug Transport" discloses methods of "conducting a reaction within a microfabricated substrate" by flowing reagent fluids into an immiscible carrier fluid to generate plugs within which the reaction occurs. Ex. 1004, 3:13-21. However, the "reactions" that occur in the plugs referenced by the '544 Application do not include PCR nor do any of the elements in the application inform the use of these methods for PCR. The '544 Application never mentions PCR.

55. I understand that Petitioner contends³ that the '544 Application's description of synthesizing a polymer in droplets provides support for a disclosure of performing PCR reactions inside of droplets. I disagree. A generic polymerization reaction is not the same thing as a polymerase chain reaction, and the '544 Application makes no mention of the latter. Polymerization simply refers assembly of monomers into a growing chain, a polymer. However, PCR or

³ Dr. Meinhart does not offer an opinion regarding the 544 Application's disclosure of reactions. He only provides an overview of the prosecution history of Ismagilov. I reserve the right to respond to additional arguments that Dr. Meinhart advances.

“polymerase chain reaction” is a very specific type of reaction that results in the amplification of a target DNA, and requires specific enzymes and primers. Furthermore, PCR requires that the device heat and cool the reaction on a cyclical basis. PCR involves three main components: (1) denaturization of double-stranded DNA into single-stranded DNA templates; (2) annealing of primers to the single stranded templates; and (3) extension of the primers via a polymerase enzyme to create a new double-stranded DNA copy of the target DNA. Each step involves a specific temperature. These three steps are repeated resulting in the exponential increase in the amount of target DNA. The '544 Application makes no mention of these details, which further supports my opinion that PCR was not contemplated.

56. In fact, the '544 Application provides a definition of the term “polymer” that includes several examples of polymers. These examples include polyamide, polyester, protein, wood, silk, polyurethane, cellulose, polyethylene, polyisobutylene, and polystyrene. Ex. 1004 at 9:7-16. There is no mention of nucleic acids, like DNA or RNA, that is the basis for PCR. This further confirms my opinion that the '544 Application does not contemplate PCR. In my opinion, if the inventors intended for “polymerization” reactions to include PCR, they would have at the very least included nucleic acid as an exemplary polymer.

57. To be sure, the '544 Application references polymer synthesis in two places. First in the summary of the invention, where it provides that the disclosed invention can include “synthesizing a polymer” by introducing a reagent fluid comprising a chemical polymerization catalyst into a carrier fluid to create plugs in which the polymerization reaction can occur. Ex. 1004 at 4:13-21. This is a chemical reaction distinct from the enzyme-dependent PCR. The '544 Application is not focused on PCR but instead on a platform to study chemical reactions on a molecular scale. Ex 1004 at 11:37-44.

58. The second reference to polymerization in the '544 Application explains that methods that involving merging two plugs together can be used to synthesize a polymer. The '544 Application explains that in “this embodiment, the first reagent fluid contains a monomer, and a second reagent fluid contains a polymerization catalyst. When the reagent fluids are combined, polymerization occurs.” Ex. 1004 at 29:26-29. PCR is much more complex and involves nucleic acids, oligonucleotides and enzymes in addition to thermocycling, and therefore would not be induced simply by combining two fluids together. The reaction is initiated by the heating of the sample to a temperature where the enzyme is active and not by the mere mixing of components. Thus, it is clear to me from reading this

description in the '544 Application that the inventors were not contemplating performing PCR in droplets at the time of the '544 Application.

59. The description goes on to explain that the method “can be adapted to form block copolymers by merging plugs with different monomers.” *Id.* at 29-31. Again, this suggests that the inventors of the '544 Application were not contemplating performing PCR in droplets. Forming block copolymers involves reacting two different monomers to form a covalent bond. This can involve a chemical polymerization catalyst, but is not similar to a complex reaction like PCR. As I have already explained, PCR involves denaturing double-stranded DNA to create two single strands of template DNA, and extending a complementary strand from each single strand using primers and a specific enzyme. This process involves a primer binding to the single-stranded template DNA, and being extended using a polymerase (which can bind to the primer) which sequentially attaches single, nucleotide bases one-by-one complementary to the single-stranded template DNA sequence. This process is much more complex than the formation of block copolymers described in the '544 Application.

60. The '544 Application also provides illustrative examples of how its disclosed methods are advantageous in the context of performing reactions in droplets, which further support my opinion that PCR was not contemplated at the

time of the '544 Application. The '544 Application states that two or more reagents can be “screened for the effects of different concentrations of reagents on some process, and the conditions under which an inhibitor can terminate the reaction of the enzyme...at various concentrations.” *Id.* at 30:13-18. The '544 Application explains that, due to the linear transport of droplets in the disclosed devices, “one can determine not only the presence or absence of an interaction or reaction, but also measure the rate at which the reaction occurs.” *Id.* at 25-27. In other words, the inventors were envisioning use of their droplet-based system to understand on a molecular and temporal time scale how various reactions occur. This type of analysis does not align with performing PCR in droplets. In general, PCR is typically used to determine the presence or absence of a particular target nucleic acid sequence in a sample. Droplet-based PCR methods, such as those disclosed in the '365 Patent (and later in the Ismagilov family of applications), were directed to assessing droplets for the presence or absence of a nucleic acid target. That is, droplet-based PCR, particularly in the early 2000's time frame, was about a binary

determination, not to assess the molecular and temporal characteristics of chemical and biological reactions.⁴

61. The '544 Application goes on to describe a specific example of how reactions can be studied in droplets on a molecular level in real time. The example the applicants chose was studying RNA folding in a solution in the presence of different concentrations of Magnesium and Hydrogen, and similar "protein/protein interaction mediation by small molecules, protein/RNA/DNA interaction by small molecules, or a protein/several small molecule binding events." Ex. 1004 at 31:5-15. This is clearly not contemplating performing PCR in droplets. Instead, the applicants were focusing on studying the folding of RNA using these devices as alternatives to other methods that did not allow for the continuous monitoring of the events. This focus on studying the kinetics of molecular events such as protein or

⁴ In fact, as of the early 2000s, PCR was a well-studied reaction, and the necessary enzymes and reagents were already developed. However, droplet-based detection would be ill suited even to the assessment of improvements to enzymes and PCR reagents. Evaluating the effectiveness of an enzyme for performing PCR would generally involve assessment of both the speed and amount of amplification of a target sequence. Particularly in this time-frame, droplet based detection would not have been a good choice to assess the amount of amplification, which would require measurements of relative intensity of fluorescent molecules. Real-time PCR would have produced more meaningful results, as measuring the relative intensity of small droplets is not as accurate as the type of measurements that could be obtained using real-time PCR.

RNA folding does not inform nor motivate a POSTIA to use this approach for performing PCR in droplets.

62. I understand that Petitioner pointed to the prosecution of a related Ismagilov patent to argue that “the Ismagilov patentee explained that the ’544 Application described conducting PCR on a single DNA molecule in a plug through the use of heat.” Petition at 13. The citation to the prosecution history by Petitioner provides several citations to the ’544 Application that allegedly disclose DNA and/or PCR. Petition at 13 (citing Ex. 1019, 2/14/14 Applicant Remarks, 9). I address each of these citations in turn.

63. First, Petitioner points to a citation to page 3 line 4 of the ’544 application for a disclosure of DNA. Page 3 line 4 of the ’544 Application is part of the background section of the ’544 Application describing problems with various types of microfluidic devices, and explains that “mixing in microfluidic systems is often slow regardless of the method used to drive the fluid because flow is laminar in these systems....Mixing in laminar flow relies on diffusion and is especially slow for larger molecules such as DNA and proteins.” This does not suggest performing PCR on droplets and refers to diffusion of solutes in the microfluidic system. DNA is simply used as an example of a type of large molecule that is slow to mix in plugs driven by laminar flow in microfluidic systems. There is no indication that the ’544

Application actually seeks to use DNA as a sample, and certainly no suggestion of performing a PCR reaction on DNA based on this citation.

64. The next citation in the portion of the prosecution history pointed to by Petitioner is page 29 lines 26-31. I have already discussed this section of the '544 Application above, which relates to performing polymerization reactions, and explained how it supports my opinion that the '544 Application does not describe or even suggest performing PCR in droplets. The last citation in the cited prosecution history is page 7, lines 22-30, allegedly describing "initiating a reaction using a stimulus such as heat or a catalyst." Petition at 13 (citing Ex. 1019, 2/14/14 Applicant Remarks, 9). Petitioner points to a statement by the applicants that "PCR is a reaction initiated which such stimulus." *Id.* I do not agree that a reference to performing reactions using a stimulus suggests performing PCR. The cited portion of the '544 Application provides a definition of the term "reagent" of "a component of a reagent fluid which undergoes or participates (e.g., by influencing the rate of a reaction) in at least one type reaction....to produce one or more reaction products or intermediates..." Ex. 1004 at 7:22-26. The definition then explains that a reagent in a reagent fluid "may also undergo a reaction in which a stimulus such as radiation, heat, temperature or pressure change, ultrasonic wave, or a catalyst induces a reaction." *Id.* at 26-30. That this list of potential reaction catalysts includes heat

does not demonstrate that the '544 Application discloses performing PCR in droplets. Countless types of reactions use heat as a catalyst—that PCR is one of them is not relevant and does not provide support for performing PCR in droplets.

65. Nothing in the '544 Application, even considered in aggregate, suggests performing PCR in droplets. The evidence that Petitioner points to at best shows that the applicants contemplated performing reactions in droplets that involved polymerization, and reactions involving heat to induce the reaction. In my opinion, this falls far short of demonstrating that the applicants considered performing PCR in droplets at the time of the '544 Application. The '544 Application does not have a single reference to PCR, and certainly does not describe any of the steps, or requisite materials needed to perform PCR in droplets. For example, the '544 Application says nothing about the use of primers, polymerase enzymes, or thermal cycling.

66. I understand that Petitioner relies on the prosecution history of the '544 Application to argue that the '544 Application discloses performing PCR in droplets. Petitioner argues that the applicants stated in prosecution that they “invented and possessed methods in which a PCR reaction was conducted on a single DNA or RNA molecule in a plug” and that the '544 Application “disclose[s] reactions in which a polymer is in a plug and a polymerization reaction is conducted on the polymer in

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the plug” and “it [was] known that DNA is a polymer and PCR is clearly a polymerization reaction.” Petitioner’s Reply to PO’s Preliminary Response. I disagree that these statements demonstrate that the ’544 Application discloses PCR. In my opinion, the applicant’s remarks during prosecution do not overcome the reality that the ’544 Application contains no reference to, much less sufficient description of, methods of performing PCR in droplets.

2. The Inventors of the ’365 Patent Conceived Of The Claimed Invention Prior To the Alleged Priority Date of Ismagilov

67. I understand that Patent Owner has argued that the inventors of the ’365 Patent conceived of the claimed invention prior to the earliest possibly priority date of Ismagilov, and diligently reduced the invention to practice thereafter. I have been asked to provide an opinion on whether Patent Owner can show conception of the invention prior to the earliest possible priority date of Ismagilov.⁵ I understand from counsel that conception requires a definite and permanent idea of the operative invention. I have reviewed the invention disclosure that was prepared by the inventors of the ’365 Patent, and I agree that it demonstrates conception of each

⁵ I have not been asked to provide an opinion on diligence in reducing the claimed invention to practice.

challenged claim of the '365 Patent. I explain the basis for my opinion in greater detail below.

68. I understand from counsel that the inventors of the '365 Patent submitted a record of invention (ROI) dated July 30, 2002, which is a business record at Lawrence Livermore. I have reviewed the ROI and it is my opinion that it demonstrates conception of each and every element of the claims challenged by Petitioner. The ROI shows that the inventors of the '365 Patent realized that microdroplet DNA detection could be used to reduce the reaction time of PCR to allow for the detection of dangerous bioagents, such as those used in biological warfare. The ROI explains that the invention “provides a unique method for performing extremely rapid nucleic acid amplification and detection...based [on] separating a chemical reagent and an input sample into a large number of microdroplets.” Ex. 2004 at 2. By amplifying selected portions of each nucleic acid inside of droplets, a more concentrated amplification product is produced, and fluorescence-based optical detection can be performed to detect droplets that contain target DNA. *Id.* The ROI provides a detailed description of how to implement this idea for rapid nucleic acid amplification, including a description of partitioning sample fluid into droplets by flowing fluid through an injection orifice, and subjecting the droplets to the appropriate number of PCR cycles needed for

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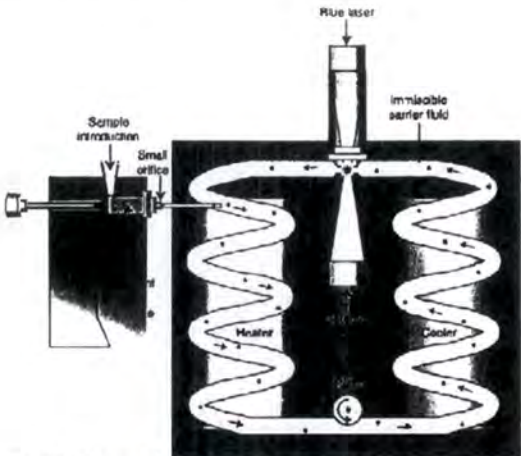
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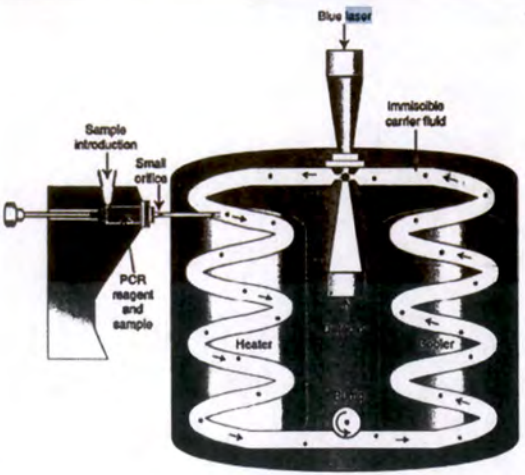
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detection. *See id.* at 9-12. In my opinion, this description demonstrates that the inventors conceived of the challenged claims of the '365 Patent as of July 30, 2002.

69. In the chart below, I provide a list of evidence from the ROI that supports my opinion that the ROI demonstrates conception of the challenged claims.

Claim (365 Patent)	Evidence of Conception
[11a] A nucleic acid amplification apparatus comprising:	The ROI discloses an apparatus for partitioning samples into droplets for amplification and detection. In particular, the invention describes performing polymerase chain reaction (PCR) in microdroplets. “This document describes an invention for performing ultra-fast PCR-based DNA detection using fluid partitioning.” Ex. 2004 at 9. “With this new bioassay technique, each partitioned DNA-containing fluid volume would contain the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” <i>Id.</i> at 9. “The conceptual design of an instrument capable of performing FPDD with PCR based detection and amplification has several potential embodiments...Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.” <i>Id.</i> at 10. <i>See also, id.</i> at Figure 1.
[11b] a microdroplet generator comprising an orifice wherein said	“Fluid-Partitioned DNA Detection (FPDD) involves dispersing the DNA-containing solution in an immiscible carrier liquid. The DNA-containing

Claim (365 Patent)	Evidence of Conception
orifice connects a sample flow pathway to a channel or tube comprising an immiscible fluid, wherein said channel or tube passes through a heating element;	solution is typically dispersed in the immiscible carrier fluid as microdroplets..." <i>Id.</i> at 9. "Here, microdroplets are formed by forcing the PCR mix (sample and reagent) through a small orifice or microjet. These microdroplets are then captured in an immiscible fluid, such as mineral oil, and flowed past heating elements." <i>Id.</i> at 10. <i>See also, id.</i> at Figure 1 (depicting an orifice to create microdroplets).  Figure 1. Diagram of fluid partitioned DNA detection apparatus using small volume micro-droplets that serve as concentrated reaction vessels for PCR amplification and detection
[11c] and wherein said apparatus further comprises a detector capable of detecting microdroplets in said immiscible fluid and a pump for moving said microdroplets through the channel or tube.	Figure 1 depicting one embodiment in which a blue laser is projected upon the microdroplets as they pass between the laser and the detector. <i>Id.</i> at 12.

Claim (365 Patent)	Evidence of Conception
	 Figure 1: The proposed platform would generate extremely small volume microdroplets that serve as concentrated reaction vessels for PCR amplification and detection. “The target DNA is detected by monitoring for the colorimetric indicator (e.g., fluorescence or optical absorption) generated with each DNA template duplication sequence.” <i>Id.</i> at 9. “An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers.” <i>Id.</i> at 10. “Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.” <i>Id.</i> at 10.
[12] The apparatus of claim 11 wherein the detector is positioned	See claim 11c.

Claim (365 Patent)	Evidence of Conception
such that generated microdroplets suspended in said immiscible fluid pass by the detector as they are moved through the channel or tube.	
[13] The apparatus of claim 11 wherein the immiscible fluid is a mineral oil.	"...microdroplets are then captured in an immiscible fluid, <i>such as mineral oil...</i> " <i>Id.</i> at 10.
[15] The apparatus of claim 11 wherein the channel is a micromachined channel.	<i>See</i> claim 11c.
[17] The apparatus of claim 11 wherein the channel or tube extends through a heater and a cooler.	"In a related embodiment of the FPDD method, <i>microdroplets of the DNA-containing solution are suspended in a carrier fluid held within a tube and the heating and cooling elements</i> , and the optical detection system are repeatedly passed over the tube to achieve the desired amplification and detection." <i>Id.</i> at 10-11.

70. Accordingly, it is my opinion that the ROI demonstrates conception of the claimed invention.

B. Quake in view of Holliger and Schneegaß Does Not Render the Challenged Claims Unpatentable

71. In my opinion, Quake in view of Holliger in further view of Schneegaß does not render the challenged claims obvious. I understand from counsel that to establish obviousness, Petitioner must demonstrate a motivation to combine the asserted references, and a reasonable expectation of success in the claimed invention. For the reasons stated below, it is my opinion that neither of these requirements are met. I first address the combination of Quake and Holliger, and subsequently address the combination of Quake, Holliger, and Schneegaß.⁶

1. There Is No Motivation To Combine Quake and Holliger

72. In my opinion, a person of skill in the art would not have been motivated to combine Quake and Holliger. Quake and Holliger are directed to solving different problems, and utilize incompatible techniques that cannot be

⁶ My declaration in this IPR, and the 780 Patent IPR are largely the same, because Dr. Meinhart's and Petitioner's arguments are nearly identical in both Petitions. The only difference is that the 365 Patent IPR asserts one combination of Quake, Holliger, and Schneegaß, whereas the 780 Patent IPR asserts two combinations involving Quake: Quake and Holliger, and Quake and Schneegaß. Thus, the opinions presented in my declaration in the 780 Patent IPR are equally applicable here.

combined without substantial modifications, and there is no motivation to pursue these modifications.

a) Quake Does Not Disclose Performing PCR In Droplets

73. Quake is not directed to performing PCR in droplets and it is my opinion that a skilled artisan would not be motivated to combine a reference like Holliger—which describes performing PCR reactions in a bulk emulsion—with the disclosure of Quake. Dr. Meinhart asserts that “both Quake and Holliger disclose performing PCR in micrometer-dimension w/o droplets (i.e., microdroplets), and assumes that a POSITA would be motivated to look to Holliger to “teach how to implement PCR” in microdroplets. Meinhart Decl. ¶ 165-169. I disagree. Quake does not disclose performing PCR in droplets. Rather, Quake reports detecting and sorting viral particles, and purports to make improvements to traditional cell sorting devices to make them suitable for detection and sorting of small particles such as viruses. *See e.g.*, Quake at 5:1-4. In fact, Quake frames its invention as *an alternative* to PCR-based techniques of viral detection, citing problems in PCR that his technology circumvents.

74. There is no disclosure in Quake of performing PCR in droplets—the entirety of Quake’s 88-page description only mentions PCR twice, and neither mention is directed to performing PCR in droplets.

75. First, Quake mentions PCR in the background section, where it explains different approaches to laboratory diagnosis of viral infection. Quake explains that techniques such as PCR “are used for the detection of viruses where cell culture techniques or serological methods are difficult, expensive, or unavailable” or to “detect viral DNA or RNA directly in clinical specimens.” Quake at 2:29-32. According to Quake, the advantage to using PCR for viral diagnostics is high sensitivity (it can “detect very low numbers of viruses in a small clinical specimen”), but the downside is a significant risk of cross-contamination from sample to sample, which “can outweigh the benefits of detecting small quantities of target viral nucleic acid” and “can also result in false positives, making interpretation of epidemiological data impossible.” *Id.* at 2:32-3:6.

76. Quake offers flow sorting techniques as a potential alternative to PCR based detection, but notes that flow sorting is often impractical for analyzing and sorting viruses due to their small size. *Id.*, at 3:7-14. Thus, Quake focuses on solving problems with cell sorting devices that traditionally did not have the resolution to detect and sort viral particles. Quake discloses techniques that could be used to sort

viral particles as an alternative to using PCR to analyze viruses. Accordingly, it is my opinion that Quake teaches away from performing PCR in droplets, because it offers droplet-based detection as an *alternative* to PCR. And in fact, employing PCR would be contradictory to Quake's teachings that the "significant risk of cross-contamination from sample to sample can outweigh the benefits of detecting small quantities of a target viral nucleic acid," as I explain in greater detail below. Quake at 3:3-5. That is, rather than teaching or suggesting the use of PCR to perform viral detection, Quake expressly states *not* to use PCR for this purpose.

77. The second time Quake mentions PCR is to explain that "microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR (13), moving cells using optical tweezer/trapping (14-16), transformation of cells by electrophoresis (17); μ TAS (18), and DNA hybridization (5)." Quake at 23:26-30. I disagree with Dr. Meinhart that this discloses or even suggests performing PCR in droplets. Quake is explaining that other technologies can be combined with flow cytometry on the same chip, and lists several other technologies that are also based on fluid flow in microchips. Quake is not suggesting that these technologies be performed in droplets, nor is there even an express function for fluid flow in carrying out some of these technologies. In fact, the reference Quake cites after stating that PCR can be integrated on the same

chip as flow cytometry, Kopp, is directed to flow through thermocycling. Flow-through thermocycling does not use droplets. Instead, it performs PCR in a bulk solution flowing through a microfluidic device, with the sample fluid being the only fluid flowing through the chip. Thus, it is my opinion that Quake's reference to Kopp suggests that PCR could be performed without encapsulation of a sample into droplets, not that PCR could be performed in droplets. Kopp does not present any teachings of how to implement PCR in droplets.

78. Rather, Kopp presents "a microfabricated device for performing PCR in a continuous flow at high speed." Ex. 1012 at 1046. In a proof-of-concept experiment, only the PCR sample mixture was flowed through channels of a microfluidic device where the mixture was heated and cooled to perform amplification. After amplification, the samples were analyzed using an off-chip polyacrylamide gel, a standard method of PCR product analysis that is distinct from optical based detect in droplets. Ex. 1012 at 1047.

79. I understand that the Board stated in its institution decision that "Kopp discloses the general principle of PCR amplification on a microfabricated device, and that one of ordinary skill would not consider this principle cabined to continuous flow system." Institution Decision at 33. I understand that the Board also asserted that, based on Dr. Meinhardt's testimony that Kopp discloses performing PCR by

thermocycling (i.e. heating and cooling), Quake “also discloses heating and cooling the microdroplets through its incorporation of Kopp.” *Id.* I do not agree with this analysis. In particular, I do not agree that Quake discloses heating and cooling *of microdroplets* based on its incorporation of Kopp’s disclosure of flow-through thermocycling.

80. Flow-through thermocycling of sample fluid is a distinct technology from performing PCR in droplets, and the ability to thermocycle on a microfluidic chip for flow-through thermocycling does not provide a disclosure of being able to perform PCR in droplets. While thermocycling is an important piece of performing PCR in droplets, it is certainly not the only necessary technology. Performing PCR in droplets is a complicated task, and was not well-studied in the early-2002 time frame. For example, to effectively perform PCR in droplets , the appropriate reagents need to be encapsulated in the droplets and distributed appropriately, and heating and cooling cycles need to be applied to the microfluidic device so that each droplet goes through the correct amount of amplification cycles to enable detection

81. Even Dr. Meinhart acknowledges that droplets present a host of considerations beyond flow through thermal cycling. For example, in his discussion of Quake in combination with Holliger and Schneegaß, Dr. Meinhart explains that the size of droplets affect “the applicable heat and mass transfer distances” which

affects the heating and cooling time of the sample material. Ex. 1009 ¶ 159. In a population of microdroplets that vary in size, the time to heat the interior contents sufficiently for PCR will vary. The same holds true for cooling. The result is a non-uniform distribution of PCR products in the microdroplet population. Further, Dr. Meinhart acknowledged that where “the cross-section of a droplet is smaller than the cross-section of a channel or tube” the reaction time may need to be altered “to compensate for any increase in heat transfer distance from the heating element to the droplet.” 780 Patent IPR, Ex. 1009 ¶ 277.⁷ This provides further support for my opinion that a skilled artisan would not have been able to simply take Kopp’s teaching of thermocycling and apply it directly to Quake’s droplets resulting in successful PCR. In my opinion, Dr. Meinhart does not correctly represent the complexity of performing PCR in droplets, particularly at the time of the invention.

82. At the time of Quake, this technology was in its early stages, and performing PCR in droplets was not simply a matter of integrating thermocycling on Quake’s device. Kopp does not teach how to perform thermocycling in droplets, and there is no indication in the references, by Dr. Meinhart, or otherwise, that

⁷ Dr. Meinhart’s declaration in the 780 Patent IPR. All other references to Ex. 1009 are to Dr. Meinhart’s declaration in the present proceeding.

Kopp's disclosure of a flow through microfluidic device (for bulk sample fluid) could be easily combined with Quake droplet-generation device with a reasonable expectation of success. Rather, Kopp's disclosure only demonstrates a proof of concept experiment with "a very simply device and no optimization" that can be used to perform PCR in continuous flow." Kopp at 2. In my opinion, this disclosure is not sufficient to provide a skilled artisan at the time of the '365 Patent with enough information to implement PCR in Quake's droplets. As I explain in greater detail below, performing thermocycling using Quake's sorting device would require a complete redesign.

83. In further support of my opinion that Quake's reference to PCR was not a suggestion to perform PCR in droplets, several of the other technologies recited in the above-referenced sentence of Quake cannot be performed in droplets. For example, references (17) and (18), above, rely on the use of electrophoresis to analyze cells. Electrophoresis is a method of separating particles (such as proteins) based on their size by drawing them through a sieve (polymer) using an electrical charge. Droplets of a uniform size would not separate into different populations. More importantly gel electrophoresis is not able to size droplets of this size.. Accordingly, some of the technologies Quake suggests that one could combine with its microfluidic device are not even compatible with droplet-based analysis. This

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confirms my opinion that Quake was simply listing technologies that also used microfluidics, not necessarily technologies that could be used with droplet-based analysis.

84. Further, as I explain in greater detail below, even if Quake did teach or suggest performing PCR in droplets, a skilled artisan would not have looked to Holliger for assistance with implementing the details of PCR, as Dr. Meinhart alleges. Holliger provides little if any detail that would be useful to a skilled artisan in implementing PCR in Quake's microfluidic device.

b) Quake Does Not Provide A Motivation To Perform PCR

85. Not only does Quake fail to make any disclosure of performing PCR in droplets, but there is no reason or motivation suggested by Quake to modify its devices for such a purpose.

86. As I discussed above, Quake is focused on analyzing and sorting viral particles for analysis. Quake purports to offer an improvement to viral analysis and detecting using flowing sorting, rather than traditional PCR-based techniques. The characteristics of viruses Quake seeks to evaluate can be detected by linking a detectable reporter molecule to a feature of the virion—generally a surface marker used to identify the virus or cell. Ex 1006 at 33:17-28. This type of detection

involves attaching a reporter molecule onto to a specific feature, and causing it to exhibit a specific characteristic (e.g., fluorescence). Performing PCR would not provide any benefit to the applications in Quake that utilize surface markers, and would be a much more complicated, expensive, and difficult manner of detecting a feature of a particle like a virus.

87. The only specific references in Quake to sorting molecules like polynucleotides suggests sorting such particles based on size or weight. *Id.* (“[T]he device can determine the size or molecular weight of molecules such as polynucleotides or polypeptides (including other enzymes and other proteins) or fragments thereof passing through the detection region.”). Of course, this type of analysis does not require or benefit from PCR. And in fact, this type of analysis would be thwarted by using PCR, not enhanced. Again, this demonstrates that Quake did not contemplate performing PCR in droplets, and would not have recognized a benefit from such an application.

88. Dr. Meinhart alleges that “Quake teaches that its droplets may be ‘used as microreactors for chemical reactions’ and asserts that this indicates that “Quake suggests PCR as a method of using its microfluidic device to identify and/or sort virions or virus particles.” Meinhart Decl. ¶ 166. I disagree. Quake primarily contains passing references to performing reactions in droplets, and none of those

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references suggest that PCR is a type of reaction that can be performed in droplets. For example, Quake states that “individual particles or molecules can be separately compartmentalized inside individual droplets. These droplets can be analyzed, combined with other droplets (e.g., to react droplet contents) and/or sorted, as desired.” Quake at 5:10-12. This reference does not contain any detail about the type of reaction contemplated or how to perform it, and certainly does not make any disclosure of PCR. Quake’s other generic references to reactions are listed below:

- “A reporter may also include any substance on or in a cell that causes a detectable reaction, for example, by acting as a starting material, reactant or a catalyst for a reaction which produces a detectable product.” Quake at 17:2-23.
- “The strength of the signal may indicate...a positive or negative reaction such as binding or hybridization of one molecule to another, or a chemical reaction of a substrate catalyzed by an enzyme.” Quake at 22:32-23:3.
- “For instance, in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at 28:20-23.
- “Enzymes can be analyzed and/or sorted by the extent to which they catalyze chemical reactions of a substrate (conversely substrate can be analyzed and/or sorted by the level of chemical reactivity catalyzed by an enzyme).” Quake at 32:10-15.

- “The introduction of droplets through the different extrusion regions may be controlled e.g., so that the droplets combine (allowing, for example, the enzyme to catalyze a chemical reaction of the substrate).” Quake at 36:21-23.
- “Droplets of aqueous solution typically contain a sample of molecules (e.g., for small scale chemical reactions) or particles (such as cells or virions).” Quake at 80:29-31.
- “By timing the release of these droplets to occur as a droplet of enzyme passes through a second droplet extrusion region, the two droplets are combined and the enzyme is able to react with the substrate.” Quake at 86:4-6.

89. None of these references to performing enzymatic reactions contain any disclosure or suggestion of performing PCR. These references do not even describe any of the necessary conditions of the reactions, suggesting that the reactions do not require conditions that would require any heating or cooling elements on a microchip, as would PCR.

90. If anything, these descriptions demonstrate that Quake does not contemplate performing PCR in droplets. The types of reactions described by Quake are passive, relying on self-enzymatic activity contained within the droplets themselves without further input from the instrument. For example, the citation at 36:21-23 states that droplets can combine, allowing the enzyme to catalyze a chemical reaction. Ex. 1006 at 36:21-23. This suggests that the only thing that is required to catalyze the reaction is combining the droplets, which would not be the

case if the reaction were PCR. In fact, Quake's techniques of reacting droplets based on droplet-merger provide no benefit to PCR. The utility of using droplet merger is so that the time of the reaction can be controlled. That is, when the droplets are merged, the reaction materials are brought together, and the reaction is catalyzed at the intended time. However, with PCR, heat is the catalyst of the reaction, and it will not start without the application of heat. Therefore, droplet merger does not provide a benefit to performing PCR in droplets—there is no need to initiate the reaction by merging droplets. Again, Quake's disclosures all point towards mechanisms that do not suggest the use of PCR.

91. Likewise, the citation at 32:10-15 states that droplets can be used to analyze the extent to which enzymes catalyze a reaction. Ex. 1006 at 32:10-15. This type of analysis would be difficult to carry out if the reaction were PCR. Using the detection techniques described in Quake, the extent to which PCR is carried out would need to be determined by the level of fluorescence (or other optical feature) of the droplet. The relative intensity of a fluorescent droplet would be a poor indicator of the extent to which a PCR reaction occurred. For example, a PCR reaction carried out 75% of the way in a droplet would be difficult to differentiate from a PCR reaction carried out 100% of the way based on optical detection systems, particularly those available at the time. Even now, droplet-based PCR primarily

relies on fluorescence to make a binary determination of whether or not the target molecule is present in a droplet, not the extent to which the target molecule is amplified.

92. Quake makes only one mention of a specific reaction, which in my opinion further demonstrates that Quake was not contemplating performing PCR in droplets. Quake explains that cells containing a certain gene “may catalyze a reaction on an aromatic substrate...with the net result that fluorescence, or another detectable property of the substrate will change.” Ex. 1006 at 50:7-11. This makes no reference to PCR, and in my opinion, suggests a type of reaction that is quite different from PCR. The reaction Quake is describing is for analyzing cells that contain a gene that enables them to produce a specific type of enzyme. This enzyme can be detected in the presence of certain substrates based on their ability to produce a reaction with a detectable product. The example Quake provides is cells that produce a P450 enzyme (often found in the liver), can be detected in the presence of naphthalene because P450 can react with naphthalene to produce a detectable reaction product. This allows the types of cells producing that particular enzyme to be detected (and presumably sorted). Thus, Quake envisions detection of cells that can encode for particular enzymes. This is nothing like PCR, which detects specific target sequences of DNA by using an enzyme to create multiple copies of template

DNA that contains that particular sequence. In the reactions referred to by Quake, the enzyme itself is the reaction product, as opposed to a PCR reaction, where amplified copies of genetic material are the reaction product.

93. I understand that in instituting this proceeding, the Board indicated that Quake is not limited to using droplets to sort viral particles, but is more broadly directed to a “microfluidic device for analyzing and/or sorting biological material (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells).” While true that Quake mentions analysis and sorting of several different types of particles, its primary focus (and the way it frames the benefits of its device) is on detecting and analyzing viral particles. Regardless, it never discloses or suggests any benefit to performing PCR to detect any of the “particles” it references.

94. I understand that the Board’s Institution decision also states that even if there were no benefit to amplifying viral nucleic acids when sorting viral particles, Quake makes clear that the same may not have been said for all laboratory diagnosis of viral infection as it describes PCR as “generally the method of choice to detect viral DNA or RNA directly in clinical specimens.” Institution Decision at 32. It appears to me that the Board has misinterpreted the purposes of Quake’s description of methods of detecting viral DNA and RNA in clinical specimens. As I have

already explained, Quake identifies a problem with traditional methods of clinical diagnosis using PCR, and purports to solve that problem by applying alternative methods of detection that do not rely on reactions that have a significant risk of cross-contamination. Quake does not suggest that problems with PCR can be solved by moving the reaction into droplets—it posits that problems with PCR can be circumvented by performing detection of viral particles encapsulated into droplets.

95. I understand that in its institution decision, the Board also stated that “Quake identifies PCR as a method of choice in detecting viral nucleic acids, but notes that the high sensitivity of PCR is balanced with a risk of cross-contamination between samples” and that Quake addresses the “problem of cross-contamination because individual particles or molecules are compartmentalized into droplets, which prevent diffusion or mixing.” Institution Decision at 32. In my opinion, this analysis by the Board misinterprets Quake. I do not agree that Quake addresses problems with cross-contamination in PCR. More importantly, as I discuss in greater detail below, cross-contamination in PCR is the result of PCR products from one reaction, contaminating a subsequent reaction. Persons of ordinary skill in the art would have understood this problem and may have referred to it as ‘carry-over’ where PCR products after the analysis of one sample are carried-over to the next

sample. Droplets nor anything that Quake discloses addresses the problem of carry-over.

96. The Board seems to infer that Quake solved problems with cross-contamination in PCR by using microfluidic devices, which is not the case. Quake acknowledges problems with cross-contamination in PCR, and notes that this is not an issue in sorting viral particles. Quake does not provide a solution to cross-contamination in the context of PCR, but rather states that cross-contamination does not occur when using the alternative techniques of viral detection suggested by Quake. That is, Quake does not disclose that its microfluidic devices and methods address the problem of cross-contamination *in PCR* by compartmentalized particles into droplets to prevent diffusion or mixing, as the Board suggests. Institution Decision at 32. The Board's analysis appears to conflate cross-contamination with Quake's references to diffusion and mixing.

97. In traditional PCR, cross-contamination is a major problem that can affect the accuracy of results. Cross-contamination occurs when unwanted PCR products (e.g., DNA fragments) from one reaction are introduced into another sample that is to be analyzed via PCR. This can happen, for example, in a lab that is analyzing multiple samples if there are PCR products left over on surfaces or equipment from previous analyses. Additionally, the process of opening the tubes

used to conduct PCR after the reaction is complete can result in aerosolization of these PCR products. Cross-contamination is difficult to deal with in the context of PCR, because PCR products are difficult to effectively remove from surfaces, equipment, lab personnel, etc. Cross-contamination is particularly problematic in the context of PCR because of the amplification of the sample. One or two PCR amplicons (e.g., fragments of DNA), can contaminate a sample, and give rise to a false positive (a result that is incorrect as appears to be positive by virtue of contamination with PCR products from any previous reaction) as a result of being amplified over hundreds of cycles. In the background section, Quake references this “significant risk of cross-contamination from sample to sample” in PCR, and points out that it can “result in false positives,” which can lead to an incorrect experimental conclusion or misdiagnosis of a patient. Ex. 1006 at 3:2-6.

98. When Quake discusses its microfluidic devices, however, it does not suggest that it has mitigated the issue of cross-contamination in PCR—Quake simply avoids the problem all together by refraining from the use of PCR. As the Board recognized, Quake explains that droplets have boundaries that “are defined by channel walls, and therefore they do not diffuse and/or mix” such that “individual particles can be separately compartmentalized inside individual droplets.” Ex. 1006 at 5:1-12. This is not addressing the type of cross-contamination Quake was

referencing in the context of PCR. If PCR were performed in Quake's droplets, even though particles would in theory be compartmentalized in droplets, cross contamination from one sample to the next sample could still occur during sample preparation, as occurs with traditional PCR. The limited diffusion and exchange between droplets deals with cross-talk (exchange of information between entities in a sample) rather than cross-contamination.

99. I also note that Quake only suggests that its droplets prevent diffusion of material the size of viruses. Quake at 5:31-32. However, DNA fragments are generally much smaller than viral particles and are more difficult to keep from diffusing in and out of droplets. Thus, it is not clear that Quake's droplets would also prevent diffusion of DNA particles.

100. The Board also points out that Quake posits that one advantage of "the sorting devices of the invention" are that they are "inexpensive and disposable, which obviates the need for cleaning and sterilization and prevents cross-contamination." Ex. 1006 at 5:25-27. Again, disposability does not solve the types of cross-contamination Quake was referencing in the context of PCR. Disposability can prevent cross-contamination resulting from residual material on surfaces of the same device. However, all fluid contact surfaces (tubes, pipette tips) are disclosed

of in any laboratory in which PCR is practiced. Thus, this is not the type of cross-contamination that creates problems for PCR.

101. When performing PCR, researchers generally do not re-use the test tubes or sample plates that PCR is performed in, as those too are relatively inexpensive and disposable. Accordingly, it is my opinion that the cross-contamination referenced in Quake as problematic in the context of PCR is not a result of reusing devices—cross contamination from other samples in PCR can come from any surface or piece of equipment that was not disposed of or thoroughly cleaned. Thus, the use of a disposable microfluidic device would not solve problems with cross-contamination in PCR. This further supports my opinion that Quake’s recitation of the alleged benefits of its devices did not purport to improve up on the PCR process by solving cross-contamination in that context, but instead used a different method of detection that relies on analyses that are less susceptible to cross-contamination.

102. Further, based on my reading of Quake, it appears the issue Quake was addressing by pointing out that its microfluidic devices were disposable is the issue of “sample carryover,” which Quake describes as occurring “when remnants of previous specimens left in the channel back-flush into the new sample stream during consecutive runs.” Ex. 1006 at 3:19-21. Quake describes sample carry-over

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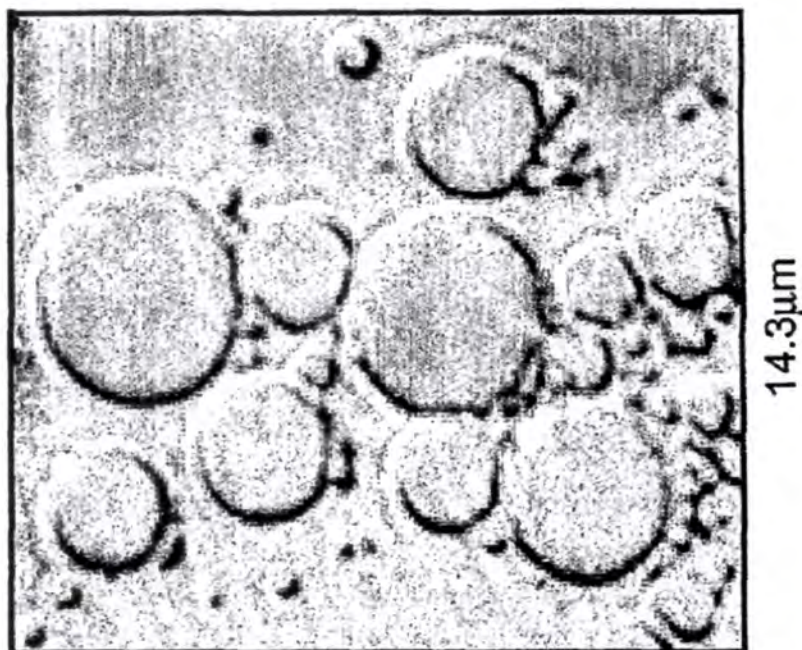
as another “major problem” with conventional flow-sorting devices and points out that even though traditional FACS devices can be sterilized in between runs “the procedure is costly, time consuming, inefficient, and results in hours of machine down time.” *Id.* at 19-25. Again, this supports my opinion that Quake did not purport to solve problems with cross-contamination *in PCR* via the use of droplets.

c) Holliger Does Not Provide Motivation To Perform PCR In Quake’s Droplets

103. Dr. Meinhart and Petitioner also allege that a POSITA seeking further instruction on how to perform PCR in Quake’s droplets would have considered Holliger, because Holliger taught methods and systems for performing PCR in droplets of the same size and formed using similar methods. Petition at 53; Ex. 1009 at ¶ 165-172. I disagree that a POSITA would have considered Quake and Holliger as teaching methods and systems for performing PCR in droplets of the same size and formed using similar methods.⁸ Accordingly, I do not agree that a POSITA would have been motivated to look to Holliger to modify Quake to perform PCR in droplets.

⁸ As I explain in greater detail below, I also do not agree that a POSITA would have expected to succeed in practicing the claimed method as a whole by performing PCR in Quake’s droplets using the implementation details provided by Holliger. Petition at 53-54; Ex. 1009 at ¶ 227-28.

104. I have already explained above why I do not agree that a POSITA would have considered Quake to teach performing PCR in droplets. However, to the extent a POSITA would somehow recognize Quake as disclosing performing PCR in droplets, I do not believe a POSITA would have considered Quake's methods as "similar" to Holliger's, as Dr. Meinhart contends. Quake and Holliger describe very different methods of creating and analyzing droplets. Holliger describes a method of creating droplets that involves adding aqueous solution manually or "dropwise" to a container of oil while stirring or agitating the mixture. This results in a heterogeneous emulsion of millions of vesicles varying in size and inconsistent with respect to their internal contents, as demonstrated by Figure 2, below.



Ex. 1007 at Figure 2. To generate the vesicles for this experiment, Holliger introduces 200 μL of PCR mixture into a mineral oil. *Id.* at 59:10-15. With an average droplet size of 15 μm and a density of 10^9 microcompartments per milliliter, this leads to millions of vesicles, far more than the thousands disclosed in Quake.⁹ *See, e.g.*, Ex. 1006 at 58:1-3 (exemplary Quake device was capable of sorting 33,000 beads per hour).

105. Quake, by contrast, describes methods of droplet formation that involves the use of microfluidics to direct a stream of sample fluid into oil at a

junction to create relatively uniform droplets in microfluidic channels, which can be precisely manipulated and sorted. Thus, the droplets in Quake and Holliger are not created by “similar” methods. To the extent “similarities” in methods of droplet creation can constitute a motivation to combine, or support for a reasonable expectation of success, I disagree that Quake and Holliger demonstrate such similarities.

106. I also disagree that a POSITA presented with Quake would look to Holliger for instruction on how to implement PCR in droplets. As I stated previously, Holliger describes performing PCR in bulk emulsions, which are formed by manually dropping sample fluid into a container of oil and agitating the mixture to form the emulsion. This bulk emulsion method of performing PCR provides no instruction on how to encapsulate the materials needed to perform PCR in droplets, and thermal cycle those droplets in order to carry out the reaction in a microfluidic device. For example, Holliger provides no information on the number of cycles that are able to or should be performed inside of droplets, methods of effectively heating and cooling droplets in tubes or channels, or how thermocycling techniques can be applied to microfluidic devices. Thus, Holliger provides no evidence that the PCR is actually occurring in a substantial number of the droplets, let alone droplets of a uniform size that would allow for predictable heating and cooling.

107. Bulk emulsion techniques like those described in Holliger were disclosed years before Quake—as were microfluidics. Emulsions have been known for hundreds of years. Despite a strong interest in PCR, and a trend towards implementing “lab on a chip technologies,” researchers had not implemented PCR in droplets on a microfluidic device. This confirms my opinion that performing PCR in droplets in a microfluidic system was not an easy task, and it would not have been obvious or straightforward to take Holliger’s teaching of performing PCR in a bulk emulsion and apply them to Quake to perform PCR in droplets. Presented with the disclosures of both Holliger and Quake, and a skilled artisan still would not have the motivation or instruction to effectively combine the PCR technology of Holliger with the microfluidics of Quake to implement PCR in droplets as disclosed by the ’365 Patent.

108. It is also my opinion that, like Quake, Holliger fails to offer any motivation to perform PCR in droplets using a microfluidic device such as that described in Quake. The purpose of Holliger is to perform PCR in droplets in bulk emulsion, and analyze the contents of the emulsified droplets using techniques that rely on breaking the emulsion. *See, e.g.*, Ex. 1007 at 59:17-21, 76:1-4. Holliger is primarily directed to selecting enzymes that exhibit certain desirable behaviors, for example, by selecting for nucleic acid sequencers that encode for enzymes which

replicate nucleic acids with a high efficiency. The “amplified sequences” are then sequenced or assayed to evaluate the “replication ability” of the evaluated enzymes.

109. The assays described in Holliger measure the amount of nucleic acid produced by mutant enzymes selecting for those enzymes that have an enhanced replication efficiency. The copy number of nucleic acids—generally determined via sequencing or gel electrophoresis—provides an indication of “the level of activity of the polymerase encoded by it.” Holliger at 27:25-28:1. These assays used to evaluate copy number rely on breaking the emulsion and extracting the amplified genetic material inside—Holliger’s methods were not aimed at nor were they capable of precisely detecting material in individual droplets. Thus, Quake’s use of microfluidics for sorting and controlling the flow of droplets would not enhance Holliger’s analysis techniques. While Quake relies on microfluidics to control the flow of viral particles encapsulated in droplets and detect the presence of reporter molecules inside of droplets, and subsequently sort the droplets using valves, channels, and fluid flow, Holliger has no use for such fluidic control and sorting.

110. In fact, it is my opinion that Holliger’s goal of analyzing the activity of polymerase enzymes in amplifying nucleic acids would be unworkable with the methods of analyzing and sorting described in Quake. It is not clear how Quake could achieve Holliger’s goal of determining which enzymes effectively amplified

nucleic acids. While Holliger performs this assessment by identifying the reaction product (and therefore the enzyme), Quake's method of optical detection would have limited utility for this purpose. Particularly at the time of the invention of the '365 Patent, only a small number of optical markers could be distinguished in a single experiment. Thus, it would have been difficult to differentiate the enzymatic activity of a large number of enzymes. The assays used in Holliger are much better suited for much a purpose. Utilizing Quake's microfluidic devices would only increase the complexity of Holliger's analysis, with no upside (and in fact, would not achieve Holliger's goal). Accordingly, it is my opinion that Holliger does not provide any motivation to use the devices described in Quake to perform PCR in droplets (and, as I explained previously, Quake provides no motivation to perform PCR in droplets). Thus, I do not believe that either Quake or Holliger provide any motivation to modify Quake with the teachings of Holliger as suggested by Petitioner.

2. There Is No Reasonable Expectation of Success In The Combination Of Quake and Holliger

111. It is also my opinion that a skilled artisan would not have a reasonable expectation of success in combining Quake and Holliger to arrive at the claimed invention. As I have alluded to above, neither Quake nor Holliger teach how to

implement PCR in droplets within a microfluidic device. I have already explained above my opinion that Quake does not disclose or suggest performing PCR in droplets and offers this work as an alternative to PCR. I also do not agree with Dr. Meinhart's assertion that a "POSA would have expected to succeed in applying Holliger's implementation details to conduct PCR in Quake's microdroplets because of the similarity between the references' microdroplets." Meinhart Decl. ¶ 172. This is Dr. Meinhart's only rationale in the single paragraph he offers on reasonable expectation of success in combining Quake and Holliger, and fails to establish a reasonable expectation of success.

112. As I described above, Quake and Holliger do not present similar methods of creating, manipulating, or analyzing microdroplets—the microdroplets in Quake and Holliger are not “similar” by any meaningful metric. For example, Holliger's microdroplets are not uniform in size because they are created by manually dropping an aqueous PCR liquid into a stationary container of oil, then shaking, whereas Quake's droplets are of uniform size because they are created by injecting a stream of sample fluid into a moving stream of oil. Accordingly, I do not agree with Dr. Meinhart that Quake and Holliger disclose similar microdroplets.

113. I also do not agree that merely mentioning microdroplets in the two references (even if there were similarities) demonstrates a reasonable expectation of

success when combined. Whether or not the droplets themselves are similar does not mean that Holliger provides any details that would allow a POSITA to have a reasonable expectation of success in implanting PCR in Quake's droplets. And it is my opinion that Holliger does not provide such details. As I stated previously, Holliger provides no detail about the necessary components of performing reactions in droplets—for example, it does not disclose the number of cycles that the droplets should be subjected to allow the detection of the incorporated target (particularly detection using Quake's methods). Moreover, neither Dr. Meinhart nor any of the asserted references addresses the fact that the droplets in Quake are constantly being circulated through a microfluidic device whereas the droplets in Holliger in a bulk, non-flowing solution. Thus, even if a skilled artisan were motivated to look to Holliger, he or she would not find the teachings needed to implement PCR in Quake's microfluidic device.

114. In my opinion, Dr. Meinhart has not established that Holliger's disclosure of performing PCR in droplets provides any indication of success in implementing PCR within microdroplets flowing through Quake's microfluidic system. A microfluidic droplet-based system used to detect cells and viral particles like that described in Quake is not similar to the bulk emulsion created by manually distributing sample material into a container of oil and agitating the mixture with a

paddle or a whisk to create droplets, as described in Holliger. That Holliger could generate a bulk emulsion of heterogeneous droplets and purportedly performed PCR across some fraction of those droplets does not suggest that anything about performing PCR in microdroplets in a microfluidic system. Holliger's method of creating millions of vesicles, and then breaking them open to analyze the reaction product says nothing about the productivity of individual droplets in facilitating reactions. Thus, there is no indication in Holliger of how effective its amplification methods are, and whether this technique would work for the smaller number of droplets disclosed in Quake. For example, Holliger may have only observed a reaction product in a small fraction of its millions of droplets, which, when translated to a system that produces only a handful of droplets, would be unworkable

115. Microfluidic systems like Quake create droplets using different mechanisms (for example, two types of moving fluids), heat, cool, and store droplets differently, and subject droplets to different forces and methods of manipulation. There is no reason for a skilled artisan to believe that the broad distribution of droplet sizes characteristic of a bulk emulsion created by stirring or agitation would compare to the homogenous population of droplets created by the flowing fluid streams in microfluidic system, and there is therefore no reason to think that the conditions

described in Holliger (to the extent they are described) would be implemented in Quake with a reasonable expectation of success.

116. Dr. Meinhart provides no explanation as to how a skilled artisan would modify Quake to conduct PCR reactions in droplets. Performing PCR using Quake's devices would require a complete redesign. Quake's microfluidic devices are geared towards simple droplet-sorting techniques, and include a droplet generation region, a droplet detection region, and in some cases a discrimination region or branch point(s) to control the fluid flow and direct droplets into different portions of the device to sort them in response to the detection step. Ex. 1006 at 22:8-23:10. In order to implement thermocycling prior to detection (such that a reaction could be performed and the product could be detected), Quake's devices would need to be redesigned to include sufficient channel length to conduct an adequate number of amplification cycles such that the amplification could be detectable (a number which is not disclosed or suggested by Quake or Holliger, as I have already discussed above). These details are not discussed by Dr. Meinhart, and are not taught by Quake or Holliger.

117. To the extent Petitioner alleges that Quake's incorporation of Kopp discloses these missing details, I disagree. Kopp does not address these details in reference to droplet-based detection. In particular, Kopp provides no indication of

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the cycles of heating and cooling needed to produce a sufficient reaction product within the droplets to be detectable.

118. To the extent Petitioner or Dr. Meinhart alleges that a POSITA could instead use an off-chip thermocycling process rather than circulate droplets through a microfluidic chip to produce a detectable reaction product, in my opinion, utilizing off-chip thermocycling is not feasible nor disclosed by any of the cited references (or Petitioner or Dr. Meinhart). In particular, off-chip thermocycling requires some mechanism for removing then reintroducing the droplets from the microfluidic chip. Of course, none of the cited references provide any disclosure of how to perform this process.

119. In my opinion, modifications to Holliger in view of Quake to arrive at the claimed invention would require a complete redesign of Holliger's apparatus, such that a skilled artisan would not have a reasonable expectation of success in such a modification. Dr. Meinhart does not offer any opinion as to how such a modification would be performed, or suggest that it could be. Modifying Holliger to perform PCR in droplets on a microfluidic device would require abandoning the bulk emulsion techniques that Holliger relies on to create droplets and perform PCR. Of course, this would require discarding Holliger's entire system in favor of a completely different system, with no motivation to do so.

120. Further, a graduate student of Quake, Todd Thorsen, attempted but failed to implement rudimentary chemical reactions in droplets. This provides further support for my opinion that a skilled artisan would not have had a reasonable expectation of success in modifying Quake to perform PCR in droplets. In Thorsen's thesis, he describes "hundreds" of failed attempts at performing reactions in droplets "in an attempt to discover the perfect operation conditions. Ex. 2015 at 101-102. Thorsen stated that "[w]orking on the development of a microfluidic crossflow assay emphasized the *difficult dynamic integration process* between biochemical and micromechanical systems." *Id.* at 108 (emphasis added). That Todd Thorsen—who was certainly beyond a person of ordinary skill in the art in the relevant field—had difficulty implementing reactions in microfluidic devices, which he would have learned about from his work in Steven Quake's lab, demonstrates to me that a skilled artisan would not have had a reasonable expectation of success in implementing PCR in Quake's droplets, particularly if her reference point for performing PCR in droplets was a bulk emulsion reference like Holliger.

C. Quake in view of Holliger in further view of Schneegaß Does Not Render the Challenged Claims Unpatentable

121. In my opinion, Schneegaß does not remedy the deficiencies of Quake in combination with Holliger. That is, Schneegaß provides no additional teachings

that render the asserted claims obvious when combined with Quake and Holliger. I do not agree that a POSITA “would have been motivated to further modify the methods and systems of Quake in view of Holliger to incorporate Schneegaß’s teachings related to flow-through thermocycling. Meinhart Decl. ¶ 174. As I explained above, Quake does not disclose PCR and does not suggest any benefit to performing PCR in its microfluidic devices, and in fact teaches away from PCR. Accordingly, I do not agree that a POSITA presented with Quake would be motivated to take Schneegaß’s disclosures of flow-through thermocycling to implement PCR in droplets. I incorporate my opinions above with respect to Quake in combination with Holliger herein.

122. Dr. Meinhart also alleges that Schneegaß explains that flow-through thermocycling offered numerous benefits over conventional thermocycling. This does not establish a motivation to perform PCR in the droplet-generation device disclosed in Quake. Dr. Meinhart does not even attempt to show that the alleged benefits of flow-through thermocycling in comparison to traditional PCR would motivate a POSITA to perform PCR in droplets (as opposed to simply performing flow-through thermocycling). Dr. Meinhart fails to identify any recognized benefits of droplet technology over flow-through thermocycling that would have motivated the asserted combination.

123. Dr. Meinhart also asserts that “[a]t the time Schneegaß was published, the industry was already trending towards miniaturized total analysis systems.” Meinhart Decl. ¶ 175. I do not agree with Dr. Meinhart’s implication that this demonstrates a motivation to combine Quake, Holliger, and Schneegaß to arrive at the claimed invention, or a reasonable expectation of success in the combination. In my opinion, a general trend toward miniaturization does not provide a specific motivation to perform PCR in droplets, as taught by the claimed invention. Miniaturization in and of itself is not a universal solution to any technical challenge and the nature of the ‘365 patent is far more innovative than the simple reduction in size of various existing components to carry out PCR in droplets.

124. I also do not agree that Schneegaß’s statement that the “ultimate manifestation of [miniaturized total analysis systems] is a fully integrated device that receives a sample, performs a multi-step process, and analyzes the result” motivates performing PCR in droplets. Meinhart Decl. ¶ 175. This generalized statement about industry trends does not provide any specific insight into how or motivation to, combine Quake, Holliger, and Schneegaß to arrive at the claimed invention. Indeed, the addition of Schneegaß does not provide any motivation to modify Quake to perform PCR in droplets when Quake teaches away from such a modification by using direct detection in small-volume droplets as an alternative method to PCR.

125. I also do not agree that Quake's statement that multiple technologies can be incorporated into a single device provides a motivation to combine Quake, Holliger, and Schneegaß. As I explained previously, this statement does not suggest that a combination of technologies into the same chip includes performing PCR in droplets. Instead, Quake referenced technologies like flow-through thermocycling, which to me indicates that the integration Quake was referring to involved performing PCR either before or after sample material was encapsulated into droplets, not on droplets containing genetic material. Thus, to the extent a POSITA would have been motivated to use Schneegaß's teachings, it would not have been to perform PCR in droplets, but to integrate flow-through thermocycling into Quake's microfluidic devices.

126. I also note that Petitioner has not asserted that Schneegaß provides any independent motivation to perform PCR in droplets. Rather, based on my understanding, Petitioner has argued that Quake includes disclosures that suggest performing PCR in droplets, and given those alleged disclosures, a POSITA would be motivated to combine Schneegaß's teachings of thermocyclers with Quake and Holliger to implement this technology. Again, I disagree with this analysis because Quake does not disclose or suggest performing PCR in droplets, but instead teaches away from doing so. Additionally, Quake teaches that it would be advantageous to

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use disposable, single-use materials like plastic. However, Schneegaß discloses the use of thermocyclers made of glass and silicone due to their thermal properties and “ability for common use in micromachining technologies.” Ex. 1008 at 107-108. Accordingly, it is my opinion that a skilled artisan would not be motivated to combine Schneegaß’s teachings with Quake, given that Schneegaß proposes the use of different materials of thermocycling devices.

127. I also disagree with Dr. Meinhart and Petitioner that a POSITA would have a reasonable expectation of success in the combination of Quake, Holliger, and Schneegaß. For the same reasons I discussed above, incorporating thermocycling into Quake’s device would require a complete redesign (as would incorporating the use of microfluidics into Holliger’s teachings). I do not agree with Dr. Meinhart’s assertion that a POSITA “would expect to succeed in integrating Schneegaß’s flow-through heating elements with Quake’s device because of the similarity between the systems.” Meinhart ¶ 177. Quake and Schneegaß do not describe similar systems, and adding Schneegaß’s thermocycling elements would certainly change the performance, transport, and formation of the droplets contrary to the Petitioner’s assertions. Adding thermocycling and the ability to perform PCR reactions to droplets would at the very least change the way that droplets are transported because it would require that droplets are moved to insure their residence time in the heating

and cooling is sufficient to enable the PCR reaction to occur. As I described above, this would require significant re-design of Quake's microfluidic device.

128. As Dr. Meinhart acknowledges, Schneegaß does not disclose picoliter sized droplets like those described in Quake. Meinhart Decl. ¶ 179-180. I do not agree with Dr. Meinhart's baseless assertion that "the small droplets of Quake and Holliger would have made it easier, not more difficult to apply the thermocycling teachings of Schneegaß to the channels of Quake." *Id.* Dr. Meinhart asserts that the smaller size of the droplets would improve heat transfer. However, he offers no explanation of how a POSITA would adjust Schneegaß's minimal teachings to account for this "improvement." While true that smaller droplets may be quicker to heat, adjusting the thermocycling machinery to a specific droplet size is not straightforward. For example, as Dr. Meinhart acknowledges, it is not just droplet size that needs to be accounted for when implementing thermocycling. Things like the diameter of the channel or tube that the droplets are circulating in need to be considered, see Meinhart Decl. ¶ 179-180, as do the materials used in the microfluidic device, which, as I noted above, are different in Quake than those used in Schneegaß, Ex. 1008 at 107-108. Further, neither reference addresses the necessary concentration of reaction materials needed to effectively perform PCR in Quake's droplets. Accordingly, it is my opinion that Dr. Meinhart has not

demonstrated that a POSITA would have a reasonable expectation of success in applying Schneegaß's teachings to smaller droplets.

129. The assertion that Schneegaß and Quake allegedly arrive “at the same solution” taught by Quake (a two phase w/o system and the use of surfactants), is not supported. First, Schneegaß's disclosure is primarily focused on flow-through thermocycling devices and makes a very limited reference to performing PCR in droplets not providing sufficient detail on how to make these droplets. As I explained above, Schneegaß's discussion of performing PCR in droplets is primarily centered around prior art references that do not disclose performing PCR reactions in droplets, and focuses mainly on the selection of appropriate materials for microfluidic devices. Second, to the extent Schneegaß discloses the successful use droplet-based technology at all, it does not use droplets for the same purpose as Quake (i.e. detection and sorting of cells and viral particles). Thus, even if the references utilized similar technology, it cannot be said that they arrived at the same solution—there was no common problem. And, as discussed previously, Dr. Meinhart has not established a reason why Quake's objectives would benefit from PCR. Lastly, neither the asserted references nor Dr. Meinhart has offered any explanation of how a POSITA would adjust the thermocycling components

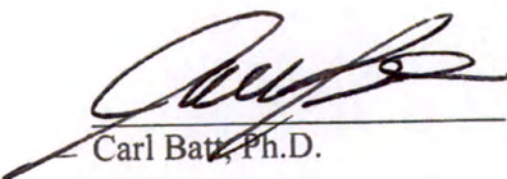
disclosed in Schneegaß to account for the different droplet sizes, channel sizes, and materials used in Quake.

130. In further support of my opinion, I note that Quake's graduate student, Todd Thorsen, attempt to perform rudimentary chemical reactions in droplets, as I discussed above. In his thesis, Dr. Thorsen documented hundreds of failures in his attempts to perform reactions in droplets such as those described in Quake. Ex. 2015. This demonstrates that performing reactions in droplets was not a straightforward task, even for someone with a skill level far beyond that of a POSITA. This also supports my opinion that altering droplet size would not have made performing reactions easier—it would have raised additional questions regarding how to effectively implement thermocycling that would require significant experimentation in order to successfully arrive at the asserted combination. In my opinion, Dr. Meinhart has not supported his assertion that Schneegaß's teachings could be directly translated to performing PCR in small droplets such as those described in Quake.

131. For at least these reasons, it is my opinion that the challenged claims of the '365 Patent are not invalid.

* * *

I hereby declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct, and that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, I understand that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001).



Carl Batt, Ph.D.

Dated: September 8, 2021