

**10X GENOMICS, INC.,**  
**Petitioner**  
**v.**  
**BIO-RAD LABORATORIES, INC.,**  
**Patent Owner**

*Inter Partes* Review Nos.  
**IPR2020-00086 and -00088**

**Patent Owner's Demonstratives**  
**Consolidated Hearing January 27, 2021**

# **Outline of Argument**

- 1) Background on U.S. Patent Nos. 9,562,837 and 9,896,722**
- 2) Claim Construction**
- 3) Background on Ismagilov and Quake**
- 4) Ismagilov and Quake Are Missing Several Elements**
- 5) Secondary Considerations**

# **Background on the '837 and '722 Patents**





US009896722B2

(12) **United States Patent**  
**Link**

(10) **Patent No.:** US 9,896,722 B2  
(45) **Date of Patent:** Feb. 20, 2018

(54) **SYSTEMS FOR HANDLING MICROFLUIDIC DROPLETS**

(71) Applicant: **Raindance Technologies, Inc.**,  
Billerica, MA (US)

(72) Inventor: **Darren Roy Link**, Lexington, MA (US)

(73) Assignee: **Raindance Technologies, Inc.**,  
Billerica, MA (US)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/415,044**

(22) Filed: **Jan. 25, 2017**

(65) **Prior Publication Data**

US 2017/0183722 A1 Jun. 29, 2017

**Related U.S. Application Data**

(63) Continuation-in-part of application No. 13/855,318, filed on Apr. 2, 2013, now Pat. No. 9,562,837, which (Continued)

(51) **Int. Cl.**  
**C12Q 1/68** (2006.01)  
**B01L 3/00** (2006.01)  
(Continued)

(52) **U.S. Cl.**  
CPC ..... **C12Q 1/6848** (2013.01); **B01L 3/502715** (2013.01); **B01L 3/502753** (2013.01);  
(Continued)

(58) **Field of Classification Search**  
CPC ..... C12Q 2563/159; C12Q 2565/629; C12Q 1/6848; C12Q 1/00; C12Q 1/6848;  
(Continued)

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435:6.11

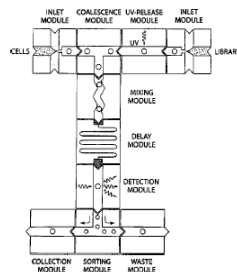
\* cited by examiner

**Primary Examiner** — Jennifer Wecker  
(74) **Attorney, Agent, or Firm** — Thomas C. Meyers;  
Brown Rudnick LLP

(57) **ABSTRACT**

The invention generally relates to assemblies for displacing droplets from a vessel that facilitate the collection and transfer of the droplets while minimizing sample loss. In certain aspects, the assembly includes at least one droplet formation module, in which the module is configured to form droplets surrounded by an immiscible fluid. The assembly also includes at least one chamber including an outlet, in which the chamber is configured to receive droplets and an immiscible fluid, and in which the outlet is configured to receive substantially only droplets. The assembly further includes a channel, configured such that the droplet formation module and the chamber are in fluid communication with each other via the channel. In other aspects, the assembly includes a plurality of hollow members, in which the hollow members are channels and in which the members are configured to interact with a vessel. The plurality of hollow members includes a first member configured to expel a fluid immiscible with droplets in the vessel and a second member configured to substantially only droplets from the vessel. The assembly also includes a main channel, in which the second member is in fluid communication with the main channel. The assembly also includes at least one analysis module connected to the main channel.

17 Claims, 40 Drawing Sheets



10X Ex. 1001, Page 1

1. A method of reducing contamination associated with sample handling, comprising:  
providing an aqueous fluid comprising a sample through a sample inlet;  
providing an immiscible fluid flowing through a main channel that is in fluidic communication with the sample inlet, wherein the main channel is in a horizontal plane;  
partitioning the aqueous fluid with the immiscible fluid to form a plurality of droplets in the main channel, wherein at least one droplet comprises a sample;  
flowing the droplets toward a downstream separation chamber that is in fluidic communication with the main channel,  
wherein the separation chamber has a wider cross-section than the main channel cross-section and the separation chamber is disposed perpendicular to the main channel;  
and  
separating the plurality of droplets from the immiscible fluid in the separation chamber based on the different densities of the droplets and the immiscible fluid.

(088 and 089 IPRs, Ex. 1001 ('722 patent) at Title, 88:2-23).

# Claim Construction



# “Separation Chamber”

Patent Owner	Petitioner
“chamber sized to accommodate multiple droplets and an immiscible fluid in separated form”	“a space that is substantially enclosed”

# “Droplet Receiving Outlet” (‘837 Patent)

Patent Owner	Petitioner
“portion of the separation chamber where droplets are collected”	Means plus function or  “a structure connected to the interior space of the chamber and configured so that substantially only droplets flow into it when exiting the chamber”



# “Coupled” (Dependent Claims 7 and 9 of the ‘837 Patent)

Patent Owner	Petitioner
“operably linked”	“fastened or joined”

# Background on Ismagilov and Quake



US007129091B2

(12) **United States Patent**  
**Ismagilov et al.**

(10) **Patent No.:** **US 7,129,091 B2**  
(45) **Date of Patent:** **Oct. 31, 2006**

(54) **DEVICE AND METHOD FOR PRESSURE-DRIVEN PLUG TRANSPORT AND REACTION**

(75) **Inventors:** **Rustem F. Ismagilov**, Chicago, IL (US); **Joshua David Tice**, Webster, NY (US); **Helen Song**, Chicago, IL (US)

(73) **Assignee:** **University of Chicago**, Chicago, IL (US)

(\*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 93 days.

(21) **Appl. No.:** **10/434,970**

(22) **Filed:** **May 9, 2003**

(65) **Prior Publication Data**  
US 2005/0272159 A1 Dec. 8, 2005

**Related U.S. Application Data**

(60) Provisional application No. 60/379,927, filed on May 9, 2002, provisional application No. 60/394,544, filed on Jul. 8, 2002.

(51) **Int. CL**  
**G01N 33/00** (2006.01)

(52) **U.S. CL** ..... **436/34; 436/55; 436/164**

(58) **Field of Classification Search** ..... **436/34; 436/55, 164**  
See application file for complete search history.

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(57) **ABSTRACT**  
The present invention provides microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid.

**32 Claims, 36 Drawing Sheets**  
**(33 of 36 Drawing Sheet(s) Filed in Color)**

10X Ex. 1004, Page 1

FIG. 3A

FIG. 7A

FIG. 38

(Ex. 1004 (Ismagilov), Figs. 3A, 4, 7A, 38; 086 Paper No. 26 (POR) at 8-9; 088 Paper No. 26 (POR) at 8-9).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

11



US 2002/0058332 A1

(19) United States  
(12) Patent Application  
Quake et al.

(54) MICROFABRICATED CROSS  
DEVICES AND METHODS

(75) Inventors: Stephen R. Quake, I  
(US); Todd Thorsen,  
(US)

Correspondence Address:  
DARBY & DARBY P.C.  
805 Third Avenue  
New York, NY 10022 (US)

(73) Assignee: California Institute of

(21) Appl. No.: 09/953,103

(22) Filed: Sep. 14, 2001

Related U.S. Application

(63) Non-provisional of provisional  
60/246,793, filed on Nov. 8, 2000,  
provisional application No. 60/23  
15, 2000.

Publication Classificat

(51) Int. Cl.<sup>7</sup>

US 2002/0058332 A1

21

May 16, 2002

is a function of the cross-sectional area of the channel and the cell concentration, the latter typically determined in view of acceptable values of  $P_{m2}$ , discussed above. From these relationships, it is then easy to calculate the maximum channel length between the detection and discrimination region which would ensure that cells do not change order or position in the line of cells. In practice, the channel length between the detection and discrimination regions is between about 1  $\mu$ m and about 100  $\mu$ m.

[0191] Shear forces may affect the velocity at which the cells move through the microfluidic device, particularly when living cells are to be sorted and collected. Experiments have shown that high electric fields, in the range of 2-4 kV/cm for human erythrocytes and 5-10 kV/cm for yeast cells can be used to introduce DNA and other substances into cells using electroporation. At these voltages there was no cell lysis, although membrane permeation was possible. To avoid membrane permeation and cell lysis, it is preferred that the electric fields applied to move cells in any of the described flow techniques is less than about 600 V/cm and most preferably less than about 100 V/cm.

#### EXAMPLE 7

[0192] Elastomeric Microfabricated Device

[0193] This Example demonstrates the manufacture and operation of a disposable microfabricated FACS device, which can function as a stand-alone device or as a component of an integrated microanalytical chip, in sorting cells or biological materials. The device permits high sensitivity, no cross-contamination, lower cost to operate and manufacture than conventional FACS machines and multiple-hour run times. In this example, the microfabricated chip had a detection volume of approximately 250 fL and single channel throughput of about 20 cells/second. The device obtained substantial enrichment of micron-sized fluorescent bead populations of different colors. In addition, populations of *E. coli* cells expressing green fluorescent protein were separated, and enriched, from a background of non-fluorescent (wild type) *E. coli* cells. The bacteria were also found to be viable after extraction from the sorting device.

[0194] Preparation of the microfabricated device

[0195] A silicon wafer was etched and fabricated as described above and in (12). After standard contact photolithography techniques to pattern the oxide surface of the silicon wafer, a  $C_2F_4/CHF_3$  gas mixture was used to etch the wafer by RIE. The silicon wafer was then subjected to further etch with KOH to expose the silicon underneath the oxide layer, thereby forming a mold for the silicone elastomer. The silicon mold forms a "T" arrangement of channels. The dimensions of the channels may range broadly, having approximately 5x4  $\mu$ m dimension.

[0196] A representative device of the invention is shown in FIG. 6. The etching process is shown schematically in FIG. 7. Standard micromachining techniques were used to create a negative master mold out of a silicon wafer. The disposable silicone elastomer chip was made by mixing General Electric RTV 615 components (36) together and pouring onto the etched silicon wafer. After curing in an oven for two hours at 80° C., the elastomer was peeled from the wafer and bonded hermetically to a glass coverslip for sorting. To make the elastomer hydrophilic the elastomer

chip was immersed in HCl (pH=2.7) at 60 degrees C for 40 to 60 min. Alternatively, the surface could have been coated with polyurethane (3% w/v in 95% ethanol and diluted 10 X in ethanol). It is noted that the master wafer can be reused indefinitely. The device shown has channels that are wide at the wells, narrowing to 3  $\mu$ m at the sorting region (discrimination region). The channel depth is 2 mm in diameter.

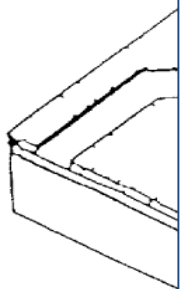
[0197] In this embodiment the cell-sorting device was mounted on an inverted optical microscope (Zeiss Axiovert 35) as shown in FIG. 8. In this system, the flow control can be provided by voltage electrodes for electro-osmotic control or by capillaries for pressure-driven control. The detection system can be photo multiplier tubes or depending upon the application. The inlet well and two collection wells were incorporated into the elastomer chip on three sides of the "T" forming three channels (FIGS. 6 and 7). The chip was adhered to a glass coverslip and mounted onto the microscope.

[0198] Three platinum electrodes were each separate wells. A water-cooled argon laser (R 100 W mercury lamp (for beads) focused the immersion objective (Olympus Plan Apo 60 X) used to excite the fluorescence and a charge-coupled (CCD) camera took the image. To select for red emission a 630 nm $\pm$ 30 emission filter (Chroma) detection region was approximately 5 to 10  $\mu$ m. The window is implemented with a Hamamatsu R928 phototube (bias -850 V) with custom current-to-voltage converter, or using photodiodes, as detectors, and voltage amplifiers (depending on the fluorescence intensity) from the detector(s) were converted to Burr-Brown OP128 optical amplifier (10<sup>7</sup> V/A). National Instrument PCI200 board and personal computer. The voltages on the electrodes are a pair of Apex PA42 HV op amps powered by A supplies. The third electrode was ground. A voltage settings on the PCI200 board analog-to-digital amplification to the platinum electrodes can switching of the directions of the fluids. Thus directed to either side of the "T" channels depending upon the voltage potential settings. Furthermore, different ways of sorting in the microfluidic device can be achieved by different computer programs, e.g., different computer-controlled procedures using known programming techniques.

[0199] Sorting Experiments

[0200] This embodiment of the microfabricated FACS system was used to sort fluorescent beads of different emission wavelengths in different ratios up to 33,000 beads per hour throughput (See FIGS. 9-12). Extra reservoir wells were incorporated into the outer side of the three wells of the chip in order to avoid ion-depletion, and platinum electrodes (with the ground electrode in the inlet well) were inserted into the reservoir wells. One micron diameter beads were suspended in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1.4 mM KH<sub>2</sub>PO<sub>4</sub>) with 10% BSA (1 g/l) and 0.5% Tween 20 in various ratios and dilutions. Samples of the different colored fluorescent beads, having ratios as indicated below, were injected into the inlet well in 10 to 30  $\mu$ l aliquots. The collection wells were filled with the same buffer.

[0197] In this embodiment the cell-sorting device was mounted on an inverted optical microscope (Zeiss Axiovert 35) as shown in FIG. 8. In this system, the flow control can be provided by voltage electrodes for electro-osmotic control or by capillaries for pressure-driven control. The detection system can be photo multiplier tubes or photodiodes, depending upon the application. The inlet well and two collection wells were incorporated into the elastomer chip on three sides of the "T" forming three channels (FIGS. 6 and 7). The chip was adhered to a glass coverslip and mounted onto the microscope.



# **Ismagilov and Quake Are Missing Several Elements**



# Ismagilov and Quake Are Missing Several Elements

- With respect to the ‘837 patent, Ismagilov and Quake are missing several elements of the claims, including but not limited to:
  - 1) “separation chamber”
  - 2) “upright to the microchannel and out of the horizontal plane”
  - 3) “wider cross-section than the microchannel cross-section to accumulate a plurality of droplets”
  - 4) “volume sufficient to separate the plurality of droplets”
  - 5) “droplet receiving outlet”

(086 Paper 26 (POR) at 29-43, 48-49).

- With respect to the ‘722 patent, Ismagilov and Quake are missing several elements of the claims, including but not limited to:
  - 1) “separation chamber”
  - 2) “disposed perpendicular to the main channel”
  - 3) “wider cross-section than the main channel cross-section”
  - 4) “separating the plurality of droplets”
  - 5) “collecting the separated droplets”

(088 Paper 26 (POR) at 22-36, 42-43).

# Ismagilov and Quake Are Missing Several Elements

## Direct Testimony of Petitioner's Expert Dr. Fair

101. There are two main theories of invalidity. First, 10X identifies prior art disclosures meeting the broad constructions implied by Bio-Rad's infringement contentions: specifically, collection wells. Ismagilov discloses a collection well (**Ground 1**) and anticipates. Further, a person of ordinary skill in the art would have been motivated to combine Quake for additional information regarding the structure of a well of a microfluidic device (**Ground 2**).

(086 Ex. 1008 (Fair Dec.) at ¶101;  
086 Paper 2 (Petition) at 23-24;  
088 Ex. 1008 (Fair Dec.) at ¶95;  
088 Paper 2 (Petition) at 22).



# Ismagilov Is Missing Several Elements

11. Ismagilov discloses collection wells or reservoirs that are a separation chamber with a droplet receiving outlet under Bio-Rad's interpretations. Ismagilov discloses an "outlet port," "an area of a substrate that *collects* or dispenses . . . plugs" and may "communicate with a *well or reservoir*." Ismagilov, 9:5-8, 14:36-38. Ismagilov describes after a manifold an "outlet" that "can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a *standard 1.5 ml centrifuge tube*. Collection can also be done *using micropipettes*." Ismagilov, 17:27-30. Collecting fluids by micropipette requires a container with a volume of liquid to pipette and an opening to insert the pipette, *i.e.* a collection well. Fair, ¶¶ 129-132 (citing Ex. 1040, Ex. 1041).

(086 Paper 2 (Petition) at 36;  
*see* 088 Paper 2 (Petition) at 36).

# Ismagilov Is Missing Several Elements

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FIG. 42 shows a schematic diagram (left side) of a microfluidic network and a photograph (right side) of the plug region of the microfluidic network in which the gradients were formed by varying the flow rates.  
FIG. 43 shows a schematic diagram (left side) of a microfluidic network and a photograph (right side) of lysoczyme crystals formed in the microfluidic network using gradients.

technology such as soft lithography. As used herein, the term "device" includes those that are called, known, or classified as microfabricated devices. A device according to the invention may have dimensions between about 0.3 cm to about 15 (for 6 inch wafer) cm per side and between about 15 micrometer to about 1 cm thick, but the dimensions of the device may also lie outside these ranges.  
The term "discrimination region" refers to a part of or a

The term "inlet port" refers to an area of a substrate that receives plug-fluids. The inlet port may contain an inlet channel, a well or reservoir, an opening, and other features that facilitate the entry of chemicals into the substrate. A substrate may contain more than one inlet port if desired. The inlet port can be in fluid communication with a channel or separated from the channel by a valve.

chemistry, or composition, or they may be separated at least to a certain extent.  
The term "inlet port" refers to an area of a substrate that receives plug-fluids. The inlet port may contain an inlet channel, a well or reservoir, an opening, and other features that facilitate the entry of chemicals into the substrate. A substrate may contain more than one inlet port if desired.  
The inlet port can be in fluid communication with a channel or separated from the channel by a valve.  
The term "nanoparticles" refers to atomic, molecular or macromolecular particles typically in the length scale of approximately 1–100 nanometer range. Typically, the novel and differentiating properties and functions of nanoparticles are observed or developed at a critical length scale of matter typically under 100 nm. Nanoparticles may be used in constructing nanoscale structures and they may be integrated into larger material components, systems and architectures.  
In some particular cases, the critical length scale for novel properties and phenomena involving nanoparticles may be under 1 nm (e.g., manipulation of atoms at approximately 0.1 nm) or it may be larger than 100 nm (e.g., nanoparticle reinforced polymers have the unique feature at approximately 200–300 nm as a function of the local bridges or bonds between the nanoparticles and the polymer).  
The term "nucleation composition" refers to a substance or mixture that includes one or more nuclei capable of growing into a crystal under conditions suitable for crystal formation. A nucleation composition may, for example, be induced to undergo crystallization by evaporation, changes

The term "detection region" refers to a part of or a location in a substrate or channel wherein a chemical is identified, measured, or sorted based on a predetermined property or characteristic.

The term "device" refers to a device fabricated or manufactured using techniques such as wet or dry etching and/or conventional lithographic techniques or a micromachining

10X Ex. 1004, Page 42

(Ex. 1004 (Ismagilov) at 8:41-44;  
086 Paper 26 (POR) at 30-31;  
088 Paper 26 (POR) at 23-24).

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The term "outlet port" refers to an area of a substrate that collects or dispenses the plug-fluid, carrier-fluid, plugs or reaction product. A substrate may contain more than one outlet port if desired.

The term "particles" means any discrete form or unit of matter. The term "particle" or "particles" includes atoms, molecules, ions, dimers, polymers, or biomolecules.  
The term "particulate" refers to a cluster or agglomeration of particles such as atoms, molecules, ions, dimers, polymers, or biomolecules. Particulates may comprise solid matter or be substantially solid, but they may also be porous or partially hollow. They may contain a liquid or gas. In addition, particulates may be homogeneous or heterogeneous, that is, they may comprise one or more substances or materials.

"Plugs" in accordance with the present invention are formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible. The flow of the fluids in the device is induced by a driving force or stimulus that arises, directly or indirectly, from the presence or application of, for example, pressure, radiation, heat, vibration, sound waves, an electric field, or a magnetic field. Plugs in accordance with the present invention may vary in size but when formed, their cross-section should be substantially similar to the cross-section of the channels in which they are formed. When plugs merge or get trapped inside plug traps, the cross-section of the plugs may change. For example, when a plug enters a wider channel, its cross-section typically increases.

Further, plugs in accordance with the present invention may vary in shape, and for example may be spherical or non-spherical. The shape of the plug may be independent of the shape of the channel (e.g., a plug may be a deformed sphere traveling in a rectangular channel). The plugs may be in the form of plugs comprising an aqueous plug-fluid containing one or more reagents and/or one or more products formed from a reaction of the reagents, wherein the aqueous plug-fluid is surrounded by a non-polar or hydrophobic fluid such as an oil. The plugs may also be in the form of a solid composition, such as a solid or a liquid, which is

different plug-fluids are used in the present invention. When both plug-fluids contain reagents, the fluids are typically miscible, but can also be partially immiscible, so long as the reagents within each plug-fluid can react to form at least one product or intermediate.

The term "polymer" means any substance or compound that is composed of two or more building blocks ("mers") that are repetitively linked to each other. For example, a "dimer" is a compound in which two building blocks have been joined together. Polymers include both condensation and addition polymers. Typical examples of condensation polymers include polyamide, polyester, protein, wool, silk, polyurethane, cellulose, and polysiloxane. Examples of addition polymers are polyethylene, polyisobutylene, polyacrylonitrile, poly(vinyl chloride), and polystyrene. Other examples include polymers having enhanced electrical or optical properties (e.g., a nonlinear optical property) such as electroconductive or photorefractive polymers. Polymers include both linear and branched polymers.

The term "protein" generally refers to a set of amino acids linked together usually in a specific sequence. A protein can be either naturally-occurring or man-made. As used herein, the term "protein" includes amino acid sequences that have been modified to contain moieties or groups such as sugars, polymers, metalloorganic groups, fluorescent or light-emitting groups, moieties or groups that enhance or participate in a process such as intramolecular or intermolecular electron transfer, moieties or groups that facilitate or induce a protein into assuming a particular conformation or series of conformations, moieties or groups that hinder or inhibit a protein from assuming a particular conformation or series of conformations, moieties or groups that enhance, enhance, or inhibit protein folding, or other moieties or groups that are incorporated into the amino acid sequence and that are intended to modify the sequence's chemical, biochemical, or biological properties. As used herein, a protein includes, but is not limited to, enzymes, structural elements, antibodies, hormones, electron carriers, and other macromolecules that are involved in processes such as cellular processes or activities. Proteins typically have up to four structural levels that include primary, secondary, tertiary, and quaternary structures.

The term "reaction" refers to a physical, chemical, biochemical, or biological transformation that involves at least one chemical or physical process, such as a reaction, reaction, or reaction.

The term "outlet port" refers to an area of a substrate that collects or dispenses the plug-fluid, carrier-fluid, plugs or reaction product. A substrate may contain more than one outlet port if desired.

which a reaction or precipitation can occur. Typically, the plug-fluid contains a solvent and a reagent although in some embodiments at least one plug-fluid may not contain a reagent. The reagent may be soluble or insoluble in the solvent. The plug-fluid may contain a surfactant. At least two

reaction with one or more components of other plug-fluids or a reagent-containing carrier-fluid in the substrate to produce one or more reaction products or intermediates which may undergo a further reaction or series of reactions. A reagent contained in a plug-fluid may undergo a reaction

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(Ex. 1004 (Ismagilov) at 9:5-8;  
086 Paper 26 (POR) at 30-31;  
088 Paper 26 (POR) at 23-24).





# Ismagilov Is Missing Several Elements



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## Using nanoliter plugs in microfluidics to facilitate and understand protein crystallization

Bo Zheng, Cory J Gerdts and Rustem F Ismagilov

Protein crystallization is important for determining protein structures by X-ray diffraction. Nanoliter-sized plugs — aqueous droplets surrounded by a fluorinated carrier fluid — have been applied to the screening of protein crystallization conditions. Preformed arrays of plugs in capillary cartridges enable sparse matrix screening. Crystals grown in plugs inside a microcapillary may be analyzed by *in situ* X-ray diffraction. Screening using plugs, which are easily formed in PDMS microfluidic channels, is simple and economical, and minimizes consumption of the protein. This approach also has the potential to improve our understanding of the fundamentals of protein crystallization, such as the effect of mixing on the nucleation of crystals.

**Addresses**  
Department of Chemistry, The University of Chicago, 5735 South Ellis Avenue, Chicago, IL 60637, USA

Corresponding author: Ismagilov, Rustem F (f-ismagilov@uchicago.edu)

**Current Opinion in Structural Biology** 2005, 15:548-555

This review comes from a themed issue on  
Biophysical methods  
Edited by Wah Chiu and Keith Moffat

Available online 18th September 2005

0959-440X/\$ — see front matter  
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DOI 10.1016/j.sbi.2005.08.009

### Introduction

This paper reviews recent applications of nanoliter plugs in protein crystallization and the resulting improvements in our understanding of the crystallization process. Macromolecular crystals are important in structural biology, both for determining the three-dimensional structures and functions of molecules and complexes [1,2,3-6], and for understanding ultrafast macromolecular dynamics [7,8]. Protein crystallization is carried out by combining and confining a protein solution with crystallization reagents. The crystallization of many proteins is still difficult [9] and, because of a lack of fundamental understanding, the optimal conditions for protein crystallization cannot be predicted *a priori* [10-12]. Mixing of solutions, phase behavior, and nucleation and growth of crystals need to be thoroughly understood in order to make protein crystallization a more rational process. In the mean time, a large number of screens are still required to find the best crystallization conditions for a protein [1].

The protein supply is usually limited, especially for eukaryotic proteins. The 0.5–1.0  $\mu$ l volume of concentrated protein solution per trial, used in traditional crystallization methods, makes screening large numbers of precipitants difficult for such proteins. It is also labor intensive to manually set up a large number of screens, and to harvest and mount the crystals. New tools for protein crystallization are required and are being developed [12-17], to both improve our understanding of and accelerate protein crystallization.

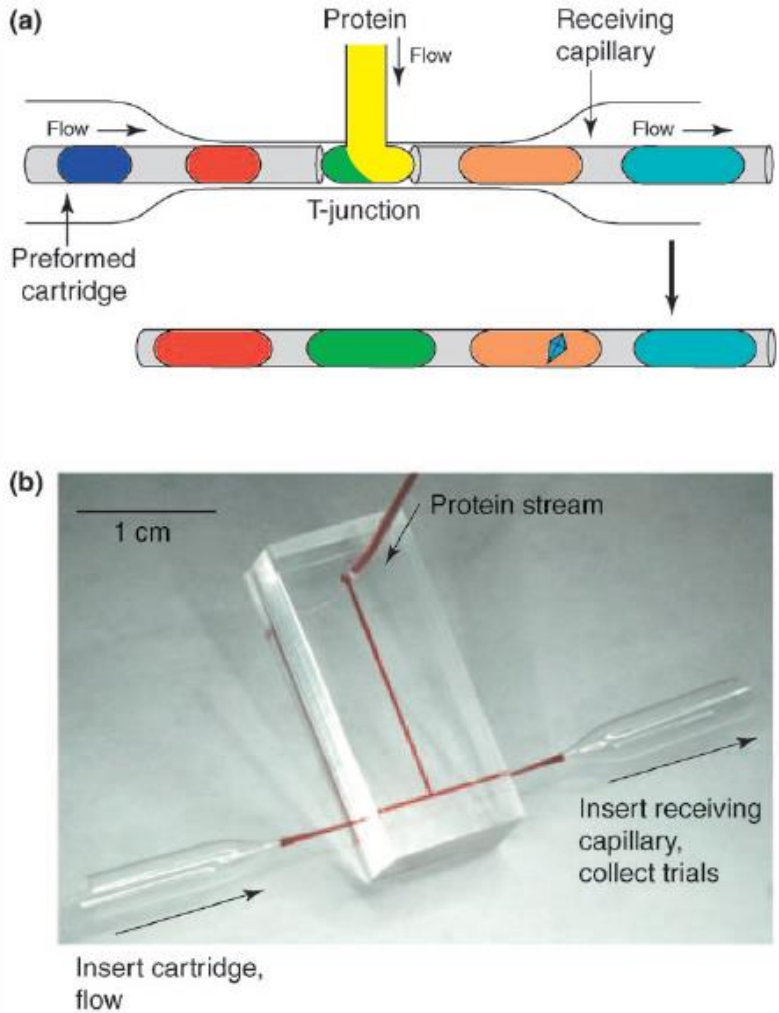
### Tools are needed to improve screening of crystallization conditions and to understand crystallization

In traditional methods of protein crystallization, the protein and reagents are deposited into crystallization wells by pipetting [1]. The volume and composition of the mixture may then be further controlled through equilibration by vapor diffusion or dialysis. By confining the mixture of protein and precipitant to a smaller volume, the consumption of the protein can be reduced. Robotic systems have been developed to automate crystallization processes and to dispense solutions into nanoliter volumes (much smaller than traditional pipetting methods) [13,14,18-20,21,22]. In robotic systems, nanoliter volumes of reagents are dispensed into microwells or microchambers. Thousands of trials can be easily set up per day. These systems are finding important applications in high-throughput protein crystallization centers [19,20,23] and in the pharmaceutical industry. However, the upfront equipment cost of these systems is higher than what many individual laboratories can justify. Lower cost, smaller systems are needed for such laboratories, which often work on difficult but scientifically very important macromolecular targets.

Microfluidics provides a method for combining and confining crystallization reagents in volumes as small as or smaller than those dispensed by robotic systems [24,25]. Microfluidic chambers combined with elastomeric PDMS [poly(dimethylsiloxane)] valves have been developed, and used for protein crystallization [15] and for studying the crystallization phase diagrams of proteins [26,27]. In these systems, crystallization requires several actively controlled microfluidic valves for each trial, necessitating the use of specialized microfluidic chips that are still rather expensive. The permeability of PDMS to water and organic molecules, which is problematic for crystallization experiments, is being addressed by the development of new fluorinated elastomeric materials [27].

**Current Opinion in Structural Biology** 2005, 15:548-555

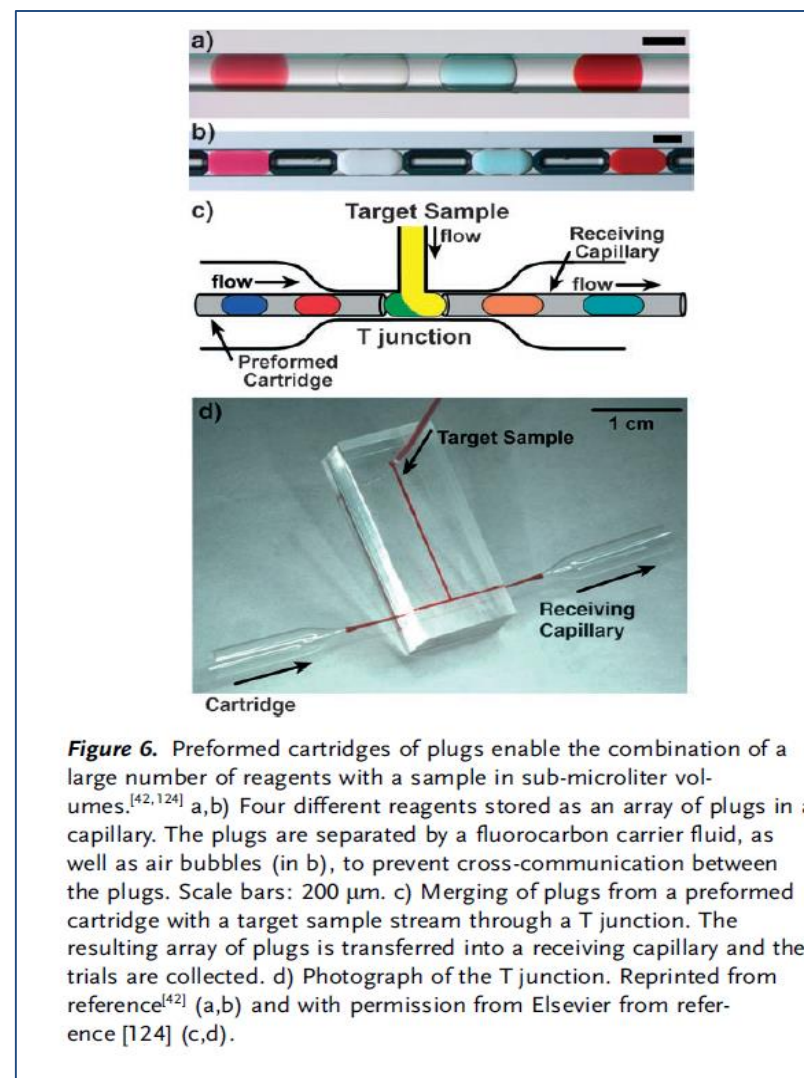
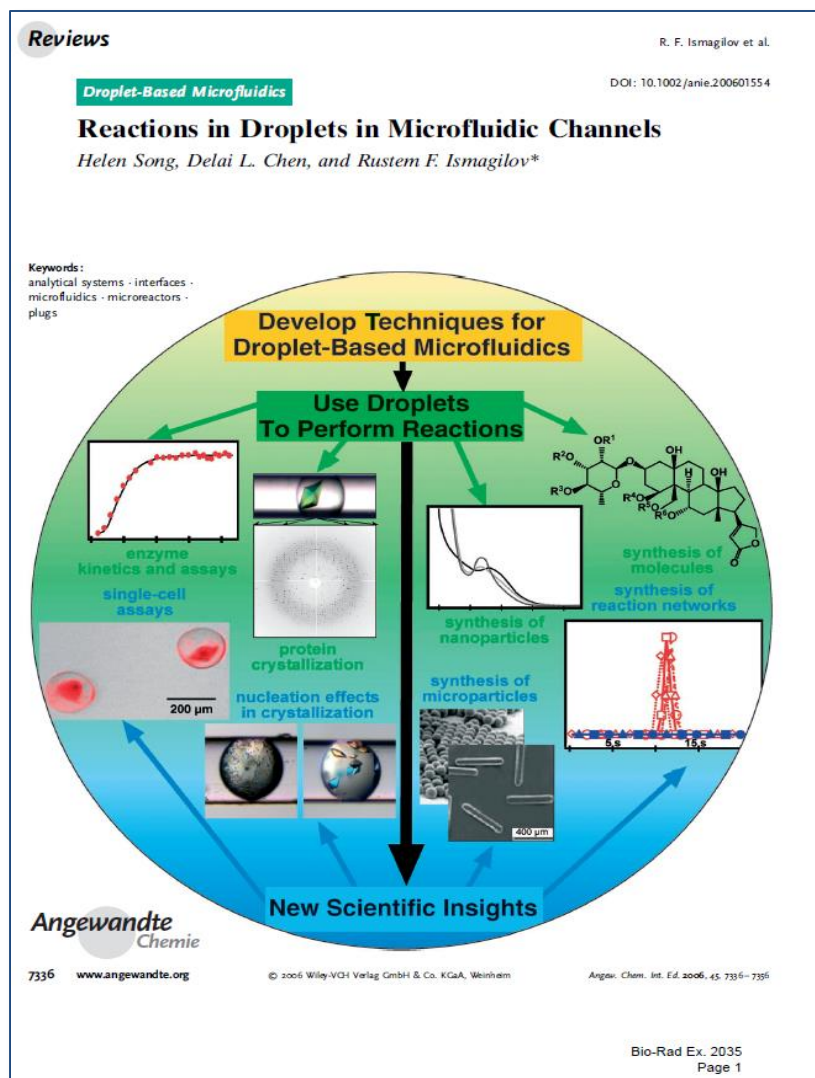
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Bio-Rad Ex. 2020  
Page 1



Current Opinion in Structural Biology

(Ex. 2020 (Ismagilov, Using Nanoliter Plugs);  
086 Paper 26 (POR) at 17, 34;  
088 Paper 26 (POR) at 16-17, 27).

# Ismagilov Is Missing Several Elements



(Ex. 2035 (Ismagilov, Reactions in Droplets);  
086 Paper 26 (POR) at 17;  
088 Paper 26 (POR) at 17).



# Ismagilov Is Missing Several Elements



US007129091 B2

(12) **United States**  
**Ismagilov et al.**

(54) **DEVICE AND METHOD**  
**PRESSURE-DRIVEN PL**  
**AND REACTION**

(75) Inventors: **Rustem F. Ism**  
(US); **Joshua D**  
(US); **Helen So**

(73) Assignee: **University of C**  
(US)

(\*) Notice: Subject to any d  
patent is extend  
U.S.C. 154(b) b

(21) Appl. No.: **10/434,970**

(22) Filed: **May 9, 2003**

(65) **Prior Public**  
US 2005/0272159 A1

**Related U.S. Appl**

(60) Provisional application No.  
9, 2002, provisional applica  
on Jul. 8, 2002.

(51) **Int. CL**  
**G01N 33/00** (2006)

(52) **U.S. CL**

(58) **Field of Classification See**

See application file for con

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**U.S. PATENT DOC**

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US 7,129,091 B2

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*Neural and Electronic Networks*, Academic Press, San Diego, Calif., 1990.) algorithms. For example, patterns that arise in blood or saliva analysis may correspond to certain normal or abnormal (e.g., disease, fatigue, infection, poisoning) conditions involving, for example, human and animals.

Moreover, it may be possible to create intelligent microfluidic systems in accordance with the invention, where the nonlinear chemical reactions perform not only detection, but also analysis using ANN algorithms. For example, after amplification, the channels of the present invention typically will contain sufficient amounts of material to operate hydrogel-based valves (Liu et al., "Fabrication and characterization of hydrogel-based microvalves," *J. Microelectromech. Syst.* 2002, vol. 11, pp. 45-53; Yu et al., "Responsive biomimetic hydrogel valve for microfluidics," *Appl. Phys. Lett.* 2001, vol. 78, pp. 2589-2591; Beebe et al., "Functional hydrogel structures for autonomous flow control inside microfluidic channels," *Nature*, 2000, vol. 404, 588.). These valves can be used to control flows inside the system as a function of the sample plug composition. Feedforward and even feedback (e.g., by using the hydrogel valves to control the flow of the input streams) networks may thus be created and used for analysis. Such nonlinear networks may be used not only to recognize patterns pre-programmed by the connectivity of the channels (Hjelmfelt et al., "Pattern Recognition in Coupled Chemical Kinetic Systems," *Science*, 1993, 260, 335-337), but also to learn patterns by reconfiguring themselves (Jackson, R. B. & T. *Neural Computing: An Introduction*, Hilger, N.Y., 1991; Zornetzer et al., *An Introduction to Neural and Electronic Networks*, Academic Press, San Diego, Calif., 1990.). Such intelligent microfluidic devices could have unprecedented capabilities for fully autonomous detection, analysis, and signal processing, perhaps surpassing those of biological and current man-made systems.

The devices and methods of the invention are also useful in genomics and proteomics, which are used to identify thousands of new biomolecules that need to be characterized, or are available only in minute quantities. In particular, the success of genomics and proteomics has increased the demand for efficient, high-throughput mechanisms for protein crystallization. X-ray structure determination remains the predominant method of structural characterization of proteins. However, despite significant efforts to understand the process of crystallization, macromolecular crystallization largely remains an empirical field, with no general theory to guide a rational approach. As a result, empirical screening has remained the most widely used method for crystallizing proteins.

The following areas also provide applications of the devices and methods according to the invention. For example, a number of problems still beset high-throughput kinetics and protein crystallization. When it comes to determining protein structure and quantitatively ascertaining protein interactions, there are at least two technological challenges: (1) most robotic technology still only automate existing methods and are often too expensive for a small research laboratory; and (2) there remains the need for conceptually new methods that provide greater degree of control over the crystallization process. In addition, setting up and monitoring crystallization trials typically involve handling of sub-microliter volumes of fluids over periods ranging from seconds to days.

Thus, various devices and methods according to the present invention are designed to provide novel and efficient means for high-throughput crystallization of soluble and

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membrane proteins. In addition to being a simple and economical method of setting up thousands of crystallization trials in a matter of minutes, a system according to the invention will enable unique time control of processes such as the mixing and nucleation steps leading to crystallization. A system according to the present invention may also be used to control protein crystallization by controlling not only short time-scale events such as nucleation but also long time-scale events such as crystal growth.

Further, the devices and methods of the present invention may be used in high-throughput, kinetic, and biophysical measurements spanning the  $10^{-5}$ - $10^2$  second time regime. Preferably, the various devices and methods according to the present invention require only between about 100 to 1000 microliters of each solution. of such devices and methods include studies of protein kinetics and RNA folding, and nanoparticle characterization and synthesis, which are discussed in detail below.

**Channels and Devices**  
In one aspect of the invention, a device is provided comprising one or more substrates comprising a channel or more secondary channels (or branch channels) communicating with the first channel, at least two plug-fluid reservoirs in fluid communication with the first channel, and a means for applying pressure to a fluid within the substrate.

A device according to the invention preferably includes one or more substrates comprising a channel wherein plugs can be trapped in the channel. The present invention may comprise connected channels.

The device may have one or more outlet ports. Each of the outlet and inlet ports may communicate with a well or reservoir. The inlet and outlet ports may be in fluid communication with the channels such that they are connecting or may contain one or more plugs. Fluid can be introduced into the channels via the inlet means. Typically, a syringe pump is used, wherein the rate of the fluid into the inlet can be controlled.

A plug-forming region generally comprises a plug-fluid inlet and a channel or carrier-fluid such that plugs form which are similar in size to each other and which have ends which are substantially similar in size to the ends of the channel in the plug-forming region. In one embodiment, the substrate may contain a plurality of plug-forming regions.

The different plug-forming regions may each be connected to the same or different channels of the substrate. Preferably, the sample inlet intersects a first channel such that the pressurized plug fluid is introduced into the first channel at an angle to a stream of carrier-fluid passing through the first channel. For example, in preferred embodiments, the sample inlet and first channel intersect at a T-shaped junction; i.e., such that the sample inlet is perpendicular (i.e. at an angle of 90°) to the first channel. However, the sample inlet may intersect the first channel at any angle.

A first channel may in turn communicate with two or more branch channels at another junction or "branch point", forming, for example, a T-shape or a Y-shape. Other shapes and channel geometries may be used as desired. In exemplary embodiments, the angle between intersecting channels is in the range of from about 60° to about 120°. Particular

The device may have one or more outlet ports or inlet ports. Each of the outlet and inlet ports may also communicate with a well or reservoir. The inlet and outlet ports may be in fluid communication with the channels or reservoirs that they are connecting or may contain one or more valves. Fluid can be introduced into the channels via the inlet by any means. Typically, a syringe pump is used, wherein the flow rate of the fluid into the inlet can be controlled.

10X Ex. 1004, Page 45

(Ex. 1004 (Ismagilov) at 14:36-42;  
086 Paper 26 (POR) at 31-32;  
088 Paper 26 (POR) at 24-25).

# Ismagilov Is Missing Several Elements

## **Direct Testimony of Patent Owner's Expert Dr. Anna**

In my opinion, the absence of wells and reservoirs from the definition of outlet port, when they are included in the definition of an inlet port just a few paragraphs earlier, indicates to a POSA that they are not part of the outlet port.

And again, had the inventors intended there to be a specific function for a well or reservoir at the outlet, namely to accumulate fluids in any manner rather than to simply facilitate movement of fluids out of the device using the standard customary techniques known in the art that I described above, they clearly could have done so.

(086 Ex. 2016 (Anna Dec.) at ¶¶121, 125;  
086 Paper 26 (POR) at 30-31;  
088 Ex. 2016 (Anna Dec.) at ¶¶ 103, 107;  
088 Paper 26 (POR) at 23-25).



# Ismagilov Is Missing Several Elements

## **Direct Testimony of Patent Owner's Expert Dr. Anna**

122. Petitioner also points to a separate disclosure in Ismagilov describing that the outlet port “may also communicate with a well or reservoir.” (Pet. 36 (citing Ex. 1004 at 14:36-38)). However, in my opinion this separate disclosure does not teach that the “well or reservoir”—which *may* be in communication with an outlet port—is functioning as a separation chamber at the outlet of the device.

123. I also do not believe that this section of the specification, or any other portion of the specification, provides a description of the structure, function, or operation of any wells or reservoirs that may communicate with an outlet port.

(086 Ex. 2016 (Anna Dec.) at ¶¶122-123;  
086 Paper 26 (POR) at 31-32;  
088 Ex. 2016 (Anna Dec.) at ¶¶ 104-105;  
088 Paper 26 (POR) at 24-25).

# Ismagilov Is Missing Several Elements

## **Direct Testimony of Patent Owner's Expert Dr. Anna**

129. I believe that Petitioner is improperly equating the term “outlet,” i.e., “manifold outlet” with Ismagilov’s prior disclosure of an “outlet port” even though Ismagilov does not actually refer to the “manifold outlet” as a “port” of any kind. As Ismagilov states, the “manifold outlet” is only described as “facilitating the movement of plugs” from different analysis units. (Ismagilov at 17:18-22).

(086 Ex. 2016 (Anna Dec.) at ¶ 129;  
086 Paper 26 (POR) at 32-33;  
088 Ex. 2016 (Anna Dec.) at ¶111;  
088 Paper 26 (POR) at 25-26).

(19) **United States**  
(12) **Patent Application Publication**  
**Quake et al.**

(54) **MICROFABRICATED CROSSFLOW DEVICES AND METHODS**

(75) Inventors: **Stephen R. Quake**, Pasadena, CA (US); **Todd Thorsen**, Pasadena, CA (US)

Correspondence Address:  
**DARBY & DARBY P.C.**  
805 Third Avenue  
New York, NY 10022 (US)

(73) Assignee: **California Institute of Technology**

(21) Appl. No.: **09/953,103**

(22) Filed: **Sep. 14, 2001**

**Related U.S. Application Data**

(63) Non-provisional of provisional application 60/246,793, filed on Nov. 8, 2000. Non-provisional application No. 60/233,037, filed 15, 2000.

**Publication Classification**

(51) Int. Cl.<sup>7</sup> **..... C12M 1/26**

US 2002/0058332 A1

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is a function of the cross-sectional area of the channel and the cell concentration, the latter typically determined in view of acceptable values of  $P_{2,2}$  discussed above. From these relationships, it is then easy to calculate the maximum channel length between the detection and discrimination region which would ensure that cells do not change order or position in the line of cells. In practice, the channel length between the detection and discrimination regions is between about 1  $\mu\text{m}$  and about 100  $\mu\text{m}$ .

[0191] Shear forces may affect the velocity at which the cells move through the microfluidic device, particularly when living cells are to be sorted and collected. Experiments have shown that high electric fields, in the range of 2-4 kV/cm for human erythrocytes and 5-10 kV/cm for yeast cells can be used to introduce DNA and other substances into cells using electroporation. At these voltages there was no cell lysis, although membrane permeation was possible. To avoid membrane permeation and cell lysis, it is preferred that the electric fields applied to move cells in any of the described flow techniques is less than about 600 V/cm and most preferably less than about 100 V/cm.

**EXAMPLE 7**

[0192] **Elastomeric Microfabricated Device**

[0193] This Example demonstrates the manufacture and operation of a disposable microfabricated FACS device, which can function as a stand-alone device or as a component of an integrated microanalytical chip, in sorting cells or biological materials. The device permits high sensitivity, no cross-contamination, lower cost to operate and manufacture than conventional FACS machines and multiple-hour run times. In this example, the microfabricated chip had a detection volume of approximately 250 fl and single channel throughput of about 20 cells/second. The device obtained substantial enrichment of micron-sized fluorescent bead populations of different colors. In addition, populations of *E. coli* cells expressing green fluorescent protein were separated, and enriched, from a background of non-fluorescent (wild type) *E. coli* cells. The bacteria were also found to be viable after extraction from the sorting device.

[0194] **Preparation of the microfabricated device**

[0195] A silicon wafer was etched and fabricated as described above and in (12). After standard contact photolithography techniques to pattern the oxide surface of the silicon wafer, a  $\text{C}_2\text{F}_4/\text{CHF}_3$  gas mixture was used to etch the wafer by RIE. The silicon wafer was then subjected to further etch with KOH to expose the silicon underneath the oxide layer, thereby forming a mold for the silicone elastomer. The silicon mold forms a "T" arrangement of channels. The dimensions of the channels may range broadly, having approximately 5-4  $\mu\text{m}$  dimension.

[0196] A representative device of the invention is shown in FIG. 6. The etching process is shown schematically in FIG. 7. Standard micromachining techniques were used to create a negative master mold out of a silicon wafer. The disposable silicone elastomer chip was made by mixing General Electric RTV 615 components (36) together and pouring onto the etched silicon wafer. After curing in an oven for two hours at 80° C., the elastomer was peeled from the wafer and bonded hermetically to a glass cover slip for sorting. To make the elastomer hydrophilic the elastomer

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chip was immersed in HCl (pH=2.7) at 60 degrees C for 40 to 60 min. Alternatively, the surface could have been coated with polyurethane (3% w/v in 95% ethanol and diluted 10 X in ethanol). It is noted that the master wafer can be reused indefinitely. The device shown has channels that are 100  $\mu\text{m}$  wide at the wells, narrowing to 3  $\mu\text{m}$  at the sorting junction (discrimination region). The channel depth is 4  $\mu\text{m}$ , and the wells are 2 mm in diameter.

**[0197]** In this embodiment the cell-sorting device was mounted on an inverted optical microscope (Zeiss Axiovert 35) as shown in **FIG. 8**. In this system, the flow control can be provided by voltage electrodes for electro-osmotic control or by capillaries for pressure-driven control. The detection system can be photo multiplier tubes or photodiodes, depending upon the application. The inlet well and two collection wells were incorporated on three sides of the "T" forming and 7). The chip was adhered mounted onto the microscope.

[198] Three platinum electrode separate wells. A water-cooled / 100 W mercury lamp (for bead immersion objective (Olympus F used to excite the fluorescence at (CCD) camera took the image. To emission a (330 nm) 30 emission detection region was approximal T-junction and has a window o dimension. The window is imple able slit. Using one or two Hamt buns (bias = 850 V) with custom for or using photodiode or de emission filters (depending on t rent) from the detector's) are Bur-Brown OP128 optical ampli National Instrument PC1200 as computer. The voltages on the el part of Asca P424 UV cop osing supplies. The third electrode v voltage settings on the PC1200 h amplification to the platinum e switching of the directions of the directed to either side of the "T" the voltage potential settings. I microfluidic in the device de trolled computer programs, e.g. ferent procedures using known and 7).

[0199] Sorting Experiments

**[200]** This embodiment of the system was used to sort fluor emission wavelengths in different per hour throughput (See FIGS. 9-12). Extra-reservoir wells were incorporated into the outer side of the three wells of the chip in order to avoid ion-depletion, and platinum electrodes (with the ground electrode in the inlet well) were inserted into the reservoir wells. One micron diameter beads were suspended in PBS (1.37 mM NaCl, 2.7 mM KCl, 4.3 mM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1.4 mM KH<sub>2</sub>PO<sub>4</sub> with 10% BSA (1 g/l) and 0.5% Tween 20 in various ratios and dilutions. Samples with different bead concentrations, having different sizes, indicated below, were injected into the inlet well in 10 to 30  $\mu$ l aliquots. The collection wells were filled with the same buffer.

is a function of the cross-sectional area of the channel and the cell concentration, the latter typically determined in view of acceptable values of  $P_{22}$ , discussed above. From these relationships, it is then easy to calculate the maximum channel length between the detection and discrimination region which would ensure that cells do not change order or position in the line of cells. In practice, the channel length between the detection and discrimination regions is between about 1  $\mu\text{m}$  and about 100  $\mu\text{m}$ .

**[0191]** Shear forces may affect the velocity at which the cells move through the microfluidic device, particularly when living cells are to be sorted and collected. Experiments have shown that high electric fields, in the range of 2–5 kV/cm, cause human erythrocytes and 5–10 V/cm for yeast cells can be used to introduce DNA and other substances into cells using electroporation. At these voltages there was no cell lysis, although membrane permeation was possible. To avoid membrane permeation and cell lysis, it is preferred that the electric fields applied to move cells in any of the described flow techniques is less than about 600 V/cm and most preferably less than about 100 V/cm.

### EXAMPLE 7

[0192] Elastomeric Microfabricated Device

**[0193]** This sample demonstrates the manufacture and operation of a disposable microfabricated FACS device, which can function as a stand-alone device or as a component of an integrated microanalytical chip, in sorting cells or biological materials. The device permits high sensitivity, no-cross-contamination, lower cost to operate and manufacture than conventional FACS machines and a multiple-hour run time. For example, approximately 250 cells were sorted in a detection volume of approximately 250 fl and single channel throughput of about 20 cells/second. The device obtained substantial enrichment of micron-sized fluorescent bead populations of different colors. In addition, populations of *E. coli* cells expressing green fluorescent protein were separated from a mixture of green fluorescent protein (wild type) (*E. coli* cells). The bacteria were also found to be viable after extraction from the sortine device.

[0194] Preparation of the microfabricated device

**[0195]** A silicon wafer was etched and fabricated as described above and in (12). After standard contact photolithography techniques to pattern the oxide surface of the silicon wafer, a  $C_2F_4/CHF_3$  gas mixture was used to etch the wafer by RIE. The silicon wafer was then subjected to further etch with KOH to expose the silicon underneath the oxide layer, thereby forming a mold for the silicone elastomer. The silicon mold forms a "T" arrangement of channels. The dimensions of the channels may range broadly, having approximately  $5 \times 4 \mu m$  dimension.

**[0196]** A representative device of the invention is shown in FIG. 6. The etching process is shown schematically in FIG. 7. Standard micromachining techniques were used to create a negative master mold out of a silicon wafer. The disposable silicone elastomer chip was made by mixing General Electric RTV 615 components (36) together and pouring onto the etched silicon wafer. After curing in an oven for two hours at 80° C., the elastomer was peeled from the wafer and bonded hermetically to a glass cover slip for sorting. To make the elastomer hydrophilic the elastomer

10X Ex. 1006, Page 45

[0197] In this embodiment the cell-sorting device was mounted on an inverted optical microscope (Zeiss Axiovert 35) as shown in FIG. 8. In this system, the flow control can be provided by voltage electrodes for electro-osmotic control or by capillaries for pressure-driven control. The detection system can be photo multiplier tubes or photodiodes, depending upon the application. The inlet well and two collection wells were incorporated into the elastomer chip on three sides of the “T” forming three channels (FIGS. 6 and 7). The chip was adhered to a glass coverslip and mounted onto the microscope.

(Ex. 1006 (Quake) ¶197;  
086 Paper 26 (POR) at 45-48;  
088 Paper 26 (POR) at 39-42).

# No Motivation to Combine Ismagilov and Quake

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q        It's your opinion that Quake used  
these wells to collect droplets; is that right?

A        No.

Q        What -- how did I get that wrong?

A        Quake did not use droplets, he  
injected dye, which then he sorted based upon  
red or green. And this is not a droplet-based  
system.

(Ex. 2017 (Fair Cross Tr.) at 141:20-142:2;  
086 Paper 26 (POR) at 45-47;  
088 Paper 26 (POR) at 39-41).

# No Motivation to Combine Ismagilov and Quake

If a POSA with Ismagilov would want additional information regarding microfluidic structures, the POSA would have looked to the work of a leader in developing early microfluidic devices: Quake. Fair, ¶ 107.

(086 Paper 2 (Petition) at 27-28;  
088 Paper 2 (Petition) at 26).





“[Petitioner] does not explain what problems exist in the web of Perkins [primary reference], or how those problems would have been solved by Mykleby [secondary reference]. By [Petitioner’s] own analysis, the problem of ‘continuous, quick production of plastic bags’ is addressed by the web and apparatus of Perkins itself; it is unclear why a person of ordinary skill in the art would have considered it desirable to further modify Perkins to include the variable perforations of Mykleby.”

(086 Paper 26 (POR) at 46;  
088 Paper 26 (POR) at 39-40).

# No Motivation to Combine Ismagilov and Quake

A POSA would have been motivated to combine Ismagilov with Quake. Stephen Quake was well-known in microfluidics, and a POSA with Ismagilov would have been motivated to combine with Quake for additional microfluidic structures, including particularly wells or reservoirs.





“Noting that the references differ only in their perforation patterns, [Petitioner] contends that a person of ordinary skill in the art would have considered the references “highly similar and completely complementary,” and would “look to both [references] to obtain advantages of the other.”

“[Petitioner’s] argument may be enough to show that the two references are analogous art, *see Wyers v. Master Lock Co.*, 616 F.3d 1231, 1238 (Fed. Cir. 2010), but simply demonstrating that a set of references are all directed to the same problem is not, by itself, a sufficient rationale to combine the references.”

(086 Paper 26 (POR) at 46;  
088 Paper 26 (POR) at 39-40).

(19) **United States**  
(12) **Patent Application Pub**  
**Quake et al.**

(54) **MICROFABRICATED CROSSFLOW DEVICES AND METHODS**

(75) Inventors: **Stephen R. Quake**, Pasadena, (US); **Todd Thorsen**, Pasadena, (US)

Correspondence Address:  
**DARBY & DARBY P.C.**  
805 Third Avenue  
New York, NY 10022 (US)

(73) Assignee: **California Institute of Technol**

(21) Appl. No.: **09/953,103**

(22) Filed: **Sep. 14, 2001**

**Related U.S. Application Data**

(63) Non-provisional of provisional application 60/246,793, filed on Nov. 8, 2000. Non-prov. provisional application No. 60/233,037, filed 15, 2000.

**Publication Classification**

(51) Int. Cl.<sup>7</sup> ..... **CL**

US 2002/0058332 A1

21

May 16, 2002

is a function of the cross-sectional area of the channel and the cell concentration, the latter typically determined in view of acceptable values of  $P_{22}$ , discussed above. From these relationships, it is then easy to calculate the maximum channel length between the detection and discrimination region which would ensure that cells do not change order or position in the line of cells. In practice, the channel length between the detection and discrimination regions is between about 1  $\mu\text{m}$  and about 100  $\mu\text{m}$ .

[0191] Shear forces may affect the velocity at which the cells move through the microfluidic device, particularly when living cells are to be sorted and collected. Experiments have shown that high electric fields, in the range of 2-4 kV/cm for human erythrocytes and 5-10 kV/cm for yeast cells can be used to introduce DNA and other substances into cells using electroporation. At these voltages there was no cell lysis, although membrane permeation was possible. To avoid membrane permeation and cell lysis, it is preferred that the electric fields applied to move cells in any of the described flow techniques is less than about 600 V/cm and most preferably less than about 100 V/cm.

**EXAMPLE 7**

[0192] **Elastomeric Microfabricated Device**

[0193] This Example demonstrates the manufacture and operation of a disposable microfabricated FACS device, which can function as a stand-alone device or as a component of an integrated microanalytical chip, in sorting cells or biological materials. The device permits high sensitivity, no cross-contamination, lower cost to operate and manufacture than conventional FACS machines and multiple-hour run times. In this example, the microfabricated chip had a detection volume of approximately 250 fl and single channel throughput of about 20 cells/second. The device obtained substantial enrichment of micron-sized fluorescent bead populations of different colors. In addition, populations of *E. coli* cells expressing green fluorescent protein were separated, and enriched, from a background of non-fluorescent (wild type) *E. coli* cells. The bacteria were also found to be viable after extraction from the sorting device.

[0194] **Preparation of the microfabricated device**

[0195] A silicon wafer was etched and fabricated as described above and in (12). After standard contact photolithography techniques to pattern the oxide surface of the silicon wafer, a  $\text{C}_2\text{F}_4/\text{CHF}_3$  gas mixture was used to etch the wafer by RIE. The silicon wafer was then subjected to further etch with KOH to expose the silicon underneath the oxide layer, thereby forming a mold for the silicone elastomer. The silicon mold forms a "T" arrangement of channels. The dimensions of the channels may range broadly, having approximately 5-8  $\mu\text{m}$  dimension.

[0196] A representative device of the invention is shown in FIG. 6. The etching process is shown schematically in FIG. 7. Standard micromachining techniques were used to create a negative master mold out of a silicon wafer. The disposable silicone elastomer chip was made by mixing General Electric RTV 615 components (36) together and pouring onto the etched silicon wafer. After curing in an oven for two hours at 80° C., the elastomer was peeled from the wafer and bonded hermetically to a glass cover slip for sorting. To make the elastomer hydrophilic the elastomer

chip was immersed in HCl (pH=2.7) at 60 degrees C for 40 to 60 min. Alternatively, the surface could have been coated with polyurethane (3% w/w in 95% ethanol and diluted 10 X in ethanol). It is noted that the master wafer can be reused indefinitely. The device shown has channels that are 100  $\mu\text{m}$  wide at the wells, narrowing to 3  $\mu\text{m}$  at the sorting junction (discrimination region). The channel depth is 4  $\mu\text{m}$ , and the wells are 2 mm in diameter.

[0197] In this embodiment the cell-sorting device was mounted on an inverted optical microscope (Zeiss Axiovert 35) as shown in FIG. 8. In this system, the flow control can be provided by voltage electrodes for electro-osmotic control or by capillaries for pressure-driven control. The detection system can be photo multiplier tubes or photodiodes, depending upon the application. The inlet well and two collection wells were incorporated on three sides of the "T" formed and 7). The chip was adhered mounted onto the microscope.

[0198] Three platinum electrodes separate wells. A water-cooled 100 W mercury lamp (for bead immersion objective (Olympus F used to excite the fluorescence at (CCD) camera took the image. It emission a 630 nm±30 emission detection region was approximal T-junction and has a window of dimension. The window is imple able slit. Using one or two Hammm tubes (bias -850 V) with custom fier, or using photodiodes, as de emission filters (depending on th rent(s) from the detector(s) were Burr-Brown OP128 optical ampl National Instrument PCI2000 b computer. The voltages on the el pair of Apex PA42 HV op amps i supplies. The third electrode w voltage settings on the PCI2000 b amplification to the platinum d switching of the directions of the directed to either side of the "T" the voltage potential settings. Fur sorting in the microfluidic devic ferent computer programs, e.g. trolled procedures using known

[0199] **Sorting Experiments**

[0200] This embodiment of t system was used to sort flow emission wavelengths in differer per hour throughput (See FIGS. 9-12). Extra reservoir wells were incorporated into the outer side of the three wells of the chip in order to avoid ion-depletion, and platinum electrodes (with the ground electrode in the inlet well) were inserted into the reservoir wells. One micron diameter beads were suspended in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1.4 mM  $\text{KH}_2\text{PO}_4$ ) with 10% BSA (1 g/l) and 0.5% Tween 20 in various ratios and dilutions. Samples of the different colored fluorescent beads, having ratios as indicated below, were injected into the inlet well in 10 to 30  $\mu\text{l}$  aliquots. The collection wells were filled with the same buffer.

[0197] In mounted on 35) as shown be provided control or by ca tion system depending u collection w on three side and 7). The mounted on

10X Ex. 1006, Page 45

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

# No Motivation to Combine Ismagilov and Quake

11X0001250001.D5

US 7,129,091 B2

**1**  
**DEVICE AND METHOD FOR  
PRESSURE-DRIVEN PLUG TRANSPORT  
AND REACTION**

This application claims priority to U.S. Provisional Application No. 60/379,927 filed on May 9, 2002 and U.S. Provisional Application No. 60/394,544 filed on Jul. 8, 2002, the entirety of which are incorporated herein by reference.

**BACKGROUND**

Taylor dispersion broadens and dilutes sample plugs. Some of the sample is frequently left behind the plug as a tail. Overlap of these tails usually leads to cross-contamination of samples in different plugs. Thus, samples are often introduced into the channels individually, separated by buffer washes. On the other hand, interleaving samples with long buffer plugs, or washing the system with buffer between samples, reduces the throughput of the system.

In EOF, flow transport is essentially linear, that is, if two reactants are introduced into a plug and transported by EOF, their residence time (and reaction time) can be calculated simply by dividing the distance traveled in the channel by the velocity. This linear transport allows precise control of

**SUMMARY ACCORDING TO THE INVENTION**

In accordance with the invention, a method of conducting a reaction within a substrate is provided that comprises introducing a carrier-fluid into a first channel of the substrate; introducing at least two different plug-fluids into the first channel; and applying pressure to the first channel to induce a fluid flow in the substrate to form substantially identical plugs comprising a mixture of plug-fluids.

charged and does not adsorb to the walls. However, this can be a serious limitation in applications that involve proteins that are often charged and tend to adsorb on charged surfaces. In addition, high voltages are often undesirable, or sources of high voltages such as portable analyzers may not be available.

Flows driven by pressure are typically significantly less sensitive to surface chemistry than EOF. The main disadvantage of pressure-driven flows is that they normally have a parabolic flow profile instead of the flat profile of EOF. Solute in the middle of the channel move much faster (about twice the average velocity of the flow) than solutes near the walls of the channels. A parabolic velocity profile normally leads to high dispersion in pressure-driven flows; a plug of solute injected into a channel is immediately distorted and stretched along the channel. This distortion is somewhat reduced by solute transport via diffusion from the middle of the channel towards the walls and back. But the distortion is made worse by diffusion along the channel (the overall dispersion is known as Taylor dispersion).

**SUMMARY ACCORDING TO THE INVENTION**

In accordance with the invention, a method of conducting a reaction within a substrate is provided that comprises introducing a carrier-fluid into a first channel of the substrate; introducing at least two different plug-fluids into the first channel; and applying pressure to the first channel to induce a fluid flow in the substrate to form substantially identical plugs comprising a mixture of plug-fluids. The plug-fluids are immiscible with the carrier-fluid. During plug formation, the cross-section of the plug is substantially similar to the cross-section of the first channel, so that the plug is substantially in contact with all walls of the first channel. After plug formation, the cross-section of the plug may be smaller than the cross-section of the channel. A thin layer of carrier-fluid typically exists between the wall of the channel and the plug, although in some cases this layer disappears. In general, each plug is substantially similar in

10X Ex. 1004, Page 39

(Ex. 1004 (Ismagilov) at 2:50-58; 086 Paper 26 (POR) at 45-48; 088 Paper 26 (POR) at 39-42).

US 2002/0058332 A1

May 16, 2002

23

(PDMS), preferably PDMS. This is cast from a mold made by etching the negative image of these channels into the same type of crystalline silicon wafer used in semiconductor fabrication. As described above, the same techniques for patterning semiconductor features are used to form the pattern of the channels. The uncured liquid silicone rubber is poured onto these molds placed in the bottom of a Petri dish. To speed the curing, these poured molds are baked. After the PDMS has cured, it is removed from on top of the mold and trimmed. In a chip with one set of channels forming a "T", three holes are cut into the silicone rubber at the ends of the "T", for example using a hole cutter similar to that used for cutting holes in cork, and sometimes called cork borers. These holes form the sample, waste and collection wells in the completed device. In this example, the hole at the bottom end of the T is used to load the sample. The hole at one arm of the T is used for collecting the sorted sample while the opposite arm is treated as waste. Before use, the PDMS device is placed in a hot bath of HCl to make the surface hydrophilic. The device is then placed onto a No. 1 (150 µm thick) (25x25 mm) square microscope cover slip. The cover slip forms the floor (or the roof) for all three channels and wells. The chip has a detection region as described above.

[0217] Note that any of or all of these manufacturing and preparation steps can be done by hand, or they can be automated, as can the operation and use of the device.

[0218] The above assembly is placed on an inverted Zeiss

[0221] Electrodes

[0222] Two of the above electrodes are driven by high voltage operational amplifiers (op-amps) capable of supplying voltages of  $\pm 150$  V. The third electrode is connected to the electrical ground (or zero volts) of the high voltage op-amp electronics. For sorting operation the driven electrodes are placed in the collection and waste wells. The ground electrode is placed in the sample well. The op-amps amplify, by a factor of 30, a control voltage generated by two digital to analog converters (DACs). The maximum voltage these DACs generate is  $\pm 5$  V, which determines the amplification factor of 30. The 150 V limit is determined by the power supply to the amplifiers, which are rated for  $\pm 175$  V. These DACs reside on a card (a Lab PC1200 Card, available from National Instruments, Austin, Tex.) mounted in a personal computer. The card also contains multiple channels of analog to digital converters (ADC's) one of which is used for measuring the signal generated by photo multiplier tubes (PMTs). This card contains two DACs. A third DAC can be used to drive the third electrode with an additional high voltage op amp. This would provide a larger voltage gradient, if desired, and some additional operational flexibility.

[0223] Without being bound by any theory, it is believed that the electrodes drive the flow of sample through the device using electro-osmotic or electrophoretic forces, or both. To start the movement of cells or particles to be sorted, a voltage gradient is established in the channels. This is done

the sample well with sample. In this embodiment, it is preferred that the flow of material through the device (i.e. the flow of sample) be driven only by the electrodes, e.g. using electro-osmotic and/or electrophoretic forces. The electrodes may be in place during loading, or they can be placed into the wells after loading, to contact the buffer.

Preferably, no other well is loaded until all the channels fill with buffer, to avoid the formation of air pockets. After the channels fill the remaining wells can be loaded with buffer, as needed, to fill or equilibrate the device. The input or sample well is typically loaded last so that the flow of liquid in the channels is initially directed towards it. Generally, equal volumes of buffer or sample are loaded into each well. This is done in order to prevent a net flow of liquid in any direction once all of the wells are loaded, including loading the sample well with sample. In this embodiment, it is preferred that the flow of material through the device (i.e. the flow of sample) be driven only by the electrodes, e.g. using electro-osmotic and/or electrophoretic forces. The electrodes may be in place during loading, or they can be placed into the wells after loading, to contact the buffer.

voltage at the electrode in one well (or multiple wells, in multi-channel embodiments) is made the same as the voltage at the junction or branch point, where the channels meet. The voltage of the electrode at one well of the two or more wells is raised or lowered, to produce a gradient between that well and the branch point. This causes the flow to move down the channel towards and into the well, in the direction produced by the gradient. Typically, the voltage of the electrode at the waste well is raised or lowered with respect to the voltage at the collecting well, to direct the flow into the waste channel and the waste well, until a particle or cell is identified for collection. The flow is diverted into the collection channel and collection well by adjusting the voltages at the electrodes to eliminate or reduce the gradient toward the waste well, and provide or increase the gradient toward the col-

10X Ex. 1006, Page 47

(Ex. 1006 (Quake) at ¶220; 086 Paper 26 (POR) at 45-48; 088 Paper 26 (POR) at 39-42).



# Ismagilov and Quake Are Missing Several Elements

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q Now, as part of your obviousness analysis, did you start with the idea that a person of ordinary skill would have recognized contamination problem and then solved it or did you start with the claim language and then analyze the prior art?

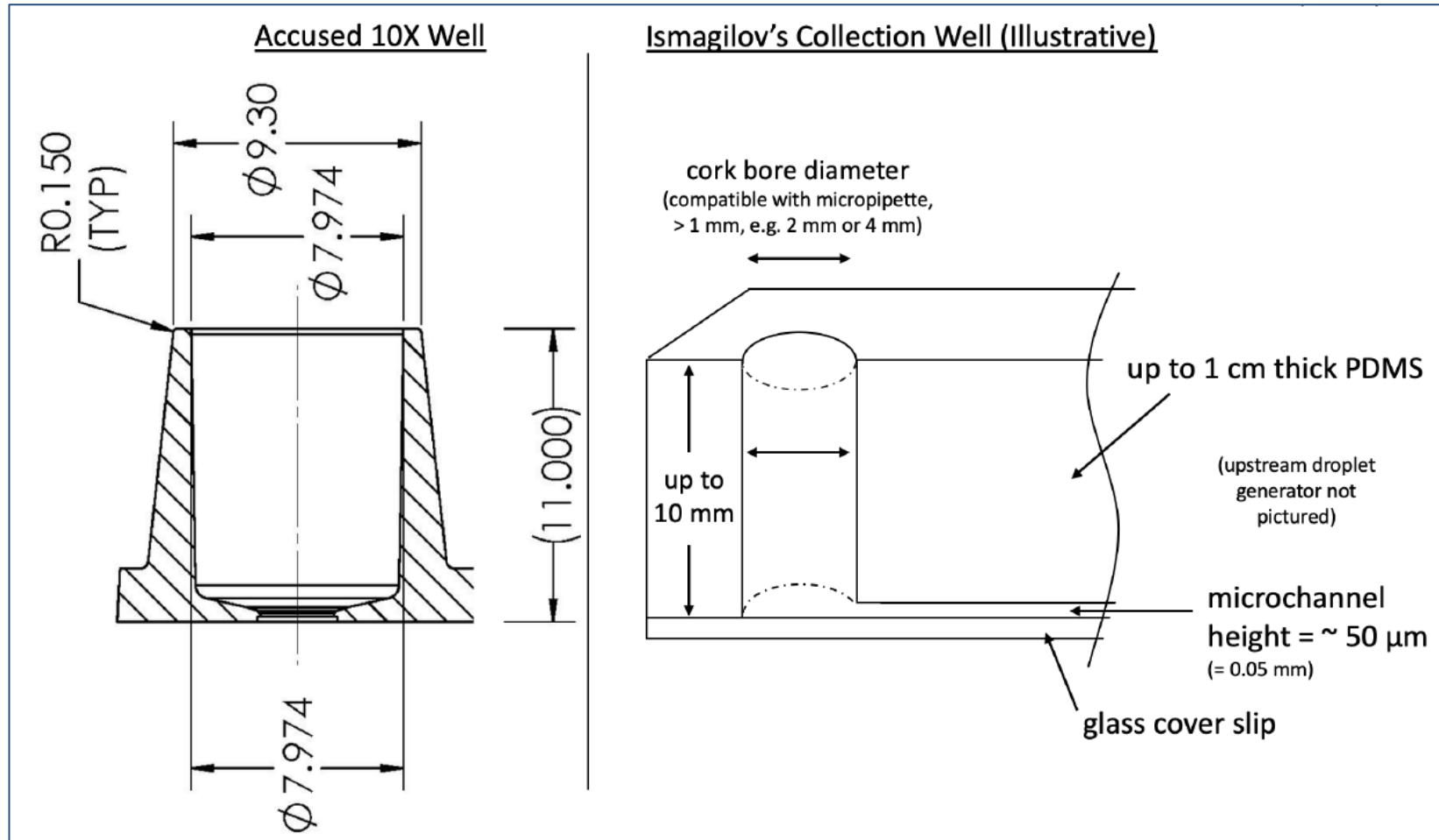
MS. JAMESON: Objection to form.

THE WITNESS: I would have to say I started with looking at the claim language and I found appropriate references to cite in an invalidity analysis.

But that was not -- first and foremost, I was looking for reading the method of the limitations on reading prior art under the methods claims -- claim elements.

(Ex. 2017 (Fair Cross Tr.) at 52:23-53:8, 54:16-19;  
086 Paper 26 (POR) at 47-48;  
088 Paper 26 (POR) at 41-42).

# Ismagilov Is Missing Several Elements



(086 Ex. 1071 (Fair Rep. Decl.) at ¶37;  
086 Paper 31 (Sur-Reply) at 10-13;  
088 Ex. 1073 (Fair Reply Decl.) at ¶33;  
088 Paper 31 (Sur-Reply) at 10-13).

# Ismagilov and Quake Are Missing Several Elements

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q      When is the first time you used a  
pipette to remove a droplet from a chip and move  
it off chip?

A      I don't think I've done that.

Q      You've never done that?

A      Not on electrowetting chips, no.

Q      On any other chips?

A      Not that I can recall.

(Ex. 2017 (Fair Cross Tr.) at 43:9-16;  
086 Paper 26 (POR) at 33-34;  
088 Paper 26 (POR) at 26-27).

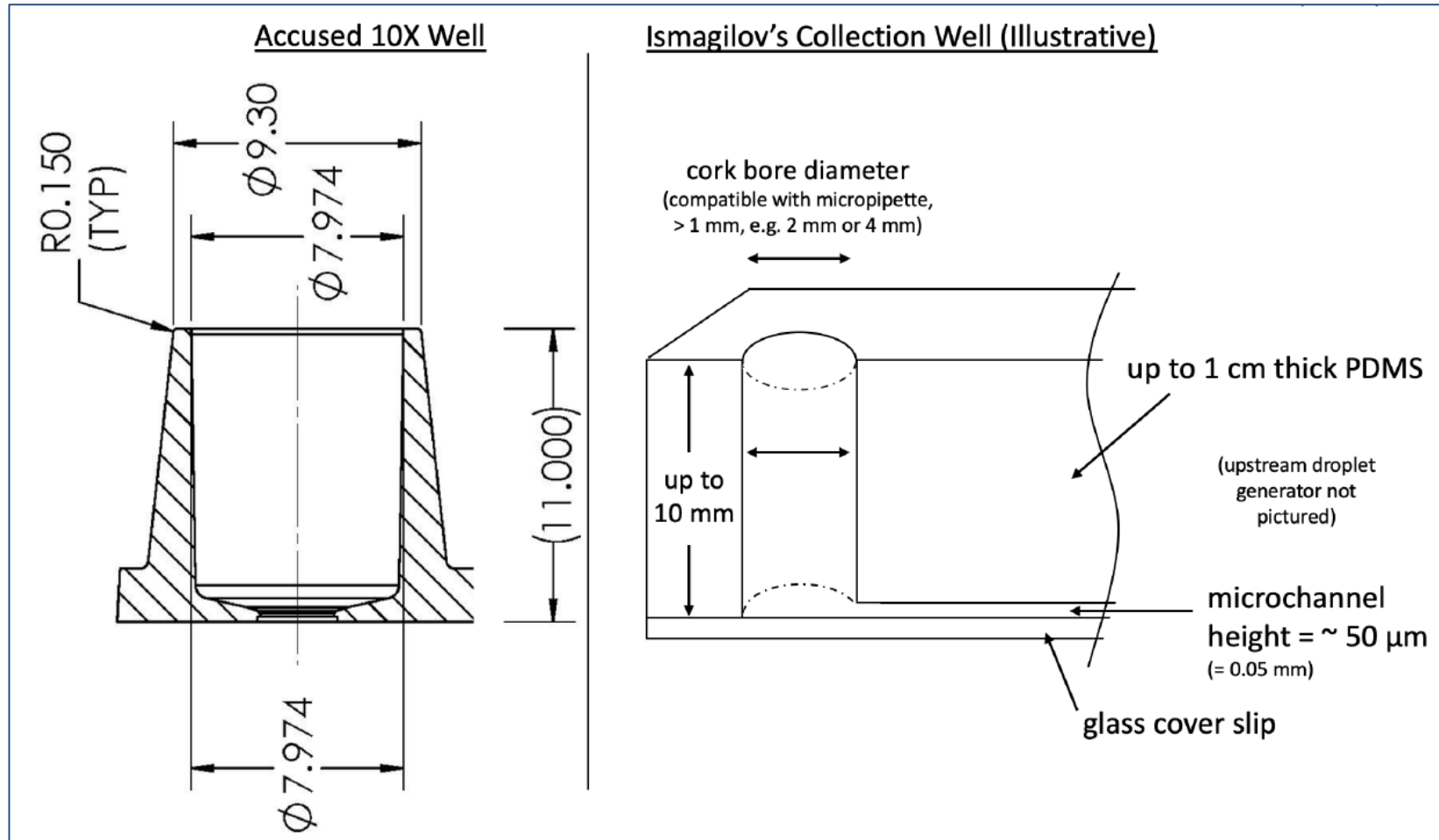


“[I]t is impermissible to use the claimed invention as an instruction manual or ‘template’ to piece together the teachings of the prior art so that the claimed invention is rendered obvious.”

(086 Paper 26 (POR) at 47-48;  
088 Paper 26 (POR) at 41-42).



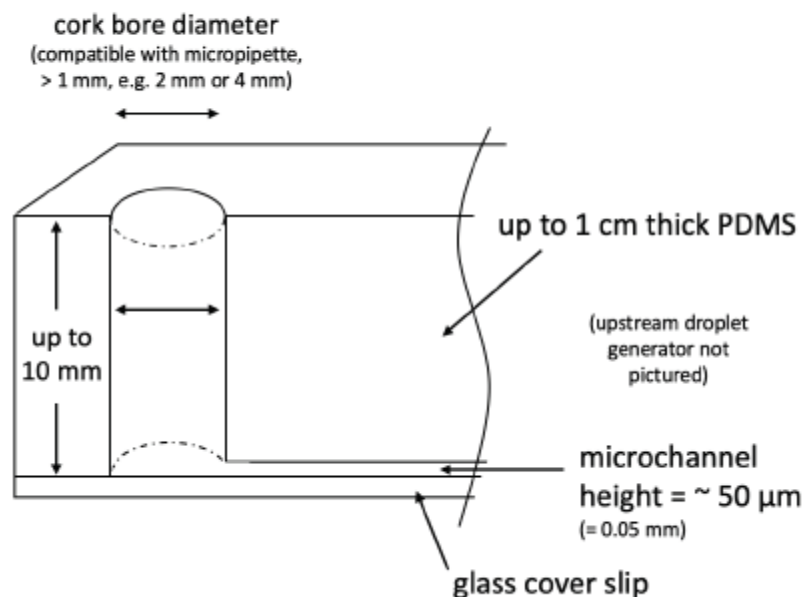
# Ismagilov Is Missing Several Elements



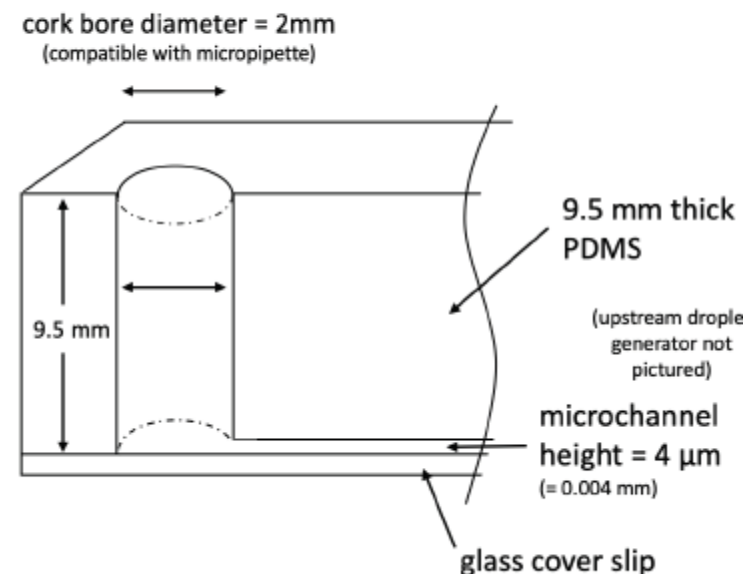
(086 Ex. 1071 (Fair Rep. Decl.) at ¶37;  
086 Paper 31 (Sur-Reply) at 10-13;  
088 Ex. 1073 (Fair Reply Decl.) at ¶33;  
088 Paper 31 (Sur-Reply) at 10-13).

# Ismagilov and Quake Are Missing Several Elements

Ismagilov's Collection Well (Illustrative)



Ismagilov's Collection Well + Quake's Dimensions



(086 Ex. 1071 (Fair. Reply Decl.) at ¶42;  
086 Paper 31 (Sur-Reply) at 15-16;  
088 Ex. 1073 (Fair Reply Decl.) at ¶38;  
088 Paper 31 (Sur-Reply) at 15-17).

# Ismagilov and Quake Are Missing Several Elements

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q      Okay. If I asked you to design a system where the outlet well was in the same plane as the channel, could you do that?

A      You could you do that, but if the collection well that you're describing has the same height as the channel, then it would be very difficult to do separation of oil and liquid and droplets.

So the early waste reservoirs that we built were, in fact, designed to have the same height as the channels in our devices. And they were just holding -- they just were there to hold the droplets, not to separate from the oil.

Q      You did not put the outlet wells vertical to the channel until you decided you wanted to separate?

A      That's correct.

(Ex. 2017 (Fair Cross Tr.) at 69:2-18;  
086 Paper 26 (POR) at 39-40;  
088 Paper 26 (POR) at 32-33).

# Secondary Considerations

# Secondary Considerations

8/11/2020

Terms and Conditions | Bio-Rad

BIO-RAD

Home > Trading Terms

## Terms and Conditions

### BIO-RAD LABORATORIES, INC. STANDARD TERMS AND CONDITIONS OF SALE

These Standard Terms and Conditions of Sale set forth the terms and conditions that apply to all sales of goods and services by means of a purchase order or other written order ("PO") received by Bio-Rad Laboratories, Inc. or an affiliate in its global group of companies ("Bio-Rad") to the entity identified in the PO as the buyer ("Buyer"). The terms and conditions included with Bio-Rad's written quotation (if any) ("Quotation") and acknowledgement of the PO or invoice with shipment ("Invoice") are incorporated herein by reference, and such terms and conditions together with these Standard Terms and Conditions of Sale and including any written contract between the parties are referred to herein as the "Agreement." Notwithstanding anything herein to the contrary, if a written contract signed by both parties is in existence covering the sale of the Goods and Services, the terms and conditions of said contract shall prevail to the extent they are inconsistent with the terms of the Quotation, PO, Invoice or these Standard Terms and Conditions of Sale. The Agreement is formed between the Bio-Rad entity listed on the Quotation or Invoice and Buyer when the Buyer receives confirmation that Bio-Rad has accepted the PO.

1. **Agreement.** Bio-Rad or its affiliate shall provide the products ("Goods" or "Products") and perform the services ("Services") described in a Quotation in accordance with the terms and conditions of this Agreement. By submitting a PO, accepting shipment of Goods or the commencement of the performance of Services, Buyer shall be bound by the provisions of this Agreement, whether Buyer acknowledges or otherwise signs this Agreement or the Invoice, unless Buyer expressly objects to such terms in writing prior to accepting the Goods or commencing the performance of Services. This Agreement may not be added to, modified, superseded, or otherwise altered, except in writing signed by an authorized Bio-Rad representative. Bio-Rad shall not be bound by any different or additional terms or conditions (a) contained in any POs, pre-printed forms, online agreements, or in any other documents or communications issued by Buyer, or (b) arising from prior courses of dealing, usages of trade, or verbal agreements not reduced to writing and signed by Bio-Rad. Any such different or additional terms or conditions are hereby rejected, and shall be deemed null and void, without the need for Bio-Rad to expressly reject such terms. To the extent that an Invoice might be treated as an acceptance of Buyer's PO, such acceptance is expressly made on condition of assent by Buyer to the terms and conditions of this Agreement, and Buyer's acceptance of the shipment of the Goods or commencement of the performance of Services shall constitute such assent. Any supplemental terms, such as label licenses or field restrictions included with the sale of goods or services hereunder, shall be in addition to, and if in conflict, shall take precedence over the conflicting terms of this Agreement.

2. **Changes.** Bio-Rad may at any time make changes in the specifications, designs, drawings, samples, qualities, prices, terms, conditions, requirements or descriptions to which the Goods or Services are to conform. Bio-Rad shall not be bound by the descriptions or specifications of the Goods or Services, or any other information contained in, any advertisement, publication, booklet or pamphlet of Bio-Rad. If any such change causes an increase or decrease in the cost of or the time required for the performance of any part of the work under the Agreement, then Bio-Rad and Buyer shall discuss in good faith whether a written modification should be made to the Agreement to adjust the price or delivery schedule, or both. Any claim by Buyer for such an adjustment must be made in writing within thirty (30) days of the receipt of any such notice provided by Bio-Rad. Nothing in this clause shall excuse Buyer from proceeding without delay to perform the PO as changed.

3. **Cancellations.** A PO constitutes a firm offer and may not be revoked or cancelled at any time. Bio-Rad hereby reserves the right to reschedule any delivery or cancel any PO issued at any time and Bio-Rad shall not be subject to any charges or other fees as a result of such cancellation.

4. **Shipment.** All Goods shall be suitably packed in Bio-Rad's standard shipment packaging, marked, and shipped in accordance with Bio-Rad's applicable specifications (or if no specifications are provided, in accordance with reasonable commercial practices) using a carrier of Bio-Rad's choice. Goods may be shipped, depending on lead time and availability, in installments. Each installment shall be invoiced and considered a separate sale. Unless otherwise agreed, Buyer shall clear any Imported Goods at the point of import and pay all relevant duties. Delivery terms (Incoterms 2010) shall be set forth in any Quotation.

5. **Delivery.** Bio-Rad shall make a commercially reasonable attempt to deliver the Goods at the time stated in the order confirmation (when applicable) but such dates are estimates only and are not guaranteed. Such dates shall not constitute a term or condition of the Agreement between Bio-Rad and Buyer. Bio-Rad accepts no liability whatsoever to meet such dates and such failure shall not entitle Buyer to repudiate or cancel a PO. If Bio-Rad delivers any Goods in quantities that are less than the quantities specified in the PO, Buyer shall notify Bio-Rad of the discrepancy and Bio-Rad shall deliver the confirmed missing amount of Goods. If Bio-Rad delivers any Goods in quantities in excess of the quantities specified in the PO, Buyer shall return the amount of the overshipment to Bio-Rad or notify Bio-Rad of intention to retain such overshipment. Buyer shall pay for those quantities of Goods actually accepted by Buyer.

6. **Inspection.** Buyer shall immediately inspect all deliveries for damage upon receipt and if any damage is noticed, Buyer shall accept the shipment only after the driver has noted the damage on both carrier's and Buyer's copies of the delivery receipt. Buyer shall promptly inspect all shipments for concealed shipping damage, defects, or shortages, and notify Bio-Rad of any such damage, defect, or shortage. Buyer's failure to notify Bio-Rad within seven (7) days of delivery (or Buyer's non-receipt of the Goods in the case of non-delivery) of defects or shortages reasonably discoverable upon inspection will be a waiver of any right to make any claim relating to the defective or missing Goods, including, without limitation, under the warranty set forth herein.

<https://www.bio-rad.com/en-us/terms-conditions?ID=1572915545863>

Bio-Rad Ex. 2022  
Page 1

1/5

**10. Limited License.** Subject to the Agreement, and to the terms and conditions of any license provided by Bio-Rad that is specific to a particular product (which shall govern with respect to such product in the event of conflict with the terms herein), Bio-Rad hereby grants to Buyer a non-exclusive, non-transferable, non-sublicensable license to use the Good(s) provided to Buyer by Bio-Rad only in accordance with the written published manuals and instructions provided by Bio-Rad. Buyer understands and agrees that except as expressly set forth in this Agreement (or in the Bio-Rad-provided license specific to a particular Good), no right or license to any patent or other intellectual property owned or licensable by Bio-Rad is conveyed or implied by this Agreement. In particular, no right or license is conveyed or implied to use any Goods provided hereunder in combination with a product not provided, licensed or specifically recommended by Bio-Rad for such use. This limited license only permits the Buyer to use the Goods in accordance with local laws and regulations in Buyer's normal course of business.

**a. Special Licensing Terms for Bio-Rad Digital PCR and Single-Cell NGS Sample Preparation Products.** Purchase of Digital PCR and Single-Cell NGS Sample Preparation products from Bio-Rad Laboratories includes a limited, non-transferable right under our intellectual property for you to use of the products for your internal research purposes only. Unless we expressly state otherwise in additional Terms and Conditions, no rights are granted for you to distribute or resell the products, or to use the products for diagnostic uses and/or patient screening activities. Development of commercial products or the sale of products for use on Bio-Rad's Digital PCR and Single-Cell NGS Sample Preparation portfolio of products requires an additional commercial license from Bio-Rad. Information concerning a license for such uses can be obtained from Bio-Rad Laboratories. It is the responsibility of the purchaser/end user to acquire any additional intellectual property rights that may be required. The Bio-Rad Digital PCR Systems and/or their use is covered by claims of U.S. patents, and/or pending U.S. and non-U.S. patent applications owned by or under license to Bio-Rad Laboratories, Inc., including, but not limited to, U.S. Patent Nos. 9,089,844; 9,126,160; 9,216,392; 9,347,059; 9,500,664; 9,562,837; 9,636,682; 9,649,635; and 9,896,722.

(Ex. 2022 (Bio-Rad T&C of Sale) at 2;  
086 Paper 26 (POR) at 53-54;  
088 Paper 26 (POR) at 47-48).



## Secondary Considerations



## Bio-Rad to Acquire RainDance Technologies

Jan 16, 2017

This article has been updated from a previous version to correct the number of droplets generated by the RainDance digital PCR system.

NEW YORK (GenomeWeb) – Bio-Rad Laboratories announced today that it has agreed to acquire rival droplet-based PCR systems manufacturer RainDance Technologies for an undisclosed amount. The acquisition is expected to close during the first quarter of 2017.

RainDance's systems include RainDrop Digital PCR, ThunderStorm, and ThunderBolts Next-Generation Sequencing Enrichment. The RainDance system partitions samples into some 10 million droplets, which is purportedly 500 times greater than the number generated by the Bio-Rad system.

The RainDance intellectual property portfolio will extend Bio-Rad's reach into applications and strengthen its position in the area of droplet digital PCR, Bl said in a statement.

"We look forward to expanding our offering to provide life science and clinical solutions for a wide range of nucleic acid detection applications," Schwartz

Droplet-based PCR from both Bio-Rad and RainDance has been developed applications, viral load monitoring, as well as copy number variation and mo

Bio-Rad announced last week the [launch](#) of a single-cell sequencing system

RainDance also has several partnerships in the clinical space. It signed a multi-year license with Myriad Genetics, for example, which uses 30 in-house RainDance ThunderStorm reactions for target amplification prior to sequencing. RainDance has also partnered with Illumina Biosciences to co-develop and commercialize solutions for de novo whole-genome sequencing.

However, RainDance also **reduced** its staff by about 10 percent last year and **poned** a planned initial public offering citing market conditions.

Schwartz had [hinted](#) in a Q3 earnings call in November that it was scoping out M&A possibilities and something might come to pass in the following three to six months. It's most recent major acquisition prior to RainDance was of [GnuBio](#) in 2014 for [\\$110 million](#). At the JP Morgan Healthcare Conference last week Bio-Rad [said](#) it is targeting late 2017 for the launch of a sequencing system developed from the GnuBio technology and that the system will initially focus specifically on targeted sequencing for the oncology market. Some U.S. state privacy laws offer their residents specific consumer privacy rights, which we

market. Some U.S. state privacy laws offer their residents specific consumer privacy rights, which we respect as described in our [privacy statement](#). To opt-out of our making available to third parties information relating to cookies and similar technologies for advertising purposes, select "Opt-Out".

**Filed Under:** [Cookies](#), [Other Rights you may have](#), [Opting out of cookies & mobile ads](#), [Privacy Request](#) link.

RainDance's systems include [RainDrop Digital PCR](#), [ThunderStorm](#), and [ThunderBolts](#) Next-Generation Sequencing Enrichment. The RainDance system partitions samples into some 10 million droplets, which is [purportedly](#) 500 times greater than the number generated by the Bio-Rad systems.

The RainDance intellectual property portfolio will extend Bio-Rad's reach into next-generation sequencing applications and strengthen its position in the area of droplet digital PCR, Bio-Rad CEO Norman Schwartz said in a statement.

Bio-Rad Ex. 2014  
Page 01

(Ex. 2014 (GenomeWeb: Bio-Rad to Acquire Raindance Technologies) at 2;  
086 Paper 26 (POR) at 53-54;  
088 Paper 26 (POR) at 47-48).



**10X GENOMICS, INC.,  
Petitioner  
v.  
BIO-RAD LABORATORIES, INC.,  
Patent Owner**

***Inter Partes* Review Nos.  
IPR2020-00087 and -00089**

**Patent Owner's Demonstratives  
Consolidated Hearing January 27, 2021**

# **Outline of Argument for**

- 1) Claim Construction**
- 2) Background on Thorsen**
- 3) Thorsen Is Missing Several Elements**
- 4) Thorsen Is Not Enabled**

# Claim Construction

# “Separation Chamber”

Patent Owner	Petitioner
“chamber sized to accommodate multiple droplets and an immiscible fluid in separated form”	“Bio-rad’s interpretation of this term must be broad enough to include an open well”

# “Droplet Receiving Outlet” (‘837 Patent)

Patent Owner	Petitioner
“portion of the separation chamber where droplets are collected”	“Bio-rad’s interpretation of this term must be broad enough to include the opening at the top of an open well”



# “Coupled” (Dependent Claims 7 and 9 of the ‘837 Patent)

Patent Owner	Petitioner
“operably linked”	“Bio-rad’s interpretation of this term must be broad enough to include the use of a pipette to move droplets from an outlet to a vessel”

Patent Owner	Petitioner
“fluid containing molecules, cells, small molecules (organic or inorganic) or particles”	“a compound, composition, and/or mixture of interest, from any suitable source(s)”

# Background on Thorsen

Microfluidic technologies for high-throughput screening applications

Citation

Thorsen, Todd (2003) *Microfluidic technologies for high-throughput screening applications*. Dissertation (Ph.D.), California Institute of Technology. <https://resolver.caltech.edu/CaltechETD:etd-12012002-092026>

Abstract

In this thesis, I present a strategy for the design and development of microfluidic devices for high-throughput screening applications, such as mutant enzyme libraries expressed in prokaryotic hosts, where a few point mutations at the DNA level translates to hundreds of thousands of enzyme variants. The work falls into three main sections. Section I addresses fundamental research in polymer chemistry, where I explore the suitability of several polymers for microfluidic applications, examining properties such as molding, fluorescence, solvent compatibility, and adhesion/sealing to glass substrates. Section II describes my development of a two-phase microfluidic device, in which I report on crossflow-based dynamic formation of picoliter-sized water droplets in a continuously flowing oil-surfactant stream. A predictive model describing the fluid dynamics of droplet formation in this model is presented as well as its applications in screening bacterial populations. Section III reports the development of multilayer soft lithography technology using silicone rubber to build addressable high-density microfluidic arrays with thousands of integrated mechanical valves. This technology, which introduces the concept of fluidic large scale integration, is presented as a high-throughput parallel method to analyze bacterial enzyme expression at the single cell level. The detection of enzymatic activity in these high-density microarrays is described, comparing a self-constructed solid-state laser apparatus with a modified scanner (Axon Industries) used for looking at DNA arrays.

Item Type:	Thesis (Dissertation (Ph.D.))
Subject Keywords:	high-throughput screening; microfluidics; soft lithography
Degree Grantor:	California Institute of Technology
Division:	Chemistry and Chemical Engineering
Major Option:	Biochemistry and Molecular Biophysics
Thesis Availability:	Public (worldwide access)
Research Advisor(s):	<ul style="list-style-type: none"><li>Roberts, Richard W. (advisor)</li><li>Arnold, Frances Hamilton</li></ul>
Thesis Committee:	<ul style="list-style-type: none"><li>Quake, Stephen R. (chair)</li><li>Arnold, Frances Hamilton</li><li>Gray, Harry B.</li><li>Richards, John H.</li><li>Roberts, Richard W.</li></ul>
Defense Date:	23 September 2002
Non-Caltech Author Email:	thorsen (AT) mit.edu
Record Number:	CaltechETD:etd-12012002-092026
Persistent URL:	<a href="https://resolver.caltech.edu/CaltechETD:etd-12012002-092026">https://resolver.caltech.edu/CaltechETD:etd-12012002-092026</a>
Default Usage Policy:	No commercial reproduction, distribution, display or performance rights in this work are provided.
ID Code:	4701
Collection:	CaltechTHESIS
Deposited By:	Imported from ETD-db

10X Ex. 1003, Page 4

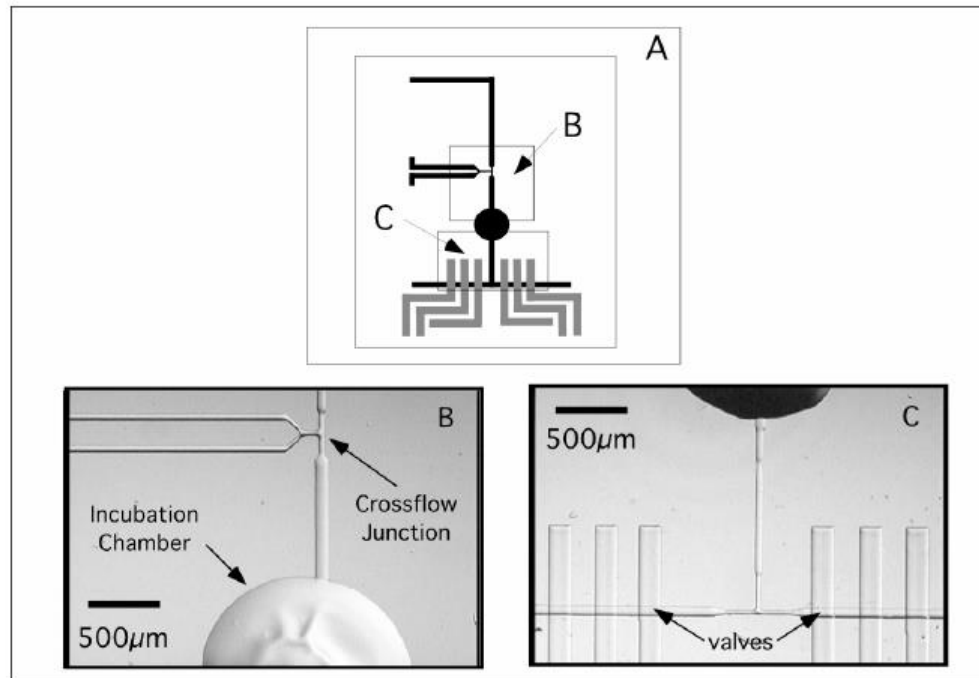
(Ex. 1003 (Thorsen)).

The 087 Petition alleges

that the Challenged Claims are anticipated or obvious in light of the incubation chamber system of Thorsen Chapter 3 or the serpentine channel system of Thorsen Chapter 4. (087 Pet. at 12-15).

(087 Paper 27 (POR) at 7;  
089 Paper 26 (POR) at 7).

# Thorsen Chapter 3 Incubation Chamber

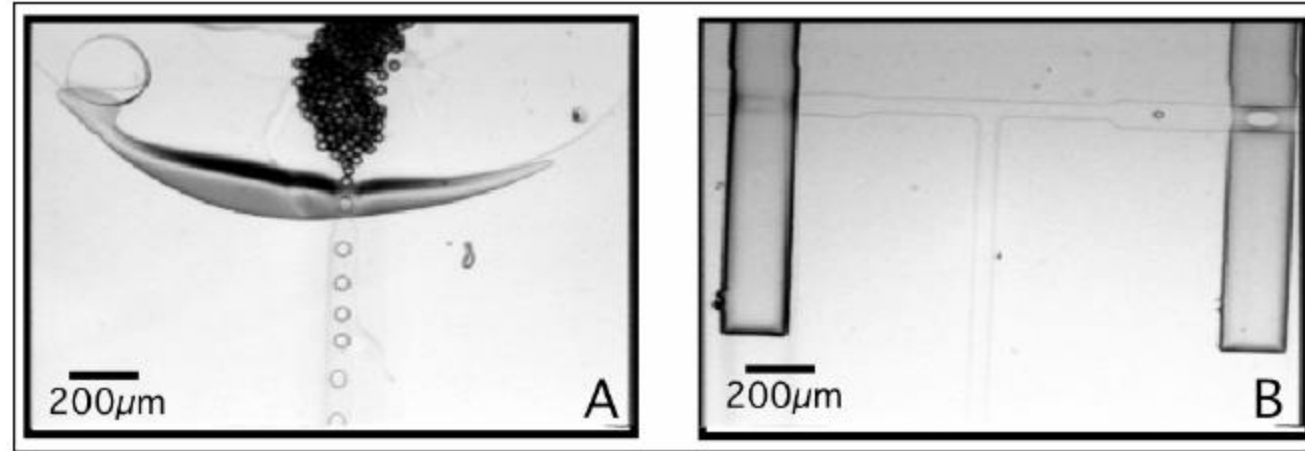


**Figure 3.28:** Schematic of silicone crossflow device with incubation cavity. A) Microfluidic chip design. Black layer - flow layer; Grey layer - control layer. B) High resolution image of actual chip showing crossflow junction and incubation chamber. C) High resolution of sorting junction in chip showing valve structures.

(Ex. 1003 (Thorsen) at 101;  
087 Paper 2 (Petition) at 31;  
089 Paper 2 (Petition) at 29).



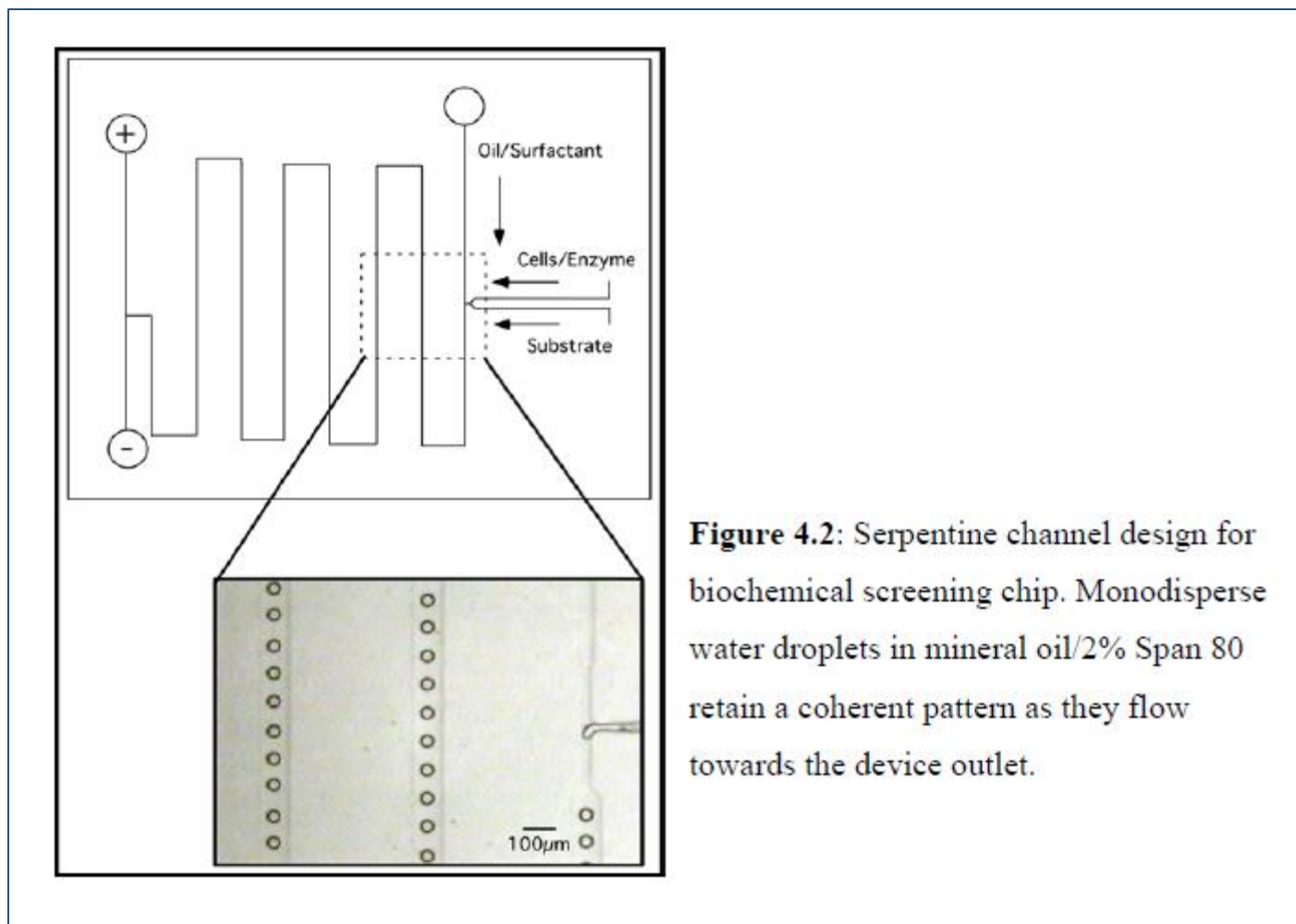
# Thorsen Chapter 3 Incubation Chamber



**Figure 3.29:** A) Water droplets entering incubation cavity of Sylgard 184 device.  
B) Sorting of droplets using elastomeric valves.

(Ex. 1003 (Thorsen) at 103;  
087 Paper 2 (Petition) at 32;  
089 Paper 2 (Petition) at 31).

# Thorsen Chapter 4 Serpentine Channel System



**Figure 4.2:** Serpentine channel design for biochemical screening chip. Monodisperse water droplets in mineral oil/2% Span 80 retain a coherent pattern as they flow towards the device outlet.

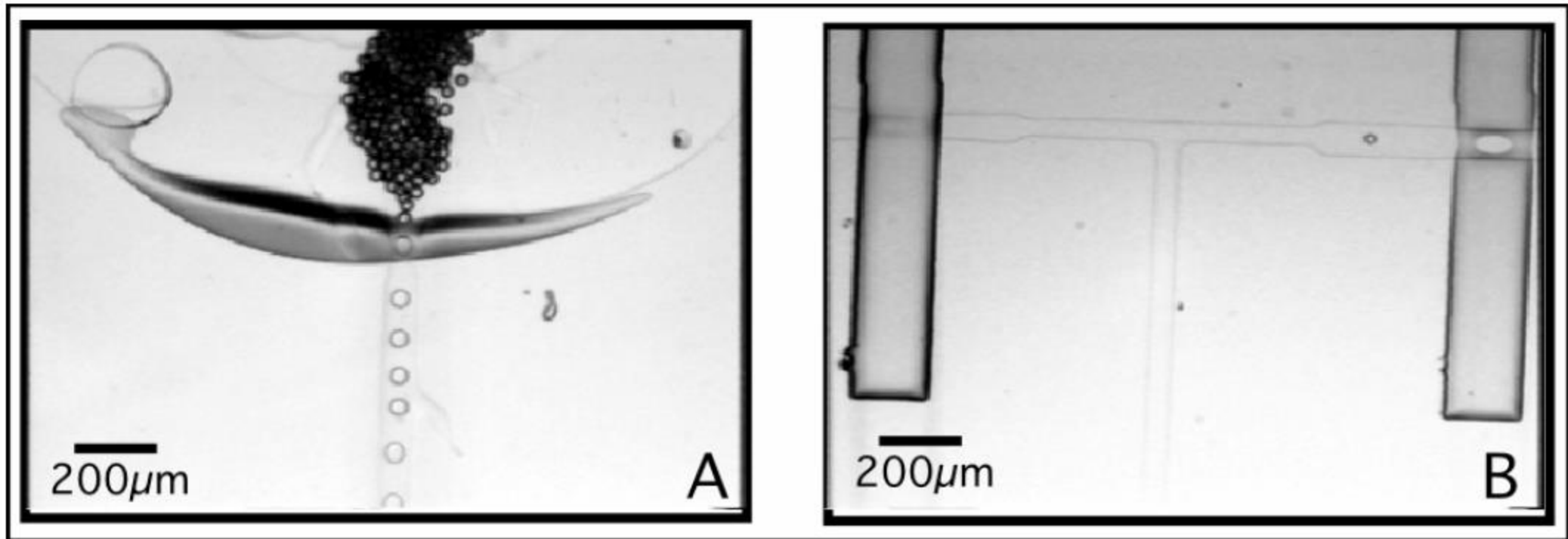
(Ex. 1003 (Thorsen) at 116;  
087 Paper 2 (Petition) at 36;  
089 Paper 2 (Petition) at 33).

# **The Thorsen Incubation Chamber Is Missing Several Elements**

# The Thorsen Incubation Chamber Is Missing Several Elements

- With respect to the ‘837 patent, the Thorsen incubation chamber is missing several elements of the claims, including but not limited to:
  - 1) “separation chamber” (087 Paper 27 (POR) at 29-31)
  - 2) “volume sufficient to separate the plurality of droplets” (087 Paper 27 (POR) at 46).
- With respect to the ‘722 patent, the Thorsen incubation chamber is missing several elements of the claims, including but not limited to:
  - 1) “separation chamber” (089 Paper 26 (POR) at 25-27)
  - 2) “separating the plurality of droplets” (089 Paper 26 (POR) at 35).

# The Thorsen Incubation Chamber Is Missing Several Elements



**Figure 3.29:** A) Water droplets entering incubation cavity of Sylgard 184 device.  
B) Sorting of droplets using elastomeric valves.

(Ex. 1003 (Thorsen) at 103;  
087 Paper 2 (Petition) at 32;  
089 Paper 2 (Petition) at 31).



# The Thorsen Incubation Chamber Is Missing Several Elements

## **Direct Testimony of Patent Owner's Expert Dr. Anna**

129. Thorsen also describes in Chapter 3 how the droplets and oil enter and leave the incubation chamber *together*. (See, e.g., Ex. 1003 at 103 (“Generated water droplets entered the ‘incubation’ cavity, where they encountered a sharp drop in flow velocity (Figure 3.29A). After ~ 1 hour, the droplets had passed entirely through the cavity and re-entered the narrow (~60 μm wide) outlet channel, where they are flowed toward the T-junction for valve-based sorting.”)). In my opinion, even if the droplets separate from the oil while inside the incubation chamber, they would no longer be separate when they leave the incubation chamber.

130. The ‘837 patent explains that the purpose of the “separation chamber” is to separate droplets from the immiscible fluid so that they may be collected at the “droplet receiving outlet.” Because the oil and droplets would re-disperse and both would exit the incubation chamber together, the separation and collection of droplets described in the ‘837 patent that takes place in a separation chamber does not occur in the incubation chamber of Thorsen Chapter 3.

(087 Ex. 2016 (Anna Decl.) at ¶¶129-30;  
087 Paper 27 (POR) at 30-31;  
089 Ex. 2016 (Anna Decl.) at ¶¶119-20;  
089 Paper 26 (POR) at 26-27).

# The Thorsen Incubation Chamber Is Missing Several Elements

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q Could you collect only droplets from the incubation chamber?

A Not with the configuration shown here. Because you need immiscible oil surrounding the droplets in the outlet flow.

So this is more or less a flow-through system where you get separation in the chamber, in the incubation chamber, and then you take the output for sorting.

Q Right. So as the droplets leave the chamber, they go out with the oil; right?

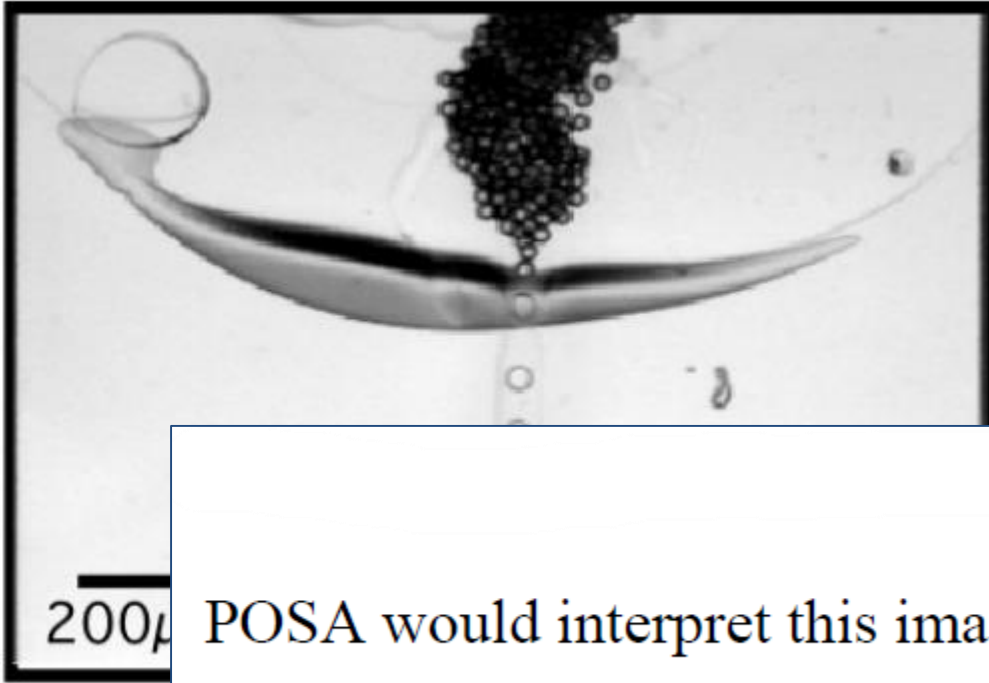
A The outlet channel is drawing some of the oil that's present in the system.

Q Okay.

A The droplets by sedimentation clump to the bottom down to the glass floor. The oil is lighter. It floats up, but the droplets are surrounded by oil. As they are leaving, you're drawing both droplets and oil.

(Ex. 2017 (Fair Cross Tr.) at 85:13-86:6;  
087 Paper 27 (POR) at 38-39).

# The Thorsen Incubation Chamber Is Missing Several Elements



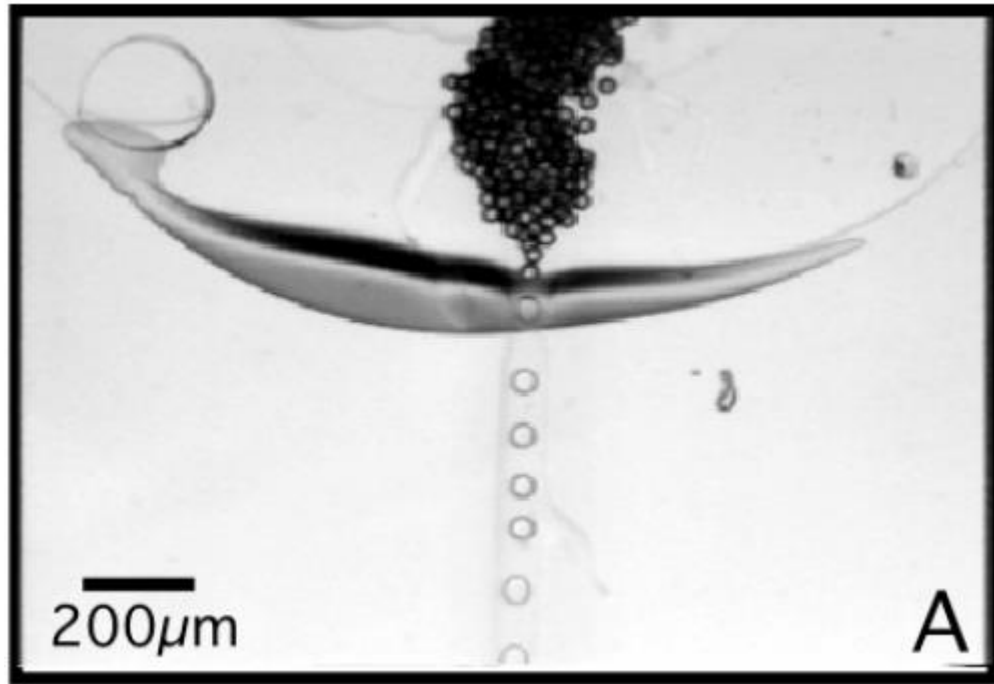
## **Direct Testimony of Patent Owner's Expert Dr. Anna**

Further, in my opinion, a

200μ POSA would interpret this image – where some of the droplets have clear centers, and some are almost completely black – to show droplets that are in multiple layers, not all resting on the bottom of the incubation chamber.

(087 Ex. 2016 (Anna Decl.) at ¶131;  
087 Paper 27 (POR) at 31;  
089 Ex. 2016 (Anna Decl.) at ¶121;  
089 Paper 26 (POR) at 27).

# The Thorsen Incubation Chamber Is Missing Several Elements



## **Direct Testimony of Patent Owner's Expert Dr. Anna**

In a planar image such as this one, droplets with curved interfaces will refract light passing through the droplet, diverting the light away from the initial incidence angle. This leads to the appearance of clear droplet centers, since light remains largely unrefracted nearest the centers of the droplets, and dark edges, since the curved edges refract light the most dramatically. The presence of multiple layers of droplets would further refract and scatter the incident light, creating dark regions of the image in which the individual droplets are difficult to distinguish and count. Placement of the droplets out of the plane is not possible in a planar microscope through-image such as this one.

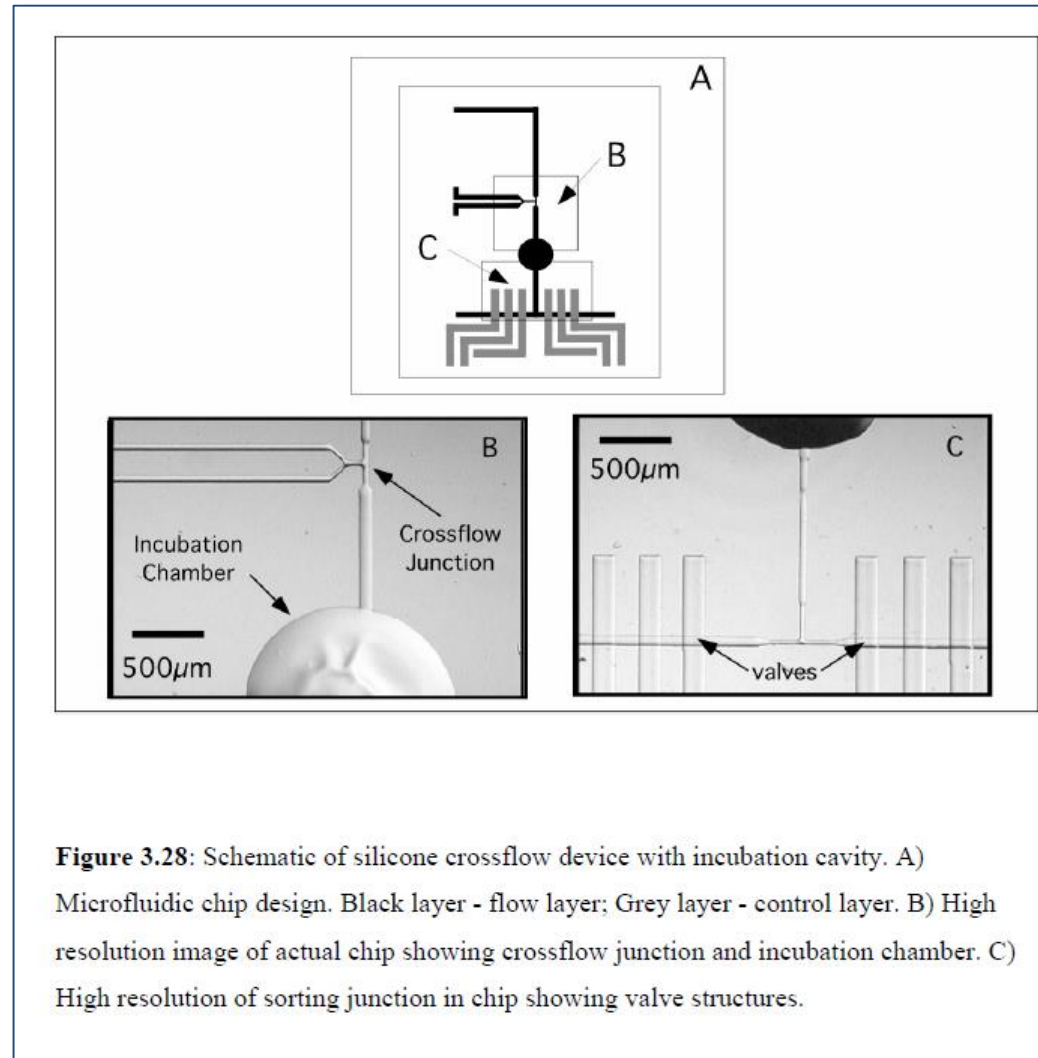
(087 Ex. 2016 (Anna Decl.) at ¶131;  
087 Paper 27 (POR) at 31;  
089 Ex. 2016 (Anna Decl.) at ¶121;  
089 Paper 26 (POR) at 27).

# The Thorsen Incubation Chamber Is Missing Several Elements

- With respect to the ‘837 patent, the Thorsen Incubation Chamber is missing a “droplet receiving outlet.” (087 Paper 27 (POR) at 37-41).
- With respect to the ‘722 patent, the Thorsen incubation chamber is missing “collecting the separated droplets” (dependent claim 2). (089 Paper 26 (POR) at 35-38).



# The Thorsen Incubation Chamber Is Missing Several Elements



(Ex. 1003 (Thorsen) at 101;  
087 Paper 2 (Petition) at 31;  
089 Paper 2 (Petition) at 29).

# The Thorsen Incubation Chamber Is Missing Several Elements

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q Could you collect only droplets from the incubation chamber?

A Not with the configuration shown here. Because you need immiscible oil surrounding the droplets in the outlet flow.

So this is more or less a flow-through system where you get separation in the chamber, in the incubation chamber, and then you take the output for sorting.

Q Right. So as the droplets leave the chamber, they go out with the oil; right?

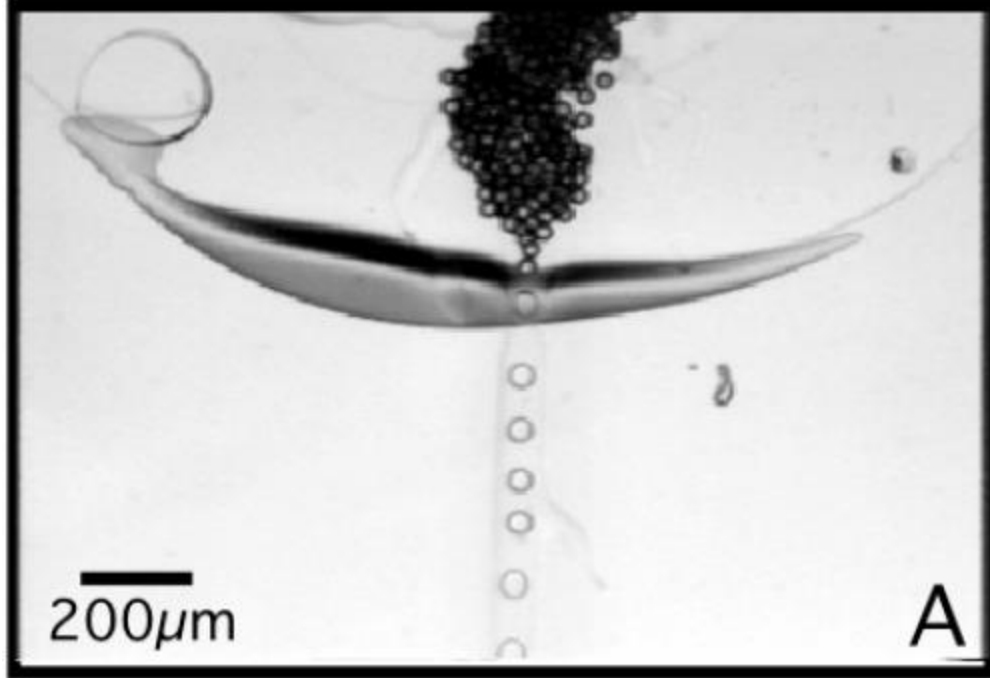
A The outlet channel is drawing some of the oil that's present in the system.

Q Okay.

A The droplets by sedimentation clump to the bottom down to the glass floor. The oil is lighter. It floats up, but the droplets are surrounded by oil. As they are leaving, you're drawing both droplets and oil.

(Ex. 2017 (Fair Cross Tr.) at 85:13-86:6;  
087 Paper 27 (POR) at 38-39).

# The Thorsen Incubation Chamber Is Missing Several Elements



## Cross-Examination Testimony of Petitioner's Expert Dr. Fair

Q So it's your belief on Page 74 of your declaration we see the droplets, there is a clump of droplets; right?

A Right.

Q And then there is what looks like a curved part of the incubation chamber; right?

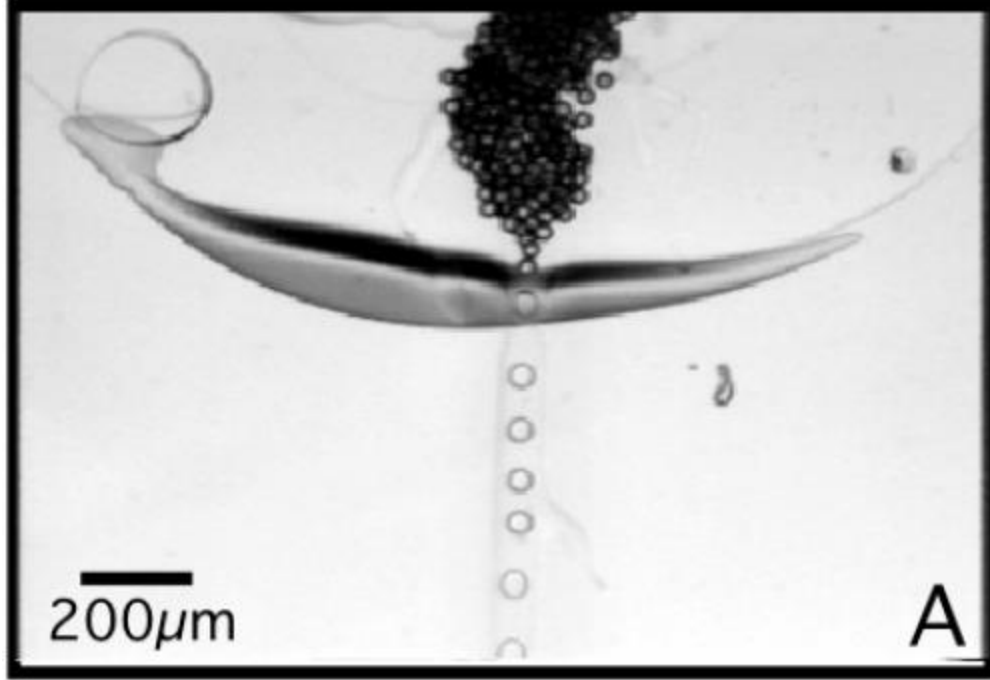
A Right.

Q And there is a single line of droplets; right?

A Right.

(Ex. 2017 (Fair Cross Tr.) at 132:21-133:5;  
087 Paper 27 (POR) at 40-41).

# The Thorsen Incubation Chamber Is Missing Several Elements



## Cross-Examination Testimony of Petitioner's Expert Dr. Fair

Q And your understanding is that that is showing the outlet of the incubation chamber?

A Correct.

Q And that's what your opinion is based on?

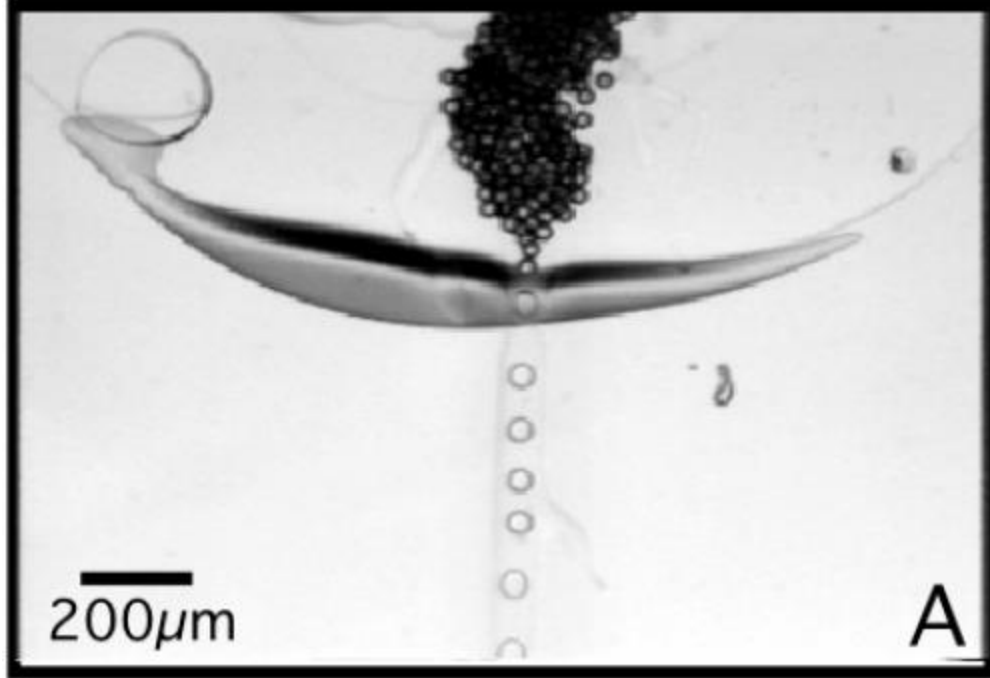
A Correct.

Q Got it. Okay. And that's what you're -- is the basis for your opinion that there is a droplet receding outlet as recited by the claims?

A Yes.

(Ex. 2017 (Fair Cross Tr.) at 133:6-16;  
087 Paper 27 (POR) at 40-41).

# The Thorsen Incubation Chamber Is Missing Several Elements



## Cross-Examination Testimony of Petitioner's Expert Dr. Fair

Q And, Dr. Fair, looking at the sentence that you read -- excuse me. Earlier on the page you were reading the sentence, "Generated water droplets entered the incubation cavity where they encountered a sharp drop in flow velocity," Figure 3.29A.

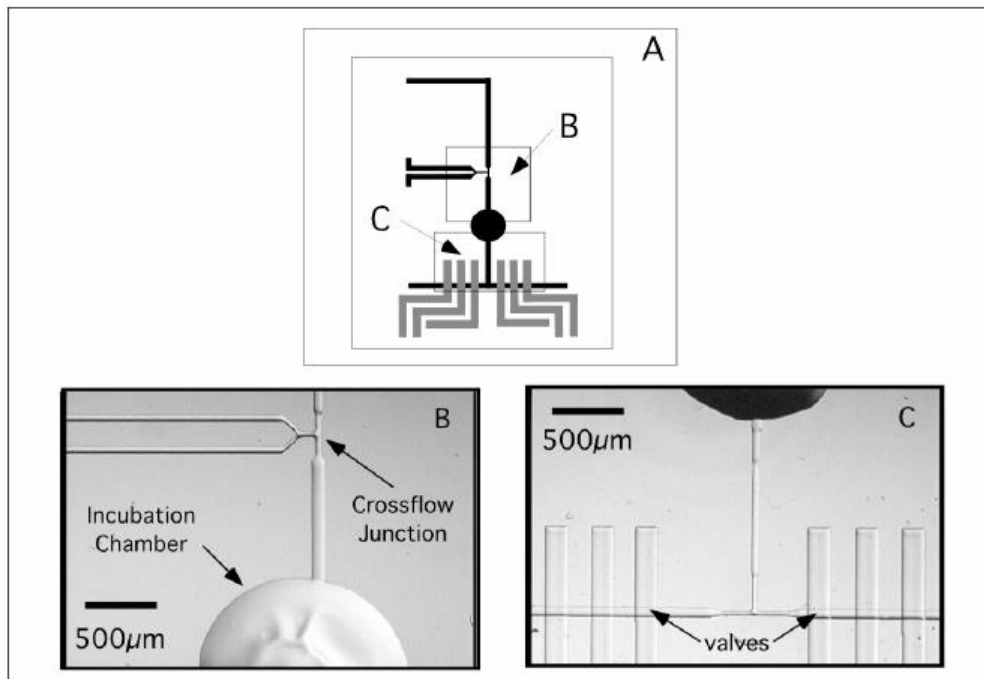
Was that the sentence you were reading from?

A Yes. Okay. So Figure A, as the caption says, water droplets entering the cavity, they slow down and accumulate. So I had that backwards.

(Ex. 2017 (Fair Cross Tr.) at 133:6-16;  
087 Paper 27 (POR) at 41).



# The Thorsen Incubation Chamber Is Missing Several Elements



**Figure 3.28:** Schematic of silicone crossflow device with incubation cavity. A) Microfluidic chip design. Black layer - flow layer; Grey layer - control layer. B) High resolution image of actual chip showing crossflow junction and incubation chamber. C) High resolution of sorting junction in chip showing valve structures.

(Ex. 1003 (Thorsen) at 101).

## Cross-Examination Testimony of Petitioner's Expert Dr. Fair

Q. Okay.

If I asked you to go in with a micropipette and collect droplets from the top schematic in figure 3.28, where would you do that?

MR. GERRITY: Objection to form and beyond the scope of reply.

A. Well, yes, I have not offered any opinion in my responsive declaration regarding how you would collect with a micropipette in this configuration.

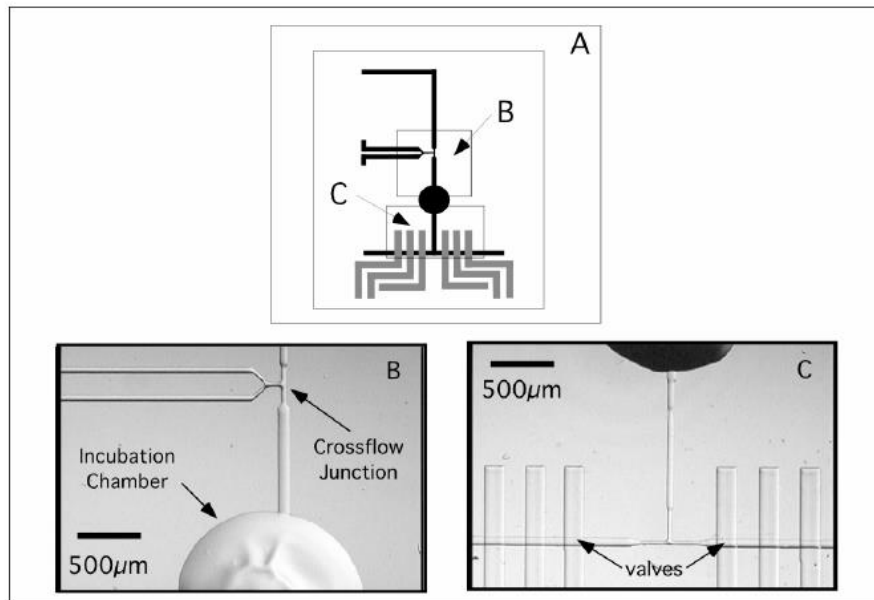
(Ex. 2041 (Fair Reply Cross Tr.) at 200:11-20;  
087 Paper 32 (PO Sur-Reply) at 7).

# **The Thorsen Outlet Wells Are Missing Several Elements**

# The Thorsen Outlet Wells Are Missing Several Elements

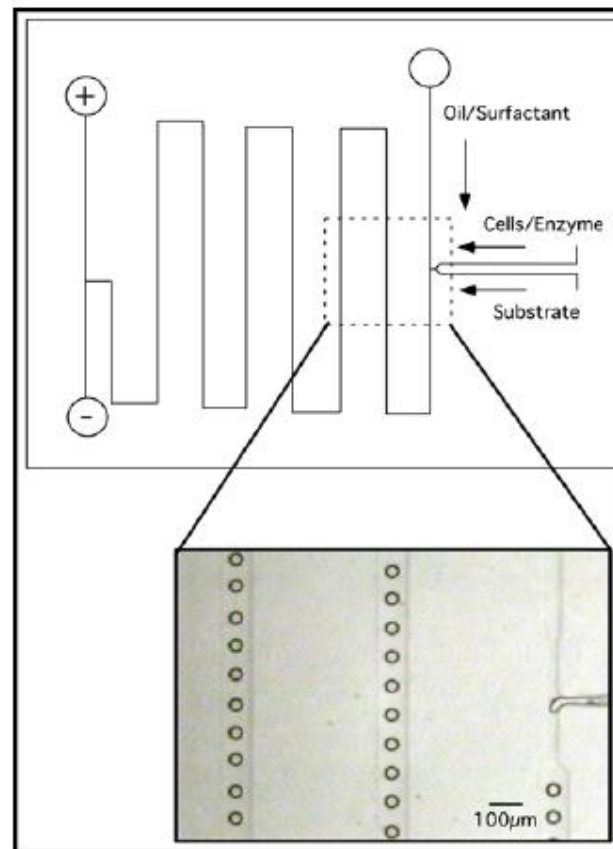
- With respect to the ‘837 patent, the Thorsen outlet wells are missing several elements of the claims, including but not limited to:
  - 1) “separation chamber” (087 Paper 27 (POR) at 32-37)
  - 2) “volume sufficient to separate the plurality of droplets” (087 Paper 27 (POR) at 46).
- With respect to the ‘722 patent, the Thorsen outlet wells are missing several elements of the claims, including but not limited to:
  - 1) “separation chamber” (089 Paper 26 (POR) at 28-33)
  - 2) “separating the plurality of droplets” (089 Paper 26 (POR) at 35).

# The Thorsen Outlet Wells Are Missing Several Elements



**Figure 3.28:** Schematic of silicone crossflow device with incubation cavity. A) Microfluidic chip design. Black layer - flow layer; Grey layer - control layer. B) High resolution image of actual chip showing crossflow junction and incubation chamber. C) High resolution of sorting junction in chip showing valve structures.

(Ex. 1003 (Thorsen) at 101;  
087 Paper 2 (Petition) at 31;  
089 Paper 2 (Petition) at 29).



**Figure 4.2:** Serpentine channel design for biochemical screening chip. Monodisperse water droplets in mineral oil/2% Span 80 retain a coherent pattern as they flow towards the device outlet.

(Ex. 1003 (Thorsen) at 116;  
087 Paper 2 (Petition) at 36;  
089 Paper 2 (Petition) at 33).

# The Thorsen Outlet Wells Are Missing Several Elements

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q      Okay. If I asked you to design a system where the outlet well was in the same plane as the channel, could you do that?

A      You could you do that, but if the collection well that you're describing has the same height as the channel, then it would be very difficult to do separation of oil and liquid and droplets.

So the early waste reservoirs that we built were, in fact, designed to have the same height as the channels in our devices. And they were just holding -- they just were there to hold the droplets, not to separate from the oil.

Q      You did not put the outlet wells vertical to the channel until you decided you wanted to separate?

A      That's correct.

(Ex. 2017 (Fair Cross Tr.) at 69:2-18;  
087 Paper 27 (POR) at 34;  
089 Paper 26 (POR) at 30).



# The Thorsen Outlet Wells Are Missing Several Elements

## **Direct Testimony of Patent Owner's Expert Dr. Anna**

135. Thorsen also does not disclose anything about the dimensions or design of the alleged “outlet wells” in Chapter 3, and depending on the design of the alleged “outlet wells,” droplets may or may not separate from surrounding oil based on density.

136. Further, I agree with the testimony Dr. Fair provided during his deposition where he explained that it was not necessary for an outlet well to be out of the horizontal plane of the channels to accommodate “thousands” of droplets.

(087 Ex. 2016 (Anna Decl.) at ¶¶135-36;  
087 Paper 27 (POR) at 33-34;  
089 Ex. 2016 (Anna Decl.) at ¶¶125-26;  
089 Paper 26 (POR) at 29-30).

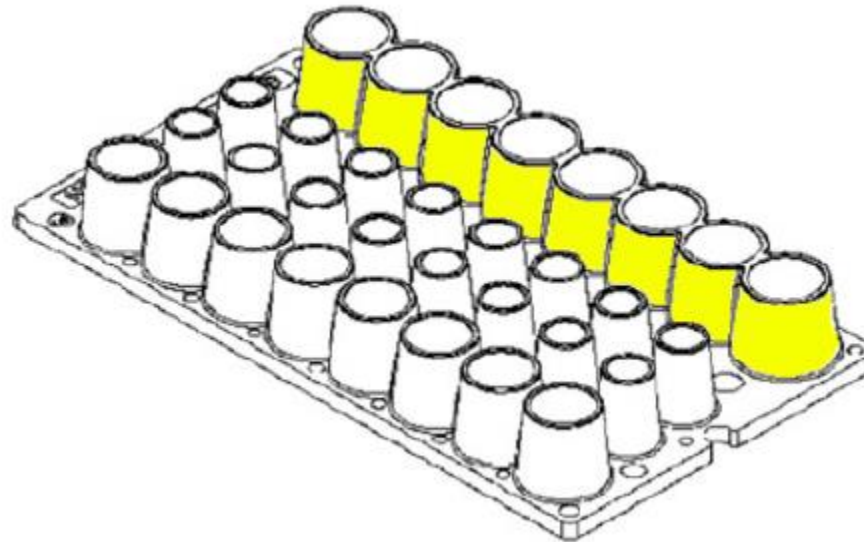
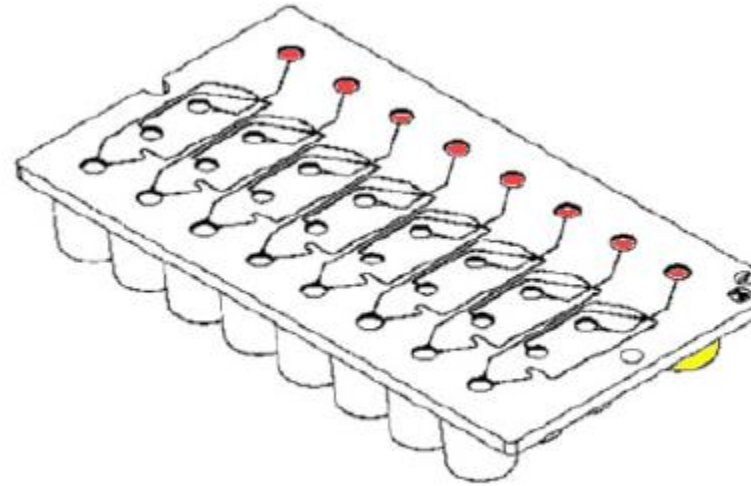
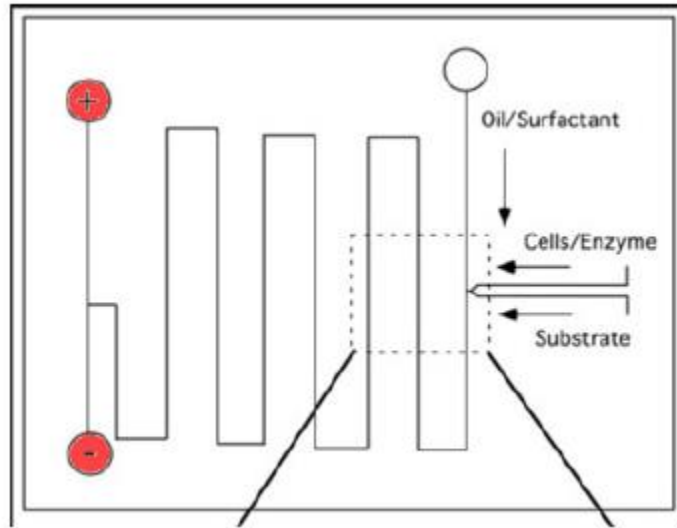
# The Thorsen Outlet Wells Are Missing Several Elements

## Patent Owner Response

*Finally*, Petitioner argues that “[u]nder Bio-Rad’s interpretation, an open well with droplets that have a different density than the immiscible fluid meets this claim limitation.” (087 Pet. at 48). Specifically, Petitioner argues that if the 10X chambers highlighted in yellow in the image below constitute “separation chambers” within the meaning of the ‘837 patent, then so too must the “outlet wells” allegedly disclosed in Thorsen Chapter 3. (*Id.*).

(087 Paper 27 (POR) at 34;  
089 Paper 26 (POR) at 30).

# The Thorsen Outlet Wells Are Missing Several Elements



(087 Paper 27 (POR) at 36;  
089 Paper 26 (POR) at 32).

# The Thorsen Outlet Wells Are Missing Several Elements

- With respect to the ‘837 patent, the Thorsen outlet wells are missing a “droplet receiving outlet.” (087 Paper 27 (POR) at 41-44).
- With respect to the ‘722 patent, the Thorsen outlet wells are missing “collecting the separated droplets” (dependent claim 2). (089 Paper 35-38).

# The Thorsen Outlet Wells Are Missing Several Elements

## **Direct Testimony of Petitioner's Expert Dr. Fair**

Thus, for a micropipette to be able to collect liquid, the liquid must first be collected in a container that (1) is of a sufficient volume for the pipette tip to be inserted, (2) has an opening through which the pipette can be inserted, and (3) the liquid is of a sufficient volume that the micropipette can collect it. This describes an outlet well (sometimes called a collection well).

(087 Ex. 1008 (Fair Decl.) at ¶130;  
087 Paper 27 (POR) at 43-44;  
089 Ex. 1008 (Fair Decl.) at ¶160;  
089 Paper 26 (POR) at 37-38).

## **Cross-Examination Testimony of Petitioner's Expert Dr. Fair**

Q      What is the minimum dimension of a micropipette?

A      The ones we have, which are the smallest, are 1 millimeter.

(Ex. 2017 (Fair Cross Tr.) at 159:13-16;  
087 Paper 27 (POR) at 43-44;  
089 Paper 26 (POR) at 38).



# The Thorsen Outlet Wells Are Missing Several Elements

## Petitioner's Reply

Dr. Anna is PO's only support for the first argument, and her opinion is a conclusory assertion without citation that 1 mm "is much larger than any of the structures described by Thorsen." Anna, ¶153. However, Thorsen discloses "inlet holes punched with a large diameter (~5mm) beveled steel punch." Thorsen, 44.

(087 Paper 30 (Reply) at 24;  
089 Paper 29 (Reply) at 20).

# The Thorsen Outlet Wells Are Missing Several Elements

## 2.2.2 Diene-based Polymers

Diene-based polymers like  
problem encountered with PDM  
petroleum cracking process, D

well as inorganic acids and bases. The polymer ranges in color from optically clear to pale yellow after polymerization, depending on the catalyst, and exhibits low background fluorescence. However, DCPD is not inherently elastomeric, exhibiting a high degree of crosslinking upon polymerization that classifies it as a duroplastic. Its mechanical toughness and chemical resistance has made it a popular polymer for use in aerospace, marine, ballistic, and electronic applications.

### Dicyclopentadiene (DCPD)

#### *Structural and Mechanical properties*

DCPD has excellent molding capabilities<sup>22</sup>, and is primarily used for injection molding of items such as hazardous chemical storage tanks, boat shells, and printed circuit boards. The neat polymer is mechanically tough, with a Young's modulus value of 1.5-3.0 GPa, depending on the catalyst employed for the crosslinking. The polymerization reaction is initiated by organometallic catalysts like the Grubb's reagent (Figure 2. 6), which opens the vinyl bonds of DCPD using a mechanism in which a covalent intermediate is formed between substrate and catalyst (Figure 2.7). The reaction is commonly referred to as ring opening metathesis polymerization, or ROMP.

10X Ex. 1003, Page 38

## 2.2.2 Diene-based Polymers

25

polymer (vs. pure DCPD), resulting in several failed polymerizations due to boil off of the methylene chloride and precipitation of the ruthenium catalyst in the mixture prior to polymerization. The co-polymerizations also leave trace amounts of uncured monomer trapped in the devices, whose potent smell is an undesirable product. However, the co-polymers with a high fraction of 1,5-COD show excellent coverslip adhesion (tested up to 30 days without delamination).

#### *Chemical Resistance: Bulk and Microfluidic*

Chemical resistance of the DCPD/COD co-polymers was comparable to polyDCPD devices. No swelling was observed for water and all alkanes ( $C_7 - C_{16}$ ) in bulk tests in which solvent was pipetted on the surfaces of the co-polymers. Microfluidic tests with pressurized solvent were still difficult to conduct because we had not developed a

### Microfluidic tests

with pressurized solvent were still difficult to conduct because we had not developed a good methodology to punch small inlet holes in the relatively tough devices. Devices cast from the silicon molds still needed to have inlet holes punched with a large diameter (~5mm) beveled steel punch, making the clamping apparatus still necessary for fluid introduction.

10X Ex. 1003, Page 44

(Ex. 1003 (Thorsen) at 38, 44;  
087 Paper 30 (Reply) at 24;  
089 Paper 29 (Reply) at 20).

# The Thorsen Outlet Wells Are Missing Several Elements

8

devices for sorting DNA and cells using electrophoretic techniques<sup>10</sup>, so this provided a natural starting material for my early studies. As PDMS proved to have inadequate chemical resistance against most oils, exhibiting swelling and degradation, my work broadened to investigate other groups of polymers, including dienes, urethanes and block co-polymers. A summary of this research follows.

## 2.2.1 Silicone-based Polymers

## 2.2.1 Silicone-based Polymers

most common and widely used elastomer is PDMS, having the basic repeating unit,  $[(CH_3)_2SiO]$  (Figure 2.1).

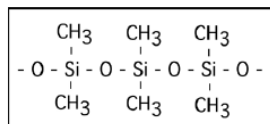


Figure 2.1: Structure of repeating PDMS subunits.

## Polydimethylsiloxane (PDMS)

## Polydimethylsiloxane (PDMS)

10X Ex. 1003, Page 27

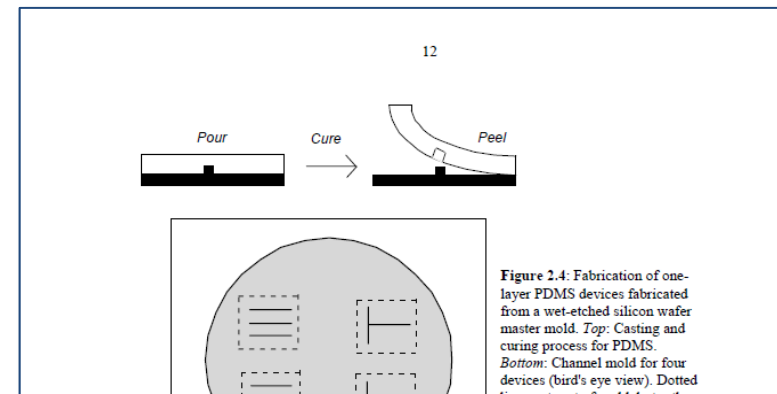


Figure 2.4: Fabrication of one-layer PDMS devices fabricated from a wet-etched silicon wafer master mold. Top: Casting and curing process for PDMS. Bottom: Channel mold for four devices (bird's eye view). Dotted lines not part of mold, but rather

Holes were then punched with

a 20G (I.D.= 585  $\mu$ m) beveled steel punch at the channel ends and the devices were sealed hermetically to No.1 glass coverslips (VWR) at either room temperature (23°C) or in the oven (80°C) for 4 hours.

a 20G (I.D.= 585  $\mu$ m) beveled steel punch at the channel ends and the devices were sealed hermetically to No.1 glass coverslips (VWR) at either room temperature (23°C) or in the oven (80°C) for 4 hours.

10X Ex. 1003, Page 31

(Ex. 1003 (Thorsen) at 27, 31;  
087 Paper 32 (Sur-Reply) at 11;  
089 Paper 31 (Sur-Reply) at 10-11).

# The Thorsen Outlet Wells Are Missing Several Elements

## Petitioner's Reply

Exs. 1077, 1078, 1079). Thus, if the Board finds Thorsen does not disclose outlet wells large enough to accommodate a micropipette tip, it would have been well within the knowledge and skill of a POSA to use a commercially available 2 mm or 4 mm cork borer (or Thorsen's "~5mm" punch) to produce an outlet well that would accommodate a micropipette tip.

087 Paper 30 (Reply) at 24-25;  
089 Paper 29 (Reply) at 21).

## Patent Owner's Sur-Reply

This argument also fails. Thorsen discloses how to make the hole punches in his Chapter 3 and 4 devices, *i.e.* by using a 20G (I.D.=585  $\mu$ m) beveled steel punch. A POSA would have had no reason to deviate from this procedure, nor does Petitioner provide any such reason. Instead, Petitioner starts with the claim element ("droplet receiving outlet") and looks backwards to craft an argument that the element would have been obvious. This is improper hindsight.

087 Paper 32 (Sur-Reply) at 12;  
089 Paper 31 (Sur-Reply) at 11).

# The Thorsen Outlet Wells Are Missing Several Elements

## Cross-Examination Testimony of Petitioner's Expert Dr. Fair

Q      When is the first time you used a  
pipette to remove a droplet from a chip and move  
it off chip?

A      I don't think I've done that.

Q      You've never done that?

A      Not on electrowetting chips, no.

Q      On any other chips?

A      Not that I can recall.

(Ex. 2017 (Fair Cross Tr.) at 69:2-18;  
087 Paper 27 (POR) at 42-43;  
089 Paper 26 (POR) at 37).





“[I]t is impermissible to use the claimed invention as an instruction manual or ‘template’ to piece together the teachings of the prior art so that the claimed invention is rendered obvious.”

# Thorsen Is Not Enabled

# Thorsen Is Not Enabled

## Microfluidic technologies for high-throughput

Thorsen, Todd (2003) *Microfluidic technologies for high-throughput screening applications*  
California Institute of Technology

In this thesis, I present a strategy for high-throughput screening applications, such as mutagenesis at the DNA level translates into main sections. Section I addresses fundamental principles of microfluidic technology, including several polymers for microfluidic applications, compatibility, and adhesion/sealing to microfluidic device, in which I report on a continuously flowing oil-surfactant stream in this model is presented as well as the development of multilayer soft lithography for microfluidic arrays with thousands of channels. The concept of fluidic large scale integration for enzyme expression at the single cell level is described, comparing a self-constructed microfluidic device used for looking at DNA arrays.

Item Type:	Thesis (Dissertation)
Subject Keywords:	high-throughput
Degree Grantor:	California Institute of Technology
Division:	Chemistry and Physics
Major Option:	Biochemistry and Molecular Biology
Thesis Availability:	Public (worldwide)
Research Advisor(s):	<ul style="list-style-type: none"><li>• Roberts</li><li>• Arnold</li></ul>
Thesis Committee:	<ul style="list-style-type: none"><li>• Quake</li><li>• Arnold</li><li>• Gray, H</li><li>• Richard</li><li>• Roberts</li></ul>
Defense Date:	23 September 2003
Non-Caltech Author Email:	thorsen (AT) n
Record Number:	CaltechETD:00000000
Persistent URL:	<a href="https://resolver.caltech.edu/CaltechETD:00000000">https://resolver.caltech.edu/CaltechETD:00000000</a>
Default Usage Policy:	No commercial use without permission provided.
ID Code:	4701
Collection:	CaltechTHESIS
Deposited By:	Imported from

## 4.6 Conclusions

Working on the development of a microfluidic crossflow assay emphasized the difficult dynamic integration process between biochemical and micromechanical systems. Progress was made in making the crossflow device a true microfluidic mixer, capable of encapsulating picoliter volumes of two aqueous components exactly at the position where the two reagents combine. pNB esterase was chosen as a model enzyme on the basis of its recombinant expression in *E. coli*, its fast kinetics and the availability of sensitive, fluorescence-based detection reagents. While problems such as non-specific cell adhesion and substrate autohydrolysis plagued the assays, the results are not intended to discourage future research in this area. Improvements in areas such as surface chemistry technology and reagent stability will promote the development of cell-based, sensitive biochemical assays in this system.

## Patent Owner's Sur-Reply

*First*, Petitioner's argument that Thorsen is presumed enabling and that "PO has not overcome that presumption" (Reply at 24) ignores the law. Where, as here, a Patent Owner makes a non-frivolous argument that the prior art is not enabled, the presumption no longer applies, and the burden shifts to the Petitioner. *In re Morsa*, 713 F.3d 104, 106 (Fed. Cir. 2013).

(087 Paper 32 (Sur-Reply) at 15-16;  
089 Paper 31 (Sur-Reply) at 15).

# Thank You