

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DROPWORKS, INC.,
Petitioner,

v.

THE UNIVERSITY OF CHICAGO,
Patent Owner.

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

PETITION FOR INTER PARTES REVIEW
OF U.S. PATENT NO. 8,304,193

TABLE OF CONTENTS

I.	INTRODUCTION	1
II.	MANDATORY NOTICES	3
	A. Real Parties-In-Interest (37 C.F.R. §42.8(b)(1)).....	3
	B. Related Matters (37 C.F.R. §42.8(b)(2)).....	3
	C. Identification of Counsel (37 C.F.R. §42.8(b)(3)) and Service Information (37 C.F.R. §42.8(b)(4))	4
III.	CERTIFICATIONS (37 C.F.R. §42.104(A)).....	4
IV.	IDENTIFICATION OF CHALLENGE AND THE RELIEF REQUESTED	4
V.	THE CHALLENGED PATENT	5
	A. Priority Date	7
	B. Prosecution History	7
	C. IPR.....	8
	D. Reexamination.....	9
VI.	CLAIM CONSTRUCTION	11
VII.	LEVEL OF ORDINARY SKILL IN THE ART	12
VIII.	SUMMARY OF THE PRIOR ART.....	13
	A. Background	13
	B. The Prior Art	17
	i. Quake	17
	ii. Holliger	19
	iii. Litborn et al., Synthesis and Analysis of Chemical Components in Nanoscale, Micro Total Analysis Systems 2000, Enschede, The Netherland, pp. 447-454 (2000) ("Litborn," EX1007).	20
	iv. Shaw Stewart.....	21

v.	Schneegaß & Köhler, Flow-through polymerase chain reactions in chip thermocyclers, 82 REV. MOL. BIOTECH. 101-121 (2001) (“Schneegaß,” EX1009).....	21
IX.	REASONS FOR THE RELIEF REQUESTED	22
A.	Ground 1: Claims 1-9 and 11 Are Obvious Over Quake in further view of Holliger.....	22
i.	Claim 1	28
ii.	Claim 2	42
iii.	Claim 3	43
iv.	Claim 4	44
v.	Claim 5	45
vi.	Claim 7	47
vii.	Claims 6 and 8.....	48
viii.	Claim 9	49
ix.	Claim 11	50
B.	<u>Ground 2:</u> Claims 1-9 and 11 Are Obvious Over Quake and Holliger In Further View of Shaw Stewart	53
C.	<u>Ground 3:</u> Claims 1-9 and 11 Are Obvious Over Quake in further view of Litborn.....	54
i.	Claim 1	58
ii.	Claim 2	67
iii.	Claim 3	67
iv.	Claim 4	68
v.	Claim 5	68
vi.	Claim 7	69
vii.	Claims 6 and 8.....	69
viii.	Claim 9	70
ix.	Claim 11	70

D.	<u>Ground 4: Claims 1-9 and 11 Are Obvious Over Quake and Litborn In Further View of Shaw Stewart</u>	70
X.	SECONDARY CONSIDERATIONS	71
XI.	NEW REFERENCES AND EVIDENCE OF UNPATENTABILITY	73
XII.	DROPWORKS’ FIRST AND ONLY PETITION SHOULD BE INSTITUTED	75
XIII.	CONCLUSION.....	76
XIV.	CERTIFICATE OF COMPLIANCE	77
XV.	PAYMENT OF FEES UNDER 37 C.F.R. §§ 42.15(A) AND 42.103	78
XVI.	APPENDIX – LIST OF EXHIBITS	79

I. INTRODUCTION

Petitioner Dropworks, Inc. requests *Inter Partes* Review of claims 1-9 and 11 of U.S. Patent No. 8,304,193 to Ismagilov *et al.* (EX1001), which is owned by The University of Chicago (“Patent Owner”). This Petition demonstrates there is a reasonable likelihood the challenged claims are obvious under 35 U.S.C. §103.

Petitioner thus requests review be instituted and the challenged claims cancelled.

The challenged claims are directed to a method for conducting an autocatalytic reaction in aqueous plugs in a microfluidic system. EX1001, 78:7-29. The method requires, *inter alia*, forming at least one plug of aqueous fluid at a junction of two channels by continuously flowing an aqueous fluid through a first channel into an oil continuously flowing through a second channel. The plug is thus a water-in-oil (w/o) droplet that is substantially surrounded by the oil. The ’193 patent asserts that the autocatalytic reaction may be polymerase chain reaction (PCR) and some dependent claims are directed to PCR. EX1001, 2:58-67, 44:58-61, 78:32-35; EX1002, ¶¶43-45.

The ’193 patent issued in error. The prosecution Examiner identified US 2002/0058332 to Quake as the closest prior art. Quake ’332 discloses forming microfluidic droplets, suggests performing PCR in the droplets, and incorporates a reference explaining how to perform PCR. The Examiner distinguished Quake ’332 on the basis that its w/o droplets “are not formed from two continuously

flowing immiscible fluids.” EX1004, 0283. That was incorrect. Both the Patent Owner and a subsequent reexamination Examiner have respectively admitted and found that the prosecution Examiner was in error about how Quake formed droplets, and that the droplets in Quake are, in fact, formed in precisely that fashion. EX1026, 13-14; EX1028, 1915-16.

However, Patent Owner has thus far avoided cancellation of the claims from 10X Genomics, Inc.’s IPR petition and reexamination request by arguing that using mineral oil and standard hydrocarbons to form the droplets, as disclosed in Quake, would preclude successful PCR amplification within them. This Petition provides new evidence that has not been considered previously by the Office, including Holliger (EX1006), Litborn (EX1007), and Schneegaß (EX1009), which demonstrate that PCR amplification of DNA was successfully achieved in sub-microliter w/o droplets formed in standard hydrocarbons before the earliest claimed priority date. These references demonstrate that Patent Owner’s assertion on which this Office relied in upholding the claims are wrong.

As explained by Dr. Meinhart, a microfluidics expert with decades of relevant experience, a skilled artisan at the relevant time would have been motivated to arrive at the claimed subject matter with a reasonable expectation of success, for example, by using the system described in Quake to form droplets in which PCR reactions could then take place. EX1002, ¶¶1-10, 22-33. The

challenged claims are not patentable, but are, at most, merely the “combination of familiar elements according to known methods.” *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 416 (2007).

II. MANDATORY NOTICES

A. Real Parties-In-Interest (37 C.F.R. §42.8(b)(1))

Dropworks, Inc. is the Petitioner and the real party-in-interest in this IPR.

B. Related Matters (37 C.F.R. §42.8(b)(2))

The ’193 patent was asserted in *Bio-Rad Laboratories, Inc. et al. v. Dropworks, Inc.*, Case No. 1:20-cv-00506-UNA (D. Del.). EX1025. The complaint was filed on April 14, 2020, and served no earlier than May 6, 2020. *Id.*

On February 15, 2015, Patent Owner asserted the ’193 patent against 10X Genomics, Inc., together with U.S. Patent Nos. 8,273,573; 8,329,407; 8,822,148; 8,889,083; and 7,129,091. 10X Genomics, Inc., an unrelated party, filed an IPR petition challenging claims 1-9 and 11 of the ’193 patent in IPR2015-01163. The Board denied institution on November 16, 2015. EX1027. 10X Genomics also filed IPR petitions challenging claims of the ’573, ’407, and ’148 patents in IPR Nos. 2015-01162, 2015-01158, and 2015-1156, each of which was denied institution. 10X Genomics filed an IPR petition challenging claims of the ’083 patent in IPR No. 2015-01157, which resulted in a final written decision not finding the challenged claims unpatentable.

On November 3, 2017, 10X Genomics requested *ex parte* reexamination of claims 1-14 of the '193 patent, Control No. 90/014,043. EX1028, 4390. A reexamination certificate issued on October 31, 2018, confirming the patentability of the claims. *Id.*, 3.

C. Identification of Counsel (37 C.F.R. §42.8(b)(3)) and Service Information (37 C.F.R. §42.8(b)(4))

Lead Counsel	Back-Up Counsel
Michael T. Rosato, Reg. No: 52,182 WILSON SONSINI GOODRICH & ROSATI 701 Fifth Avenue, Suite 5100, Seattle WA 98104-7036 Tel.: 206-883-2529 Fax: 206-883-2699 Email: mrosato@wsgr.com	Jad A. Mills, Reg. No: 63,344 WILSON SONSINI GOODRICH & ROSATI 701 Fifth Avenue, Suite 5100, Seattle WA 98104-7036 Tel.: 206-883-2554 Fax: 206-883-2699 Email: jmills@wsgr.com

Please direct all correspondence to lead counsel and back-up counsel at the contact information above. Dropworks consents to electronic mail service at the email addresses above. A power of attorney accompanies this Petition.

III. CERTIFICATIONS (37 C.F.R. §42.104(A))

Dropworks certifies the '193 patent is available for IPR and Dropworks is not barred or estopped from requesting IPR on the identified grounds.

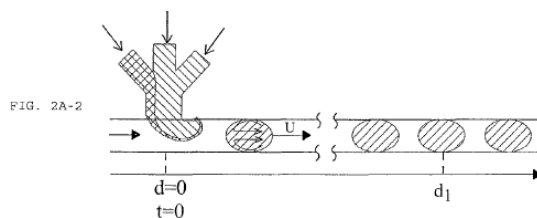
IV. IDENTIFICATION OF CHALLENGE AND THE RELIEF REQUESTED

Dropworks requests review of claims 1-9 and 11 of the '193 patent under 35 U.S.C. §311 and AIA §6.

Ground	Claims	Obvious under 35 U.S.C. §103 over:
1	1-9, 11	International Publication No. WO 02/23163 (“Quake,” EX1005) in view of International Publication No. WO 02/022869 (“Holliger, EX1006).
2	1-9, 11	Quake (EX1005) and Holliger (EX1006) in further view of International Publication No. WO 84/02000 (“Shaw Stewart,” EX1008).
3	1-9, 11	Quake (EX1005) in view of Litborn (EX1007).
4	1-9, 11	Quake (EX1005) and Litborn (EX1007) in further view of Shaw Stewart (EX1008)

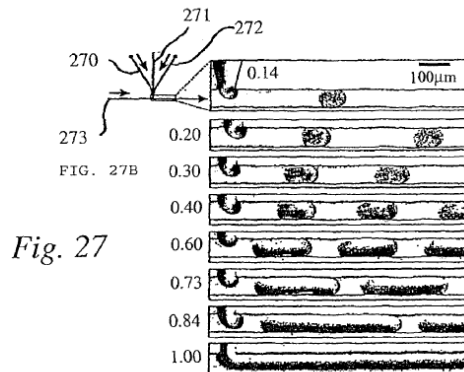
V. THE CHALLENGED PATENT

The '193 patent relates to conducting an autocatalytic reaction in plugs in a microfluidic system. EX1001, Title; EX1002, ¶¶34-45. The patent purports to address cross-contamination between reactions by forming a plug (illustrated in Figure 2A-2 below) that is substantially surrounded by an oil carrier fluid. EX1001, 1:56-2:10, 2:54-58, 3:39-40, 17:37-38 & Fig. 2A-1; EX1002, ¶¶35-37, 42.



The '193 patent contemplates a wide range of plug volumes ranging from “femtoliters to hundreds of nanoliters.” EX1001, 11:24-53, 19:45-57; EX1002,

¶¶38, 40. As illustrated in Figures 3, 12, and 27 (reproduced below), the plugs may be short or elongated:



The '193 patent uses syringe pumps to form the plugs but teaches that fluid flow may cease after plug formation, such as before amplification. EX1001, 3:50-56, 18:38-46, 20:37-39, 21:33-38, 21:45-47, 55:7-8, 55:17-20; *id.*, 1:25-32, 14:38-41, 71:53-59; EX1002, ¶39. It identifies TweenTM, SpanTM, and ZonylTM as preferred surfactants. EX1001, 20:31-52; EX1002, ¶41-42. It issued with 14 claims. EX1001, 78:8-29; EX1002, ¶¶44-45.

A. Priority Date

The '193 patent claims priority to Provisional Application Nos. 60/394,544 (July 8, 2002) and 60/379, 927 (May 9, 2002). EX1001, cover; EX1002, ¶46. All asserted references qualify as prior art as of the earliest claimed priority date, May 9, 2002.¹

B. Prosecution History

During prosecution, the Examiner asserted the claims lacked priority before 2011 and rejected them using a 2007 reference. EX1004, 0496-0499. Applicants antedated the 2007 reference with a 2004 priority date. EX1004, 0282, 0331-0386; EX1002, ¶¶47-50. The Notice of Allowance stated that US 2002/0058332 to Quake “disclose[s] a system with two channels and the method of forming plugs

¹ As discussed below, the asserted references discuss PCR in considerably more detail than the '193 patent specification, which contains only high-level disclosure of PCR. EX1002, ¶43. The provisional applications reference “polymerization” and “DNA” in different portions of the specification, but never PCR. EX1002, ¶43. Nonetheless, Patent Owner argued that this provided §112 support for a related claim requiring providing conditions suitable for a polymerase-chain reaction in the at least one plug such that the target DNA or RNA is amplified. EX1038, 0282, 0287-0289; EX1039, cover.

(droplets) with reagents in immiscible oil” but that “the droplets in oil are not formed from two continuously flowing immiscible fluids.” EX1004, 0282-0283; EX1002, ¶¶51-53.

C. IPR

In IPR2015-01163, 10X Genomics challenged certain claims as obvious over Haff (EX1010) and US 2002/0058332 (EX1029) or in further view of Ramsey (EX1037). EX1025, i, 1-2; EX1002, ¶¶54-55. In response, Patent Owner conceded that Quake forms droplets “by introducing *a flow of a fluid* that may ‘contain a biological sample (e.g., molecules of an enzyme or a substrate, or one or more cells, or one or more viral particles)’ *into a main channel through which a fluid is flowing* that is immiscible with the first fluid.” EX1026, 13-14 (emphasis added); EX1002, ¶56. Patent Owner thus abandoned the original basis for issuance of the patent.

Seeking a new basis to preserve the claims, Patent Owner argued the 10X petition “fail[ed] to identify a single reference that teaches chemical reactions in microfluidic droplets,” and that the “use of microfluidic droplets as chemical microreactors was at most an aspiration until Ismagilov and co-workers made this goal a reality.” EX1026, 1, 7-8. Patent Owner thus argued that PCR and other autocatalytic reactions “could, for the first time, be carried out using microfluidic droplets” only with the benefit of the “Ismagilov Patents.” *Id.*, 8-9. Patent Owner

distinguished Haff as operating “on a size-scale” of 10 microliters to 1.5 milliliters, and as being “expressly direct[ed] to *increasing* the volume of a PCR reaction, the exact opposite of what one hopes to achieve by using droplets[.]” *Id.*, 3, 11-12; EX1002, ¶¶57-65.

The Board denied institution, finding 10X failed to adequately address reasonable expectation of success. EX1027, 15-17 (“simply stating it would have been ‘routine’ is insufficient.”). The Board noted that 10X had “not explained why” conducting PCR in a *stream* of reaction mixture in a microfluidic device would make it routine to conduct PCR in *droplets* in a microfluidic device. *Id.*, 17. The Board found that 10X’s expert testimony was an “unexplained opinion” that did not disclose the underlying facts on which it was based. *Id.*, 17-18. The Board thus denied institution. EX1002, ¶¶66-67.

D. Reexamination

10X subsequently filed an *ex parte* reexamination request. EX1028, 4399; EX1002, ¶¶68-70. The Examiner rejected claims 1-14 as obvious over WO 2002/023163 (“Quake,” EX1005) in view of Kopp (EX1011) and GB 2,097,692 (EX1020). EX1028, 1899-1900. In ordering reexamination, the Examiner found Quake’s microfluidic device forms plugs in the manner claimed. *Id.*, 1899-1900, 1915-16. The Examiner also found that it would have been obvious to provide the substrate molecule and reagents specific to PCR and to provide the necessary

conditions (temperature cycling) for PCR because Quake specifically points to Kopp for PCR. *Id.*, 1901. The Examiner found that the remaining limitations of the dependent claims were disclosed in the prior art and were obvious. *Id.*, 1901-1903. Patent Owner never overcame these findings.

Instead, Patent Owner argued that the conditions disclosed in the cited art did not enable autocatalytic reactions in microfluidic droplets. EX1002, ¶¶71-73. Specifically, Patent Owner asserted that “traditional hydrocarbons, such as decane, tetradecane, hexadecane, and mineral oil” used as a carrier fluid in Quake “are not remotely biocompatible and will destroy proteins.” *Id.*, 261, 1882. Patent Owner cited no objective evidence to corroborate the *ipse dixit* testimony of its expert.

The Examiner confirmed the claims. EX1002, ¶74. As to Kopp, the Examiner concluded it does not teach a microfluidic droplet or system or use oil as a carrier fluid. *Id.*, 9. The Examiner also credited Patent Owner’s argument that the inventors provided suitable conditions for PCR, including through the use of fluorinated oils and surfactants. *Id.*, 7-8. Relying on Patent Owner’s attorney argument and expert *ipse dixit*, and without commenting on the absence of fluorinated oils and surfactants in the independent claim, the Examiner stated: “Instead of using biocompatible fluorinated oils and fluorosurfactants, Quake teaches traditional hydrocarbons which are not biocompatible and will destroy proteins.” *Id.*, 8-9. As explained in detail below, Patent Owner’s unsupported

assertion that Quake's traditional hydrocarbons would have prevented successful PCR amplification in microfluidic droplets is not correct and is contradicted by prior art evidence not previously presented to the Office.

VI. CLAIM CONSTRUCTION

Claim terms should be interpreted according to their ordinary and customary meaning to a POSA at the relevant priority date, consistent with the specification. 37 C.F.R. §42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (en banc). This Petition applies the prior art to the claims using this standard. EX1002, ¶¶75-85.

Patent Owner has previously construed the claims of the '193 patent before Judge Andrews in the District of Delaware using the *Phillips* standard. EX1031, 1-2. Patent Owner proposed, and Judge Andrews agreed, that the claims of the '193 patent do not require that amplification occur in the substrate or on a chip in the "microfluidic system," and do not require the reaction to take place while the plug is flowing through the channel. EX1031, 6-7; EX1032, 3-5. On appeal, however, the Federal Circuit concluded that the reaction must take place in the droplets while the droplets are inside the microfluidic system (*i.e.*, on-chip reactions). EX1035, 20-24 & n.3-4; EX1002, ¶¶77-79, 81.

Patent Owner argued, and Judge Andrews agreed, that the term "providing conditions suitable for" the reaction means "providing a set of physical and

chemical conditions that allow” the reaction to occur. EX1031, 10-11; EX1002, ¶80. Judge Andrews construed the term “polymerase chain reaction.” EX1033; EX1002, ¶82. Judge Andrews also construed reagent, reaction, autocatalytic reaction, fluorinated oil, fluorinated surfactant, plug, and surfactant. EX1034, 2-3; EX1002, ¶¶80, 83. The analysis of the challenged claims below adopts and applies these claim constructions.

Moreover, as discussed above in Section V.B, the claim language and the prosecution history demonstrate that claim 1 and all dependent claims require that the droplets in oil be “formed from two continuously flowing immiscible fluids.” EX1004, 0282-0283. During droplet formation, as claimed, the aqueous fluid thus must continuously flow from the first channel into the oil, which is flowing continuously in the second channel. EX1001, 78:8-26; EX1002, ¶85.

VII. LEVEL OF ORDINARY SKILL IN THE ART

The '193 patent relates to microfluidic systems and conducting reactions in microfluidic systems. EX1001, 1:15-2:50. Dr. Carl D. Meinhart is a Professor of Engineering at the University of California Santa Barbara who specializes in microfluidics and in surface interactions at the micro-scale and nano-scale. Dr. Meinhart was well-versed in microfluidic systems and conducting reactions in microfluidic systems at the relevant times. EX1002, ¶¶2-10; EX1003.

As Dr. Meinhart explains, a person of ordinary skill in the art (POSA) at the relevant times would have been a researcher interested in microfluidics with at least a bachelor's degree in a science such as bioengineering, chemical engineering, chemistry, physics, mechanical or aeronautical engineering, or biochemistry; or a bachelor's or master's degree in a relevant discipline and three to five years of relevant experience. EX1002, ¶¶86-88; EX1026, 15. A POSA may have worked as part of a multidisciplinary team, which may have included a biochemist, biologist, or a PCR specialist. The prior art cited in this Petition, including the background art, demonstrates information with which a POSA would have been familiar at the relevant time. EX1002, ¶¶88-119.

VIII. SUMMARY OF THE PRIOR ART

A. Background

PCR is a conventional amplification reaction technique that has been well known in the art for nearly four decades. EX1002, ¶¶89-93; EX1010, 1:16-45; EX1011, 1046-47. PCR reactions involve three steps. First, the double stranded DNA is separated into two single strands. This is usually done by heating. Second, short polynucleotide molecules called primers hybridize to a portion of the DNA. Third, an enzyme called polymerase adds additional nucleotides to the end of the primers based on the DNA template, thereby creating a second strand of DNA that is complementary to the template strand. Four main reagents are needed: a

polymerase enzyme, primers, free nucleotides, and a buffer in which the polymerize can function. All of this was well-known decades before the priority date. *Id.*; EX1009, 103.

Scientists have long recognized that autocatalytic reactions such as PCR could occur in extremely small aqueous compartments. PCR thermocyclers originally operated on reaction tubes or mm-scale capillary tubes. EX1002, ¶¶94-105; EX1010, 1:50-2:6. PCR reaction mixture volumes thus generally were no smaller than 10 µl. EX1010, 2:23-27; EX1016, 484; EX1012, 138; EX1013, 271. Haff's non-microfluidic PCR capillary tube, for example, had an inner diameter of 1-3 mm. EX1010, Cover, Abstract, 3:6-9, 8:28-37. In Haff's system, the capillary tube passes through various heat zones to perform the melting, primer annealing, and extension phases of PCR on an aqueous PCR mixture flowing through the tube. *Id.* Haff's system permitted mL-scale PCR reactions. *Id.*, 7:9-11, 7:27-31. Haff disclosed that thermocycling generally can be completed faster for smaller reaction volumes. *Id.*, 2:27-49. EX1002, ¶¶96-98. Haff taught that capillary tube PCR advantageously provided more rapid thermal incubation transitions than a comparable reaction volume in a stationary reaction chamber. EX1010, 2:50-53, 5:11-40. Haff injected air bubbles or an immiscible fluid bubble such as an oil into the flow stream of the reagent mixture in the tubing to demark the ends of the reaction mixture sample volume. *Id.*, 8:37-42, 10:29-34.

Microfluidic PCR devices have been available since the 1990s. EX1002, ¶¶99-105; EX1012, 138 & n.3-12. Microfluidic PCR systems were desirable to reduce the sample size, which used less reagents, reduced the cost, reduced the time required, and reduced the number of template copies required. EX1012, Abstract, 138-39, 44; EX1013, 271; EX1002, ¶¶101-105. These microfluidic PCR devices performed PCR amplification by thermocycling the PCR reaction either in stationary chambers or using continuous flow. EX1012, 138; EX1009, 104-05, 108. In each case, the aqueous PCR mixture would cycle through the same well-known melting, annealing, and extension phases as for all PCR reactions. EX1012, 142, 144 (Fig. 5 caption); EX1013, 274, 277; EX1011, 1046; EX1014, 11:1-24 & Fig. 4. PCR microreactors originally had volumes on the order of 1 µl, though reaction volumes between 250-280 nL were reported by 2001. EX1012, Abstract, 144 (microchamber); EX1013, 280 (microfluidic capillary).

POSAs recognized that it was important to isolate individual PCR reaction mixtures from subsequent samples. EX1002, ¶¶106-08. Practitioners thus injected volumes of air or immiscible fluid between PCR reaction mixtures. EX1010, 10:20-37; EX1015, [0095]-[0116]. In 1998, for example, Kopp reported continuous-flow PCR amplification in a microfluidic channel on a chip. EX1011, 1046. Kopp used syringe pumps to alternately inject the PCR reaction mixture or just the aqueous buffer into the channels on the chip at a ratio of 1:0.8. *Id.*, 1047.

It was shown before 2002 that PCR could be performed successfully in microfluidic water-in-oil (w/o) droplets formed in standard hydrocarbons such as octane and mineral oil. EX1006; EX1007; EX1009; EX1002, ¶¶108, 137-146. In December 2001, for example, Schneegaß disclosed successful PCR DNA amplification in microfluidic w/o droplets flowing through a microfluidic device. EX1009, Abstract, 109-10, 115, 118, Fig. 6 & caption; EX1002, ¶¶149-159. Schneegaß concluded the amplification was “efficient and highly selective for all templates.” EX1009, 118.

As shown in the literature, POSAs knew quite well that fluorinated oils and fluorinated surfactants were useful for forming emulsions containing biological materials because they were known to be chemically and biologically inert and to form stable and strong droplet interfaces. EX1002, ¶¶109-119. For example, the Shaw Stewart Thesis disclosed using fluorinated oil as an inert, immiscible carrier fluid for forming and transporting microfluidic w/o droplets as small as 10 nL for biochemical enzymatic microreactions. EX1023, Abstract, 2, 18, 21, 89-92, 147-48; EX1002, ¶¶110-14. Rüdiger taught that fluorinated oils, and particular perfluorodecalin, were suitable for use in biological applications because they are highly chemically inert, strongly hydrophobic, and also oleophobic, such that they are immiscible in water and poorly miscible in all but fluorophilic substances (*i.e.*, other fluorocompounds). EX1017, 209, 212-213; EX1002, ¶¶114-17. Rüdiger

explains that fluorinated oils thus should be used in combination with fluorinated surfactants to create a water-in-oil emulsion, and that such surfactants arrange themselves at the interface to stabilize the microemulsion. EX1017, 213. Krafft similarly reports fluorinated surfactants were desirable for making colloidal systems (*e.g.*, water-in-fluorinated oil emulsions) with biological applications because they are chemically and biologically inert and have a tendency to organize at the oil/water interface. EX1018, Summary, 489-90, 492, 496, 510, Fig. 6; *see also* EX1006, 47:7-8; EX1002, ¶¶118-19.

B. The Prior Art

As Dr. Meinhart explains, and as addressed in further detail below, the challenged claims would have been obvious to a POSA at the relevant time. EX1002, ¶¶22-33. For example, Quake discloses forming microfluidic droplets in the manner claimed, suggests performing PCR in the droplets, and incorporates a reference discussing how to perform PCR in a microfluidic environment. EX1005, 23:26-27, 89:31. Other prior art printed publications also specifically taught how to perform PCR in the microfluidic droplets present in Quake. EX1006; EX1007; EX1009.

i. Quake

Quake, published on March 21, 2002, based on a U.S. PCT Patent Application filed on September 14, 2001, claims priority to U.S. Provisional

Applications filed in November and September 2000. EX1005, cover; EX1030.

Quake is prior art to the challenged claims at least under pre-AIA 35 U.S.C.

§§102(a), (e).

Quake provides a microfluidic device for analyzing, sorting, and reacting biological materials, including polynucleotides. EX1005, Abstract, 5:1-12, 5:29-32. Quake's microfluidic device compartmentalizes small droplets of aqueous solution within oil continuously flowing in the main microfluidic channel by continuously flowing a stream of aqueous solution from a sample inlet channel into the main channel via a junction. EX1005, Abstract, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16, Fig. 16A (reproduced below); EX1002, ¶¶120-24.

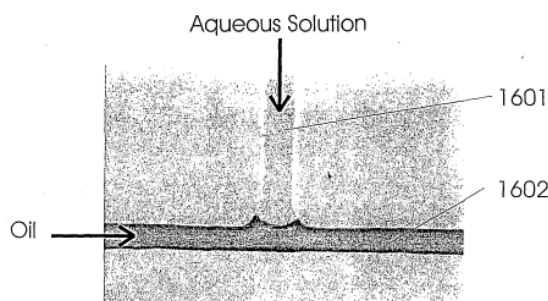


FIG. 16A

The droplets preferably have a volume of 0.1-100 picoliters. *Id.*, 1:15-28. Quake suggests performing PCR in the droplets. EX1002, ¶¶125-28. EX1005, 1:29-2:9. 2:29-3:6, 23:26-30.

ii. Holliger

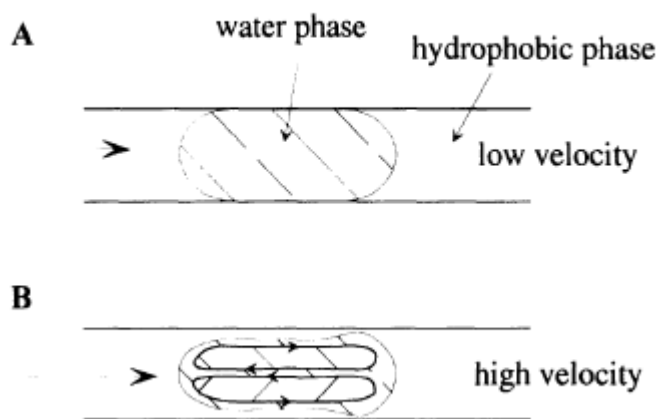
Holliger, published on March 21, 2002, based on a Great Britain PCT Patent Application filed on September 13, 2001, claims priority to a Great Britain application filed in September 2000 and U.S. Provisional Patent Applications filed in April 2001. EX1006, cover. Holliger is prior art to the challenged claims at least under pre-AIA 35 U.S.C. §§102(a), (e).

Holliger discloses compartmentalizing DNA template with polymerase and PCR reagents in w/o droplets, performing PCR amplification in the droplets, and detecting the amplified DNA. EX1006, Abstract, 2:4-11, 3:14-20, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-20; EX1002, ¶¶129-34. Holliger discloses forming the droplets by adding the aqueous phase to the oil phase in the presence of a surfactant and mechanical force. EX1006, 6:11-15, 7:16-20, 47:9-11, 52:7-15. Holliger discloses that mineral oil was a suitable oil. *Id.*, 44:19-21. 47:7-48:5, 48:22-26. Holliger teaches how to perform PCR amplification, including PCR thermocycling. *Id.*, 5:7-10, 5:19-23, 6:20-21, 10:20-25, 21:23-22:20, 25:24-26:22. Holliger reports successfully amplifying DNA templates in sub-microliter sized w/o droplets. EX1006, 8:21-9:6, 9:12, 12:21-26, 18:3-7, 49:20-27, 52:25-53:2, 59:4-23, 63:9-13. & Figs. 3-4, and 7; EX1002, ¶¶134-36.

- iii. *Litborn et al., Synthesis and Analysis of Chemical Components in Nanoscale, Micro Total Analysis Systems 2000, Enschede, The Netherlands, pp. 447-454 (2000) (“Litborn,” EX1007).*

Litborn published in 2000 and was cited in 2001 in another periodical publication. EX1007, 447; EX1009, 120. Litborn is prior art to the challenged claims at least under pre-AIA 35 U.S.C. §102(b).

Litborn describes the simplest version of “nanoscale reactions” in “[m]icrofluidic systems,” which involves transporting a “pre-mixed sample through a channel, which has the desired reaction conditions,” such as elevated temperature zones. EX1007, 451. Litborn discloses performing successful PCR amplification in water droplets in flowing oil in a microfluidic capillary channel to reduce cross-contamination as compared to separating samples with air or gas bubbles. *Id.*, 452-53 & Fig. 7 (reproduced below); EX1002, ¶¶137-46.



Litborn reports that “[a]mplification was successful...” EX1007, 453.

iv. *Shaw Stewart*

Shaw Stewart published in 1984. EX1008, cover. Shaw Stewart is prior art to the challenged claims at least under pre-AIA 35 U.S.C. §102(b).

Shaw Stewart discloses a microfluidic system for performing nanoliter-sized chemical reactions with the aim of reducing or eliminating contamination of the reactants. EX1008, Abstract, 2 (objects 1 and 5); EX1002, ¶¶147-48. Shaw Stewart discloses forming an aqueous droplet by flowing aqueous fluid into a channel flowing with an inert, immiscible carrier fluid. EX1008, Abstract, 3. Shaw Stewart discloses the carrier fluid may be mineral oil or a fluorinated hydrocarbon. *Id.*, Abstract, 4. Shaw Stewart discloses the invention can be used to perform biological or chemical assays. *Id.*, 8.

v. *Schneegaß & Köhler, Flow-through polymerase chain reactions in chip thermocyclers, 82 REV. MOL. BIOTECH. 101-121 (2001) (“Schneegaß,” EX1009).*

Schneegaß published in 2001. EX1009, 101. Schneegaß is thus prior art to the challenged claims at least under pre-AIA 35 U.S.C. §102(a).

Schneegaß discloses successful droplet PCR DNA amplification in a microfluidic chip device. EX1009, Abstract, 109-10, 115, 118, Fig. 6 & caption; EX1002, ¶¶149-59. Schneegaß’s microfluidic PCR amplification was performed in aqueous droplets formed in mineral oil to reduce “cross-talk” between samples. EX1009, 102, 108-09; EX1002, ¶¶155-59. Schneegaß reports that “non-volatile

mineral oil...led to the ideal sample transport and showed the expected amplification results.” *Id.*, 109. Schneegaß “supplemented the PCR mixture” with Tween 20 to reduce surface tension, stabilize the droplets, and stabilize the polymerase during PCR. *Id.*, 110. Schneegaß also notes using PEG to prevent protein (polymerase) adsorption was known. *Id.*, 110.

IX. REASONS FOR THE RELIEF REQUESTED

A. Ground 1: Claims 1-9 and 11 Are Obvious Over Quake in further view of Holliger

1) Motivation to Combine and Reasonable Expectation of Success

As discussed here and throughout this Petition, a POSA would have had good reason, with a reasonable expectation of success, for arriving at the ’193 patent claimed subject matter in view of the prior art.

As 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. Quake and Holliger create microscopic water-in-oil (w/o) droplets and are directed to performing enzymatic reactions (including specifically PCR) in the droplets. EX1002, ¶¶161-64. Quake discloses forming the w/o droplets at the junction of two channels of a microfluidic device as recited in the challenged claims and expressly suggests performing PCR. EX1005, 1:18-24, 5:6-7, 5:21-23, 6:4-8, 6:23-27, 7:30-8:5, 11:20-12:12, 22:6-16, 23:26-30, 30:1-2; EX1002, ¶¶161-63. Quake teaches that this droplet formation methodology is beneficial because it

creates monodisperse (*i.e.*, uniformly sized) droplets with high-throughput.

EX1005, 27:27-32 & Figs 17A, C and 19J, L; EX1002, ¶¶124, 166, 219. Holliger discloses successfully performing PCR in microscopic w/o droplets made using the same oil and similar surfactants as described in Quake. EX1006, Abstract, 2:4-11, 3:14-20, 6:11-15, 7:16-20, 8:21-9:6, 9:12, 18:3-7, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-20, 49:20-27, 52:25-53:2, 52:7-15, 59:4-23, 63:9-13 & Figs. 3-4, and 7; EX1002, ¶¶164, 167. Holliger teaches that microscopic w/o droplets are useful for performing PCR on a massive scale, including digital PCR and multiplex PCR. EX1006, 12:21-26; EX1002, ¶¶134, 177. Quake and Holliger thus individually and collectively teach microscopic w/o droplets were suitable and useful for performing PCR analyses.

Additionally, the cited references together disclose all aspects of the challenged claims and expressly contemplate performing PCR in the w/o droplets in a microfluidic device, as claimed. EX1002, ¶¶165, 176-222; *KSR*, 550 U.S. at 418-19 (TSM not required but useful). As discussed in detail in below, 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 (*e.g.*, decane or mineral oil). EX1005, 1:18-24, 5:6-7, 5:21-23, 6:4-8, 6:23-27, 7:30-8:5, 11:20-12:12, 22:6-16, 30:1-2; EX1002, ¶161. Quake teaches using these water-in-oil (w/o) droplets for analysis

and as microreactors (including as enzyme bioreactors). EX1005, 6:10-16, 7:15-21, 8:14-21, 15:2-6, 22:27-23:3, 35:9-14. Quake's plugs are substantially surrounded by the oil flowing through the second channel. EX1005, Abstract, 5:1-12; *Id.*, 5:29-32, 7:6-15, 29:33-30:2, 36:9-28, 85:9-86:6 & Figs. 17A-C, 19J, L, 22. 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. *Id.* 2:25-3:6, 8:19-23, 13:16-26, 15:18-21, 23:26-30; EX1002, ¶¶162-63. Quake also cites and incorporates Kopp, which further describes performing PCR in microfluidic systems using thermocycling. EX1005, 23:26-27, 89:31; EX1011, 1046-47. Holliger teaches successful PCR amplification of DNA in microscopic aqueous droplets in mineral oil formed using standard surfactants (*e.g.*, Span/Tween/Triton) similar to those used in Quake. EX1006, Abstract, 2:4-11, 3:14-20, 6:11-15, 7:16-20, 8:21-9:6, 9:12, 18:3-7, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-20, 49:20-27, 52:25-53:2, 52:7-15, 59:4-23, 63:9-13 & Figs. 3-4, and 7; EX1002, ¶¶164, 167. Holliger expressly discloses including the PCR reagents and DNA substrate in the aqueous fluid *before* droplet formation. EX1006, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27. Holliger teaches that PCR amplification is then initiated by thermocycling. *Id.*, 18:3-7, 19:3-4, 21:23-22:23; 59:4-8, 75:20-26 A POSA thus

would have good reason to combine the teachings of Quake and Holliger to successfully achieve PCR amplification in the droplets. EX1002, ¶¶164-67.

Furthermore, 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. EX1002, ¶¶166-67. Although Quake described initiating certain enzymatic reactions using droplet merger, Quake also broadly teaches the aqueous fluid used to form the droplets may contain enzyme substrate and reactants. EX1005, 6:11-15, 8:14-22 (aqueous sample fluid may contain biological materials, including polynucleotides, enzymes, substrate, or mixtures thereof). Holliger does exactly this. EX1006, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27. It was well-known that PCR is initiated by thermocycling, not simply by substrate contact with the enzyme. Holliger expressly teaches as much. EX1006, 18:3-7, 19:3-4, 21:23-22:23; 59:4-8, 75:20-26; EX1002, ¶¶164-67. Elsewhere in the literature, Litborn, for example, expressly explains that using a pre-mixed aqueous solution and initiating the reaction by heating was the “simplest version.” EX1007, 451. Holliger thus exemplifies a known practice for PCR reactions of using a pre-mixed aqueous solution containing the substrate, enzyme, and the remaining reagents to form the droplets. EX1002, ¶¶162-66. In view of Holliger’s express disclosure of this well-known, simple, and convenient way to perform PCR in microscopic droplets (further

corroborated by Litborn), a POSA would have had good reason to do the same when forming Quake's droplets. *Id.*; *see also* Sections IX.A.i.a, c, e, h below.

In addition, a POSA would have viewed Quake as providing technical benefits for droplet formation. EX1002, ¶168. Quake teaches forming droplets at the junction of the two microchannels using continuous flow of aqueous solution into continuously flowing oil produced uniformly-sized (monodisperse) droplets with high throughput. EX1005, 1:18-24, 5:6-7, 5:21-23, 6:4-8, 6:23-27, 7:30-8:5, 11:20-12:12, 22:6-16, 27:27-32 & Figs 17A, C and 19J, L; EX1002, ¶¶124, 161-63, 219. Another prior art reference that performed PCR in microfluidic w/o droplets, Schneegaß, suggested using "T-shaped injector channel" to form microfluidic PCR droplets precisely to increase throughput. EX1009, 109. Moreover, it was well-known that emulsion PCR helped to reduce cross-talk between PCR samples. EX1002, ¶¶108, 124; EX1009, 108; EX1005, 5:1-12, 5:25-27. As Dr. Meinhart explains, a POSA would have had good reason to make w/o droplets at the microchannel junction as disclosed in Quake to create consistently-sized droplets that would have relatively uniform sizes and thus uniform sample concentrations, at high throughput, especially to perform digital PCR as expressly suggested by Holliger. EX1002, ¶¶166, 219.

Moreover, the fact that multiple prior art references (including, *inter alia*, Quake and Holliger) describe PCR analysis in microscopic w/o droplets further

underscores that a POSA reasonably would expect to successfully arrive at the '193 patent claimed subject matter in view of the prior art. *See, e.g.*, discussion above; Section VIII. Like other scientific literature available at the time, Holliger's disclosure that PCR amplification was successful in sub-microliter aqueous droplets formed in mineral oil using standard surfactants confirms that this approach would have been expected to work. EX1002, ¶¶168-74; *see also* EX1007, EX1009 (performing PCR in sub-microliter aqueous droplets). *In re Antor Media Corp.*, 689 F.3d 1282, 1289, 1292 (Fed. Cir. 2012); *Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1355 (Fed. Cir. 2003) (prior art presumed enabled for what it discloses). In addition to teaching the PCR reagents and thermocycling conditions, Holliger also teaches that mineral oil was a suitable oil for forming the PCR droplets and does not abolish the enzyme functionality. *Id.*, 5:7-10, 5:19-23, 6:20-21, 10:20-25, 12:21-26, 21:23-22:20, 25:24-26:22, 44:19-21. 47:7-48:5, 48:22-26. As Dr. Meinhart explains, Holliger thus confirms a POSA had a reasonable expectation of success in performing the claimed method as a whole, whether PCR was performed in a microfluidic reaction chamber or via microfluidic continuous flow. EX1002, ¶¶160, 166-74; *see also* EX1009, 109; EX1007, 451-453; Sections VI above (on-chip PCR need not occur in continuous flow), and VIII.A (conditions for PCR well known including when using standard hydrocarbons as carrier fluid).

The discussion below provides claim-by-claim analysis demonstrating that Quake in view of Holliger discloses every element of each of claims 1-9 and 11, and that these claims are each obvious as a whole. EX1002, ¶175. Exemplary prior art text is reproduced in the charts below.

i. Claim 1

a) 1.[preamble] A method for...

Quake in view of Holliger discloses a method for conducting an autocatalytic reaction (*e.g.*, PCR) in plugs in a microfluidic system. EX1002, ¶¶175-79. For example, Quake discloses a microfluidic device for reacting biological materials, including specifically DNA polynucleotides, in w/o droplets preferably having a volume of 0.1-100 picoliters. EX1005, Abstract; 1:15-28, 5:11-12, 6:10-16, 7:6-15, 8:1-5, 8:14-21, 8:19-23, 13:16-26, 15:18-21, 26:30-27:32, 35:9-14, 36:9-28, 85:9-86:6. Quake expressly suggests using the microfluidic device for on-chip PCR reactions. EX1005, 23:26-30 (citing EX1011); *see also* Section VIII.A above.

Holliger discloses successfully performing PCR amplification in sub-microliter scale w/o droplets like Quake's. EX1006, 8:21-9:6, 18:3-7, 49:20-27, 52:25-53:2, 59:4-23, 63:9-13 & Figs. 3-4, and 7. A POSA thus would have had good reason and a reasonable expectation of success for conducting the autocatalytic reaction PCR in plugs in a microfluidic system. EX1002, ¶¶177-79.

Claim Element	Obvious over Quake and Holliger
<p>1.[preamble] A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:</p>	<p><u>Quake discloses w/o droplets for conducting enzymatic reactions in a microfluidic system:</u></p> <p>“The microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region.” EX1005, [Abstract]; EX1030, Abstract.</p> <p>“[D]roplets of the sample fluid containing the biological material for analysis, reaction or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” EX1005, 8:1-5; EX1030, 8:16-20.</p> <p>“[T]he strength of the signal may indicate...a chemical reaction of a substrate catalyzed by an enzyme.” EX1005, 22:30-23:3; EX1030, 23:15-20; <i>see also</i> EX1005, 7:17-21; EX1030, 8:1-5.</p> <p><u>Quake teaches the enzymatic reaction may be PCR:</u></p> <p>“Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR ([EX1011])” EX1005, 23:26-27, 89:31; EX1030, 24:12-13, 90:13.</p>

	<p><u>Holliger discloses conducting autocatalytic PCR amplification in microscopic w/o droplets:</u></p> <p>“When combined in solution, both templates are amplified. When emulsified separately, prior to mixing, only PCR1 is amplified.” EX1006, 8:21-9:6.</p> <p>“PCR thermocycling then leads to the amplification of the polymerase genes.” EX1006, 18:3-7.</p> <p>“[P]olymerase chain reaction proceeded efficiently within the compartments of this water-in-oil composition,.... Average aqueous compartment dimensions in the water-in-oil emulsion...are on average 15µm [<2 picoliters] in size.” EX1006, 52:25-53:2; EX1002, chart following ¶179.</p>
--	--

b) 1.[a] providing the microfluidic system...

Quake discloses providing the microfluidic system comprising at least two channels having at least one junction, as recited in element 1.[a]. EX1005, 1:18-24, 6:4-8, 7:22-26, 11:17-21, 22:6-12 & Figs. 16A-B, 17C; EX1002, ¶180.

Claim Element	Obvious over Quake and Holliger
[1.a] Providing the microfluidic system comprising at least two channels having at least one junction;	<p>“A microfluidic device provided by the invention comprises a main channel and at least one inlet region which is in communication with the main channel at a droplet extrusion region.” EX1005, 6:4-6; EX1030, 6:17-19;</p>

“[T]he **micro fabricated substrate** has at least one main channel, an **inlet which meets the main channel at a droplet extrusion region**[.]” EX1005, 7:22-26; EX1030, 8:6-10; *see also* EX1005, 1:18-27; EX1030, 1:14-2:2.

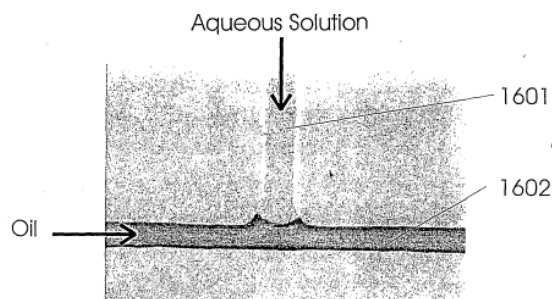


FIG. 16A

EX1005, Fig. 16A; EX1030, Fig. 16A; *see also* EX1005, 6:23-25, 79:23-31; EX1030, 7:6-10, 83:8-16.

c) 1.[b] flowing an aqueous fluid...

Quake in view of Holliger teaches claim element 1.[b]. Quake discloses flowing an aqueous fluid containing a substrate and/or reagents for conducting an enzymatic reaction through a first channel. EX1002, ¶¶181-83. For example, Quake discloses forming microfluidic w/o droplets at the junction by continuously flowing an aqueous fluid through a first channel into an oil continuously flowing in a second channel. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. Quake discloses that the aqueous fluid may comprise a biological sample (*e.g.*, DNA), a substrate, an enzyme, buffer, and/or reaction reagents. EX1005, 6:10-16, 8:14-23, 13:16-26, 15:18-21, 35:9-14. Quake teaches using the droplets formed

from the aqueous fluid for conducting enzyme-catalyzed bioreactions and suggests performing on-chip PCR. EX1005, 7:17-21, 8:1-5, 22:30-23:3, 23:26-27.

Holliger discloses forming microfluidic droplets from an aqueous fluid containing a substrate **and** reagents for conducting PCR. EX1006, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27. Holliger also teaches that PCR thermocycling activates polymerase amplification but that *Taq* polymerase is inactive at low temperature. EX1006, 18:3-7, 19:3-4, 21:23-22:23; 59:4-8, 75:20-26. As Dr. Meinhart explains, a POSA thus would have had good reason to combine the substrate and PCR reagents in Quake’s aqueous fluid before forming the w/o droplets because this was a well-known, simple and convenient way to proceed, as in Holliger, and the reaction would be initiating by thermocycling. EX1002, ¶183. Quake’s ability to generate generally monodisperse PCR droplets with high-throughput would have been very attractive to a POSA reading Holliger. EX1002, ¶¶168, 220.

Claim Element	Obvious over Quake and Holliger
[1.b] 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;	<p><u>Quake discloses forming the droplets by continuously flowing an aqueous fluid containing biological materials through the first channel:</u></p> <p>“The devices...comprise...at least one sample inlet channel, through which a pressurized stream of</p>

	<p>aqueous solution is passed.” EX1005, 1:18-24; EX1030, 1:14-17.</p> <p>“Fluid flows continuously through the system....” EX1005, 5:21-23; EX1030, 6:3-5.</p> <p>“The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample.” EX1005, 8:1-5; EX1030, 8:14-18.</p> <p><u>Holliger teaches the DNA substrate and PCR reagents are combined before forming the w/o droplets:</u></p> <p>“200 µl of PCR reaction mix (<i>Taq</i> expression plasmid (200ng), primers 3 and 4 (1µM each), dNTPs (0.25mM), <i>Taq</i> polymerase (10 units) is added dropwise (12 drops/min) to the oil phase (mineral oil (Sigma))...and PCR carried out[.]” EX1006, 59:10-16.</p>
--	--

d) 1.[c] flowing an oil...

Quake discloses continuously flowing an oil through the second channel as required by element 1.[c]. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16; EX1002, ¶184.

Claim Element	Obvious over Quake and Holliger
[1.c] Flowing an oil through the second channel of the at least two channels;	<p>“[T]he first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, mineral oil).” EX1005, 6:8-10; <i>see also id.</i>,</p>

	7:30-8:1, 8:12-13, 34:17-21; EX1030, 6:21-23, 8:14-16, 34:26-29. “Fluid flows continuously through the system...” EX1005, 5:21-23; EX1030, 6:3-5.
--	--

e) 1.[d] forming at least one plug...

Quake in view of Holliger teaches element 1.[d]. EX1002, ¶¶185-89. Quake discloses forming at least one plug by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16; *see also id.*, 19:26-32, 78:17-79:31, 81:31-82:8, Figs. 16-22 & accompanying text. As discussed above for element 1.[b], Quake also discloses that the aqueous fluid may contain a substrate molecule, enzyme, and reagents and teaches using the droplets formed from the aqueous fluid for conducting enzyme-catalyzed bioreactions. EX1005, 6:10-16, 7:17-21, 8:1-5, 8:14-23, 13:16-26, 15:18-21, 22:30-23:3 35:9-14. As discussed above for element 1.[b], Holliger discloses forming microfluidic droplets from an aqueous fluid already containing a substrate and reagents for conducting autocatalytic PCR and initiating PCR via thermocycling. EX1006, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27 (PCR mixture before droplet formation); *id.*, 18:3-7, 19:3-4, 21:23-22:23; 59:4-8, 75:20-26 (thermocycling initiates PCR). Holliger thereby provides good reason to combine the substrate and PCR reagents in Quake’s aqueous fluid before forming the w/o droplets at the junction as claimed. EX1002, ¶189

Claim Element	Obvious over Quake and Holliger
<p>[1.d] Forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels,</p>	<p><u>Quake discloses forming the droplets by partitioning the aqueous fluid with the flowing oil at the junction:</u></p> <p>“A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel....[A] pressure difference can be established between the two channels such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets.” EX1005, 1:21-27; EX1030, 1:17-2:2; <i>see also</i> EX1005, 6:23027, 78:18-19, 79:7-17; EX1030, 7:7-10, 82:23-83:4.</p> <div data-bbox="721 909 1263 1205" data-label="Image"> <p>The diagram shows a cross-section of a microfluidic device. A horizontal channel contains an 'Oil' stream, indicated by an arrow pointing from the left. A vertical channel from above introduces an 'Aqueous Solution' into the oil stream. The point of entry is labeled '1601'. As the aqueous solution enters the oil stream, it is sheared into small droplets, which are labeled '1602'.</p> </div> <p>FIG. 16A</p> <p><u>Holliger discloses forming microfluidic w/o droplets containing substrate molecule and reagents for conducting PCR:</u></p> <p>“200 µl of PCR reaction mix (<i>Taq</i> expression plasmid (200ng), primers 3 and 4 (1µM each), dNTPs (0.25mM), <i>Taq</i> polymerase (10 units) is added dropwise (12 drops/min) to the oil phase (mineral oil (Sigma))...and PCR carried out.... EX1006, 59:10-16.</p>

f) 1.[d.1] substantially surrounded by an oil...

Quake discloses the plug being substantially surrounded by the oil flowing through the channel, as required by claim element 1.[d.1]. EX1002, ¶¶190-191.

Quake depicts its droplets as being substantially surrounded by the oil flowing through the channel. EX1005, Figs. 17A-C, 19J, L. Quake explains that the droplets are separated from one another and from the channel walls by oil. *Id.*, Abstract, 5:1-12; *Id.*, 5:29-32, 7:6-15, 29:33-30:2, 36:9-28, 85:9-86:6 & Fig. 22.

As discussed above, Holliger confirms the reasonable expectation of success for PCR amplification in microscopic aqueous droplets that are substantially surrounded by oil. EX1006, 8:21-9:6, 47:16-24; EX1002, ¶191.

Claim Element	Obvious over Quake and Holliger
[1.d.1] the plug being substantially surrounded by an oil flowing through the channel,	<p>“[A]queous droplets are encapsulated or separated from each other by oil.” EX1005, 29:32-30:2; EX1030, 30:2-3.</p> <p>“[T]he surfactant in the extrusion fluid coats the channel walls.” EX1005, 28:24-29; EX1030, 28:21-26.</p>

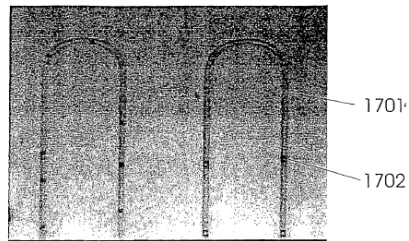


FIG. 17A

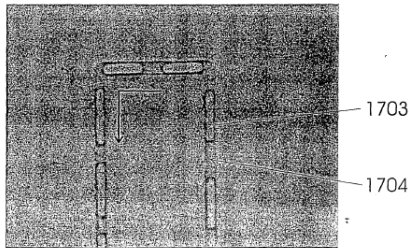


FIG. 17B

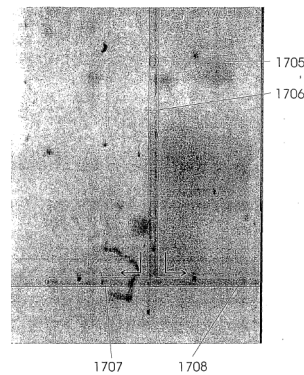


FIG. 17C

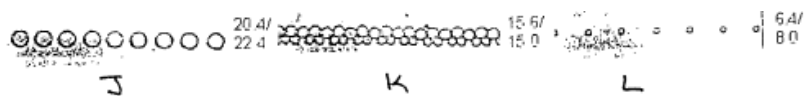


FIG. 19

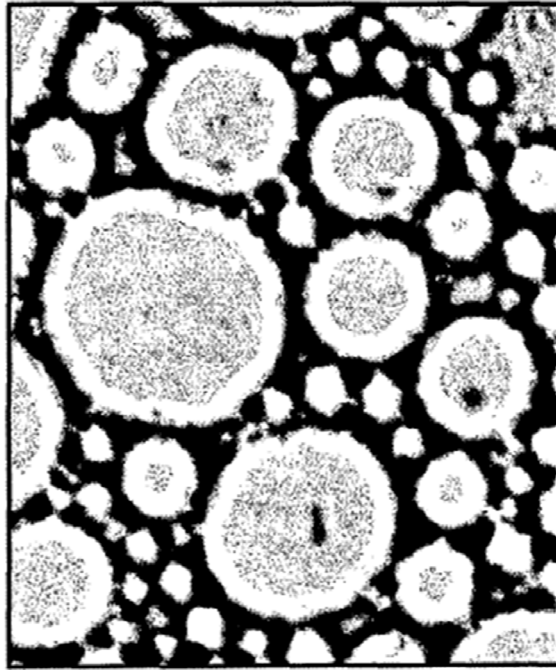
EX1005, Figs. 17A, 17B, 17C, 19J, 19L

Holliger's PCR droplets were substantially surrounded by oil:

“[T]he entire aqueous phase containing the biochemical components is compartmentalized in

discrete droplets (the internal phase).” EX1006, 47:16-24; *see also* EX1006, 59:10-16.

B



15.7 μ m

FIG. 2

EX1006, Fig. 2.

g) 1.[d.2] substrate molecule and reagents...

As discussed above for element 1.[b] and 1.[d] and included here, Quake in view of Holliger discloses the “at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule” as required by claim element 1.[d.2]. EX1002, ¶¶192-94.

h) 1.[e] providing conditions suitable...

As discussed above, the '193 patent specification discusses PCR at a high level. EX1002, ¶43. Its Provisional applications do not even mention PCR. EX1002, ¶¶43, 196. Thus, according to Patent Owner, a document need not detail specific conditions suitable for PCR to provide adequate disclosure for this claim element. EX1038, 0282, 0287-0289; EX1039, cover. The prior art references identified herein provide far more detail than what is contained in Patent Owner's own documents, and certainly disclose conditions suitable for PCR in the plugs such that the DNA molecule is amplified.

Holliger, for example, discloses successfully performing PCR amplification in sub-microliter water-in-oil droplets like Quake's. EX1006, 8:21-9:6, 9:12, 18:3-7, 49:20-27, 52:25-53:2, 59:4-23, 63:9-13 & Figs. 3-4, and 7. Holliger specifically discloses combining the nucleic acid substrate, the polymerase, and other PCR reagents in the aqueous solution *before* forming the droplets in oil. *Id.*, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27. Holliger also teaches how to perform PCR amplification in sub-microliter w/o droplets. *Id.*, 5:7-10, 5:19-23, 6:20-21, 10:20-25, 21:23-22:20, 25:24-26:22. Holliger discloses that mineral oil was a suitable oil and discloses suitable surfactants as well. *Id.*, 44:19-21. 47:7-48:5, 48:22-26. Holliger thus teaches claim element 1.[e] and demonstrates the successful

performance of PCR in microscopic w/o droplets formed in mineral oil, just as suggested by Quake. EX1002, ¶¶197-98.

Quake also discloses the conditions suitable for PCR amplification in the droplets and, indeed, provides more direction about PCR than the '193 patent itself by incorporating Kopp (EX1011) by reference. EX1002, ¶¶199-200; EX1005, 1:10-12, 23:26-30, 89:31. Quake, by incorporating Kopp, discloses that the aqueous droplet should contain at least one substrate molecule and PCR reagents, such as polymerase, primers, and dNTPs in aqueous buffer, to perform PCR. EX1011, 1046-47. It also discloses that PCR is performed by thermocycling. *Id.* As Dr. Meinhart confirms, the conditions suitable for PCR in the plug such that the DNA molecule is amplified were well-known in the art. EX1002, ¶200. Each of Quake and Holliger thus discloses element 1.[e]. EX1002, ¶¶195-201.

Claim Element	Obvious over Quake and Holliger
[1.e] providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.	<p>“Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR ([EX1011])” EX1005, 23:26-27, 89:31; EX1030, 24:12-13, 90:13.</p> <p>“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50°C to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.” EX1011, 1046.</p>

“Gene amplification techniques **requiring thermal cycling** such as PCR and LCR may also be used....” EX1006, 50:21-22.

“200 µl of **PCR reaction mix** (*Taq* expression plasmid (200ng), primers 3 and 4 (1µM each), dNTPs (0.25mM), *Taq* polymerase (10 units) **is added** dropwise (12 drops/min) **to the oil phase (mineral oil (Sigma))** in the presence of 4.5% (v/v) Span 80 (Fluka), 0.4% (v/v) Tween 80 (Sigma) and 0.05% (v/v) Triton X100 (Sigma) under constant stirring (1000rpm) in 2ml round bottom biofreeze vials (Costar, Cambridge MA)...Emulsified mixtures are then transferred to 0.5 ml thin-walled PCR tubes (100µl/tube) and **PCR carried out using 25 cycles of the profile 94 °C (1 min), 60 °C (1 min), 72 °C (3min)** after an initial 5 minute incubation at 94 °C. EX1006, 59:10-16.

“Suitable oils include **light white mineral oil** and **non-ionic surfactants**...(SpanTM80, ICI) and...(TweenTM 80; ICI)....” EX1006, 47:25-48:5.

“**[PCR] proceeded efficiently within the compartments of this water-in-oil composition**, to approach the rates observed in solution PCR.” EX1006, 52:25-53:2.

“**PCR1 is amplified.**” EX1006, 8:21-9:6.

“Emulsified mixtures are then transferred...and PCR carried out....**Amplified product** is visualized...]” EX1006, 59:16-21

“Example 9. Standard PCR in Aqueous Compartments Within an Emulsion

To establish whether **conditions** in the aqueous compartment present in an emulsion are **permissive for catalysis, a standard reaction mix is emulsified and PCR carried out. This leads to amplification....**” EX1006, 63:9-13.

As discussed above, Quake in view of Holliger discloses each element of claim 1 in the claimed configuration and renders claim 1 obvious. EX1002, ¶¶175-202.

ii. Claim 2

As discussed above and included here, Quake in view of Holliger renders claim 1 obvious. *See* Section IX.A.i above. Quake also discloses the substrate molecule is a single biological molecule. EX1005, 5:7-8, 6:16-21, 8:5-8, 9:5-9, 36:29-37:28.; EX1002, ¶203. Holliger provides relevant disclosure and corresponding motivation to do so by teaching that emulsion PCR (ePCR) was useful for “Digital PCR” and that each w/o droplet preferably contains substantially one nucleic acid member. EX1006, 12:21-26, 13:30-14:1. Accordingly, Quake in view of Holliger renders claim 2 obvious. EX1002, ¶203; *see also* Section VIII.A above.

Claim Element	Obvious over Quake and Holliger
2. The method of claim 1, wherein the at least one substrate molecule is a single biological molecule.	<p><i>See</i> discussion of claim 1 above.</p> <p>“[T]he sample concentration should be dilute enough that most of the droplets contain no more than a single molecule...with only a small statistical chance that a droplet will contain two or more molecules....This is to ensure that...the level of reporter measured in each droplet...corresponds to a single molecule....” EX1005, 36:29-37:4; EX1030, 37:12-19.</p> <p>“In preferred embodiments the second fluid includes a biological sample that comprises one or more molecules, cells, virions or particles. In exemplary</p>

	<p>embodiments for detecting and sorting droplet contents, the droplets of the second fluid each contains, on average, no more than one particle.” EX1005, 6:16-20; EX1030, 6:29-7:5;</p> <p>“ePCR)...may also have applications for large-scale diagnostic PCR applications like ‘Digital PCR[.]’” EX1006, 12:21-26.</p> <p>“In preferred embodiments, each compartment contains substantially one nucleic acid member....” EX1006, 13:30-14:1.</p>
--	---

iii. Claim 3

As discussed above and included here, Quake in view of Holliger addresses PCR in detail and renders claim 2 obvious. *See* Section IX.A.i-ii above. Quake and Holliger also each disclose the substrate molecule is DNA and the autocatalytic reaction is PCR. *See, e.g.*, EX1005, 2:29-3:6, 23:26-27, 89:31; EX1006, 5:19-20, 8:5-13, 8:21-9:6, 12:21-26, 18:3-7, 21:23-22:20, 25:24-26:22, 50:21-22, 52:25-53:2, 59:10-16, 59:16-21, 63:9-13. Accordingly, Quake in view of Holliger renders claim 3 obvious. EX1002, ¶204; *see also* Section VIII.A above.

Claim Element	Obvious over Quake and Holliger
3. The method of claim 2, wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.	<p><i>See</i> discussion of claim 2 above.</p> <p><i>See</i> discussion of claim element 1.[e] above.</p> <p>“PCR is also generally the method of choice to detect viral DNA or RNA directly in clinical specimens.” EX1005, 2:31-32; EX1030, 3:1-14.</p> <p>“Microfabrication permits other technologies to be integrated..., such as PCR” EX1005, 23:26-27,</p>

	<p>89:31 (citing and incorporating EX1011); EX1030, 24:12-13, 90:13; EX1011, 1046-47.</p> <p>“Two standard PCR reactions, differing in template size... PCR1 is amplified.” EX1006, 8:21-9:6.</p> <p>“PCR thermocycling then leads to the amplification of the polymerase genes by the polypeptides they encode....” EX1006, 18:3-7; <i>see also</i> EX1006, 63:9-13.</p> <p>“amplification of nucleic acid by methods such as...(PCR)” EX1006, 21:23-22:20.</p> <p>“200 µl of PCR reaction mix (<i>Taq</i> expression plasmid (200ng)...and PCR carried out.... EX1006, 59:10-16.</p>
--	---

iv. Claim 4

As discussed above and included here, Quake in view of Holliger renders claim 1 obvious. *See* Section IX.A.i above. Heating was a well-known aspect commonly included in PCR. *See* Section VIII.A above. Quake and Holliger also each disclose the providing step includes heating. For example, Quake cites and incorporates by reference Kopp for its discussion of microfluidic PCR. EX1005, 23:26-27, 89:31 (citing EX1011). Kopp discloses that PCR is “simply performed by heating and cooling” and gives optimal temperatures for each step. EX1011, 1046. Holliger also discloses that PCR requires thermal cycling and uses temperatures within the ranges provided in Kopp to successfully perform PCR

DNA amplification in microfluidic w/o droplets. EX1006, 50:21-22, 59:10-16.

Accordingly, Quake in view of Holliger renders claim 4 obvious. EX1002, ¶¶205-06; *see also* Section VIII.A above.

Claim Element	Obvious over Quake and Holliger
4. The method of claim 1, wherein the providing step includes heating.	<p><i>See</i> discussion of claim 1 above.</p> <p><i>See</i> discussion of claim element 1.[e] above.</p> <p>EX1005, 23:26-27, 89:31 (citing and incorporating EX1011); EX1030, 24:12-13, 90:13.</p> <p>“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.” EX1011, 1046.</p> <p>“Gene amplification techniques requiring thermal cycling such as PCR and LCR may also be used....” EX1006, 50:21-22.</p> <p>“PCR carried out using 25 cycles of the profile 94 °C (1 min), 60 °C (1 min), 72 °C (3min) after an initial 5 minute incubation at 94 °C. EX1006, 59:10-16.</p>

v. *Claim 5*

As discussed above and included here, Quake in view of Holliger renders claim 1 obvious. *See* Section IX.A.i above. Quake discloses the additional element of claim 5. For example, Quake discloses the microfluidic device comprises an

optical detector downstream of the junction to detect a signal created when a substrate reacts with an enzyme within the droplets. EX1005, 6:28-31, 7:15-21, 15:2-6, 22:27-23:3, 32:1-34:11, 17:3-25, 49:13-25, 50:7-23, 51:13-21, 52:6-21. Quake in view of Holliger thus renders claim 5 obvious. EX1002, ¶¶207-09; *see also* Section VIII.A above.

Claim Element	Obvious over Quake and Holliger
<p>5. The method of claim 1, further comprising the step of providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.</p>	<p><i>See</i> discussion of claim 1 above.</p> <p>“The device may also have a detector...associated within the detection region.” EX1005, 6:28-31, 7:19-21; EX1030, 7:11-14, 8:3-5.</p> <p>“[T]he first biological material may be an enzyme and the second biological material may be a substrate for the enzyme. The interaction of the first and second biological materials may produce a signal that can be detected, e.g., as the droplet passes through a detection region associated with the device.” EX1005, 7:17-21; EX1030, 8:1-5.</p> <p>“As each droplet passes into the detection region, it is examined for a predetermined characteristic (<i>i.e.</i>, using the detector) and a corresponding signal is produced....That is, the amount of the signal can be measured....For example, the strength of the signal may indicate...a chemical reaction of a substrate catalyzed by an enzyme.” EX1005, 22:27-23:3; EX1030, 23:12-20.</p>

vi. *Claim 7*

As discussed above and included here, Quake in view of Holliger renders claim 1 obvious. *See* Section IX.A.i above. Quake teaches the oil carrier (extrusion) fluid comprises a surfactant. EX1005, 8:15-18, 9:4-5; 28:24-29, 35:18-24. Quake teaches using a surfactant in the oil to form the w/o droplets. EX1005, 35:18-24, 35:27-29. Quake also discloses using the surfactant in the carrier oil to coat the channel walls. EX1005, 28:6-29, 35:18-24, 35:27-29. Quake’s exemplary surfactants include Tween, Span, and fluorinated oil surfactants *Id.*, 28:7-29, 35:18-24, 35:27-29, 82:2-3. Holliger discloses successful PCR amplification in w/o droplets formed using Tween and Span surfactants. EX1006, 47:25-48:5, 52:7-15. Quake in view of Holliger thus renders obvious claim 7 as a whole. EX1002, ¶¶210-211; *see also* Section VIII.A above.

Claim Element	Obvious over Quake and Holliger
7. The method of claim 1, wherein the carrier fluid further comprises a surfactant.	<p><i>See</i> discussion of claim 1 above.</p> <p>“In one particularly preferred embodiment, the extrusion fluid is an oil...[that] contains a surfactant.... [A] user preferably causes the extrusion fluid to flow through channels of the microfluidic device so that the surfactant in the extrusion fluid coats the channel walls.” EX1005, 28:24-29; EX1030, 28:21-26; <i>see also</i> EX1005, 28:1-6; EX1030, 28:21-26.</p>

	<p>“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.” EX1005, 35:18-24, 35:27-29; <i>see also id.</i>, 8:15-18, 9:2-5, 28:7-29; EX1030, 28:21-23, 35:28-36:5, 36:8-10)</p> <p>“The emulsion may be stabilized by addition of one or more surface-active agents (surfactants). These surfactants are termed emulsifying agents and act at the water/oil interface.... Suitable oils include light white mineral oil and non-ionic surfactants...such as...(SpanTM80, ICI) and ...(TweenTM 80; ICI)....” EX1006, 47:25-48:5.</p> <p>“The water-in-oil emulsion is made by adding an aqueous phase dropwise to an oil phase in the presence of a surfactant....” EX1006, 52:7-10; <i>see also</i> EX1006, 59:10-16.</p>
--	--

vii. *Claims 6 and 8*

As discussed above and included here, Quake in view of Holliger renders obvious each of claims 1 and 7. *See* Section IX.A.i, IX.A.vi above. As discussed above for claim 7, Quake discloses the surfactant may be a fluorinated oil and that the surfactants may be used in the carrier oil to coat the channel walls and to form droplets. EX1005, 28:6-29, 35:18-24, 35:27-29. Quake thus discloses the method of claim 7 wherein the surfactant is fluorinated surfactant, as required by claim 8. EX1002, ¶212; *see also* Section VIII.A above.

As Dr. Meinhart explains, a POSA would have had good reason to use a fluorinated oil as the carrier fluid when using a fluorinated oil surfactant, as disclosed by Quake, because fluorinated oils are chemically compatible with fluorinated surfactants and because fluorinated oils were known to be biologically compatible and to have favorable chemical properties for forming w/o emulsions. EX1002, ¶¶212-216; EX1017, 209, 212-213; EX1018, Summary; EX1023, Abstract, 18, 21, 89-92, 147-48; *see* Section VIII.A above. Accordingly, Quake in view of Holliger renders each of claims 6 and 8 obvious.

Claim Element	Obvious over Quake and Holliger
<p>Claim 6. The method of claim 1, wherein the oil is fluorinated oil.</p> <p>Claim 8. The method of claim 7, wherein the surfactant is fluorinated surfactant.</p>	<p><i>See</i> discussion of claims 1 and 7 above.</p> <p>“Exemplary surfactants include ... fluorinated oils.” EX1005, 35:18-24, 35:27-29; <i>see also id.</i>, 8:15-18, 9:2-5, 28:7-29; EX1030, 28:21-23, 35:28-36:5, 36:8-10.</p>

viii. Claim 9

As discussed above and included here, Quake in view of Holliger renders claim 1 obvious. *See* Section IX.A.i above. Quake discloses the w/o droplets can be combined with other droplets to facilitate a reaction or analysis, such as to add additional reagents to the droplets. EX1005, 5:11-12, 7:15-17, 15:22-25, 36:9-28,

85:9-86:6; EX1002, ¶217. Accordingly, Quake in view of Holliger renders claim 9 obvious. *Id.*; *see also* Section VIII.A above.

Claim Element	Obvious over Quake and Holliger
9. The method of claim 1, wherein the at least one plug is a merged plug.	<p><i>See</i> discussion of claim 1 above.</p> <p>“These droplets can be analyzed, combined with other droplets (e.g. to react droplet contents) and/or sorted, as desired.” EX1005, 5:11-13; EX1030, 5:21-22,16; <i>see also</i> EX1005, 7:15-17, 15:22-25, 36:21-28, 85:9-86:6; EX1030, 7:22-8:1, 16:4-7, 37:3-5, 84:28-85:10.</p>

ix. Claim 11

As discussed above and included here, Quake in view of Holliger renders claim 1 obvious. *See* Section IX.A.i above. Quake discloses using substantially spherical plugs in rounded channels. EX1005, 26:30-27:3, 27:9-32 & Figs. 17, 19. Quake Figures 17A, C and 19J, L disclose making monodisperse, round droplets, and Quake teaches these round droplets are preferred, especially in high throughput devices. *Id.*, 27:27-32. Holliger demonstrated successful PCR amplification in substantially spherical w/o droplets such as those disclosed in Quake. EX1002, ¶221; EX1006, Fig. 2. Accordingly, Quake in view of Holliger renders claim 11 obvious. EX1002, ¶¶218-22; *see also* Section VIII.A above.

As discussed above, Quake in view of Holliger renders obvious each of claims 1-5, 7, 9, and 11. EX1002, ¶¶160-223.

Claim Element	Obvious over Quake and Holliger
<p>11. The method of claim 1, wherein the at least one plug is substantially spherical in shape.</p>	<p>See discussion of claim 1 above.</p> <p>“[D]roplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes.... ¶More preferably, however, the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19).” EX1005, 27:9-32, Fig. 19; EX1030, 28:19-20, Fig. 17A.</p> <p>“[T]he channels of the device are preferably round....This geometry facilitates an orderly flow of droplets in the channels.” <i>See e.g.</i> FIG. 16B.” EX1005, 26:30-27:3; <i>see</i> EX1030, 27:22-27.</p> <p><i>See also</i> EX1005, Figs. 17A, 17C, 19J, 19L & accompanying text.</p> <div data-bbox="816 1157 1218 1394" data-label="Image"> <p>The image shows a grayscale micrograph of two parallel channels. Each channel has a rounded, semi-circular top and a narrow, straight bottom section. The left channel is labeled with the reference numeral 1701, and the right channel is labeled with 1702. The channels appear to be part of a larger microfluidic device structure.</p> </div> <p>FIG. 17A</p>

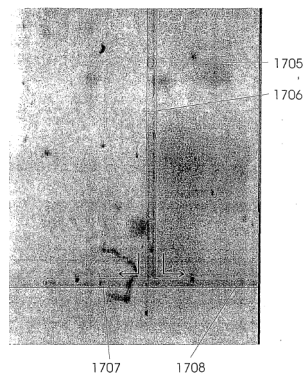


FIG. 17C

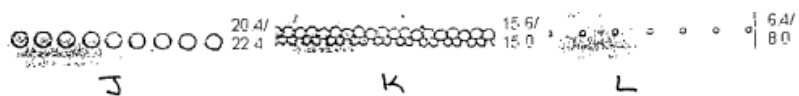
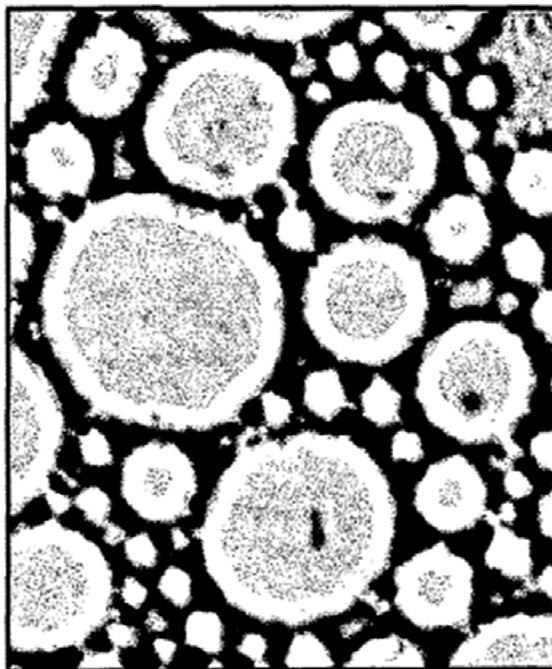


FIG. 19

B



15.7 μ m

FIG. 2

B. Ground 2: Claims 1-9 and 11 Are Obvious Over Quake and Holliger In Further View of Shaw Stewart

As discussed above in Ground 1 and included here, Quake and Holliger render obvious the method of claims 1-9 and 11. *See* Sections IX.A above; EX1002, ¶224. To the extent PO argues need for fluorinated oil, Shaw Stewart provides additional teachings demonstrating obviousness. For example, Shaw Stewart teaches using a fluorinated oil as a carrier fluid for forming aqueous droplets containing biological materials for biological or chemical assays. EX1008, Abstract, 2-4, 8; EX1002, ¶¶225-26. It states, for example: “This reactant liquid, hereafter referred to as a reactant, will be supported, defined and moved by another, immiscible liquid, referred to hereafter as the carrier phase. **Suitable carrier phases include** mineral oils, light silicon oils, water, and **fluorinated hydrocarbons.**” EX1008, 4:3-7. By disclosing fluorinated oils as a viable alternative to using mineral oil as the immiscible carrier fluid for forming microfluidic droplets for biological materials, Shaw Stewart provided a POSA with good reason to use a fluorinated oil as the carrier fluid for forming Quake’s PCR droplets. EX1002, ¶¶227-28; *see also* Section VIII.A above (discussing suitable properties of fluorinated oils and surfactants for biological w/o emulsions). Shaw Stewart thus provides additional evidence supporting the obviousness of the challenged claims with respect to their limitations about providing conditions

suitable for autocatalytic amplification reactions in the droplet (all claims), PCR (claim 3), fluorinated oil (claim 6), and fluorinated surfactant (claim 8).

As Dr. Meinhart explains, a POSA would have had a reasonable expectation of success for using a fluorinated oil as the carrier fluid, including with a fluorinated surfactant, for PCR droplet microreactors because fluorinated oils were known to be compatible with biological materials, to be sufficiently hydrophobic to encapsulate aqueous droplets when the aqueous solution is flowed into the flowing fluorinated oil, and because fluorinated surfactants were known to be indicated for use with fluorinated oils. EX1002, ¶228-29; EX1017, 209, 212-213; EX1018, Summary; EX1023, Abstract, 2, 18, 21, 89-92, 147-48.

Accordingly, each of claims 1-9 and 11 as a whole would have been obvious over Quake and Holliger in further view of Shaw Stewart. EX1002, ¶223-28; *see also* Section VIII.A above.

C. Ground 3: Claims 1-9 and 11 Are Obvious Over Quake in further view of Litborn

1) Motivation to Combine and Reasonable Expectation of Success

As discussed here and throughout this Petition, a POSA would have had good reason, with a reasonable expectation of success, for arriving at the '193 patent claimed subject matter in view of the prior art.

As an initial matter, a POSA at the critical date would reasonably have looked to Quake and Litborn in the context of performing PCR in w/o droplets in a

microfluidic device. Quake and Litborn create microscopic water-in-oil (w/o) droplets and are directed to performing enzymatic reactions (including specifically PCR) in the droplets. EX1002, ¶¶230-34; *see* Section X.A (discussing Quake), Sections VIII.B.iii above and IX.C.i below (discussing Litborn). Like other scientific literature available at the time, Litborn's disclosure that PCR amplification was successful in nanoscale aqueous droplets formed in standard hydrocarbons flowing through a microfluidic channel confirms that this approach would have been expected to work. EX1002, ¶234; EX1007, 447, 451-53; *see also* EX1009, 109, 120; EX1006 (performing PCR in sub-microliter aqueous droplets). Litborn teaches w/o droplets help to address cross-contamination between reactions and also provides "effective mixing" within each reaction. EX1007, 451-53; EX1002, ¶234. Quake and Litborn thus individually and collectively teach microscopic w/o droplets were suitable and useful for performing PCR analyses.

Additionally, the cited references together disclose all aspects of the challenged claims and expressly contemplate performing PCR in the w/o droplets in a microfluidic device, as claimed. EX1002, ¶¶235, 245-287; *KSR*, 550 U.S. at 418-19 (TSM not required but useful). As discussed above in Section X.A and included here, Quake teaches the claimed elements for forming the droplets, specifically teaches its microfluidic devices may be used to conduct on-chip PCR, and incorporated Kopp, which discloses thermocycling. EX1002, ¶¶231-33;

EX1005, 23:26-27, 89:31; EX1011, 1046-47. Litborn teaches successful PCR amplification of DNA in microfluidic aqueous droplets in standard hydrocarbons similar to those used in Quake. EX1007, 447, 451-53; *see also* EX1009, 109, 120. Litborn discloses the “pre-mixed” PCR reaction mixture is preferably included in the aqueous fluid before droplet formation, after which the PCR amplification reaction is induced by thermocycling. EX1007, 451 (“simplest version”). EX1002, ¶235. A POSA thus would have good reason to combine the teachings of Quake and Litborn to successfully achieve PCR amplification in the droplets. EX1002, ¶¶235.

Furthermore, a POSA would have viewed the combined teachings of Quake and Litborn as a reasonable and convenient approach to performing PCR, consistent with the scientific literature at the time. EX1002, ¶¶236-38. Quake broadly teaches the aqueous fluid used to form the droplets may contain enzyme substrate and reactants. EX1005, 6:11-15, 8:14-22. Litborn explains that using a pre-mixed aqueous solution and initiating the reaction by heating was the “simplest version.” EX1007, 451. Litborn thus exemplifies a known practice for PCR reactions of using a pre-mixed aqueous solution containing the substrate, enzyme, and the remaining reagents to form the droplets. EX1002, ¶¶235-37. This practice is corroborated by Holliger (EX1006), discussed above. In view of Litborn’s disclosure of this well-known, simple, and convenient way to perform PCR in

microscopic droplets, a POSA would have had good reason to do the same when forming Quake's droplets. *Id.*; *see also* Sections IX.C.i.a, c, e, h below.

In addition, a POSA would have viewed Quake as providing technical benefits for droplet formation. Quake formed uniformly-sized (monodisperse) droplets with high throughput at the microchannel junction. EX1005, 1:18-24, 5:6-7, 5:21-23, 6:4-8, 6:23-27, 7:30-8:5, 11:20-12:12, 22:6-16, 27:27-32 & Figs 17A, C and 19J, L; EX1002, ¶¶124, 162-64, 220, 237. Litborn specifically contemplated performing droplet PCR at "massive scale" in the sub-microliter w/o droplets. EX1007, 453; *see also* EX1006, 12:21-26 (high-throughput w/o droplet ePCR desirable for digital PCR and multiplex PCR). Schneegaß, suggested using "T-shaped injector channel" to form microfluidic w/o PCR droplets precisely to increase throughput. EX1009, 109. Moreover, it was well-known that w/o droplet PCR helped to reduce cross-talk between PCR samples. EX1002, ¶¶108, 124; EX1009, 108; EX1005, 5:1-12, 5:25-27. As Dr. Meinhart explains, a POSA would have good reason to make w/o droplets at the microchannel junction as disclosed in Quake to create consistently-sized droplets (and thus comparable sample concentrations) at high throughput. EX1002, ¶¶239, 286.

Moreover, the fact that multiple prior art references (including, *inter alia*, Quake and Litborn) describe PCR analysis in microscopic w/o droplets further underscores that a POSA reasonably would expect to successfully arrive at the

'193 patent claimed subject matter in view of the prior art. *See, e.g.*, discussion above; Section VIII. Like other scientific literature available at the time, Litborn's disclosure that PCR amplification was successful in sub-microliter aqueous droplets formed in standard hydrocarbon oil confirms that this approach would have been expected to work. EX1002, ¶240; *see also* Section VIII.A; EX1006, EX1009 (performing PCR in sub-microliter aqueous droplets). As Dr. Meinhart explains, a POSA had a reasonable expectation of success whether thermocycling was performed in a microfluidic reaction chamber or performed via continuous flow. EX1002, ¶¶174, 241-43; EX1006, 8:21-9:6, 52:25-53:2, 59:16-21, 63:9-13; EX1009, 109.

The discussion below provides further claim-by-claim analysis demonstrating that claims 1-9, and 11 are obvious over Quake in view of Litborn. EX1002, ¶244.

i. Claim 1

a) 1.[preamble] A method for...

Quake in view of Litborn discloses a method for conducting an autocatalytic reaction in plugs in a microfluidic system. As discussed above in Section IX.A.i.a and included here, Quake discloses a microfluidic device for analyzing, sorting, and/or reacting biological materials, including specifically enzymatic reactions and reactions on DNA polynucleotides, in w/o droplets, and suggests performing PCR,

which is an autocatalytic reaction, in its microfluidic system. EX1002, ¶¶245-43; *see also* Section VIII.A above.

Litborn discloses successfully performing PCR amplification in sub-microliter scale w/o droplets like Quake's. EX1002, ¶¶247-48; EX1007, 447, 451-53 & Fig. 7; *see also* EX1009, 109, 120. Regarding PCR amplification in these w/o droplets, Litborn reports that "[a]mplification was successful, and the results were fully comparable with the results of the commercial standard procedure." *Id.*, 453. In view of Litborn's disclosures, a POSA would have had good reason and a reasonable expectation of success for conducting PCR amplification in plugs in a microfluidic system. EX1002, ¶¶247-48.

Claim Element	Obvious over Quake and Litborn
1.[preamble]:	<p><i>See</i> discussion of Quake's disclosures for claim element 1.[preamble] in Ground 1 above.</p> <p>"A droplet of water in a continuous flow of a hydrocarbon in a hydrophobized channel....(Figure 7).</p>

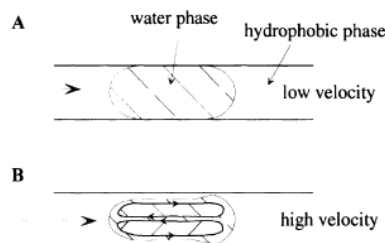


Figure 7: A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at a high velocity.

...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....[T]**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.” EX1007, 452-53 & Fig. 7.

b) 1.[a] providing the microfluidic system...

As discussed above in Section IX.A.i.b and included here, Quake discloses providing the microfluidic system comprising at least two channels having at least one junction. *See* discussion of Quake’s disclosures for claim element 1.[a] in Ground 1 above; EX1002, ¶249.

c) 1.[b] flowing an aqueous fluid...

Quake in view of Litborn teaches claim element 1.[b]. EX1002, ¶¶250-52. As discussed above in Section IX.A.i.c and included here, Quake discloses continuously flowing an aqueous fluid containing a substrate and/or reagents for conducting an enzymatic reaction through a first channel. EX1002, ¶250.

Litborn discloses forming microfluidic droplets from an aqueous fluid containing a substrate and reagents for conducting PCR. For example, Litborn describes formation of “a **droplet of water** in a continuous flow of a hydrocarbon in a hydrophobized channel...at high velocities.” *Id.*, 452 (emphasis added) & Fig. 7. Litborn teaches the PCR reaction mixture is preferably included in the aqueous fluid **before** droplet formation, after which the PCR amplification reaction is induced by thermocycling. EX1007, 451 (“simplest version”). Litborn thus motivates combining the substrate and PCR reagents in the aqueous solution **before** w/o droplet formation as a straightforward, simple, and convenient way to perform PCR. EX1002, ¶¶251-52.

Claim Element	Obvious over Quake and Litborn
[1.b]	<p>See discussion of Quake’s disclosures for claim element 1.[b] in Ground 1 above.</p> <p>“The simplest version of a fluidic reactor is the transport of a pre-mixed sample through a channel...” EX1007, 451.</p> <p>“A droplet of water in a continuous flow of a hydrocarbon...well separated from an adjacent water droplet....”</p>

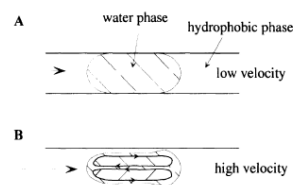


Figure 7: A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at a high velocity.

....the reaction mixture is interspaced with a hydrocarbon, as described above.” EX1007, 453 & Fig. 7.

d) 1.[c] flowing an oil...

As discussed above in Section IX.A.i.d and included here, Quake discloses continuously flowing an oil through the second channel. See discussion of Quake’s disclosures for claim element 1.[c] in Ground 1 above; EX1002, ¶253.

e) 1.[d] forming at least one plug...

As discussed above in Section IX.A.i.e and included here, Quake teaches forming at least one plug of the aqueous fluid containing the at least one substrate molecule and/or reagents for an enzymatic reaction by partitioning the aqueous fluid with the continuously flowing oil at the junction of the at least two channels. See discussion of Quake’s disclosures for claim element 1.[e] in Ground 1 above; EX1002, ¶¶254-56. As discussed for element 1.[b] in Ground 3 and included here, Litborn discloses forming microfluidic droplets from an aqueous fluid already containing a substrate and reagents for conducting autocatalytic PCR and initiating

PCR via thermocycling. *See* claim element 1.[b] above; EX1002, ¶¶257-58. Quake in view of Litborn thus teach claim element 1.[d].

f) 1.[d.1] substantially surrounded by an oil...

As discussed above in Section IX.A.i.f and included here, Quake discloses the plug being substantially surrounded by an oil flowing through the channel.

EX1002, ¶256. Litborn discloses successful PCR amplification in microfluidic droplets that are substantially surrounded by an oil flowing through the channel.

EX1007, 451-53 & Fig. 7. Quake and Litborn thus disclose claim element 1.[d.1].

EX1002, ¶¶259-60.

Claim Element	Obvious over Quake and Litborn
[1.d.1]	<p>See discussion of Quake's disclosures for claim element 1.[d.1] in Ground 1 above.</p> <p>“ A droplet of water in a continuous flow of a hydrocarbon in a hydrophobized channel will keep well separated from an adjacent water droplet, even at high velocities....[T]he water droplet detaches from the surface while the hydrocarbon creates a layer between the channel surface and the droplet (Figure 7).</p> <div data-bbox="860 882 1153 1071"> <p>The figure consists of two schematic diagrams, A and B, illustrating the transport of a water droplet in a hydrophobic solvent. Diagram A, labeled 'low velocity', shows a water droplet (labeled 'water phase') in contact with a hydrophobic surface (labeled 'hydrophobic phase'). Diagram B, labeled 'high velocity', shows the same water droplet detached from the surface, with a layer of hydrocarbon (labeled 'hydrocarbon') between the droplet and the surface. Arrows indicate the direction of flow in both diagrams.</p> </div> <p>Figure 7: A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at a high velocity.</p> <p>Thus, there is no more surface contact of the water (and no more risk for surface interactions).” EX1007, 452-53 & Fig. 7.</p>

g) 1.[d.2] plug comprises at least one substrate molecule and reagents...

As discussed above for elements 1.[b] and 1.[d], Quake in view of Litborn discloses the plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule as required by claim element 1.[d.2]. EX1002, ¶¶261-63.

h) 1.[e] providing conditions suitable...

As discussed above in Section IX.A.i.h, Patent Owner has asserted that a document that references PCR without detailing specific conditions suitable for PCR provides adequate disclosure for this claim element. EX1002, ¶¶43, 196. The references identified herein provide far more disclosure than what is contained in Patent Owner's own documents of conditions suitable for PCR in the plugs such that the DNA molecule is amplified. EX1002, ¶¶264-67.

Litborn, for example discloses providing conditions suitable for the autocatalytic reaction in the plug such that the substrate molecule is amplified. EX1002, ¶¶266. Litborn teaches successful nanoscale PCR amplification reactions in aqueous droplets in standard hydrocarbons (*e.g.*, octane) flowing through a microfluidic channel. EX1007, 447, 451-53 & Fig. 7; *see also* EX1009, 109, 120. Litborn reports that “[a]mplification was successful, and the results were fully comparable with the results of the commercial standard procedure.” *Id.*, 453. Litborn states 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.” *Id.*, 453. Litborn teaches the reaction proceeds simply by transporting the pre-mixed sample through a channel having the desired conditions, “*e.g.*, an elevated temperature zone”). EX1007, 451. Litborn thus teaches providing

conditions suitable in the plug for an autocatalytic reaction such that the DNA template is amplified.

As discussed above in Section IX.A.i.h, Quake also discloses the conditions suitable for PCR amplification in the droplets and, indeed, provides more direction about PCR than the '193 patent itself by incorporating Kopp (EX1011) by reference. EX1005, 1:10-12, 23:26-30, 89:31; EX1011, 1046-47. As Dr. Meinhardt confirms, conditions suitable for PCR in the plug such that DNA molecule is amplified were well-known in the art. EX1002, ¶¶196, 265-66. Each of Quake and Litborn thus discloses element 1.[e]. EX1002, ¶¶264-67.

Claim Element	Obvious over Quake and Litborn
[1.e]	<p><i>See discussion of Quake's disclosures for claim element 1.[e] in Ground 1 above.</i></p> <p>“4. Flow-based reactors Microfluidic systems also offer exciting possibilities for nanoscale reactions. The simplest version of a fluidic reactor is the transport of a pre-mixed sample through a channel, which has the desired reaction conditions, e.g., an elevated temperature zone....” EX1007, 451.</p> <p>“A droplet of water in a continuous flow of a hydrocarbon in a hydrophobized channel will keep well separated from an adjacent water droplet...[and] the hydrocarbon creates a layer between the channel surface and the droplet (Figure 7).</p>

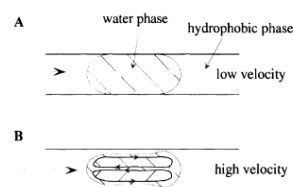


Figure 7: A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at high velocity.

....An additional benefit of this situation is that the friction between the hydrocarbon and the water leads to an intrasegmental movement within the droplet, which means **that an effective mixing action is obtained.**

...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...."**
EX1007, 452-53 & Fig. 7.

As discussed above, Quake in view of Litborn discloses each and every element of claim 1 and renders claim 1 obvious as a whole. EX1002, ¶268.

ii. Claim 2

As discussed above and included here, Quake in view of Litborn renders claim 1 obvious. *See* Section IX.C.i above. As discussed in Section IX.A.ii and included here, Quake also discloses the substrate molecule is a single biological molecule. Accordingly, Quake in view of Litborn renders claim 2 obvious.

EX1002, ¶269; *see also* Section VIII.A above.

iii. Claim 3

As discussed above and included here, Quake in view of Litborn addresses PCR in detail and renders claim 2 obvious. *See* Section IX.C.ii above. Quake and

Litborn also each disclose the substrate molecule is DNA and the autocatalytic reaction is PCR. *See, e.g.*, EX1005, 2:29-3:6, 23:26-27, 89:31; EX1007, 447, 451-53; *see also* EX1009, 109, 120 (Litborn “reported a successful DNA amplification”). Accordingly, Quake in view of Litborn renders claim 3 obvious. EX1002, ¶270; *see also* Section VIII.A above.

iv. Claim 4

As discussed above and included here, Quake in view of Litborn renders claim 1 obvious. *See* Section IX.C.i above. Heating was a well-known aspect commonly included in PCR. *See* Section VIII.A above. Quake and Litborn also each disclose the providing step includes heating. For example, Quake cites and incorporates by reference Kopp for its discussion of microfluidic PCR, which includes heating steps. EX1005, 23:26-27, 89:31 (citing EX1011); EX1011, 1046. Litborn also discloses that PCR employs heating. EX1007, 451 (“elevated temperature zone”). Accordingly, Quake in view of Litborn renders claim 4 obvious. EX1002, ¶¶271-72; *see also* Section VIII.A above.

v. Claim 5

As discussed above and included here, Quake in view of Litborn renders claim 1 obvious. *See* Section IX.C.i above. As discussed in Section IX.A.v and included here, Quake discloses providing a detector to detect, analyze, characterize and monitor one or more properties of the reaction during and after it has occurred.

Accordingly, Quake in view of Litborn renders claim 5 obvious. EX1002, ¶¶273-75; *see also* Section VIII.A above.

vi. Claim 7

As discussed above and included here, Quake in view of Litborn renders claim 1 obvious. *See* Section IX.C.i above. As discussed in Section IX.A.vi and included here, Quake teaches the oil carrier (extrusion) fluid comprises a surfactant. Quake in view of Litborn thus renders obvious claim 7 as a whole. EX1002, ¶¶276-77; *see also* EX1009, 110 (using Tween surfactant for flow-through droplet PCR to stabilize the sample droplets in the oil flow and to simultaneously stabilize the polymerase during PCR); Section VIII.A above.

vii. Claims 6 and 8

As discussed above and included here, Quake in view of Litborn renders obvious each of claims 1 and 7. *See* Sections IX.C.i and X.C.vi above. As discussed in Section IX.A.vi-vii and included here, Quake discloses the surfactant may be fluorinated oil and that the surfactants may be used in the carrier oil to coat the channel walls and to form droplets. Quake thus discloses the method of claim 7 wherein the surfactant is fluorinated surfactant and renders obvious the use of a fluorinated oil for compatibility with the fluorinated surfactant. EX1002, ¶¶278-82; *see also* Section VIII.A above (discussing EX1017, 209, 212-213; EX1018,

Summary; EX1023, Abstract, 18, 21, 89-92, 147-48). Accordingly, Quake in view of Litborn renders each of claims 6 and 8 obvious.

viii. Claim 9

As discussed above and included here, Quake in view of Litborn renders claim 1 obvious. *See* Section IX.C.i above. As discussed in Section IX.A.viii and included here, Quake discloses the w/o droplets can be combined with other droplets to facilitate a reaction or analysis. Accordingly, Quake in view of Litborn renders claim 9 obvious. EX1002, ¶283; *see also* Section VIII.A above.

ix. Claim 11

As discussed above and included here, Quake in view of Litborn renders claim 1 obvious. *See* Section IX.C.i above. As discussed in Section IX.A.ix and included here, Quake discloses using plugs that are substantially spherical in shape. Accordingly, Quake in view of Litborn renders claim 11 obvious. EX1002, ¶¶284-87; *see also* Section VIII.A above.

As discussed above, Quake in view of Litborn renders obvious each of claims 1-9 and 11. EX1002, ¶¶230-87.

D. Ground 4: Claims 1-9 and 11 Are Obvious Over Quake and Litborn In Further View of Shaw Stewart

As discussed above in Ground 3 and included here, Quake and Litborn render obvious the methods of claims 1-9 and 11. *See* Sections IX.C. above. EX1002, ¶288. As discussed in Section IX.B and included here, Shaw Stewart

provides additional teachings regarding using a fluorinated oil as a carrier fluid for forming aqueous droplets containing biological materials. EX1002, ¶¶289-93; *see also* Section VIII.A above (discussing EX1017, 209, 212-213; EX1018, Summary; EX1023, Abstract, 2, 18, 21, 89-92, 147-48). In view of Stewart's disclosure of fluorinated oils as a known alternative to mineral oil as the immiscible carrier fluid and prior art disclosure that fluorinated oil were chemically compatible with Quake's fluorinated surfactants, a POSA would have had good reason and a reasonable expectation of success for using a fluorinated oil as the carrier fluid for forming Quake's PCR droplets. EX1002, ¶¶290-92; *see also* Section VIII.A above. Shaw Stewart thus provides additional evidence supporting the obviousness of the challenged claims with respect to their limitations about providing conditions suitable for autocatalytic amplification reactions in the droplet (all claims), PCR (claim 3), fluorinated oil (claim 6), and fluorinated surfactant (claim 8). Accordingly, each of claims 1-9 and 11 as a whole would have been obvious over Quake and Litborn in further view of Shaw Stewart. EX1002, ¶293; *see also* Section VIII.A above.

X. SECONDARY CONSIDERATIONS

Patent Owner argued against 10X that the "tiny" size of sub-microliter droplets prevented practitioners from conducting biological reactions in them before the '193 patent because of alleged difficulties with mixing reagents,

adherence of droplet material to channel walls and the droplet/carrier-fluid interface, and “environments incompatible with biological molecules.” EX1026, 48-49, 7-8. As demonstrated above, however, the prior art had already overcome each of these challenges. EX1002, ¶¶294-96; EX1007, 453 & Fig. 7B (intradroplet mixing); EX1005, 28:1-29 (coating channel walls to prevent adherence); EX1009, 110 (describing channel coatings, surfactants, and PEG for preventing protein adsorption). As demonstrated by at least Holliger, Litborn, and Schneegaß, successful PCR amplification in microfluidic droplets already was achieved before the ’193 patent. EX1002, ¶¶294-97.

Patent Owner argues specific fluoroamines and fluorinated oils “unlocked microfluidic droplets as systems for studying autocatalytic biological reactions.” EX1026, 44, 55-58; EX1028, 254-260, 277. But these specific surfactants and fluoroamines are not claimed in the ’193 patent. EX1001, claims. EX1002, ¶¶298-99. Moreover, PEG and OEG “*are known* to reduce non-specific adsorption of proteins on surfaces” and were useful in the claimed invention for the same purpose. EX1001, 35:63-65 (emphasis added); EX1002, ¶300. Moreover, as just discussed, the asserted prior art demonstrates these surfactants were not “crucial” to performing microfluidic droplet PCR.

Patent Owner also argued that the 2003 Thorsen Thesis reports a failed enzymatic droplet assay. EX1026, 58-60 (citing EX1036, 94-108). These

arguments miss the mark. Thorsen did not report any failed attempt at PCR. EX1002, ¶¶301-02. Nor does Thorsen indicate droplets were unsuitable for enzymatic reactions. *Id.*; EX1036, 95, 100, 104 107. Thorsen supports a reasonable expectation of success for the claimed invention. EX1002, ¶¶302.

XI. NEW REFERENCES AND EVIDENCE OF UNPATENTABILITY

35 U.S.C. §325(d) discretionary denial is not appropriate here and the Board's precedential factors favor institution. *See Becton, Dickinson & Co. v. B. Braun Lesungen AG*, IPR2017-01586, Paper 8 (precedential); *Advanced Bionics, LLC v. Med-El Elektromedizinische Geräte GmbH*, IPR2019-01469, Paper 6 at 8 (precedential).

Factors (a), (b), and (d) all favor institution because of the significant material differences between the new references and those previously considered. The art previously considered by the Office in evaluating the challenged claims did not teach performing PCR in sub-microliter droplets. Holliger, Litborn, and Schneegaß, unlike the previously considered Haff and Kopp references, disclose successful PCR amplification of DNA in sub-microliter w/o droplets formed in the same or very similar oils (standard hydrocarbons, such as mineral and octane) as Quake discloses (*e.g.*, mineral oil or decane). *See* Sections V.B-D, VIII.B.ii-iii, v, and IX above. Litborn and Schneegaß disclose successful microfluidic w/o droplet PCR amplification in continuous oil flow in a microfluidic device. *Id.* This Petition

thus challenges the claims based on materially-different prior art references (factor a) that are not cumulative to previously considered references (factor b). The Petition's arguments are also materially different (factor d) because they rely on microscopic w/o droplet PCR references to show that the prior art taught a reasonable expectation of success for performing PCR in Quake's droplets, as Quake suggested. Because the asserted references and arguments are materially different from and non-cumulative to the references and arguments considered previously by the Office, discretionary denial is not appropriate here. *See Oticon Medical AB v. Cochlear Limited*, IPR2019-00975, Paper 15, at 10-17 (precedential).

Factors c, e, and f also favor institution. As discussed above in Sections V.B-D, the '193 patent issued based on the erroneous belief, since corrected by Patent Owner and the reexamination Examiner, that the Quake reference did not disclose droplet formation by flowing a continuous stream of aqueous fluid into a continuous stream of oil. EX1026, 13-14; EX1028, 1915-16. The 10X IPR petition was denied on the basis that the petitioner failed to meaningfully address reasonable expectation of success and failed to identify references demonstrating such success. A shortcoming of a completely different party should not prejudice petitioner here. Patent Owner similarly obtained confirmation of the claims during reexamination by convincing the Office that "traditional hydrocarbons" used to

make Quake's microfluidic w/o droplets were bioincompatible, will "destroy proteins," and will result in "protein adsorption on the surface of the droplet" making successful PCR amplification within the droplet essentially impossible. EX1028, 0008-0009; Sections V.C-D. In evaluating those arguments, the Office never had the benefit of prior art references, such as those asserted here, disclosing successful DNA PCR amplification in microfluidic w/o droplets. Had the Office considered the asserted teachings of any of Holliger, Litborn, or Schneegaß, which actively contradict Patent Owner's arguments, the challenged claims would not have issued or would not have been confirmed. Factors c (extent of consideration), e (Examiner error) and (f) (new evidence warrants reconsideration of prior arguments) thus each favor institution. Institution is therefore warranted here. *See Oticon*, IPR2019-00975, Paper 15, at 17-20.

XII. DROPWORKS' FIRST AND ONLY PETITION SHOULD BE INSTITUTED

All considerations of fairness, efficiency, and patent quality favor institution. *See General Plastic Co., Ltd. v. Canon Kabushiki Kaisha*, IPR2016-01357, Paper 19 at 16–17 (PTAB Sept. 6, 2017) (precedential as to § II.B.4.i). Dropworks has never filed any petition to the challenged patent, is unrelated to 10X, and has no special relationship with 10X. *See Oticon Medical AB v. Cochlear Limited*, IPR2019-00975, Paper 15, at 10-20 (precedential) (General Plastic concerns "are not directly raised...because this is the first Petition that Petitioner has filed").

The timing of the Petition (factors 2, 3, 4, and 5) also favors institution. Dropworks did not even exist in 2015 when 10X filed its petition or when that petition was denied. Dropworks evaluated the patents and the prior art and prepared this petition for filing in the middle of a global pandemic in the few short months since Patent Owner served the complaint in May 2020. Dropworks was not, and had no reason to be, aware of the new references presented herein at the time of 10X's petition or reexamination request. This is not the type of case contemplated by the *General Plastic* framework where a petitioner laid in wait to file a successive petition for strategic advantage.

Efficiency considerations (factors 6 and 7) also favor institution because there is no instituted case against this patent, the Board's resources will be well employed in cancelling the unpatentable claims, and the Board will be fully capable of issuing a final decision. The *General Plastic* factors favor institution.

XIII. CONCLUSION

Petitioner requests that the challenged claims be found unpatentable and canceled.

Respectfully submitted,

Dated: October 22, 2020

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182
WILSON SONSINI GOODRICH &
ROSATI

XIV. CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), the undersigned certifies that this Petition complies with the type-volume limitation of 37 C.F.R. §42.24(a). The word count application of the word processing program used to prepare this Petition indicates that the Petition contains 13,991 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Respectfully submitted,

Dated: October 22, 2020

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

XV. PAYMENT OF FEES UNDER 37 C.F.R. §§ 42.15(A) AND 42.103

The required fees are submitted herewith. If any additional fees are due at any time during this proceeding, the Office is authorized to charge such fees to Deposit Account No. 23-2415.

XVI. APPENDIX – LIST OF EXHIBITS

Exhibit No	Description
1001	U.S. Patent No. 8,304,193 to Ismagilov, <i>et al.</i>
1002	Declaration of Carl D. Meinhart, Ph.D
1003	<i>Curriculum Vitae</i> of Carl D. Meinhart, Ph.D.
1004	Prosecution History of U.S. Patent No. 8,304,193 to Ismagilov, <i>et al.</i>
1005	International Publication No. WO 02/23163 to Quake and Thorsen.
1006	International Publication No. WO 02/022869 to Holliger and Gahdessy.
1007	Litborn <i>et al.</i> , <i>Synthesis and Analysis of Chemical Components in Nanoscale</i> , MICRO TOTAL ANALYSIS SYSTEMS 2000, Enschede, The Netherland, 447-454 (2000).
1008	International Publication No. WO 84/02000 to Shaw Stewart
1009	Ivonne Schneegaß & Johann Michael Köhler, <i>Flow-through polymerase chain reactions in chip thermocyclers</i> , 82 REV. MOL. BIOTECH. 101-121 (2001)
1010	U.S. Patent No. 6,033,880 to Haff <i>et al.</i>
1011	Kopp <i>et al.</i> , <i>Chemical Amplification: Continuous-Flow PCR on a Chip</i> , 280 SCIENCE 1046-1048 (1998)
1012	Lagally <i>et al.</i> , <i>Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system</i> , 63 SENSORS AND ACTUATORS B: CHEMICAL 136-146 (2000).

Exhibit No	Description
1013	He <i>et al.</i> , <i>Capillary-based fully integrated and automated system for nanoliter polymerase chain reaction analysis directly from cheek cells</i> , 924 J. CHROMATOGR. A 271-284 (2001).
1014	U.S. Patent No. 6,171,850 to Nagle <i>et al.</i>
1015	U.S. Patent Appl. Pub. No. 2001/0041357 to Fouillet <i>et al.</i>
1016	Burns <i>et al.</i> , <i>An Integrated Nanoliter DNA Analysis Device</i> , 282 Science 484-487 (1998).
1017	Rudiger <i>et al.</i> , <i>Perfluorocarbons—Useful Tools for Medicine</i> , 5 EUR. J. MED. RES. 209-216 (2000).
1018	Krafft <i>et al.</i> , <i>Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field</i> , 80 BIOCHIMIE 489-514 (1998).
1019	<i>Reserved</i>
1020	UK Patent Application GB 2,097,692
1021	U.S. Provisional Application No. 60/379,927
1022	U.S. Provisional Application No. 60/394,544
1023	Shaw Stewart, P., <i>The Development and Applications of a New Liquid Handling Device</i> , Univ. of London Master's Thesis (1988).
1024	<i>Bio-Rad Labs., Inc. and The University of Chicago v. Dropworks, Inc.</i> , Case No. 20-cv-506-UNA, District of Delaware, Complaint, D.I. 1 (April 14, 2020).
1025	<i>10X Genomics, Inc. v. The University of Chicago</i> , IPR2015-01163, Paper 2.

Exhibit No	Description
1026	<i>10X Genomics, Inc. v. The University of Chicago</i> , IPR2015-01163, Paper 9.
1027	<i>10X Genomics, Inc. v. The University of Chicago</i> , IPR2015-01163, Paper 14.
1028	Reexamination File for Control No. 90/014,043
1029	U.S. Patent Application 2002/0058332 to Quake and Thorsen
1030	U.S. Provisional Application 60/233,037
1031	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Memorandum Order re Claim Construction, D.I. 116 (Jan. 26, 2017).
1032	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Memorandum Order re Summary Judgment, D.I. 351 (June 26, 2018).
1033	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Claim Construction Order, D.I. 179 (May 30, 2017).
1034	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Claim Construction Order, D.I. 469 (Nov. 13, 2018).
1035	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Appeal Nos. 2019-2255, 2019-2285, Slip Op., (Fed. Cir. Aug. 3, 2020).
1036	Thorsen, T., <i>Microfluidic Technologies for High-Throughput Screening Applications</i> , Caltech Ph.D Thesis (2003).

Exhibit No	Description
1037	U.S. Patent No. 6,524,456 to Ramsey <i>et al.</i>
1038	February 14, 2014 Amendment and Response to December 16, 2013 Non-Final Office Action from Prosecution History of U.S. Patent No. 8,822,148 to Ismagilov, <i>et al.</i> (US. Application No. 13/563,347).
1039	U.S. Patent No. 8,822,148 to Ismagilov, <i>et al.</i>

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105(a), this is to certify that I caused to be served a true and correct copy of the foregoing Petition for *inter partes* review of U.S. Patent No. 8,304,193 (and accompanying Exhibits 1001-1039) by overnight courier (Federal Express or UPS), on this 22nd day of October, 2020, on the Patent Owner at the correspondence address of the Patent Owner as follows:

Weil, Gotshal & Manges LLP
ATTN: Derek C. Walter
Edward R. Reines
201 Redwood Shores Parkway
Red Shores, CA 94065-1134
(650) 802-3000

Respectfully submitted,

Dated: October 22, 2020

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182