

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DROPWORKS, INC.,
Petitioner,

v.

THE UNIVERSITY OF CHICAGO,
Patent Owner.

Case IPR2021-00100
U.S. Patent No. 8,304,193

**PRELIMINARY RESPONSE TO PETITION FOR *INTER PARTES*
REVIEW OF U.S. PATENT NO. 8,304,193**

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TABLE OF CONTENTS

	Page
TABLE OF AUTHORITIES	v
PATENT OWNER’S EXHIBIT LIST	vii
I. INTRODUCTION	1
II. Background.....	5
A. The ’193 Patent	5
B. The Level Of Ordinary Skill In The Art	10
C. The Prior Art Cited In The Petition.....	12
1. Quake	12
2. Holliger	16
3. Litborn.....	18
4. Shaw-Stewart	21
5. Schneegaß	21
D. The Prior Attempts To Invalidate The ’193 Patent Before The Patent Office.....	22
1. The Previous IPR Of The ’193 Patent	22
2. The Reexamination Of The ’193 Patent	23
III. Reasons Why The Petition Should Be Denied	24
A. The Petition Should be Rejected Under § 325(d)	25
1. Dropworks Advances The Same Or Substantially The Same Art That Was Previously Presented To The Office	26

a)	Factor (a): The Similarities And Material Differences Between The Asserted Art And The Prior Art Involved During Examination. Factor (b): The Cumulative Nature Of The Asserted Art And The Prior Art Evaluated During Examination.....	26
2.	Factor D: The Extent Of The Overlap Between The Arguments Made During Examination And The Manner In Which Petitioner Relies On The Prior Art	28
3.	Dropworks Fails To Identify Material Error In The Patent Office’s Previous Evaluation Of The Art	30
a)	Factor C: The Extent To Which The Asserted Art Was Evaluated During Examination, Including Whether The Prior Art Was The Basis For Rejection	30
	Factor E: Whether Petitioner Has Pointed Out Sufficiently How The Examiner Erred In Its Evaluation Of The Asserted Prior Art Factor F: The Extent To Which Additional Evidence And Facts Presented In The Petition Warrant Reconsideration Of The Prior Art Or Arguments.	30
B.	The ’193 Patent Is Not Obvious In View Of The Prior Art.....	31
1.	The Cited Prior Art Fails To Teach Or Suggest Several Key Claim Elements	32
a)	The Prior Art Does Not Teach Autocatalytic Reactions In Droplets In A Microfluidic System.....	33
b)	The Cited Prior Art Does Not Describe Two Channels, One Of Which Contains Flowing Aqueous Fluid Containing Both Substrate And Reagent	40
c)	The Cited Prior Art Does Not Disclose Conditions For Performing Autocatalytic Reactions In A Microfluidic System	42

2.	The Skilled Artisan Would Not Be Motivated To Combine Quake With Either Holliger Or Litborn.....	47
3.	A POSA Would Not Have A Reasonable Expectation Of Succeeding In Combining Quake’s System With Holliger Or Litborn	51
IV.	Secondary Considerations	58
A.	Long Felt Need And Industry Praise.....	59
B.	Failure Of Others.....	63
V.	CONCLUSION.....	64

TABLE OF AUTHORITIES

	Page(s)
Cases	
<i>ActiveVideo Networks, Inc. v. Verizon Commc'ns, Inc.</i> , 694 F.3d 1312 (Fed. Cir. 2012).....	55
<i>Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH</i> , IPR2019-01469, Paper 6 (PTAB Feb. 13, 2020).....	24, 25, 26
<i>Apple Inc. v. Samsung Elecs. Co.</i> , 839 F.3d 1034 (Fed. Cir. 2016).....	31
<i>ATD Corp. v. Lydall, Inc.</i> , 159 F.3d 534 (Fed. Cir. 1998).....	46
<i>Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.</i> , 796 F.2d 443 (Fed. Cir. 1986).....	35
<i>Bio-Rad v. 10X Genomics, Inc.</i> , C.A. 15-cv-152-RGA D.I. 477.....	62
<i>Cheese Sys., Inc. v. Tetra Pak Cheese & Powder Sys., Inc.</i> , 725 F.3d 1341 (Fed. Cir. 2013).....	46
<i>Clim-A-Tech Ind., Inc. v. William A. Ebert</i> , IPR2017-01863, Paper No. 13 (PTAB Feb. 12, 2018).....	28
<i>Honeywell Int'l Inc. v. Mexichem Amanco Holding S.A. DE C.V.</i> , 865 F.3d 1348 (Fed. Cir. 2017).....	51
<i>In re Fritch</i> , 972 F.2d 1260 (Fed. Cir. 1992).....	47
<i>In re O'Farrell</i> , 853 F. 2d 894 (Fed. Cir. 1988).....	56
<i>In re Ratti</i> , 270 F.2d 810 (C.C.P.A. 1959)	54

<i>In re Rijckaert</i> , 9 F.3d 1531 (Fed. Cir. 1993).....	32
<i>Intelligent Bio-Systems, Inc. v. Illumina Cambridge, Ltd.</i> , 821 F.3d 1359 (Fed. Cir. 2016).....	46
<i>Kinetic Concepts, Inc. v. Smith & Nephew, Inc.</i> , 688 F.3d 1342 (Fed. Cir. 2012).....	51
<i>PAR Pharm., Inc. v. TWI Pharm., Inc.</i> , 773 F.3d 1186 (Fed. Cir. 2014).....	46
<i>Procter & Gamble Co. v. Teva Pharm. USA, Inc.</i> , 566 F.3d 989 (Fed. Cir. 2009).....	56
<i>R.J. Reynolds Vapor Co. v. Fontem Holdings I B.V.</i> , IPR2018-00626, Paper No. 7 (PTAB Sept. 27, 2018).....	28
<i>Rambus Inc. v. Rea</i> , 731 F.3d 1248 (Fed. Cir. 2013).....	57
<i>St. Jude Med., Inc. v. Access Closure, Inc.</i> , 729 F.3d 1369 (Fed. Cir. 2013).....	31
<i>Standard Oil Co. v. Am. Cyanamid Co.</i> , 774 F.2d 448 (Fed. Cir. 1985).....	11
<i>Stratoflex, Inc. v. Aeroquip Corp.</i> , 713 F.2d 1530 (Fed. Cir. 1983).....	57
<i>Trustees of Columbia Univ. in City of New York v. Illumina, Inc.</i> , 620 F.App’x 916 (Fed. Cir. 2015)	50
<i>WMS Gaming, Inc. v. Int’l Game Tech.</i> , 184 F.3d 1339 (Fed. Cir. 1999).....	46
Statutes and Regulations	
35 U.S.C. § 325(d)	24, 28, 31
M.P.E.P. § 2143	54

PATENT OWNER'S EXHIBIT LIST

<u>Exhibit Number</u>	<u>Description</u>
2001	Whitesides, G., <i>The origins and the future of microfluidics</i> , Nature, Vol. 442, 368-373 (July 27, 2006)
2002	Teh, S.Y. et al., <i>Droplet microfluidics</i> , Lab Chip, 2008, 8, 198-220
2003	Curio, M., <i>Continuous Segmented-Flow Polymerase Chain Reaction for High-Throughput Miniaturized DNA Amplification</i> , Analytical Chemistry 2003, 75, 1-7, Bates-stamped DROPWORKS006407- DROPWORKS006413
2004	Request for <i>Ex Parte</i> Reexamination of U.S. Patent No. 8, 304,193 (Nov. 3, 2017)
2005	Ex Parte Reexamination Communication Transmittal Form (Sept. 28, 2018)
2006	Response to First Office Action in <i>Ex Parte</i> Reexamination of U.S. Patent No. 8,304,193 (June 12, 2018)
2007	Collins et al., <i>The Poisson distribution and beyond: methods for microfluid droplet production and single cell encapsulation</i> , Lap on a Chip (July 20, 2015)
2008	Seemann et al., <i>Droplet based microfluidics</i> Report On Progress Physics (December 22, 2011)
2009	Payne et al., <i>High-throughput screening by droplet microfluidics: perspective into key challenges and future prospects</i> , Lab on a Chip, Royal Society of Chemistry (May 28, 2020)
2010	Leng, et al., <i>Microfluidic crystallization</i> , Lab on a Chip, May 6, 2008, 24-34
2011	Zhu, et al., <i>Analytical detection technique for droplet microfluidics—A review</i> , Analytica Chimica Acta 787 (2013), 24-35
2012	Dropwork's Continuum Digital Flow PCR System, https://www.dropworks.com/introducing-continuum

I. INTRODUCTION

Patent Owner The University of Chicago submits this Preliminary Response to Petition For *Inter Partes* Review of U.S. Patent No. 8,304,193 (“the Petition”). For the reasons stated below, the Board should not institute review.

The inventions of U.S. Patent No. 8,304,193 (“the ’193 Patent”) were made by Rustem Ismagilov and co-workers at the University of Chicago in the 2002 time frame.¹ The ’193 Patent describes and claims complete systems for carrying out a variety of chemical reactions in tiny droplets in microfluidic systems. Using the techniques pioneered in the ’193 Patent, researchers are able package chemical reagents into tiny microfluidic chambers in controllable ways and reliably carry out thousands of individualized chemical reactions in parallel. This approach has found widespread use across the medical and research space, and dozens of companies have sprouted up based on the use of microfluidic droplet technology, including the Petitioner here, which goes by the name “Dropworks.”

The instant Petition represents the fifth time Quake has been presented as a basis for invalidating U.S. Patent No. 8,304,193 (“the ’193 Patent.”). First, during prosecution, Quake was identified as a basis for rejection by the Examiner. The

¹ Dr. Ismagilov is now a professor of Chemistry and Chemical Engineering at the California Institute of Technology.

rejection was overcome. Second, after the '193 Patent was asserted in district court litigation, an IPR was filed in which Quake was the primary reference. The Board did not even institute the IPR. Third, after the first IPR of the '193 Patent failed, an *ex partes* reexamination based on Quake was filed. That reexamination failed as well. Fourth, after the IPR and reexamination failed, the validity of the '193 Patent in view of Quake was put before a Delaware jury. The jury upheld the validity of the '193 Patent, as did the district court judge following post-trial briefing.

Notably, several other patents in the same family as the '193 Patent have also subject to both IPR and *ex partes* reexamination, including U.S. Patent Nos. 7,129,091; 8,822,148; and 8,329,407. For all of these patents, the Board did not institute review and the reexamination unit upheld all claims. *See, e.g.*, IPR2015-01560 Paper 13 (PTAB Jan. 25, 2016); IPR2015-01156 Paper 14 (PTAB Nov. 16, 2015); IPR2015-01158 Paper 14 (PTAB Nov. 16, 2015); Reexamination Control No. 90/014,201; Reexamination Control No. 90/014,045; Reexamination Control No. 90/014,042. Yet another patent in the same family as the '193 Patent, U.S. Patent No. 8,889,083, was also subject to *inter partes* review, and, although the Board instituted review, all claims were upheld at the final written decision stage. *See* IPR2015-01157, Paper 51 (PTAB Nov. 15, 2016).

In each case, Quake was the primary prior art reference relied upon. These prior determinations were not due to error or oversight, but are attributable to the

strength of the inventions made by Ismagilov and co-workers and the fact that there is no invalidating prior art. Certainly, Quake is nowhere close to the '193 Patent. While Petitioner contends that the skilled artisan would have naturally been motivated to apply the disclosure of Quake for the purpose of performing PCR in droplets, this was the exact same rationale presented in the previous IPRs and reexaminations.

Far from suggesting PCR in droplets, the Quake reference teaches that the droplet technique is an *alternative to PCR* to sort cells or particles (e.g., viral particles). Quake is thus wholly oblivious to the parameters for achieving PCR in droplets in microfluidic devices. Notably, there is no evidence that Quake ever performed any meaningful reactions in droplets, let alone PCR. Unlike the '193 Patent, Quake hardly mentions any kind of reaction in droplets. To the extent Quake makes brief mention of reactions, it proposes a speculative technique based on droplet merger that is nothing like the claimed invention of the '193 Patent.

The Petition's flawed reasoning is glaringly obvious when one examines the references that the Petition purports to combine with Quake. The Petitioner's first combination reference, Holliger, has nothing to do with microfluidics but is rather about water in oil emulsions. In Holliger, a random mixture of over 113 million water vesicles containing PCR mix and having a range of sizes is formed simply by stirring or agitating a solution of water-in-oil with a paddle or whisk, much like one

would shake salad dressing. This bulk emulsion is then subject to thermal cycling, and a PCR product is allegedly recovered.

Holliger, however, provides no indication as to what fraction or type of droplets actually yielded a product. Holliger's alleged ability to capture some PCR product from 113 million water containers in a bulk emulsion says nothing about the conditions for doing PCR across a much smaller number of droplets of tightly controlled size in microfluidic devices, provides no motivation to perform such an experiment, and does not establish a reasonable expectation of being able to successfully do so.

Petitioner's second combination reference, Litborn, is a survey paper that describes potential future applications for microfluidics. Litborn includes a passing reference to "preliminary" experiments supposedly involving PCR in a flow-based reactor. No results or data are shown, let alone the experimental setup. Nevertheless, Petitioner contends that these "preliminary" experiments involved droplets. Petitioner is wrong. The Litborn group eventually published their work on flow-based reactors, and it did not involve droplets. Rather, the Litborn work involved prior art systems of precisely the type that were relied upon as secondary references in the previous IPR and reexamination of the '193 Patent.

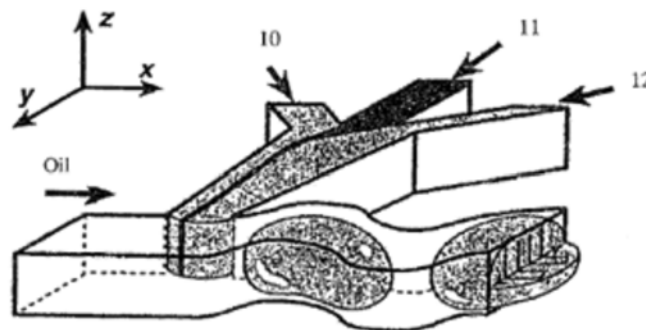
The fact is that the '193 Patent represents a revolutionary invention in a nascent field. It is not invalid in view of Quake, and the Petition should be denied.

II. BACKGROUND

A. The '193 Patent

Microfluidic systems involve the transport and manipulation of fluids on a tiny scale, using channels that typically have micrometer dimension. *See, e.g.*, Ex. 1001, '193 Patent at 1:20-22. While microfluidic systems were commonly based on uninterrupted flow of liquids through microfluidic channels, the inventions of the '193 Patent are based on the use of discrete microfluidic droplets, one type of which the patent refers to as “plugs.” The '193 Patent describes complete systems for carrying out a variety of chemical reactions in droplets in microfluidic systems.

The '193 Patent includes a detailed description of how plugs are formed. *See id.* at 17:24-21:67. Plugs are formed by introducing a stream of a plug fluid into the flow of a carrier fluid immiscible with the plug fluid. For instance, the plug fluid may be an aqueous solution and the carrier fluid may be oil. The figure below from the '193 Patent depicts the formation of droplets in this manner:



Id. at Fig. 10A. The plug fluids are introduced in channels 10, 11, and 12, and droplets of plug fluids are created upon interaction with the oil that is flowing through the perpendicular channel. The droplet volumes are typically in the picoliter to nanoliter range, but may be as small as a few femtoliters. *See id.* at 19:45-57. Importantly, the droplets that are generated in such a system are completely surrounded by the oil such that the interior of the droplet does not come into contact with the channel walls.

The use of droplets were a breakaway from prior art methods in microfluidics, which were based on either segmented or continuous flow. Continuous flow systems involve continuous streams of liquid. In segmented flow, there are simply segments of fluid that, unlike droplets, contact channel walls. The segments are separated from one another by a segmenting fluid, which can be a gas such as air. As documented below, the Litborn reference identified by Petitioner is an example of such a system. During the previous reexamination, extensive evidence was presented regarding the major difference between segmented flow and droplet systems. *See, e.g.*, Ex. 1028 at 5941-43, 5944-52.

While microfluidic systems had been known, researchers had not successfully used droplets as “microreactors” for chemical reactions. Given their tiny size, microfluidic droplets have a far higher surface to volume ratio than vessels previously used for carrying out chemical reactions. *See id.* at 35:59-63 (“Control

of surface chemistry is particularly important in microfluidic devices because the surface-to-volume ratio increases as the dimensions of the systems are reduced. In particular, surfaces that are generally inert to the adsorption of proteins and cells are invaluable in microfluidics.”).

Carrying out chemical reactions on this minuscule scale and in the environment of a microfluidic droplet presented a host of unique problems inapplicable to bulk scale chemical experiments. As Petitioner acknowledges, during the prior IPR and reexamination, extensive evidence regarding these issues was submitted and considered. *See, e.g.*, Ex. 1028 at 5944-52. Thus, while those in the field dreamed that chemical reactions could one day be carried out in microfluidic droplets, until the ’193 Patent, microfluidic droplets had only been used for rudimentary applications, such as sorting of particles.

The ’193 Patent presented a range of innovations in microfluidics, the combination of which overcame problems that had previously prevented researchers from using microfluidic droplets as chemical reactors and discouraged them from this approach. For instance, the ’193 Patent presents techniques for overcoming at least the following historical problems:

- Dispersion of solutes in microfluidic devices that increased reagent consumption and prevented experiments or measurements over long time scales (e.g., minutes to hours). *See id.* at 11:54-63.

- Difficulties in preparing and controlling complex microfluidic droplets containing a mixture of reagents with precise compositions. *Id.* at 33:27-29; *see also id.* at 18:38-19:6.
- Unresolved issues associated with microfluidic channel substrate material and carrier fluids that caused droplet material to adhere to channels walls. *See id.* at 20:59-21:20.
- The inability to quickly mix reagents in droplets, which precluded the study of reactions that take place on short time scales. *See id.* at 11:64-67; 22:1-27:5.
- The inability to accurately optically detect and monitor reaction products due to measurement distortion introduced by carrier fluid interference. *See id.* at 29:62-30:4.
- Pronounced surface chemistry problems at the droplet/carrier-fluid interface in the high surface-to-volume environment of a droplet that led to adsorption of reagents and environments incompatible with biological molecules. *See id.* at 35:59-36:8.

The '193 Patent includes numerous exemplary techniques for fabricating microfluidic devices with complex structures, creating plugs, varying the concentrations of reagents in plugs, manipulating (e.g., splitting and merging) plugs, and monitoring and analyzing the composition of plugs so that researchers could, for the first time, use microfluidic droplets as chemical reactors. *See id.* at 61:16-69:8. The '193 Patent further includes examples demonstrating a range of different

chemical reactions and phenomena that could, for the first time, be carried out using microfluidic droplets, including autocatalytic reactions (such as PCR), enzyme kinetics, RNA folding kinetics, synthesis of nanoparticles, and protein crystallization. *See id.* at 67:13-78:6. The research and development of these techniques and applications were reported in a series of frequently-cited (over 4,000 times) publications that came from the Ismagilov research group.

Each of the Ismagilov Patents includes claims directed to specific aspects of the described techniques for carrying out chemical reactions in microfluidic droplets. The '193 Patent specifically includes claims directed to preparing plugs of aqueous fluid substantially surrounded by an oil flowing through a microfluidic channel. *Id.* at claim 1. The claimed microfluidic system requires at least two channels: one for oil and another for carrying both a substrate molecule and reagents. *Id.* The formed plugs thus include at least one substrate molecule (such as a DNA or RNA molecule) and reagents for conducting an autocatalytic reaction, for example a PCR reaction. *Id.* at claim 1, 3. Dependent claims add further structural requirements related to carrying out reactions in droplets, including, for instance, the use of fluorinated oil (claim 6), the use of a surfactant in the carrier fluid (claim 7), the ability to trap plugs (claim 11), and methods for mixing reagents in droplets (claims 12-14).

B. The Level Of Ordinary Skill In The Art

Petitioner offers a brief resume-style description of the level of ordinary skill in the art. *See* Petition at 12-13. Petitioner’s description, however fails to adequately capture the specific competencies and know-how that would have characterized the person of ordinary skill.

As late as 2006, researchers characterized microfluidics as a field in “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.”). The skilled artisan would have viewed microfluidics as a breakaway from prior art techniques and would have understood that researchers had made only simple proof-of-concept demonstrations for microfluidic techniques related to introducing reagents and samples, moving fluids, and mixing fluids. *Id.* at 369 (describing “[t]he present” state of microfluidics in 2006).

Because these techniques had not moved beyond proof-of-concept, these techniques would not have been routine to the skilled artisan, and their use in applications would not have been reflexive. *See id.* at 371 (“Above all, [microfluidics] must become successful commercially, rather than remain a field based on proof-of-concept demonstrations and academic papers.”).

Further, one of skill in the art would have understood microfluidics to be largely limited to continuous flow microfluidics, as opposed to droplet microfluidics, which was barely in the proof-of-concept stage. As of 2002, the skilled artisan would have had little, if any, experience with conducting reactions in microfluidic 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.”). Accordingly, the skilled artisan interested in analyzing biological and chemical samples would have most likely attempted to follow these prior art techniques. *See Standard Oil Co. v. Am. Cyanamid Co.*, 774 F.2d 448, 454 (Fed. Cir. 1985) (“A person of ordinary skill in the art is also presumed to be one who thinks along the line of conventional wisdom in the art.”).

Roughly consistent with Petitioner’s proposal, Patent Owner agrees that in the 2002 time frame, the 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. Yet, Patent Owner wishes

to supplement this resume with the foregoing information to better capture the relevant knowledge the skilled artisan would have had.

C. The Prior Art Cited In The Petition

The Petition relies on the following references for its proposed invalidity grounds:

- Ex. 1005, International Publication No. WO 02/23163 (“Quake”)
- Ex. 1006, International Publication No. WO 02/22869 (“Holliger”)
- Ex. 1007, “Synthesis and Analysis of Chemical Components in Nanoscale (“Litborn”)
- Ex. 1008, International Publication No. WO 84/02000 (“Shaw Stewart”)
- Ex. 1009, “Flow-through polymerase chain reactions in chip thermocyclers” (“Schneegaß”).

Importantly, the primary reference in every proposed ground of invalidity is Quake.

A summary of the references relied upon in the Petition is provided below as relevant background for understanding why the obviousness grounds presented in the Petition are unsustainable. As documented below and further herein, the cited prior art is far from the claimed invention.

1. Quake

Petitioner’s main reference is Quake, which, as documented above, has been used as a primary reference to challenge the ’193 Patent four previous times.

Quake is not about autocatalytic reactions, but about sorting particles. *See, e.g.*, Ex. 1005 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.”). Quake purports to address the problems associated with conventional sorting devices such as FACS and flow cytometry. *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.

The Quake reference itself only discloses the production of small numbers of droplets, on the order of a few dozen. *See, e.g.*, Ex. 1005 at Figs. 18-19. An inventor on the Quake reference, Todd Thorsen, subsequently indicated in his doctoral dissertation that the system of Quake is suitable for generating a number of droplets on the order of thousands. *See, e.g.*, Ex. 1036 at 91 (“As switching between the two dye streams was accomplished manually, several hundred droplets were generated for each color type each time the relative water pressures were changed. Using this process, thousands of fluorescein/Rphycoerythrin droplets were generated with a rough population distribution of $\sim 1:1$.”).

Quake does not teach methods for carrying out autocatalytic chemical reactions, such as PCR, in microfluidic droplets. PCR is mentioned only twice in the entirety of Quake's disclosure. First, Quake discusses PCR, an existing method for detecting viruses, which Quake identifies as defective and attempts to improve upon with his sorting system. Ex. 1005 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.").

Second, PCR is described in a background section regarding the application 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 discussion does not describe use of PCR in Quake's system, but simply acknowledges that microfabrication techniques may be useful in a bunch of different contexts. Although Quake is about droplets, it makes no mention of the issues associated with the interface between the droplet and the carrier fluid that must be addressed to carry out PCR in droplets.

To the extent Quake includes any disclosure of conducting reactions in droplets, the disclosure speculates about an approach in which one provides

substrates and enzymes in *separate* channels that are introduced to the carrier fluid in two inlets.

Quake's proposed two-inlet system forms two kinds of droplets: one with an enzyme and a one with a substrate. *See id.* at 36:9-28, 85:26-31 ("an aqueous solution containing an enzyme" is introduced into a first inlet and "aqueous solution containing a substrate for the enzyme" is introduced into a second inlet). Quake shows this idea in Figure 22, a pencil drawing that depicts the two solutions separately entering an immiscible oil stream, via separate inlets, in the microchannel and forming two separate droplets:

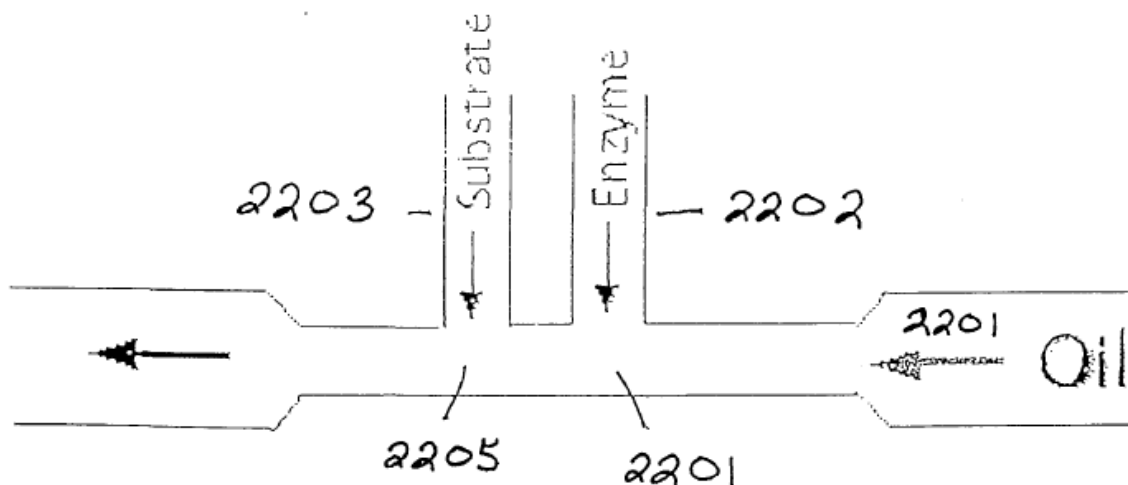


FIG. 22

Id. at Fig. 22. The two droplets may eventually be merged (somehow) in the system if and when one droplet catches up with the other droplet. A reaction is then

supposed to happen. *Id.* at 85:9-86:2.

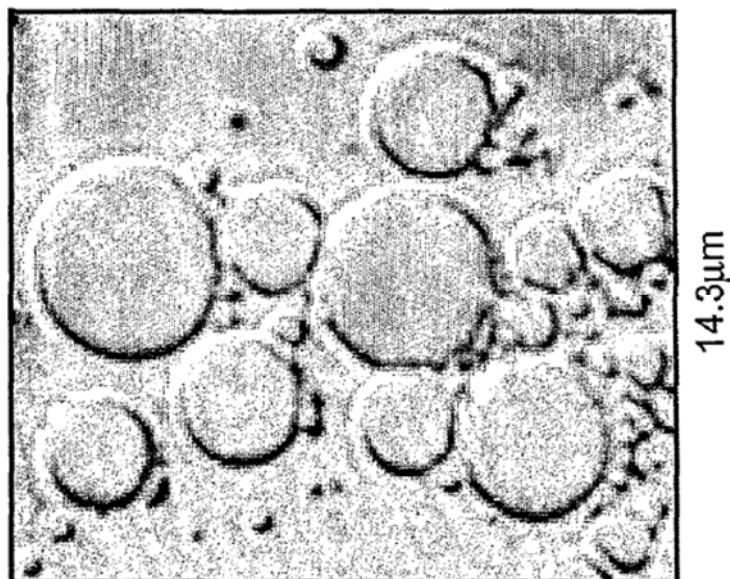
2. Holliger

Holliger is nothing like Quake. Rather than teaching microfluidics, Holliger teaches bulk emulsions, formed by mechanical agitation of an oil/water 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.

Ex. 1006 at 48:14-19. These types of emulsions are non-specific, and owe their creation simply to the general chemical characteristics of the solvent (oil) and solute (water plus reagents) when mixed together.

Figure 2 of Holliger provides an example of the types of inhomogenous mixtures of water vesicles formed using Holliger’s process:



Id. at Fig. 2. 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.

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 113 million vesicles,² far more than the few thousand droplets that are created in the microfluidic droplet system in Quake. Elsewhere, Holliger teaches

² This can be computed straightforwardly by assuming an average radius of 7.5 μm and determining the number of spheres of radius 7.5 μm that can be contained within a volume of 200 μL .

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.”)

Holliger then transfers the 113 million vesicles in bulk to a standard PCR tube and then subjects the emulsion to thermal cycling in a standard thermal cycler. *Id.* at 59:16-17. Following thermal cycling, ether is introduced to the emulsion and it is vortexed to recover the aqueous portion of the emulsion in bulk. *Id.* at 59:18-23. Holliger then analyzes this fraction using gel electrophoresis to assess whether any PCR products were formed. *Id.*

Holliger, however, includes nothing to establish what fraction of the 113 million vesicles actually yielded a PCR product, or whether the PCR products can be attributed to vesicles of a particular size that might be characteristic of the droplets in a microfluidic device.

3. Litborn

Litborn is a survey paper published in the year 2000 that describes various proposed methods for performing chemical reactions. In one section, Litborn discusses the possibility of using microfluidics as chemical reaction vessels. Litborn recognizes that much of the work that had occurred until then was driven by capillary type devices, which used a continuous flow of reagents in channels within chips. *See Ex. 1007 at 451.* Litborn explains that this technology caused problems because

surface tension could cause some parts of each droplet to be left behind, causing contamination.

Litborn briefly refers to water-in-oil droplets as a solution to the contamination problem, but includes no disclosures of PCR in droplets. Rather, Litborn refers briefly to some preliminary experiments that were supposedly done in PCR in a “flow based” reactor:

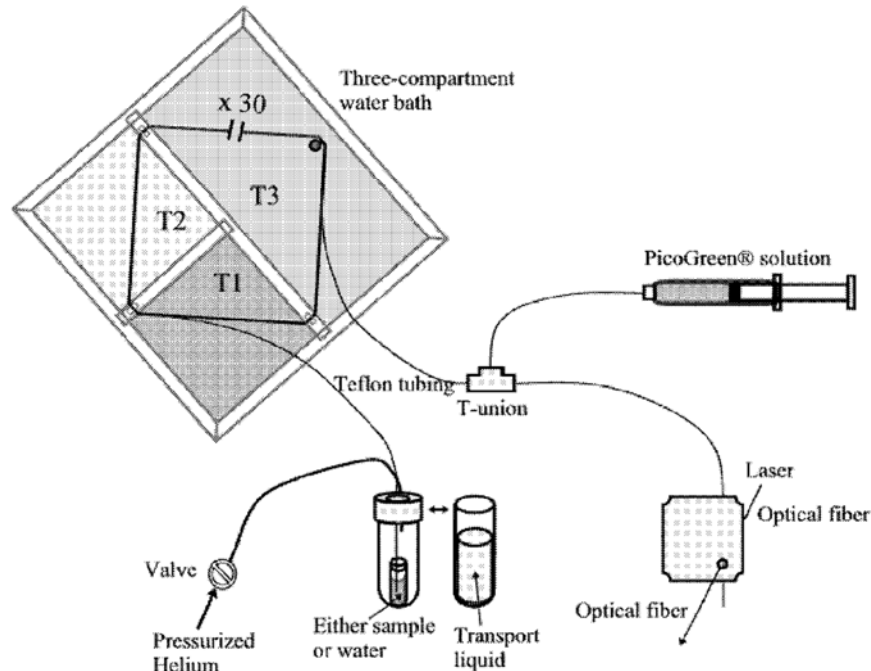
We have carried out preliminary experiments with a flow based PCR reaction, where the reaction mixture is interspaced with a hydrocarbon, as described above. Amplification was succesful, and the results were fully comparable with the results of the commercial standard procedure. It seems that this mode of operation could hold the potential to carry out a massive number of PCR reactions in nanoliter or even sub-nanoliter plugs of solution. This would have fundamental impacts on throughput bottle-necks in molecular biology.

Id. at 453. Litborn provides no details on these “preliminary experiments” in the “flow based” reactor.

Nevertheless, the Litborn research group ultimately did publish their “flow based” PCR reactor in the journal *Analytical Chemistry*. *See* Ex. 2003. It was not a droplet system. Rather than involving droplets surrounded by oil, their method involved alternating injections of sample and carrier fluid into a channel. *See id.* at 4 (“After each injection, the cap with the tubing in place was switched onto a second vial filled with carrier fluid.”). The process resulted in segments of sample fluid that

were roughly 1 cm in length. *Id.* (“Typically, using the fluorocarbon as transport fluid, injection during 2 s at 30 psi corresponded to a sample plug of 1 cm and a volume of 300 nL.”). To avoid the contamination problems associated with such systems (but not droplet systems), water is introduced between the samples. *Id.* at 1. (“[In order] to reduce carryover between samples, an intermediate water plug between two consecutive samples was required.”).

As the image below shows, a two-headed arrow is depicted between the carrier fluid (“transport liquid”) and sample to indicate that both are never connected to the system at the same time:



Id. at Fig. 1. As such, droplets of aqueous fluid surrounded by oil cannot form.

Litborn is nothing more than the type of segmented flow system disclosed in the Haff and Kopp references that were unsuccessfully relied upon in the previous IPR and reexamination of the '193 Patent, respectively.

4. Shaw-Stewart

Shaw-Stewart, like Quake, also discloses an approach based on droplet merger, and includes no working examples or data. *See, e.g.*, Ex. 1008 at 3 (“moving the immiscible liquid and discrete volumes through the conduit system in such a sequence that predetermined discrete volumes individually coalesce with each other, thereby combining the reagents”). As such, Shaw-Stewart does not teach conditions suitable for conducting a PCR reaction in droplets, and is oblivious to the relevant surface chemistry issues. In fact, PCR was not even invented until after the Shaw-Stewart application was filed in 1982.

5. Schneegaß

Finally, the Petition cites repeatedly to Schneegaß as allegedly teaching “successful PCR DNA amplification in microfluidic w/o droplets flowing through a microfluidic device.” Petition at 16. Schneegaß, however, is only relied upon as background art and is not one of the references that makes up Petitioner’s proposed invalidity grounds. *See id.* at 5.

Schneegaß, like Litborn, is a review article that primarily provides a survey review of past research. Contrary to Petitioner’s assertion, Schneegaß does not teach

PCR in microfluidic droplets. Petitioner points to Figure 6 of Schneegaß as allegedly showing results of such experiments. Yet, the text accompanying Figure 6 confirms that in Schneegaß the reagents are all in contact with the channel wall. Multiple stabilizers are thus included to avoid negative interactions with the channels. *See* Ex. 1009 at 110 (“The stabilization of the annealing of primers and the polymerase to the DNA template was supported by glycerol and bovine serum albumin (BSA), which also serves in preventing denaturation of the polymerase on the *channel surface* by deactivating of possibly available reactive groups on the surface.”).

Thus, whatever results are reported, in Schneegaß, they do not involve droplets, where the reagents do not contact the channel walls. Rather, they pertain to prior art segmented flow systems like Kopp and Haff that were previously considered in the prior IPR and reexamination.

D. The Prior Attempts To Invalidate The '193 Patent Before The Patent Office

As noted above, there have been multiple failed attempts to invalidate the '193 Patent based on the Quake reference, two of which were before this office. A brief summary of these proceedings is below.

1. The Previous IPR Of The '193 Patent

The '193 Patent was asserted in litigation against 10X Genomics, Inc. (“10X”) in 2015 in the United States District Court for the District of Delaware. Shortly thereafter, 10X filed an IPR petition that was based on two references: Quake and

Haff. The Haff reference was not a droplet system, but rather a system based on segmented flow in which slugs of fluid are separated from one another by air. *See* Ex. 1026 at 10-11. PCR is purportedly carried out in Haff by forcing a bulk PCR reaction mixture through a capillary tube which is routed through alternating hot and cool zones. *Id.* at 38-39.

During the IPR, 10X argued that the skilled artisan would have combined Quake with Haff for the purpose of performing PCR. *See* Ex. 1025 at 31 (“Haff and Quake are both directed to performing enzymatic reactions, such as PCR, in small volumes of aqueous fluids.”). The Board rejected this theory and denied institution, explaining that “Petitioner has not explained why that—or any teaching in Haff or Quake—would establish a reasonable expectation of success in providing conditions that are suitable to amplify a substrate molecule in a microfluidic plug.” *See* Ex. 1027 at 17.

2. The Reexamination Of The '193 Patent

After 10X's IPR did not succeed, 10X filed an ex partes reexamination request that was again based primarily on Quake. As a secondary reference, 10X relied on Kopp, which is cited in Quake as disclosing the use of microfabrication for PCR. 10X also relied upon Shaw Stewart. *See* Ex. 1028 at 5901-5902. Kopp, however, is not a droplet system, but a segmented flow system like Haff. In Kopp, discrete 10 μ l slugs of PCR mixture are injected one-by-one into the channels of the microfluidic

device where they contact the channel walls and travel down the channel for collection. *Id.* at 5903. During the reexamination, extensive evidence was presented detailing why Quake was far from the '193 Patent and why segmented flow systems like Kopp would not render the '193 Patent obvious. *See generally* Ex. 1028 at 5930-43.

Again, 10X's proposed motivation to combine the references was to perform PCR in droplets. As 10X argued, a "skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Quake, Burns I or Shaw Stewart because doing so have provided the substantial benefits known to be associated with such microfluidic reactors." *See* Ex. 2004 at 4. The examiner rejected the proposed combination and upheld the claims. As the Examiner explained, "none of Quake, Kopp, or Shaw-Stewart, either alone or in combination, teach or suggest a method for conducting an autocatalytic reaction in plugs in a microfluidic system and providing conditions suitable for the autocatalytic reaction in at least one plug such that at least one substrate molecule is amplified." Ex. 2005 at 4.

III. REASONS WHY THE PETITION SHOULD BE DENIED

The reasons why the instant Petition should be denied are unusually strong in this case. As documented above the '193 Patent has already been through multiple rounds of Patent Office review, not to mention a jury trial, and the Petition presents

little that is substantially different from the previous rounds of review. This warrants denial pursuant to § 325(d). Likewise, because the Petition presents theories that are little different from the previously presented defective theories, the instant Petition is again deficient on the merits.

A. The Petition Should be Rejected Under § 325(d)

The Board should exercise its discretion under 35 U.S.C. § 325(d) to deny institution because this Petition presents “the same or substantially the same prior art or arguments previously were presented to the Office.” The Board’s precedential decision in *Advanced Bionics* establishes a two-part framework for deciding whether the Board should deny institution under § 325(d). First, the Board determines “(1) whether the same or substantially the same art previously was presented to the Office or whether the same or substantially the same arguments previously were presented to the Office; and (2) if either condition of first part of the framework is satisfied, whether the petitioner has demonstrated that the Office erred in a manner material to the patentability of challenged claims.” *Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH*, IPR2019-01469, Paper 6 at 8 (PTAB Feb. 13, 2020) (precedential).

In *Becton, Dickinson*, the Board enumerated the following six non-exhaustive factors that helpfully guide the two-pronged analysis under *Advanced Bionics*:

- a. the similarities and material differences between the asserted art and the prior art involved during examination;
- b. the cumulative nature of the asserted art and the prior art evaluated during examination;
- c. the extent to which the asserted art was evaluated during examination, including whether the prior art was the basis for rejection;
- d. the extent of the overlap between the arguments made during examination and the manner in which petitioner relies on the prior art;
- e. whether petitioner has pointed out sufficiently how the examiner erred in its evaluation of the asserted prior art;
- f. the extent to which additional evidence and facts presented in the petition warrant reconsideration of the prior art or arguments.

Id. at 9 n.10 citing *Becton, Dickinson*, Paper 8 at 17-18. Factors a, b, and d pertain to the first prong, while factors c, e, and f pertain to the second prong. As documented below, consideration of the *Becton Dickinson* factors overwhelmingly favors denial of institution.

1. Dropworks Advances The Same Or Substantially The Same Art That Was Previously Presented To The Office

a) Factor (a): The Similarities And Material Differences Between The Asserted Art And The Prior Art Involved During Examination.

Factor (b): The Cumulative Nature Of The Asserted

Art And The Prior Art Evaluated During Examination.

Petitioner's art is the same or substantially the same as the art previously considered by the Office during examination. *See, Advanced Bionics*, Paper 6 at 7-8. (“[p]reviously presented art includes art made of record by the Examiner, and art provided to the Office by an applicant, such as on an Information Disclosure Statement (IDS), in the prosecution history of the challenged patent.”).

Petitioner relies first and foremost on Quake as its primary reference for all of its grounds. Indeed, Quake forms the basis of the majority of Petitioner's claim charts, including all limitations concerning the generation of plugs in a microfluidic system. *See, e.g.*, Petition at 28 (regarding Quake and Holliger, “Quake discloses a microfluidic device for reacting biological materials, including specifically DNA polynucleotides, in w/o droplets”). As Petitioner admits, however, Quake was considered during the prosecution of the '193 Patent, a failed IPR of the '193 Patent, and a failed ex partes reexamination request of the '193 Patent. Petition at 7-9.

Beyond this Quake was also the primary reference used in a failed effort to attack the '193 Patent during a jury trial. In each of those circumstances, Quake, either by itself or in combination with other references, was found not to render the '193 Patent obvious. Given how frequently Quake has been considered, factors (a) and (b) overwhelmingly weigh against institution.

2. Factor D: The Extent Of The Overlap Between The Arguments Made During Examination And The Manner In Which Petitioner Relies On The Prior Art

Not only is Quake the primary reference, the Petition relies on Quake to disclose all of the elements related to microfluidics technology. *See, e.g.*, Petition at 23 (“Quake teaches a microfluidic device for making microfluidic aqueous droplets in oil at the junction of two microfluidic channels by continuously flowing the aqueous solution into continuously flowing oil.”); *id.* at 53 (Shaw Stewart’s teachings limited to “need for fluorinated oil.”); *id.* at 55 (“Quake teaches the claimed elements for forming the droplets”).

The Patent Office, however, has repeatedly stated that there is no *a priori* reason to believe that Quake is suitable as a microfluidics device for PCR. For instance, in the previous IPR, the Board explained as follows:

Petitioner contends that a skilled artisan would have had a reasonable expectation of success because Quake teaches how to obtain encapsulated aqueous plugs with a single substrate molecule. Pet. 31. But even with that teaching, Petitioner has not explained why that—or any teaching in Haff or Quake—would establish a reasonable expectation of success in providing conditions that are suitable to amplify a substrate molecule in a microfluidic plug.

Ex. 1027 at 17. The reexamination unit arrived at a similar conclusion. *See*, Ex. 2005 at 3 (“Quake, while teaching a microfluidic device, a carrier fluid, and an aqueous sample fluid, does not teach or suggest PCR or a carrier fluid/surfactant

system that would provide conditions suitable for an autocatalytic reaction such that at least one substrate molecule is amplified.”).

While the Petition includes a few new secondary references, the Board has commonly rejected Petitions under § 325(d) even if the particular prior art in question was not directly addressed before. *See, e.g., R.J. Reynolds Vapor Co. v. Fontem Holdings I B.V.*, IPR2018-00626, Paper No. 7 at 19-21 (PTAB Sept. 27, 2018) (denying institution under Section 325(d) where Petitioner's asserted references were the same as or similar to references cited during prosecution); *Clim-A-Tech Ind., Inc. v. William A. Ebert*, IPR2017-01863, Paper No. 13 at 18-19 (PTAB Feb. 12, 2018) (denying institution of a petition even though the “Examiner did not base a rejection” on the reference asserted in the petition).

Regardless, Petitioner's new references fail to cure the fundamental deficiencies with the prior attempts to invalidate the '193 Patent based on Quake. Most important in this regard is that the overall rationale of the prior attacks based on Quake is identical to the rationale of the instant Petition, which is to identify a reference that includes a disclosure of PCR and argue that the skilled artisan would be motivated to combine that disclosure with Quake to do PCR in droplets. *See supra* Part II.C.4. While Haff and Kopp played the role of disclosing PCR in the prior IPR and reexamination, Holliger and Litborn fill that role here. In both the prior art attacks, the secondary reference involved an approach (segmented flow)

that was nothing like the approach in Quake. *See* Exs. 2005 at 4, 1027 at 17. The same is true here, where the Petitioner relies Holliger, which has nothing to do with microfluidics, and Litborn, which is another segmented flow system.

There is no meaningful difference between the positions previously submitted to the Patent Office and those in the Petition.

3. Dropworks Fails To Identify Material Error In The Patent Office's Previous Evaluation Of The Art

a) Factor C: The Extent To Which The Asserted Art Was Evaluated During Examination, Including Whether The Prior Art Was The Basis For Rejection

Becton, Dickinson factor (c) clearly favors denial of the Petition. Petitioners do not deny that Quake has been the subject of examination, IPR, and reexamination. Patent Owner overcame objections based on Quake in all three previous proceedings. Here, Quake is relied upon for a majority of claim limitations yet again.

Factor E: Whether Petitioner Has Pointed Out Sufficiently How The Examiner Erred In Its Evaluation Of The Asserted Prior Art

Factor F: The Extent To Which Additional Evidence And Facts Presented In The Petition Warrant Reconsideration Of The Prior Art Or Arguments.

Becton, Dickinson factors (e) and (f) also favor denial. The petition fails to address the fundamental reason why the previous challenges to the '193 Patent were rejected. Petitioner argues that the Board erred in its previous findings because of a mistaken assumption that PCR could not be performed in water-in-oil emulsions

where the oil comprised standard hydrocarbons such as mineral oil. *See* Petition at 2.

Accordingly, the Petition presents new art to supposedly overcome this shortcoming and show that PCR could be performed in a microfluidic system like the one disclosed in Quake where mineral oil and harsh ionic surfactants were used. This evidence, however, is unavailing. As documented above, Litborn is not even a droplet reference, but rather a segmented flow system of exactly the type that was previously relied upon in the previous IPR and reexamination. The same is true with respect to the Schneegaß reference. Holliger is not even a microfluidic system. As documented herein, Holliger is a system where 113 million aqueous vesicles of disparate size distributions were prepared and subjected in bulk to thermal cycling to perform PCR. Whether a researcher is able to recover a PCR product across such a system says nothing whatsoever about whether PCR could be done in the microfluidic system of Quake.

B. The '193 Patent Is Not Obvious In View Of The Prior Art

While there is ample reason to deny institution pursuant to § 325(d), there is also ample reason to deny institution on the merits. In this case, because the main prior art relied upon in the previous unsuccessful challenges of the '193 Patent is identical to the prior art relied upon in the instant Petition, the arguments under §

325(d) and on the merits are mutually reinforcing, clearly establishing that institution should be denied.

1. The Cited Prior Art Fails To Teach Or Suggest Several Key Claim Elements

A threshold requirement for a patent to be rendered obvious is that the prior art teach or suggest all limitations in a claim. The claims of the '193 Patent cannot be regarded as obvious unless all claim elements are disclosed or suggested in the art in some way. *See, e.g., Apple Inc. v. Samsung Elecs. Co.*, 839 F.3d 1034, 1062 (Fed. Cir. 2016) (denying JMOL of invalidity, explaining that “Apple also presented substantial evidence on which a reasonable jury could find that the combination of Robinson and Xrgomics failed to disclose every claimed element.”); *St. Jude Med., Inc. v. Access Closure, Inc.*, 729 F.3d 1369, 1381 (Fed. Cir. 2013) (“We see no error in the district court’s legal conclusion of nonobviousness. Neither Takayasu nor Smiley discloses a balloon configured to operate as a positioning device to prevent a plug from entering a blood vessel as claimed in the Fowler patents. Takayasu discloses no balloon at all, and Smiley discloses a balloon that is used to control the bleeding of a gunshot wound or an abdominal aneurism.”); *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993) (reversing obviousness rejection where prior art did not teach or suggest all claim limitations).

As documented below, as a consequence of how far the prior art is from the claimed invention, it fails to suggest several key claim elements. For this reason alone the Petition should be denied.

a) The Prior Art Does Not Teach Autocatalytic Reactions In Droplets In A Microfluidic System

Fundamental to the claims of the '193 Patent is the performance of autocatalytic reactions in “plugs” in a microfluidic system. As the Petition acknowledges, a “plug” is a “water-in-oil (w/o) droplet that is substantially surrounded by the oil.” Petition at 1. The requirement that the plugs be “substantially surrounded” by oil is expressly recited in the claims. None of the cited prior art teaches the performance of autocatalytic reactions in such plugs.

(i) Quake Does Not Teach Autocatalytic Reactions In Droplets In Microfluidic Systems

The Petition relies first and foremost on Quake, asserting that Quake “expressly suggests performing PCR” in droplets. *Id.* at 22. Quake suggests no such thing. In the previous reexamination of the '193 Patent based on Quake, the Examiner confirmed that this was the case. *See* Ex. 2005 at 4 (“nor does Quake specify the conditions are suitable for an autocatalytic reaction”).

Nevertheless, the primary portion of Quake relied upon by Petitioner is as follows:

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).

Ex. 1005 at 23:26-29. This sentence does nothing more than point out that PCR—and a bunch of other techniques—are possibilities for “microfabrication.”

Any suggestion that this is an “express” teaching of carrying out PCR in droplets is undermined by the remainder of the specification. The Background of the Invention begins with a lengthy description of detecting viruses and cells. *See id.* at 2:12-3:14. Quake describes two basic approaches that had been used in the prior art. One prior art approach was PCR. Quake explains, however, that PCR had the disadvantage of leading to false positives given its extreme sensitivity:

The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen. However, this sensitivity of detection can also cause significant problems in routine viral diagnostics. The significant risk of cross contamination from sample to sample can outweigh the benefits of detecting small quantities of a target viral nucleic acid. Cross contamination can also result in false positives, making interpretation of epidemiological data impossible.

Id. at 2:32-3:6. Other than the passing reference to how microfabrication can be adapted for PCR among several other techniques, there is no further mention of PCR in the entire Quake reference.

The second approach for detecting viruses and cells suggested by Quake in the Background of the Invention is particle sorting, using devices such as FACS and flow cytometry. *See id.* at 3:7-8 (“Flow sorting devices have been used to analyze and separate larger biological materials, such as biological cells.”). Quake devotes nearly two full pages to discussing the details of problems associated with these devices. *See id.* at 3:7-4:30.

Then, immediately thereafter, in the very first sentence of the Summary of the Invention, Quake states that he is offering an improved version of FACS and flow cytometry for sorting that avoids the particular problems he identified:

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. The invention provides microfabricated devices having channels that form the boundary for a fluid instead of using a sheath flow employed by conventional FACS.

Id. at 5:1-4. There is no mention of PCR. Rather, throughout the specification, Quake states repeatedly that it provides a technique for sorting particles that is an improved version of FACS or flow cytometry. *See id.* at Abstract (“A microfluidic device for analyzing and/or sorting biological materials....”); *id.* 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 34:14-17. (“According to the invention, molecules (such as DNA, protein, enzyme

or substrate) or particles (i.e., cells, including virions) are sorted dynamically....”). “Sorting” (or some variation thereof) is mentioned 274 times in Quake.

As the foregoing proves, Quake is not directed to performing PCR at all, let alone PCR in droplets. Quake is focused on providing an alternative to PCR based on sorting, not a better way of doing PCR. Nowhere does Quake explain why it would make sense to do PCR in droplets, or why this would offer any sort of improvement over prior art PCR techniques.

“It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art.” *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 796 F.2d 443, 448 (Fed. Cir. 1986). Petitioner’s reliance on Quake fails to heed this guidance.

**(ii) Holliger Does Not Teach Autocatalytic Reactions
In Droplets In Microfluidic Systems**

Petitioner contends that “Holliger, for example, discloses successfully performing PCR amplification in sub-microliter water-in-oil droplets like Quake’s.” Petition at 39. The ’193 patent, however, requires performance of PCR not just in a water-in-oil droplet, but in such a droplet in a microfluidics system. Holliger teaches nothing but bulk water-in-oil (“w/o”) emulsions created by general stirring and agitation, using device such as a whisk or paddle.

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 utilize 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.

Ex. 1006 at 48:14-19. Nothing in Holliger suggests microfluidic systems, and Holliger provides no teachings about how PCR could be adopted to a microfluidics device.

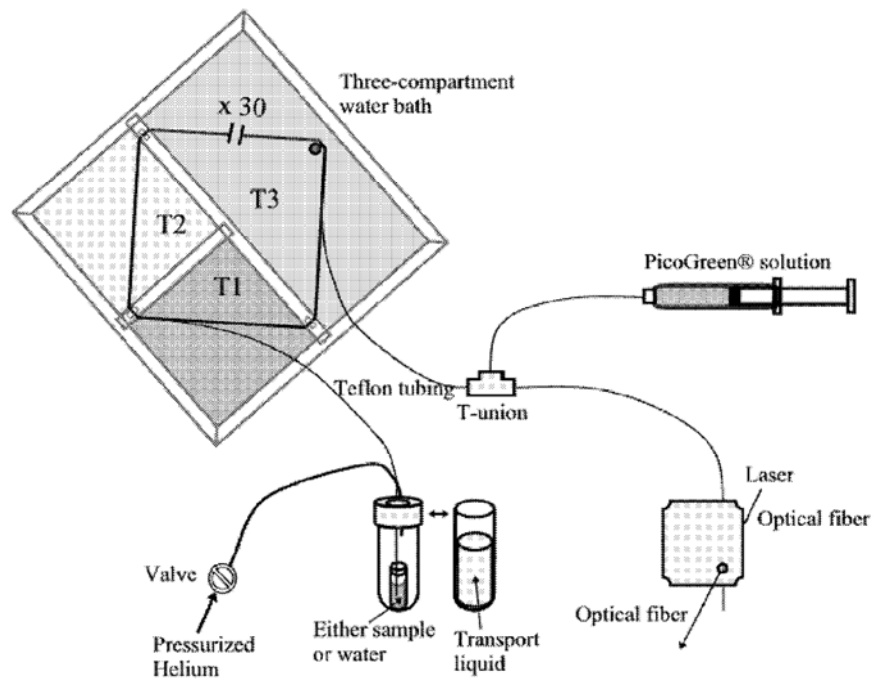
**(iii) Litborn Does Not Teach Autocatalytic Reactions
In Droplets In Microfluidic Systems**

Litborn does not disclose methods for performing autocatalytic reactions in a microfluidic system. Litborn is a survey paper that describes future applications of microfluidics, which have not yet been realized. For example, Litborn explains that using water-in-oil droplets as a “concept” that may have potential future uses. Ex. 1007 at 452. (“A concept which should have one of the best potentials for high throughput sequential flow reactions is the use of systems with two immiscible fluids.”).

The authors of Litborn states that they performed some “preliminary” PCR experiments pertaining to a “flow based PCR reaction, where the reaction mixture is interspaced with a hydrocarbon, as described above.” *Id.* at 453. The methods

described “above,” however, were not necessarily droplet experiments. The cited portion of Litborn is a general description of “flow-based” reactors and, in fact, focuses on non-droplet systems in which the reaction fluid is not surrounded by oil and instead simply consists of discrete segments of aqueous fluid. *See id.* at 451 (discussing the use of small “zones” of reactive fluid). There is thus no reason to believe the “preliminary” experiments in Litborn involved droplets.

In fact, it is known that Litborn did not use droplets. As documented above, in 2003, the research group associated with the Litborn reference actually published their work on “flow based PCR” in the journal *Analytical Chemistry*. *See Ex. 2003*. Their method did not use droplets surrounded by oil. Rather, their method involved alternating injections of sample and carrier fluid into the system. *See id.* at 4 (“After each injection, the cap with the tubing in place was switched onto a second vial filled with carrier fluid.”). As the image below shows, there is a double-headed arrow between the sample and carrier fluid vial to indicate that they are alternatively attached to the single channel that passes through the hot and cold zones:



Id at Fig. 1.

This system is nothing more than the type of segmented flow system disclosed in the Haff and Kopp references that were unsuccessfully relied upon in the previous IPR and reexamination of the '193 Patent, respectively. Indeed, as Petitioner acknowledges, during the reexamination the Examiner previously considered extensive evidence regarding why such references would not render obvious the '193 Patent. *See* Ex. 1028 at 5941-52.

b) The Cited Prior Art Does Not Describe Two Channels, One Of Which Contains Flowing Aqueous Fluid Containing Both Substrate And Reagent

The claims of the '193 Patent all require “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.” Again, none of the cited prior art suggests this claim element.

To the extent Quake includes any disclosure of conducting reactions in droplets, it is based on the exact opposite of the claimed approach. Rather than having the substrate molecule and reagents in a single channel, Quake discloses providing substrates and enzymes in *separate* channels that are introduced to the carrier fluid in two inlets. Quake’s two-inlet system forms two kinds of droplets: one with an enzyme and a one with a substrate. *See* Ex. 1005 at 36:9-28, 85:26-31 (“an aqueous solution containing an enzyme” is introduced into a first inlet and “an aqueous solution containing a substrate for the enzyme” is introduced into a second inlet). Quake shows this embodiment in Figure 22, which depicts the two solutions separately entering an immiscible oil stream, via separate inlets, in the microchannel and forming two separate droplets:

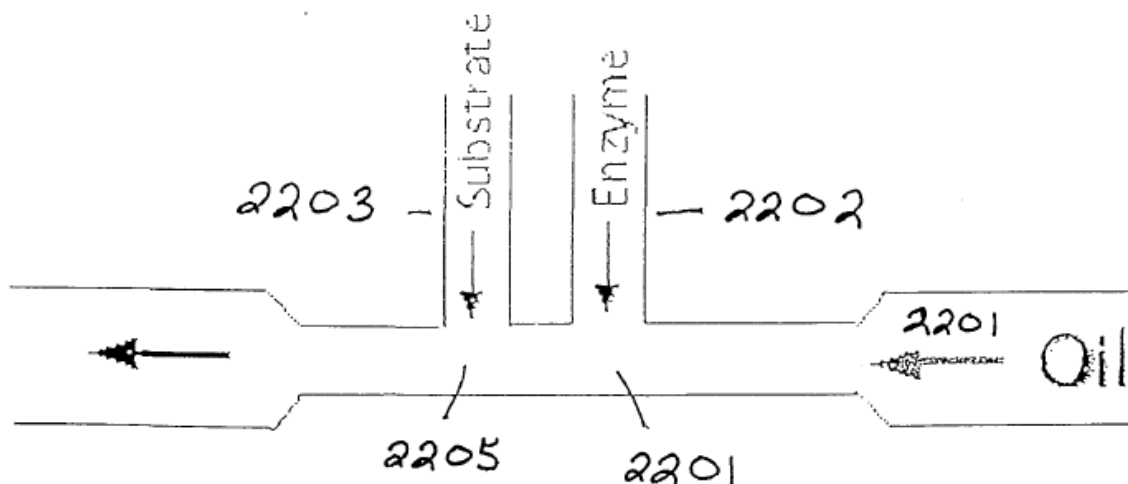


FIG. 22

Ex. 1005 at Fig. 22. The two droplets may eventually be merged (somehow) in the system, and a reaction is supposed to happen. *Id.* at 85:9-86:2. Regardless, this approach is the exact opposite of what the claims require.

Holliger, like Quake, also discloses nothing that corresponds to “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.” Holliger does not have any channels whatsoever because it just a bulk emulsion system. Rather than preparing droplets through the use of microfluidic channels, a random bulk emulsion is created using a whisk or paddle. *See* Ex. 1006 at 48:14-19

Finally, Litborn does not disclose this claim element either. While the claimed invention requires two channels and the formation of droplets using those channels

Litborn has only a single channel. The syringe containing the carrier fluid and the syringe containing the aqueous solution are alternatively connected to the *same channel*. See Ex. 2003 at 4 (“After each injection, the cap with the tubing in place was switched onto a second vial filled with carrier fluid.”). As documented above, Litborm is not referring to a droplet system. See *supra* Part II.C.3

c) The Cited Prior Art Does Not Disclose Conditions For Performing Autocatalytic Reactions In A Microfluidic System

As discussed above, Quake does not teach any autocatalytic reactions, let alone PCR or the conditions for performing such reactions in droplets in a microfluidic system. During the reexamination, the Examiner made this clear, explaining that “Quake, while teaching a microfluidic device, a carrier fluid, and an aqueous sample fluid, does not teach or suggest PCR or a carrier fluid/surfactant system that would provide conditions suitable for an autocatalytic reaction such that at least one substrate molecule is amplified.” See Ex. 2005 at 3. This finding was not in error.

In the reexamination, Patent Owner pointed to literature documenting the importance of selecting the appropriate surfactants and carrier oil when attempting to perform PCR in droplets, which have an extremely high surface to volume ratio such that the reagents within the droplet will frequently encounter the droplet

surface. Extensive evidence was submitted on this issue. *See, e.g.*, Ex. 1028 at 5944-52.

Citing a paper written by the requester's expert in the IPR proceedings, Patent Owner explained that a POSA may not simply apply traditional reaction conditions as a whole to microfluidic droplets because of this issue and the possibility that the reagents will not survive interactions with the walls of the droplet or channel. Rather, the "biocompatibility" of a droplet's surface must be considered. Ex. 2006 at 11 ("With each biological experiment biocompatibility of the reagents has to be tested anew. For examples, oligoethylene glycol-terminated surfactants prevent nonspecific adsorption of protein to the water-oil interface.[71] This is an important consideration as the large surface-to volume ratio can strongly affect the outcome of enzymatic reactions in droplets.").

The inventors of the '193 Patent solved this problem through their choice of carrier fluid and an ingenious surfactant to coat the wall of the droplet. The inventors described their technique in a figure in one of their publications. As panel (a) of the figure below shows, an incorrect surfactant will cause proteins within the droplet to stick to the droplet surface and ultimately be destroyed. On the other hand, as shown in panel (b), through the use of fluorinated surfactants with non-polar ethylene glycol heads, the proteins will remain stable and reactions, such as PCR, will be able to proceed:

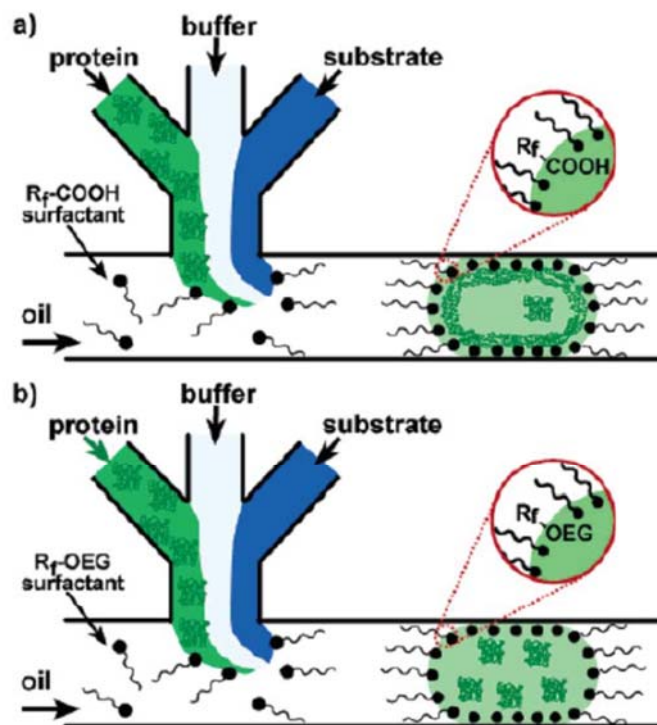


Figure 1. Controlling surface chemistry in plug-based microfluidic devices with surfactants at the aqueous-fluorous interface. (a, b) Schematic of surfactant molecules at the interface of an aqueous plug of protein in a microfluidic channel. (a) R_f -COOH surfactant provided a noninert interface prone to protein adsorption. (b) R_f -OEG surfactant provided an inert, biocompatible interface.

Ex. 2006 at Fig. 1.

Importantly, these are the very methods described in the '193 Patent. *See, e.g.,* Ex. 1001 at 35:59-36:19 (“Control of surface chemistry is particularly important in microfluidic devices because the surface-to-volume ratio increases as the dimensions of the systems are reduced. In particular, surfaces that are generally inert to the adsorption of proteins and cells are invaluable in microfluidics. Polyethylene glycols (PEG) and oligoethylene glycols (OEG) are known to reduce non-specific adsorption of proteins on surfaces.”), 55:45-50 (“In FIG. 17, for example, the following can be introduced into the various inlets: buffers into inlets 171, 172; PEG

into inlet 173; salt into inlet 174; solvent into inlet 175; and protein into inlet 176.”), 73:48-62 (“In accordance with the invention, neutral surfactants that are soluble in perfluorinated phases are preferably used to create positively and negatively-charged interfaces.”); *see also id.* at 52:26-34; 57:56-59. Quake, on the other hand, is totally silent on such issues, and instead advocates using harsh, highly polar surfactants that destroy proteins and that are not used in microfluidic system

Petitioner asserts that Holliger fixes the flaw in Quake identified by Patent Owner in the reexamination—that the simple water-in-oil droplets described in Quake would not have provided adequate conditions for PCR reactions in a microfluidic device. Petition at 39 (“Holliger, for example, discloses successfully performing PCR amplification in sub-microliter water-in-oil droplets like Quake’s.”). Holliger, however, does not fix the defects in the prior obviousness case.

Holliger purports to describe experiments involving PCR in a mess of random vesicles that are dispersed throughout an emulsion. To generate the vesicles for this experiment, Holliger introduces 200 μ L of PCR mixture into a mineral oil, allegedly creating vesicles that are on average 15 μ m in size. Ex. 1006 at 59:10-15. This leads to the creation of over 113 million vesicles, far more than the few thousand droplets that are created in the microfluidic droplet system in Quake. Along these lines, Dropworks’ current commercial system generates only 30,000 droplets per sample.

See Ex. 2012 at 4. This illustrates the great difference in scale between a bulk emulsion system like Holliger's and an actual microfluidic droplet system.

It is unsurprising that, across 113 million vesicles in a bulk fluid, Holliger was able to recover some PCR product. Holliger, however, includes nothing to establish what fraction of the 113 million droplets actually yielded a PCR product, or whether the PCR products can be attributed to droplets of a particular size that might be characteristic of the droplets in a microfluidic device. There is no reason to believe that in Holliger the PCR product arose from small vesicles as opposed to large vesicles, that latter of which would not present the surface chemistry problems associated with droplets that are addressed in the '193 Patent.

In other words, the conditions that allegedly worked in Holliger are irrelevant to the conditions that are seen in a microfluidic device. In Holliger, the PCR experiments were performed using mineral oil along with a combination of Span, Tween, and Triton surfactants. These are cheap, off-the-shelf reagents that were commercially available. *See* Ex. 1006 at 47:25-48:5, 59:10-15. If such conditions were truly appropriate for PCR in droplets in a microfluidic systems, Petitioner surely would have pointed to some exemplary microfluidic droplet system that utilized such readily available conditions for PCR.

Yet, Petitioner points to no such art. The fact is that when practitioners wish to perform PCR in droplets in microfluidic systems, they use radically different

biocompatible conditions that correspond to those pioneered in the '193 Patent and related patents. They do not use the reagents of Holliger. This proves that there was no error in the previous determinations from the Patent Office that the prior art does not teach conditions appropriate for PCR in droplets in microfluidic systems.

2. The Skilled Artisan Would Not Be Motivated To Combine Quake With Either Holliger Or Litborn

To establish obviousness, the Petitioner must demonstrate that a “skilled artisan would have had reason to combine the teaching of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success from doing so.” *PAR Pharm., Inc. v. TWI Pharm., Inc.*, 773 F.3d 1186, 1193 (Fed. Cir. 2014) (internal citations omitted). “When an obviousness determination relies on the combination of two or more references, there must be some suggestion or motivation to combine the references.” *Intelligent Bio-Systems, Inc. v. Illumina Cambridge, Ltd.*, 821 F.3d 1359, 1368 (Fed. Cir. 2016) (quoting *WMS Gaming, Inc. v. Int'l Game Tech.*, 184 F.3d 1339, 1355 (Fed. Cir. 1999)).

“Obviousness ‘cannot be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the patented invention.’” *Cheese Sys., Inc. v. Tetra Pak Cheese & Powder Sys., Inc.*, 725 F.3d 1341, 1352 (Fed. Cir. 2013) (citing *ATD Corp. v. Lydall, Inc.*, 159 F.3d 534, 546 (Fed. Cir. 1998)). The Federal Circuit has been clear that “it is impermissible to use the claimed

invention as an instruction manual or ‘template’ to piece together the teachings of the prior art so that the claimed invention is rendered obvious.” *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992).

Given how radically different the cited prior art references are from not just each other but also the claimed invention, Petitioner’s argument can only be regarded as based on improper hindsight. Indeed, the very first sentence of Petitioners’ argument regarding motivation to combine states “[a]s an initial matter, a POSA at the critical date reasonably would have looked to Quake and Holliger in the context of performing PCR in w/o droplets in a microfluidic device.” Petition at 22. But, the question at issue here is why a skilled artisan would be interested in the “context of performing PCR in w/o droplets in a microfluidic device” in the first place.

Petitioner’s proposed motivation *presumes* that the skilled artisan was already motivated to make the very combination (“PCR in w/o droplets in a microfluidic device”) that supposedly would have been motivated by the prior art. The Petition goes on to identify alleged similarities between the prior art references. *See id.* at 22-23. In this regard, the Petition presumes beforehand that the skilled artisan would be motivated to do “PCR in w/o droplets in a microfluidic device” and then works backward from alleged similarities in prior art references. This epitomizes an improper hindsight-based approach.

The thrust of Petitioner’s hindsight-based motivation to combine is that the skilled artisan would be motivated to combine Quake with Holliger or Litborn because both references are “directed to performing enzymatic reactions (including specifically PCR) in the droplets.” Petition at 22, *see also* Petition at 54-55 (same rationale for Quake and Litborn). This, however, is a poor motivation not just because it was previously presented and rejected multiple times over, but also because it mischaracterizes Quake.

As documented above Quake is not directed to PCR at all. *See supra* Part III.B.1.a)(i). Quake includes no teachings regarding how to heat droplets for PCR, no illustration of how to stabilize droplets for PCR, and no disclosure of how to load reagents for PCR into droplets, let alone any examples of PCR. To the extent Quake discloses any enzymatic reactions whatsoever, it discloses no autocatalytic reactions but rather spontaneously occurring enzymatic reactions that might happen upon merger of droplets. *See, e.g.*, Ex. 1005 at 32:9-12. Such reactions are nothing like PCR, which requires carefully selected thermal cycling conditions.

If anything, Quake teaches away from using droplets for PCR. Quake teaches the ***drawbacks*** of PCR and purports to provide an ***alternative*** to PCR (i.e., sorting), not a better way of doing PCR. Nevertheless, Petitioner asserts that “Quake specifically teaches the droplets can contain biological materials such as DNA and that its microfluidic devices may be used to conduct PCR (an autocatalytic reaction),

such as for identification of viral DNA.” This is wrong. Here is what Quake actually says about using PCR for detecting viral DNA:

The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen. However, this sensitivity of detection can also cause significant problems in routine viral diagnostics. *The significant risk of cross contamination from sample to sample can outweigh the benefits of detecting small quantities of a target viral nucleic acid.*

Ex. 1005 at 2:32-3:5 (emphasis added). Far from teaching the use of PCR in droplets for detecting viral DNA, this is an express instruction *not* to use PCR for such a purpose.

Petitioner further asserts that “a POSA would have viewed the combined teachings of Quake and Holliger as a reasonable and convenient approach to performing PCR, consistent with scientific literature at the time.” Petition at 25. The “literature at the time,” however, simply confirms that the skilled artisan would not have made the proposed combination.

According to Petitioner’s expert, both PCR and the use of microfluidics for PCR were well-known in the 1980s and 1990s. *See, e.g.*, Ex. 1002 ¶ 90 (“The PCR reaction was published in the mid-1980s and was extremely well known, routine, and conventional, and was used widely by 2002 and 2003.”); *id.* ¶ 99 (“Recognizing that reducing reaction volume permits faster thermocycling using less energy and

otherwise saves time and expensive reagents, microfluidic practitioners began creating microfluidic PCR devices in the 1990s.”). Petitioner, however, identifies no reference that discloses PCR in droplets in a microfluidic system, despite these concepts having been available for a decade before the ’193 Patent. *See supra* Part II.C. This undermines Petitioner’s assertions that their proposed combination of art would not have been “reasonable” or “convenient.”

Petitioner further argues that Holliger provides motivation to use microfluidics because there is an advantage to performing PCR on massive scales. Petition at 23 (Holliger “teaches that microscopic w/o droplets are useful for performing PCR on a massive scale, including digital PCR and multiplex PCR.”). But even if that were true, that is not a particularized reason to combine Holliger with Quake. Indeed, Holliger already teaches methods for making bulk emulsions comprising large numbers of droplets, so a POSA would not have seen a need to adapt a separate method of forming droplets. *See* Ex. 1006 at 48:14-19.

3. A POSA Would Not Have A Reasonable Expectation Of Succeeding In Combining Quake’s System With Holliger Or Litborn

To render a claim obvious, a skilled artisan “must have had not only a reason to combine the teaching of the prior art references to achieve the claimed invention, but also a reasonable expectation of success from doing so.” *Trustees of Columbia Univ. in City of New York v. Illumina, Inc.*, 620 F.App’x 916, 928 (Fed. Cir. 2015)

(internal quotation marks and citations omitted). The “standard is not whether the patent owner can persuasively show that one of ordinary skill would have expected failure.” *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. DE C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017) (emphasis in original). Rather, it is the Petitioner’s burden to show “that a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *Kinetic Concepts, Inc. v. Smith & Nephew, Inc.*, 688 F.3d 1342, 1360 (Fed. Cir. 2012) (internal quotation marks and citations omitted).

The primary reference in the Petition is Quake. Quake, however, discloses no PCR reactions and, if anything, discourages the use of PCR. *See supra* Part III.B.1.a)(i). Quake thus does not establish a reasonable expectation of successfully performing PCR in droplets in a microfluidic system.

Notably, after filing their patent application, the named inventors of the Quake reference attempted reactions in droplets. But never at any point did they tackle a complex reaction like PCR in which the droplets include a complex mix of reagents and are subject to repeated cycles of heating and cooling. Instead, as Dr. Quake’s graduate student explained in his dissertation, they simply tried to package a cell in a droplet and see if it could do a simple enzymatic reaction.

As Quake’s graduate student explains in his thesis, however, the “crossflow

assay was unreliable,” Ex. 2006 at 19, and resulted in “hundreds” of failed efforts “ultimately discarded in an attempt to discover the perfect operating conditions.” *Id.* As he stated, “[w]orking on the development of a microfluidic crossflow assay emphasized the difficult dynamic integration process between biochemical and micromechanical systems.” *Id.* In other words, it is very challenging to do biochemical reactions in a microfluidic droplet system.

While Petitioner’s expert tries to explain away these results as irrelevant because they did not involve PCR, the fact that they did not involve PCR just confirms that there was no reasonable expectation of success. Indeed, as Petitioner contends, “PCR is a conventional amplification reaction technique that has been well known in the art for nearly four decades.” Petition at 13. According to Petitioner, “POSAs recognized that it was important to isolate individual PCR reaction mixtures from subsequent samples.” *Id.* at 15. If this is true, then it speaks volumes that Quake never at any point even attempted PCR in droplets

Thus, Petitioner points to secondary references such as Holliger and argues that the skilled artisan would take some portion of the bulk droplets from Holliger and simply use them in Quake’s system to do PCR in droplets in a microfluidic system. Petitioner, however, says little about whether this approach would be successful. *See* Petition at 26-27. What little Petitioner says about reasonable expectation of success boils down to the notion that because PCR had been

performed in droplets in bulk emulsions, it should obviously be easy to make it work in a microfluidic system. *See id.*

Yet, a sensitive microfluidic droplet system is nothing like a bulk emulsion that is created with a paddle or whisk. To the extent Holliger was able to generate a bulk emulsion with aqueous particles distributed across a broad spectrum of size ranges and then allegedly achieve PCR across an unspecified fraction of droplets, this says nothing about whether one could expect to be able to achieve such a result in a microfluidic system. In a microfluidic system, the droplets are created by different means, stored by different means, heated by different means, and subject to different forces. The broad distribution of droplet sizes characteristic of bulk emulsions that are created by a paddle or whisk has no comparison to the narrow distributions of sizes characteristic of a microfluidic system. As shown above, there is no reason to think the conditions of Holliger would work in a microfluidic system. *See supra* Part III.B.2.

Faced with these difference, Petitioner says nothing about how or why one would adapt the droplet system of Holliger for use in the microfluidic system of Quake. For instance, would the thermal cycling method used for a bulk emulsion be adequate for PCR in the microfluidic channels of Quake? The Petition does not address this and offers no explanation as to how the droplets would be thermally cycled in the Quake system. Indeed, the Petition is totally silent on this point. *See*

Petition at 39-40. Likewise, the droplets in Quake are in constant motion as they travel through the microchannels, while the droplets in Holliger are stationary. How would this be dealt with vis-a-vis thermal cycling for PCR? Quake says nothing about this, and the Petition is equally silent.

As yet another example, while it is relatively straightforward to include all of the necessary reagents for PCR in a bulk emulsion, it is much more difficult to consistently package all of the reagents at the right concentrations into microfluidic droplets. Neither Quake nor Holliger teaches this, and the Petition ignores the issue. The '193 Patent, on the other hand, includes pages of teaching on how to form plugs, mix reagents in plugs, and vary the concentrations of reagents in plugs. *See, e.g.*, '193 Patent at 17:24-27:5, 33:26-35:9.

While the Petition does not explain how Holliger would need to be modified or adapted for use with Quake, doing so would require abandoning the bulk emulsion techniques upon which Holliger operates and would require a complete redesign of Holliger along with the addition of new and unspecified componentry to the Quake system. This fact pattern weighs against obviousness. *See, e.g., In re Ratti*, 270 F.2d 810, 813 (C.C.P.A. 1959) (reversing obviousness rejection where the “suggested combination of references would require a substantial reconstruction and redesign of the elements shown” in the prior art “as well as a change in the basic principles

under which the [prior art] construction was designed to operate”); *see also* M.P.E.P. § 2143.

Petitioner’s failure to include any explanation of how Holliger would be adapted for use with Quake illustrates that its obviousness theory is “fraught with hindsight bias.” *ActiveVideo Networks, Inc. v. Verizon Commc’ns, Inc.*, 694 F.3d 1312, 1327 (Fed. Cir. 2012) (affirming finding of nonobviousness where the “expert failed to explain how specific references could be combined, which combination(s) of elements in specific references would yield a predictable result, or how any specific combination would operate or read on the asserted claims.”).

Petitioner further relies upon Litborn. Litborn does not help establish reasonable expectation of success. Indeed, as documented above, Litborn is not even a droplet system. *See supra* Part III.B.1.a)(iii). Whatever results are allegedly disclosed in Litborn, they do not pertain to the successful performance of PCR in droplet systems.

Even assuming Litborn did pertain to droplets, it still cannot establish reasonable expectation of success. Every essential detail regarding the structure of the Litborn system is undisclosed. The entirety of Litborn’s discussion of its microfluidic system is:

We have carried out preliminary experiments with a flow based PCR reaction, where the reaction mixture is interspaced with a hydrocarbon, as described above. Amplification was successful, and the results were

fully comparable with the results of the commercial standard procedure. It seems that this mode of operation could hold the potential to carry out a massive number of PCR reactions in nanoliter or even sub-nanoliter plugs of solution. This would have fundamental impacts on throughput bottle-necks in molecular biology.

Ex. 1007 at 453.

Litborn does not describe the types of microfluidic channels used to generate its plugs. Nor does Litborn describe the types of oil, water, or surfactants used inside the plugs. Unlike the '193 Patent, Litborn solves none of the issues that faced researchers in the microfluidics field. Litborn provides no information regarding how droplets can be maintained in a microfluidic device. While Litborn says that “this mode of operation could hold the potential to carry out a massive number of PCR reactions in nanoliter or even sub-nanoliter plugs of solution,” Litborn’s teachings amount to little more than a proof of concept for unrelated technology.

This scant disclosure falls far short of what is required to establish obviousness. Indeed, “patents are not barred just because it was obvious ‘to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.’” *Procter & Gamble Co. v. Teva Pharm. USA, Inc.*, 566 F.3d 989, 997 (Fed. Cir. 2009) (quoting *In re O’Farrell*, 853 F. 2d 894, 903 (Fed. Cir. 1988)).

For the many reasons stated above, the skilled artisan would not have been motivated to utilize water-in-oil emulsions to perform PCR reactions in a microfluidic device in 2002.

IV. SECONDARY CONSIDERATIONS

As documented above, Petitioner has failed to make a prima facie showing of obviousness under the cited prior art combinations. Even if Petitioner had made such a prima facie showing, however, secondary considerations must be taken into account. “Such objective evidence can establish that ‘an invention appearing to have been obvious in light of the prior art was not.’” *Rambus Inc. v. Rea*, 731 F.3d 1248, 1256 (Fed. Cir. 2013) (quoting *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538 (Fed. Cir. 1983)).

The evidence shows that the work in developing the technologies claimed in the '193 Patent were revolutionary at the time. Dr. Ismagilov received extensive praise by the scientific community for his work, and the community acknowledged that he solved issues that had long felt needs. *See* Ex. 1026 at 48-58. The Petition attempts to address the evidence of secondary considerations that Patent Owner discussed in the previous *inter partes* review proceeding by stating that the prior art already addresses the stated challenges. However, Petitioner's inclusion of references predating the '193 Patent by many years if not decades further exacerbates the secondary considerations that render the '193 Patent nonobvious.

A. Long Felt Need And Industry Praise

Dr. Ismagilov and his team received widespread industry attention and acclaim for their series of publications between 2003 and 2006 describing the techniques claimed in the '193 Patent. *See* Ex. 1026 at 49-52 (describing the Ismagilov group's publications authored by the named inventors of the '193 Patent, detailing the techniques, figures, data, and results in the publications that are reported in the '193 Patent). Today, their first publication, a 2003 article in the journal *Angewendte Chemie* entitled "A Microfluidic System for Controlling Reaction Networks in Time," has been cited more than 800 times, and others continue to be cited by researchers in the field, illustrating the continued impact of the Ismagilov group's work. In total, Dr. Ismagilov's publications regarding his work on droplet-based microfluidics and performing reactions in such systems have been cited over 6,000 times.

Contrary to Petitioner's argument, the asserted prior art did not address the issues felt in the droplet microfluidics industry. As one 2015 article notes, "[a]lthough droplet production is ubiquitous in the microfluidic literature today," microfluidic droplet generation was "developed little more than a decade ago [83–86]. Because of its ubiquity, one can be forgiven for forgetting how remarkable the process is." Ex. 2007 at 3 (citing to Song et al., *Applied Physics Letters* 2003 as reference 84). In the years following the Ismagilov group's initial 2003 publication,

researchers in the field described the “seminal” impact of their “elegant” work. *See* Ex. 1026 at 53-54 (citing articles describing the work of Ismagilov and co-workers as highly influential). If, as Petitioner contends, it would have been obvious to create conditions suitable for doing PCR in droplets, researchers would not have characterized it as a pioneering and prominent work.

The Petition also misses the mark on the contributions Dr. Ismagilov provided to specific environmental conditions necessary for conducting the autocatalytic biological reactions in microfluidic droplets. In regards to the mixing of reagents in droplets, a 2012 article stated that Ismagilov’s work “started an emerging field to conduct chemical reactions in droplets.” Ex. 2008 at 23. In addition, the ’193 Patent explicitly claims “conditions suitable for the autocatalytic reaction in the at least one plug....” ’193 Patent at claim 1. The prior art does not teach these specific conditions, for which there was a long felt need prior to Ismagilov.

In 2005, Ismagilov and co-workers’ published one such solution, which was based on the use of specific fluorinated surfactants including a hydrophilic oligoethylene glycol head group (Rf-OEG surfactants) to provide an inert, biocompatible interface:

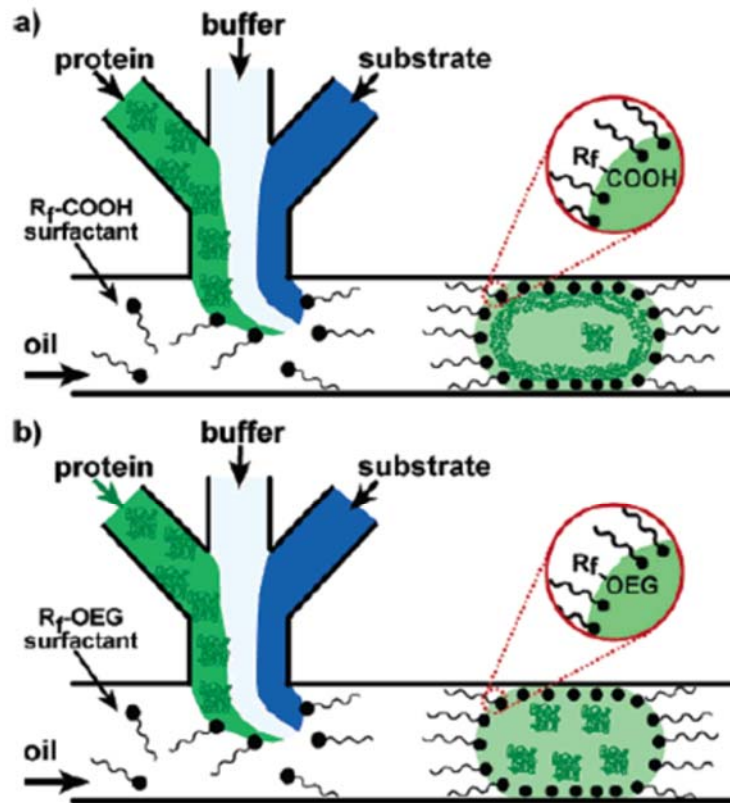


Figure 1. Controlling surface chemistry in plug-based microfluidic devices with surfactants at the aqueous–fluorous interface. (a, b) Schematic of surfactant molecules at the interface of an aqueous plug of protein in a microfluidic channel. (a) R_f -COOH surfactant provided a noninert interface prone to protein adsorption. (b) R_f -OEG surfactant provided an inert, biocompatible interface.

Ex. 2006 at Fig. 1. This solution to the surface chemistry issue has been cited to as recently as 2020:

To effectively process and analyze samples discretely, it is critical that droplets reliably maintain their contents through the course of any incubation or manipulation without cross contamination or sample loss. Experiments have revealed conditions where droplets allow cross-talk. Early research with mineral oil as the continuous phase for droplet segmentation revealed that some analytes demonstrated transfer into

this hydrophobic oil. [68] Fluorinated oil and surfactants offered an alternative carrier phase to contain analytes. [69].

Ex. 2009 at 2251.

Similarly, a 2009 article described this contribution as “major work”:

Ismagilov *et al.* did major work concerning droplet micro-fluidics [19] and concerning the chemistry of the surfactants and oils used to perform the above experiments. [61] Indeed, in order to mimic perfect microbatch conditions, they identified fluorinated oils that were nearly impermeable to water, and synthesized oligoethylene glycol-capped perfluorinated surfactants that prevented protein adsorption at the interface of the droplets.

Ex. 2010 at 29 (reference 61 is L. S. Roach, H. Song, R. F. Ismagilov, *Anal. Chem.* 2005, 77, 785).

The “crucial” need to avoid protein adsorption was stated in a 2013 article, pointing to two publications by the Ismagilov group documenting their synthesis of a “new surfactant” that solved the problem:

Fluorescence imaging has outstanding advantages in studying the spatial distribution of interested molecules in droplets and surrounding phase. The control of protein adsorption on the interface between droplet and carrier phase is crucial in enzyme kinetics study and biochemical assay. Roach et al. [59] studied the protein adsorption on droplet–oil interface with fluorescently labeled proteins and confocal fluorescence microscopy (Fig. 3C). Their study indicated the nonspecific protein adsorption could be prevented by using

oligoethylene-glycol capped surfactants in carrier phase. In the subsequent study, they designed a new surfactant to induce specific adsorption of proteins at the interface and used it to improve the success rate of protein crystallization

Ex. 2011 at 28 (reference 59 is L. S. Roach, H. Song, R. F. Ismagilov, *Anal. Chem.* 2005, 77, 785). If these surfactants were not “crucial” as Petitioner contends, then researchers in the field of microfluidics would not consistently cite to and praise Ismagilov’s work.

Furthermore, a jury has previously found the ’193 Patent valid despite a similar attack by 10X in 2019. *See Bio-Rad v. 10X Genomics, Inc.*, C.A. 15-cv-152-RGA D.I. 477 at 5. Despite Patent Owner extensively addressing various secondary considerations in multiple proceedings, Petitioner is unable to adequately rebut and show obviousness.

B. Failure Of Others

The ’193 Patent succeeded in solving the issues with performing PCR in microfluidics devices despite the failure of others. As outlined above, Quake’s graduate student attempted rudimentary chemical reactions. The results were poor. In fact, Thorsen describes “hundreds” of failed attempts “ultimately discarded in an attempt to discover the perfect operating conditions.” Ex. 1036 at 101-102. Thorsen further stated, “[w]orking on the development of a microfluidic crossflow assay emphasized the difficult dynamic integration process between biochemical and

micromechanical systems.” *Id.* at 108. The Petition states that Thorsen “supports a reasonable expectation of success for the claimed invention” with no further explanation. Petition at 73. Petitioner, however, fails to address how Thorsen would lead to a successful enzymatic droplet assay when it pointed in the wrong direction.

V. CONCLUSION

For these reasons, Dropwork’s Petition for *Inter Partes* Review should be denied.

Respectfully submitted,

Dated: February 16, 2021

/s/ Derek C. Walter

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CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24 *et seq.*, the undersigned certifies that this document complies with the type-volume limitations. This document contains 13,479 words as calculated by the “Word Count” feature of Microsoft Word 2016, the word processing program used to create it.

Dated: February 16, 2021

/s/ *Derek C. Walter*

Derek C. Walter

Reg. No. 74,656

CERTIFICATE OF SERVICE

I hereby certify that on February 16, 2021 a copy of the foregoing
PRELIMINARY RESPONSE TO PETITION FOR *INTER PARTES* REVIEW
OF U.S. PATENT NO. 8,304,193 pursuant to 37 CFR § 42.107 was served by filing
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