

charged. For OEG-terminated surfactants, high values of n (≥ 16) are preferred for making these surfactants oil-soluble and preventing them from entering the aqueous phase. In FIG. 24, compounds that have between about 3 to 6 EG units attached to a thiol are sufficient to prevent the adsorption of proteins to a monolayer of thiols on gold, and are thus preferred for inertness. In addition, surfactants that have been shown to be biocompatible in fluorocarbon blood substitutes may also be used as additives to fluorinated carrier fluids.

APPLICATIONS: KINETIC MEASUREMENTS AND ASSAYS

The devices and methods of the invention can be also used for performing experiments typically done in, for example, a microtiter plate where a few reagents are mixed at many concentrations and then monitored and/or analyzed. This can be
5 done, for example, by forming plugs with variable composition, stopping the flow if needed, and then monitoring the plugs. The assays may be positionally encoded, that is, the composition of the plug may be deduced from the position of the plug in the channel. The devices and methods of the invention may be used to perform high-throughput screening and assays useful, for example, in diagnostics and drug
10 discovery. In particular, the devices and methods of the invention can be used to perform relatively fast kinetic measurements.

The ability to perform fast measurements has revolutionized the field of biological dynamics. Examples include studies of protein C folding and cytochrome C folding. These measurements are performed using fast kinetics instruments that
15 rely on turbulence to mix solutions rapidly. To achieve turbulence, the channels and the flow rates normally have to be large, which require large sample volumes. Commercially available instruments for performing rapid kinetics studies can access times on the order of 1 ms. The improved on-chip version of a capillary glass-ball mixer gives a dead time of about 45 μ s with a flow rate of more than about 0.35
20 mL/sec. The miniaturization of these existing methods is generally limited by the requirement of high flow rate to generate turbulence. Miniaturization afforded by devices and methods according to the invention is advantageous because it allows, for example, quantitative characterization, from genetic manipulation and tissue isolation, of a much wider range of biomolecules including those available only in minute
25 quantities, *e.g.*, microgram quantities. In addition, these new techniques and instruments afford a wide range of accessible time scales for measurements.

Time control is important in many chemical and biochemical processes. Typically, stopped-flow type instruments are used to measure reaction kinetics. These types of instruments typically rely on turbulent flow to mix the reagents and
30 transport them while minimizing dispersion. Because turbulent flow occurs in tubes with relatively large diameters and at high flow rates, stopped-flow instruments tend to use large volumes of reagents (*e.g.*, on the order of mL/s). A microfluidic analog of a stopped-flow instrument that consumes small volumes of reagents, *e.g.*, on the order of μ L/min, would be useful in various applications such as diagnostics. Thus far,

microfluidic devices have not been able to compete with stopped-flow instruments because EOF is usually too slow (although it has less dispersion), and pressure-driven flows tend to suffer from dispersion. In addition, mixing is usually very slow in both systems.

5 Stopped-flow instruments typically have sub-millisecond mixing, and could be useful for experiments where such fast mixing is required. The devices and methods of the invention allow sub-millisecond measurements as well. In particular, the present invention can be advantageous for reactions that occur on a sub-second but slower than about 1 or about 10 millisecond (ms) time scale or where the primary
10 concern is the solute volume required to perform a measurement.

 Further, if a plug is generated with two reactive components, it can serve as a microreactor as the plug is transported down a channel. A plug's property, such as its optical property, can then be measured or monitored as a function of distance from a given point or region of a channel or substrate. When the plugs are transported at a
15 constant flow rate, a reaction time can be directly determined from a given distance. To probe the composition of the plug as it exits a channel, the contents of the plugs may be injected into a mass spectrometer (e.g., an electrospray mass spectrometer) from an end of the channel. The time corresponding to the end of the channel may be varied by changing the flow rate. Multiple outlets may be designed along the
20 channels to probe, for example, the plug contents using a mass spectrometer at multiple distance and time points.

 An advantage of the devices and methods of the invention is that when plugs are formed continuously, intrinsically slow methods of observation can be used. For example, plugs flowing at a flow rate of about 10 cm/s through a distance of about 1
25 mm from a point of origin would be about 10 ms old. In this case, the invention is particularly advantageous because it allows the use of a relatively slow detection method to repeatedly perform a measurement of, for example, 10 ms-old plugs for virtually unlimited time. In contrast, to observe a reaction in a stopped-flow experiment at a time, say, between about 9 and 11 ms, one only has about 2 ms to take
30 data. Moreover, the present invention allows one to obtain information involving complex reactions at several times, simultaneously, simply by observing the channels at different distances from the point of origin.

 The reaction time can be monitored at various points along a channel – each point will correspond to a different reaction or mixing time. Given a constant fluid

flow rate u , one may determine a reaction time corresponding to the various times $t_1, t_2, t_3, \dots, t_n$ along the channel. Thus, if the distance between each pair of points n and $n-1$, which correspond to time t_n and t_{n-1} , are the same for a given value of n , then the reaction time corresponding to point n along the channel may be calculated from $t_n =$
 5 nl/u . Thus, one can conveniently and repeatedly monitor a reaction at any given time t_n . In principle, the substrate of the present invention allows one to cover a greater time period for monitoring a reaction by simply extending the length of the channel that is to be monitored at a given flow rate or by decreasing the flow rate over a given channel distance (see, for example, Fig. 22). In FIG. 22, the following can be
 10 introduced into the following inlets: enzyme into inlets 2201, 2205, 2210, 2215; buffer into inlets 2202, 2206, 2211, 2216; substrate into inlets 2203, 2207, 2212, 2217; buffer into inlets 2204, 2208, 2213, 2218; inhibitor into inlets 2228, 2209, 2214, 2219. In FIG. 22, a carrier fluid flows through the channel portions 2220, 2221, 2222, 2223 from left to right. The channel portions enclosed by the dotted square
 15 2224, 2225, 2226, 2227 represent fields of view for the purpose of monitoring a reaction at various points along the channel.

The same principle applies to an alternate embodiment of the present invention, where the distance corresponding to a point n from a common point of origin along the channel differs from that corresponding to another channel by a
 20 power or multiples of 2. This can be seen more clearly from the following discussion. Given a constant fluid flow rate u , one may determine a reaction time corresponding to the various times $t_1, t_2, t_3, \dots, t_n$ along the channel. Thus, if the distance between each pair of points n and $n-1$, which correspond to time t_n and t_{n-1} , are the same for a given value of n , then the reaction time corresponding to point n along the channel
 25 may be calculated from $t_n = nl/u$. In a relatively more complex channel geometry such as the one shown in FIG. 22(c), the corresponding equation is given by $t_n = 2^{(n-1)l}/u$, which shows that the reaction times at various points n varies as a power or multiples of 2.

In one aspect, channels according to the invention are used that place into a
 30 field of view different regions that correspond to different time points of a reaction. The channels according to the invention allow various measurements such as those of a complete reaction profile, a series of linearly separated time points (such as those required for the determination of an initial reaction velocity in enzymology), and a series of exponentially separated time points (*e.g.*, first-order kinetic measurements or

other exponential analysis). Time scales in an image frame can be varied from microseconds to seconds by, for example, changing the total flow rate and channel length.

FIG. 22A-D show various examples of geometries of microfluidic channels according to the invention for obtaining kinetic information from single optical images. The illustrated channel systems are suitable for studies such as measurements of enzyme kinetics in the presence of inhibitors. The device shown in FIG. 22D has multiple outlets that can be closed or opened. In the device shown in FIG. 22D, preferably only one outlet is open at a time. At the fastest flow rates, the top outlet is preferably open, providing reduced pressure for flow through a short fluid path l . As flow rates are reduced, other outlets are preferably opened to provide a longer path and a larger dynamic range for measurements at the same total pressure.

In FIG. 22, n is the number of segments for a given channel length l traveled by the reaction mixture in time t_n (see p. 73, second full paragraph for a related discussion of reaction times and channel lengths). These systems allow the control of the ratio of reagents by varying the flow rates. The systems also allow a quick quantification of enzyme inhibition.

For example, ribonuclease A can be used with known inhibitors such as nucleoside complexes of vanadium and oxovanadium ions and other small molecules such as 5'-diphosphoadenosine 3'-phosphate and 5'-diphosphoadenosine 2'-phosphate. The kinetics may be characterized by obtaining data and making Lineweaver-Burk, Eadie-Hofstee, or Hanes-Wolfe plots in an experiment. The experiment can be accomplished using only a few microliters of the protein and inhibitor solutions. This capability is particularly useful for characterizing new proteins and inhibitors that are available in only minute quantities, *e.g.*, microgram quantities.

Kinetic measurements of reactions producing a fluorescent signal can be performed according to the invention by analyzing a single image obtained using, for example, an optical microscope. Long exposures (*i.e.*, about 2 seconds) have been used to measure fast (*i.e.*, about 2 milliseconds) kinetics. This was possible because in a continuous flow system, time is simply equal to the distance divided by the flow rate. In the continuous flow regime in accordance with the invention, the accessible time scales can be as slow as about 400 seconds, which can be extended to days or weeks if the flow is substantially slowed down or stopped. Typically, the time scale

depends on the length of the channel (e.g., up to about 1 meter on a 3-inch diameter chip) at a low flow rate of about 1 mm/s, which is generally limited by the stability of the syringe pumps, but may be improved using pressure pumping. The fastest time scale is typically limited by the mixing time, but it may be reduced to about 20 μ s in the present invention. Mixing time is generally limited by two main factors: (1) the mixing distance (e.g., approximately 10-15 times the width of the channel); and (2) the flow rates (e.g., approximately 400 mm/s, depending on the capillary number and the pressure drop required to drive the flow). Mixing distance is normally almost independent of the flow rate. By using suitable designs of microfluidic channels, or networks of microfluidic channels, a wide range of kinetic experiments can be performed.

Reducing the channel size generally reduces the mixing time but it also increases the pressure required to drive a flow. The equation below describes the pressure drop, ΔP (in units of Pa), for a single-phase flow in a rectangular capillary:

$$\Delta P = 28.42 U \mu l / a b \quad \text{Eqn. (9)}$$

where U (m/s) is the velocity of the flow, μ (kilogram/meter-second, $\text{kg m}^{-1} \text{s}^{-1}$) is the viscosity of the fluid, l (m) is the length of the capillary, a (m) is the height of the capillary, and b (m) is the width of the capillary. There is generally a physical limitation on how much pressure a microfluidic device can withstand, e.g., about 3 atm for PDMS and about 5 atm for glass and Si. This limitation becomes crucial for very small channels and restricts the total length of the channel and thus the dynamic range (the total distance through which this flow rate can be maintained at a maximum pressure divided by the mixing distance) of the measurement.

FIG. 23 depicts a microfluidic network according to the invention with channel heights of 15 and 2 μ m. The channel design shown in FIG. 23 illustrates how a dynamic range of about 100 can be achieved by changing the cross-section of the channels. Under these conditions, mixing time in the winding channel is estimated to be about 25 μ s and observation time in the serpentine channels are estimated to be about 3 ms.

As FIG. 23 shows, rapid mixing occurs in the 2 μ m x 1 μ m (height x width) channels and measurements are taken in the 2 μ m x 3 μ m channels. The table in FIG. 23 shows the distribution of the pressure drop, flow velocity, and flow time as a function of the channel cross-section dimensions. A transition from a 1- μ m wide to 3- μ m wide channels should occur smoothly, with plugs maintaining their stability and

decreasing their velocity when they move from a 20- μm wide into a 50- μm wide channel. Changing the width of the channel can be easily done and easily incorporated into a mask design. The height of the channel can be changed by, for example, using photoresist layers having two different heights that are sequentially spun on, for example, a silicon wafer. A two-step exposure method may then be used to obtain a microfluidic network having the desired cross-section dimensions.

In another example of the application of the devices and methods of the present invention, the folding of RNase P catalytic domain (P RNA C-domain) of *Bacillus subtilis* ribozyme can be investigated using channels according to the invention. RNA folding is an important problem that remains largely unsolved due to limitations in existing technology. Understanding the rate-limiting step in tertiary RNA folding is important in the design, modification, and elucidation of the evolutionary relationship of functional RNA structures.

The folding of P RNA C-domain is known to involve three populated species: unfolded (U), intermediate (I), and native (N, folded) states. Within the first millisecond, the native secondary structure and some of the tertiary structure would have already folded (the RNA is compacted to about 90% of the native dimension) but this time regime cannot be resolved using conventional techniques such as stopped-flow. Using channels and substrates according to the invention, the time-dependence of the P RNA folding kinetics upon the addition of Mg^{2+} can be studied.

Various types of assays (e.g., protein assays) known in the art, including absorbance assays, Lowry assays, Hartree-Lowry assays, Biuret assays, Bradford assays, BCA assays, etc., can be used, or suitably adapted for use, in conjunction with the devices and methods of the invention. Proteins in solution absorb ultraviolet light with absorbance maxima at about 280 and 200 nm. Amino acids with aromatic rings are the primary reason for the absorbance peak at 280 nm. Peptide bonds are primarily responsible for the peak at 200 nm. Absorbance assays offer several advantages. Absorbance assays are fast and convenient since no additional reagents or incubations are required. No protein standard need be prepared. The assay does not consume the protein and the relationship of absorbance to protein concentration is linear. Further, the assay can be performed using only a UV spectrophotometer.

The Lowry assay is an often-cited general use protein assay. It was the method of choice for accurate protein determination for cell fractions, chromatography fractions, enzyme preparations, and so on. The bicinchoninic acid

(BCA) assay is based on the same principle, but it can be done in one step. However, the modified Lowry is done entirely at room temperature. The Hartree version of the Lowry assay, a more recent modification that uses fewer reagents, improves the sensitivity with some proteins, is less likely to be incompatible with some salt solutions, provides a more linear response, and is less likely to become saturated.

In the Hartree-Lowry assay, the divalent copper ion forms a complex with peptide bonds under alkaline conditions in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue. In addition to standard liquid handling supplies, the assay only requires a spectrophotometer with infrared lamp and filter. Glass or inexpensive polystyrene cuvettes may be used.

The Biuret assay is similar in principle to that of the Lowry, however it involves a single incubation of 20 minutes. In the Biuret assay, under alkaline conditions, substances containing two or more peptide bonds form a purple complex with copper salts in the reagent. The Biuret assay offer advantages in that there are very few interfering agents (ammonium salts being one such agent), and there were fewer reported deviations than with the Lowry or ultraviolet absorption methods. However, the Biuret consumes much more material. The Biuret is a good general protein assay for batches of material for which yield is not a problem. In addition to standard liquid handling supplies, a visible light spectrophotometer is needed, with maximum transmission in the region of 450 nm. Glass or inexpensive polystyrene cuvettes may be used.

The Bradford assay is very fast and uses about the same amount of protein as the Lowry assay. It is fairly accurate and samples that are out of range can be retested within minutes. The Bradford is recommended for general use, especially for determining protein content of cell fractions and assessing protein concentrations for gel electrophoresis. Assay materials including color reagent, protein standard, and instruction booklet are available from Bio-Rad Corporation. The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change. The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range. In addition

to standard liquid handling supplies, a visible light spectrophotometer is needed, with maximum transmission in the region of 595 nm, on the border of the visible spectrum (no special lamp or filter usually needed). Glass or polystyrene cuvettes may be used, but the color reagent stains both. Disposable cuvettes are recommended.

5 The bicinchoninic acid (BCA) assay is available in kit form from Pierce (Rockford, IL). This procedure is quite applicable to microtiter plate methods. The BCA is used for the same reasons the Lowry is used. The BCA assay is advantageous in that it requires a single step, and the color reagent is stable under alkaline conditions. BCA reduces divalent copper ion to the monovalent ion under alkaline
10 conditions, as is accomplished by the Folin reagent in the Lowry assay. The advantage of BCA is that the reagent is fairly stable under alkaline condition, and can be included in the copper solution to allow a one step procedure. A molybdenum/tungsten blue product is produced as with the Lowry. In addition to standard liquid handling supplies, a visible light spectrophotometer is needed with
15 transmission set to 562 nm. Glass or inexpensive polystyrene cuvettes may be used.

 The range of concentrations that can be measured using the above assays range from about 20 micrograms to 3mg for absorbance at 280, between about 1-100 micrograms for absorbance at 205 nm, between about 2-100 micrograms for the Modified Lowry assay, between about 1-10 mg for the Biuret assay, between about 1-
20 20 micrograms for the Bradford assay, and between about 0.2-50 micrograms for BCA assay. Many assays based on fluorescence or changes in fluorescence have been developed and could be performed using methods and devices of the invention.

 A detailed description of various physical and chemical assays is provided in *Remington: The Science and Practice of Pharmacy*, A. R. Gennaro (ed.), Mack
25 Publishing Company, chap. 29, "Analysis of Medicinals," pp. 437-490 (1995) and in references cited therein while chapter 30 of the same reference provides a detailed description of various biological assays. The assays described include titrimetric assays based on acid-base reactions, precipitation reactions, redox reactions, and complexation reactions, spectrometric methods, electrochemical methods,
30 chromatographic methods, and other methods such as gasometric assays, assays involving volumetric measurements and measurements of optical rotation, specific gravity, and radioactivity. Other assays described include assays of enzyme-containing substances, proximate assays, alkaloidal drug assays, and biological tests

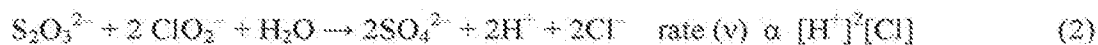
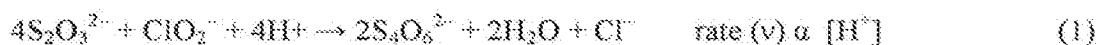
such as pyrogen test, bacterial endotoxin test, depressor substances test, and biological reactivity tests (*in-vivo* and *in-vitro*)

In addition, *Remington: The Science and Practice of Pharmacy*, A. R. Gennaro (ed.), Mack Publishing Company, chap. 31, "Clinical Analysis," pp. 501-533 (1995) and references cited therein provide a detailed description of various methods of characterizations and quantitation of blood and other body fluids. In particular, the reference includes a detailed description of various tests and assays involving various body fluid components such as erythrocytes, hemoglobin, thrombocyte, reticulocytes, blood glucose, nonprotein nitrogen compounds, enzymes, electrolytes, blood-volume and erythropoietic mechanisms, and blood coagulation.

NONLINEAR AND STOCHASTIC SENSING

Stochastic behavior has been observed in many important chemical reactions, *e.g.*, autocatalytic reactions such as inorganic chemical reactions, combustion and explosions, and in polymerization of sickle-cell hemoglobin that leads to sickle-cell anemia. Crystallization may also be considered an autocatalytic process. Several theoretical treatments of these reactions have been developed. These reactions tend to be highly sensitive to mixing.

Consider the extensively studied stochastic autocatalytic chemical reaction between NaClO_2 and $\text{Na}_2\text{S}_2\text{O}_3$ (chlorite-thiosulfate reaction). The mechanism of this reaction can be described by reactions (1) and (2),



where $[\text{H}^+]$ stands for the concentration of H^+ . At a slightly basic $\text{pH} = 7.5$, the slow reaction (1) dominates and maintains a basic pH of the reaction mixture (since the rate of this reaction v is directly proportional $[\text{H}^+]$, this reaction consumes H^+ and is auto-inhibitory). Reaction (2) dominates at acidic pH (since the rate of this reaction varies in proportion to $[\text{H}^+]^2[\text{Cl}]$, this reaction produces both H^+ and Cl^- and is superautocatalytic). FIG. 21 shows the reaction diagram for two reactions corresponding to the curves 211, 212. The rates of the two reactions (referred to here as reaction 211 and reaction 212) are equal at an unstable critical point at a certain pH. The lifetime of the reaction mixtures of NaClO_2 and $\text{Na}_2\text{S}_2\text{O}_3$ at this critical point

crucially depends on stirring. In the absence of stirring, stochastic fluctuations of $[H^+]$ in solution generate a localized increase in $[H^+]$. This increase in $[H^+]$ marginally increases the rate of reaction 212, but it has a much stronger accelerating effect on reaction 211 because of the higher-order dependence on $[H^+]$ of this reaction.

5 Therefore, in the region where local fluctuations increase local $[H^+]$, reaction 211 becomes dominant, and more H^+ is produced (which rapidly diffuses out of the region of the initial fluctuation). The initiated chemical wave then triggers the rapid reaction of the entire solution. Unstirred mixtures of $NaClO_2$ and NaS_2O_3 are stable only for a few seconds, and these fluctuations arise even in the presence of stirring.

10 FIG. 21 depicts a reaction diagram illustrating an unstable point in the chlorite-thiosulfate reaction. At $[H^+]$ values below the critical point, the slow reaction (1) dominates. At $[H^+]$ values above the critical point, the autocatalytic reaction (2) dominates. The reaction mixture at the $[H^+]$ value equal to the critical point is metastable in the absence of fluctuations. Under perfect mixing, the effects of small
15 fluctuations average out and the system remains in a metastable state. Under imperfect mixing, fluctuations that reduce $[H^+]$ grow more slowly than those that increase $[H^+]$ due to the autocatalytic nature of reaction (2), and the reaction mixture thus rapidly becomes acidic.

It is known that chaotic flows should have a strong effect on diffusive
20 transport within the fluid ("anomalous diffusion"). It is also known that chaotic dynamics can lead to non-Gaussian transport properties ("strange kinetics"). In one aspect according to the invention, these highly unstable mixtures are stabilized in the presence of chaotic mixing using channels according to the invention because this mixing can effectively suppress fluctuations. This invention can be used to
25 understand the effects of mixing on the stochastic behavior of such systems, including for example, the chlorite thiosulfate system.

In a laminar flow, the flow profile in the middle of the channel is flat and there is virtually no convective mixing. Fluctuations involving $[H^+]$ that arise in the middle of the channel can grow and cause complete decomposition of the reaction mixture.
30 Slow mixing reduces the probability of fluctuations in plugs moving through straight channels. When fluctuations that occur in the centers of vortices are not efficiently mixed away, one or more spontaneous reactions involving some of the plugs can take place. In the present invention, chaotic mixing in plugs moving through winding

channels efficiently mix out fluctuations, and thus substantially fewer or no spontaneous reactions are expected to occur.

In a simple laminar flow, there is normally very little or no velocity gradient and substantially no mixing at the center of the channel. Thus, fluctuations that arise in the chlorite-thiosulfate reaction mixture prepared at the critical $[H^+]$ are able to grow and lead to rapid decomposition of the reaction mixture. Propagation of chemical fronts in autocatalytic reactions occurring in laminar flows has been described with numerical simulations, and back-propagation has been predicted (that is, a reaction front traveling upstream of the direction of the laminar flow). Using the method of the present invention, this back-propagation involving the reaction between $NaClO_2$ and $Na_2S_2O_3$ under laminar flow conditions was observed.

In accordance with the invention, chaotic flow within plugs that flow through winding channels suppresses fluctuations and gives rise to stable reaction mixtures. There exists, of course, a finite probability that fluctuations can arise even in a chaotically stirred plug. In one aspect according to the invention, the details of the evolution of these reactions are monitored using a high-speed digital camera. The plugs are preferably separated by the oil and are not in communication with each other, so the reaction of one plug will not affect the behavior of the neighboring plug. Statistics covering the behavior of thousands of plugs can be obtained quickly under substantially identical experimental conditions.

Whether a fluctuation would be able to trigger an autocatalytic reaction depends on factors such as the magnitude of a fluctuation and its lifetime. The lifetime of a fluctuation is typically limited by the mixing time in the system. In an unstirred solution, mixing is by diffusion and quite slow, and fluctuations may persist and lead to autocatalytic reactions. In a stirred solution, the lifetime of a fluctuation is relatively short, and only large fluctuations have sufficient time to cause an autocatalytic reaction.

Mixing time and the lifetime of fluctuations typically depend on the size of the plugs. As plug size decreases, mixing is accelerated and fluctuations are suppressed. However, very small plugs (e.g., about $1 \mu m^3$ or 10^{-15} L) in a solution containing about 10^{-8} mole/liter concentration of H^+ (pH = 8) will contain only a few H^+ ions per plug (about 10^{-23} moles or about 6 H^+ ions). When such small plugs are formed, the number of H^+ ions in them will have a Poisson distribution.

An important experimental challenge is to establish that the stochastic behavior in these systems is due mainly to internal fluctuations of concentrations. Other factors that may act as sources of noise and instability are: (1) temporal fluctuations in the flow rates of the incoming reagent streams, which can lead to the formation of plugs with varying amounts of reagents; (2) temperature fluctuations in solutions in a microfluidic device, which may arise due to, for example, illumination by a microscope; and (3) fluctuations due to impurities in carrier-fluids leading to variations in the surface properties of different plugs.

Microfluidic systems according to the invention may be used to probe various chemical and biochemical processes, such as those that show stochastic behavior in bulk due to their nonlinear kinetics. They can also be used in investigating processes that occur in systems with very small volumes (e.g., about $1\ \mu\text{m}^3$, which corresponds to the volume of a bacterial cell). In systems with very small volumes, even simple reactions are expected to exhibit stochastic behavior due to the small number of molecules localized in these volumes.

Autocatalytic reactions present an exciting opportunity for highly sensitive detection of minute amounts of autocatalysts. Several systems are known to operate on this principle, silver-halide photography being the most widely used. In silver-halide photography, the energy of photons of light is used to decompose an emulsion of silver halide AgX into nanometer-sized particles of metallic silver. A film that is embedded with the silver particles is then chemically amplified by the addition of a metastable mixture of a soluble silver(I) salt and a reducing agent (hydroquinone). Metallic silver particles catalyze reduction of silver(I) by hydroquinone, leading to the growth of the initial silver particles. Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.

However, a dilemma occurs when designing systems with very high sensitivity and amplification. To achieve a very highly sensitive amplification, the system typically has to be made very unstable. On the other hand, an unstable system is very sensitive to noise and has a very short lifetime. Also, in unstable systems, it is difficult to distinguish between spontaneous decomposition and a reaction caused by the analyte. In one aspect, microfluidic devices according to the invention, which allow chaotic mixing and compartmentalization, are used to overcome this problem.

To demonstrate the potential of microfluidic systems according to the present invention, a microfluidic system according to the invention is used to handle unstable mixtures. In one application, a microfluidic system according to the invention is preferably used to control a stochastic reaction between NaClO_2 and NaS_2O_3 . In particular, this reaction is preferably used for a highly sensitive amplification process.

If a plug containing an unstable reaction mixture of NaClO_2 and NaS_2O_3 is merged with a small plug containing an amount of H^+ sufficient to bring the local concentration of H^+ above critical, a rapid autocatalytic reaction is generally triggered. This autocatalytic reaction typically leads to the production of large amounts of H^+ . Thus, a weak chemical signal, e.g., a small amount of H^+ , is rapidly amplified by an unstable reaction mixture. Thus, for example, this approach can be used to investigate biological reactions such as those that involve enzymes, in which small amounts of H^+ are produced.

The above autocatalytic system possesses several features that contribute to its novelty and usefulness. In one aspect, an unstable amplifying reaction mixture is prepared in-situ and is used within milliseconds before it has a chance to decompose. Preferably, the system is compartmentalized so a reaction that occurs in one compartment does not affect a reaction in another compartment. This compartmentalization allows thousands of independent experiments to be conducted in seconds using only minute quantities of samples. Importantly, chaotic mixing in the system reduces fluctuations and stabilizes the reaction mixture.

The applications of controlled autocatalytic amplification in accordance with the invention are not limited to the detection of protons or Co^{2+} ions. For example, the $(\text{Co(III)}-5\text{-Br-PAPS})/\text{peroxomonosulfate}$ oxidation reaction can also be used indirectly, for example, for a detection of small amounts of peroxidase, which can be used as a labeling enzyme bound to an antibody. The $(\text{Co(III)}-5\text{-Br-PAPS})/\text{peroxomonosulfate}$ oxidation reaction, which has been characterized analytically, involves the autocatalytic decomposition of violet bis[2-(5-bromopyridylazo)-5-(N-propyl-N-sulfopropyl-amino-phenolato)] cobaltate, $(\text{Co(III)}-5\text{-Br-PAPS})$, upon oxidation with potassium peroxomonosulfate to produce colorless Co^{2+} ions, which serve as the autocatalyst (the order of autocatalysis has not been established for this reaction). (Endo *et al.*, "Kinetic determination of trace cobalt(II) by visual autocatalytic indication," *Talanta*, 1998, vol. 47, pp. 349-353; Endo *et al.*, "Autocatalytic decomposition of cobalt complexes as an indicator system for the

determination of trace amounts of cobalt and effectors," *Analyst*, 1996, vol. 121, pp. 391-394.)

$\text{Co(III)-[5-Br-PAPS]reduced} + \text{HSO}_5^- \rightarrow$

$\text{Co}^{2+} + \text{[5-Br-PAPS]oxidized} + \text{HSO}_4^-$

5 Addition of small amounts of Co^{2+} to the violet mixture of the (Co(III)-5-Br-PAPS and peroxomonosulfate produces an abrupt loss of color to give a colorless solution. The time delay before this decomposition depends on the amount of the Co^{2+} added to the solution. This reaction has been used to detect concentrations of Co^{2+} as low as 1×10^{-10} mole/L. The reaction shows good selectivity in the presence of
10 other ions (V(V), Cr(III), Cr(VI), Mn(II), Fe(II), Ni(II), Cu(II) and Zn(II)).

 The devices and methods according to the invention may be applied to other autocatalytic reactions, some of which have been described in inorganic, organic and biological chemistry. Reactions of transition metal ions such as Cr(III) (B82) Mn^{2+} or colloidal MnO_2 , and reactions of halides and oxohalides are often autocatalytic.
15 Autocatalysis involving lanthanides (Eu^{2+}) and actinides (U^{4+}) has also been reported. All of these elements are potential targets for detection and monitoring in chemical waste, drinking water, or biological fluids. Intriguing possibilities arise from using asymmetric autocatalytic reactions to detect minute amounts of optically active, chiral impurities, such as biomolecules.

20 It is also possible to design new autocatalytic reactions. Autocatalysis is abundant in biology, and many enzymes are autocatalytic (*e.g.*, caspases involved in programmed cell death, kinases involved in regulation and amplification, and other enzymes participating in metabolism, signal transduction, and blood coagulation. Emulsions of perfluorocarbons such as perfluorodecaline (PFD) are used as blood
25 substitutes in humans during surgeries and should be compatible with a variety of biological molecules. Since the feasibility of quantitative measurements of enzyme kinetics has been demonstrated using plugs formed according to the invention, plugs formed according to the invention may also be applied to the detection of biological autocatalysts.

30 The devices and methods according to the present invention are not limited to the detection of the autocatalyst itself. For example, the labeling of an analyte using an autocatalyst is also within the scope of the present invention. Biomolecules are often labeled with metallic nanoparticles. Such metallic nanoparticles are highly effective autocatalysts for the reduction of metal ions to metals. Preferably, the

systems and methods of the present invention are used in the visual detection of a single molecule of DNA, RNA, or protein labeled with nanoparticles via an autocatalytic pathway. In preliminary experiments in accordance with the invention, clean particle formation and transport within plugs were observed.

5 In addition, the generation of metal (e.g., copper, silver, gold, nickel) deposits and nanoparticles upon chemical reduction also proceed by an autocatalytic mechanism. These reactions are commonly used for electroless deposition of metals and should be useful for the detection of minute amounts of metallic particles. The presence of metallic particles in water can be indicative of the presence of operating
10 mechanical devices. In one aspect according to the invention, devices and methods according to the invention are used to detect the presence of minute or trace quantities of metallic particles.

 The devices in accordance with the present invention are simple in design, consume minute amounts of material, and robust. They do not require high voltage
15 sources and can be operated, for example, using gravity or a pocket-sized source of compressed air. In one aspect, the systems according to the invention are used in portable and hand-held devices.

 Autocatalytic reactions show a threshold response, that is, there is a very abrupt temporal change from unreacted mixture to reacted mixture. In the case where
20 time is equal to distance, this abrupt transition over a short distance can be observed using the devices and methods of the invention. The time (and distance) is very sensitive to the initial concentration of the catalyst, and thus it should be easy to determine the concentration of the autocatalyst in the sample by noting how far the reaction system traveled before it reacted.

25 One example of an autocatalytic process is blood coagulation. It is very sensitive to flow and mixing, therefore experimenting with it in the absence of flow gives unreliable results or results that have little relevance to the real function of the coagulation cascade. A typical microfluidic system may be difficult to use with blood because once coagulation occurs, it blocks the channel and stops the flow in the
30 microfluidic device. In addition, coagulated blood serves as an autocatalyst; even small amounts of coagulated blood in the channels can make measurements unreliable.

 These problems can be overcome using the devices of the present invention. Using plugs, autocatalytic reactions can be easily controlled, and the formation of

solid clots would not be a problem because any solids formed will be transported inside the plugs out of the channel without blocking the channel and without leaving autocatalytic residue. In addition, flow inside plugs can be easily controlled and adjusted to resemble flow under physiological conditions.

5 To address the sensitivity of blood coagulation to surfaces (the cascade is normally initiated on the surface), microscopic beads containing immobilized tissue factor (the cascade initiator) on the surface may be added to one of the streams and transported inside the plugs. Also, surfactants may be used to control surface chemistry.

10 Thus, the devices and methods of the invention may be used, for example, to test how well the coagulation cascade functions (*e.g.*, for hemophilia or the tendency to form thrombus) under realistic flow conditions. This test would be particularly valuable in diagnostics. Blood may be injected in one stream, and a known concentration of a molecule known to induce coagulation (*e.g.*, factor VIIa) can be
15 added through another stream prior to plug formation. At a given flow rate, normal blood would coagulate at a certain distance (which corresponds to a given time), which can be observed optically by light scattering or microscopy. Blood of hemophiliac patients would coagulate at a later time. This type of testing would be useful before surgical operations. In particular, this type of testing is important for
20 successful child delivery, especially when hemophilia is suspected. Fetal testing may be performed since only minute amounts of blood are required by systems according to the invention. The blood may be injected directly from the patient or collected in the presence of anticoagulating agent (for example EDTA), and then reconstituted in the plug by adding Ca^{2+} . In some cases, the addition of Ca^{2+} may be sufficient to
25 initiate the coagulation cascade.

 The devices and methods of the invention may also be used to evaluate the efficacy of anticoagulating agents under realistic flow conditions. Plugs can be formed from normal blood (which may be used directly or reconstituted by adding Ca^{2+} or other agents), an agent known to induce coagulation, and an agent (or several
30 agents that need to be compared) being tested as an anticoagulation agent. The concentrations of these agents can be varied by varying the flow rates. The distance at which coagulation occurs is noted, and the efficacy of various agents to prevent coagulation is compared. The effects of flow conditions and presence of various compounds in the system on the efficacy of anticoagulation agents can be investigated

quickly. The same techniques may also be used to evaluate agents that cause, rather prevent, coagulation. These tests could be invaluable in evaluating drug candidates.

SYNTHESIS

5 In accordance with the present invention, a method of conducting a reaction within a substrate is provided. The reaction is initiated by introducing two or more plug-fluids containing reactants into the substrate of the present invention.

 In one aspect, the plug-fluids include a reagent and solvent such that mixing of the plug-fluids results in the formation of a reaction product. In another embodiment,
10 one of the plug-fluids may be reagent free and simply contain fluid. In this embodiment, mixing of the plug-fluids will allow the concentration of the reagent in the plug to be manipulated.

 The reaction can be initiated by forming plugs from each plug-fluid and subsequently merging these different plugs.

15 When plugs are merged to form merged plugs, the first and second set of plugs may be substantially similar or different in size. Further, the first and second set of plugs may have different relative velocities. In one embodiment, large arrays of microfluidic reactors are operated in parallel to provide substantial throughput.

 The devices and methods of the invention can be used for synthesizing
20 nanoparticles. Nanoparticles that are monodisperse are important as sensors and electronic components but are difficult to synthesize (Trindade *et al.*, *Chem. Mat.* 2001, vol. 13, pp. 3843-3858.). In one aspect, monodisperse nanoparticles of semiconductors and noble metals are synthesized under time control using channels according to the invention (Park *et al.*, *J. Phys. Chem. B*, 2001, vol. 105, pp. 11630-
25 11635.). Fast nucleation is preferably induced by rapid mixing, thereby allowing these nanoparticles to grow for a controlled period of time. Then their growth is preferably quickly terminated by passivating the surfaces of the particles with, for example, a thiol. Nanoparticles of different sizes are preferably obtained by varying the flow rate and therefore the growth time. In addition, devices according to the
30 invention can be used to monitor the synthesis of nanoparticles, and thus obtain nanoparticles with the desired properties. For example, the nanoparticle formation may be monitored by measuring the changes in the color of luminescence or absorption of the nanoparticles. In addition, the growth of nanoparticles may be

stopped by introducing a stream of quenching reagent at a certain position along the main channel.

Rapid millisecond mixing generated in channels according to the invention can help ensure the formation of smaller and much more monodisperse nanoparticles than nanoparticles synthesized by conventional mixing of solutions. FIG. 13 shows the UV-VIS spectra of CdS nanoparticles formed by rapid mixing in plugs (lighter shade spectrum with sharp absorption peak) and by conventional mixing of solutions (darker shade spectrum). The sharp absorption peak obtained for synthesis conducted in plugs indicates that the nanoparticles formed are highly monodisperse. In addition, the blue-shift (shift towards shorter wavelengths) of the absorption peak indicates that the particles formed are small.

FIG. 14A-B illustrates the synthesis of CdS nanoparticles performed in PDMS microfluidic channels in single-phase aqueous laminar flow (FIG. 14A) and in aqueous plugs that were surrounded by water-immiscible perfluorodecaline (FIG. 14B). In FIGS. 14A-B, Cd^{2+} was introduced into inlets 1400, 1403, aqueous stream was introduced into inlets 1401, 1404, and S^{2-} was introduced into inlets 1402, 1405. In FIG. 14A, an aqueous stream flowed through channel 1406 while in FIG. 14B, oil flowed through channel 1407. FIG. 14A shows portions of the channels 1408 and 1410 at time $t=6$ minutes and portions of the channels 1409, 1411 at time $t=30$ minutes. It can be seen in FIG. 14A that when laminar flow is used in the synthesis, large amounts of CdS precipitate form on the channel walls. When plugs were used for the synthesis, all CdS formed inside the plugs, and no surface contamination was observed. FIG. 15 illustrates a technique for the synthesis of CdS nanoparticles, which is discussed in detail in Example 13 below.

The following methods according to the invention can be used in synthesis involving nanoparticles:

(a) using self-assembled monolayers to nucleate nanoparticles with crystal structures not accessible under homogeneous nucleation conditions (*e.g.*, controlling polymorphism by controlling the surface at which nucleation takes place).

(b) using merging of plugs to create core-shell nanoparticles with a range of core and shell sizes. In a stream of plugs of a first channel, small core nanoparticles such as CdSe particles can be synthesized in a matter of few milliseconds. The CdSe particles can then be used as seeds for mixing with solutions such as those containing Zn^{2+} and S^{2-} . The CdSe particles, acting as seeds for the formation of ZnS, thus allow

the formation of CdSe(core)/ZnS(shell) nanoparticles. Core-shell particles with more than two layers may be obtained by simply repeating the merging process more than once.

5 (c) using merging of plugs to create composite nanoparticles. For example, small nanoparticles of CdSe and ZnS can be formed using streams of plugs from two separate channels. Merging of these streams leads to aggregation of these particles to form larger nanoparticles containing CdSe/ZnS composite. The composite nanoparticles that contain only a few of the original nanoparticles can be made non-centrosymmetric, which may have interesting photophysical properties.

10 (d) using the devices and methods according to the invention to synthesize medically important nanoparticles, such as encapsulated drugs and composite drugs.

(e) combinatorial synthesis of core-shell particles and other complex systems. For example, the luminescence of CdSe/ZnS particles may be monitored and the conditions adjusted to produce particles with various core and shell sizes, various
15 doping impurities in the core and shell, and various ligand composition on the surface of the particles. These can be conducted in real time using a device according to the invention. The entire process can also be automated.

The devices and methods according to the present invention may also be used for synthesizing polymers. Since the invention allows precise control of the timing of
20 a polymerization reaction, one or more properties of a polymer such as molecular weight, polydispersity and blockiness can be readily controlled or adjusted. In addition, use of the substrate of the present invention allows the user to precisely form block copolymers by merging plugs within a device, since the path length of the channel will correspond to a specific duration of the polymerization reaction.

25 Similarly, a living polymer chain can be terminated with a specific end group to yield polymers with a discrete subset of molecular weights.

In addition, combinatorial libraries of drug candidates may be synthesized using similar approaches. The library may be encoded using the position of plugs in a channel. Plugs of variable composition may be created by varying flow rates.

30 Combination of synthesis of the library may be combined with screening and assays performed on the same microfluidic chip according to the present invention. In some embodiments, merging, splitting and sorting of plugs may be used during synthesis, assays, etc.

All of the above synthesis methods of the present invention can be used to form macroscopic quantities of one or more reaction products by running multiple reactions in parallel.

5 **PARTICLE SEPARATION/SORTING USING PLUGS**

The flow within the moving plugs can be used for separation of polymers and particles. Plugs can be used for separation by first using flow within a moving plug to establish a distribution of the polymers or particles inside the plug (for example, an excess of the polymer inside the front, back, right or left side of the plug) and then
10 using splitting to separate and isolate the part of the plug containing higher concentration of the polymers or particles. When two polymers or particles are present inside the plug and establish different distributions, splitting can be used to separate the polymers or particles. This approach may be useful, for example, in achieving on a microfluidic chip any of, but not limited to, the following: separation,
15 purification, concentration, membrane-less dialysis, and filtration.

CRYSTALLIZATION

The devices and methods of the invention allow fast, inexpensive miniaturization of existing crystallization methods and other methods that can be
20 adapted into, for example, novel protein screening and crystallization techniques. The crystallization methods according to the invention may be applied to various drugs, materials, small molecules, macromolecules, colloidal and nanoparticles, or any of their combinations. Many relevant protein structures remain undetermined due to their resistance to crystallization. Also, many interesting proteins are only available
25 in microgram quantities. Thus, a screening process must permit the use of small amounts protein for analysis. Current crystallization screening technologies generally determine the ideal conditions for protein crystallization on a milligram scale. Devices and methods according to the invention improve current bench-top methodology available to single users, and enables higher throughput automated
30 systems with improved speed, sample economy, and entirely new methods of controlling crystallization.

A microfluidic system according to the invention can be applied to the crystallization of small molecules or macromolecules and their complexes.

For example, systems and methods in accordance with the present invention may include but are not limited to: (1) biological macromolecules (cytosolic proteins, extracellular proteins, membrane proteins, DNA, RNA, and complex combinations thereof); (2) pre- and post-translationally modified biological molecules (including but not limited to, phosphorylated, sulfolated, glycosylated, ubiquitinated, etc. proteins, as well as halogenated, abasic, alkylated, etc. nucleic acids); (3) deliberately derivatized macromolecules, such as heavy-atom labeled DNAs, RNAs, and proteins (and complexes thereof), selenomethionine-labeled proteins and nucleic acids (and complexes thereof), halogenated DNAs, RNAs, and proteins (and complexes thereof); (4) whole viruses or large cellular particles (such as the ribosome, replisome, spliceosome, tubulin filaments, actin filaments, chromosomes, etc.); (5) small-molecule compounds such as drugs, lead compounds, ligands, salts, and organic or metallo-organic compounds; (6) small-molecule/biological macromolecule complexes (e.g., drug/protein complexes, enzyme/substrate complexes, enzyme/product complexes, enzyme/regulator complexes, enzyme/inhibitor complexes, and combinations thereof); (7) colloidal particles; and (8) nanoparticles.

Preferably, a general crystallization technique according to the present invention involves two primary screening steps: a crude screen of crystallization parameters using relatively small channels with a large number of small plugs, and a fine screen using larger channels and larger plugs to obtain diffraction-quality crystals. For example, ten crude screens performed using channels with a $(50\text{ }\mu\text{m})^2$ cross-sectional dimension and with more or less one thousand 150-picoliter (pL) plugs corresponding to 10 mg/mL final concentration of a protein (10,000 trials total) will typically require about 1.5 μL of solution, produce crystals up to about $(10\text{ }\mu\text{m})^3$ in size, and will consume approximately 15 μg of protein. Up to 300 or more of such plugs can be formed in about 1 second in these microfluidic networks. A fine screen around optimal conditions in $(500\text{ }\mu\text{m})^2$ channels is expected to use more or less 50 plugs. Another $\sim 5\text{ }\mu\text{L}$ of solution and another 50 μg of the protein are expected to be consumed. This can produce crystals up to $(100\text{ }\mu\text{m})^3$ in size. Approximately 30 plugs can be formed about every second or so. The throughput of the system will generally be determined by the rate of plug formation, and may be limited by how rapidly the flow rates can be varied. Pressure control methods that operate at frequencies of 100 Hz are available and may be applied to PDMS microfluidic

networks (Unger *et al.*, "Monolithic fabricated valves and pumps by multilayer soft lithography," *Science* 2000, vol. 288, pp. 113-116.).

Crystal properties such as appearance, size, optical quality, and diffractive properties may be characterized and measured under different conditions. For example, a Raxis Ilc X-ray detector mounted on a Rigaku RU 200 rotating anode X-ray generator, which is equipped with double focusing mirrors and an MSC cryosystem, may be used for at least some of the characterizations and measurements. A synchrotron beam may be useful for characterization of small crystals. Also, these devices and methods may be used to build microfluidic systems according to the invention that are compatible with structural studies using x-ray beams.

A significant problem involving current crystallization approaches is determining the conditions for forming crystals with optimal diffractive properties. Normally crystals have to be grown, isolated, mounted, and their diffractive properties determined using an x-ray generator or a synchrotron. Microfluidic systems with thin, non-scattering walls would be desirable for determining the diffractive properties of crystals inside a microfluidic system. Preferably, crystallization is carried out inside this system using methods according to the invention, which are described herein. The crystals are exposed to x-ray beams either to determine their structure or diffractive properties (the screening mode). For example, a PDMS membrane defining two side walls of the channels could be sandwiched between two very thin glass plates (defining the top and bottom walls of the channels) that do not significantly scatter X-rays. Thus, the devices of the invention offer a further advantage in that structural characterization could be conducted while the sample is inside the microfluidic device. Thus, the sample can be characterized without the need to take out the sample, *e.g.*, crystal, from the device.

The present system enables higher throughput automated systems with improved speed, sample economy, and entirely new methods of controlling crystallization. Microfluidic versions of microbatch, vapor phase diffusion and FID techniques may be carried out using the present invention, as described below, or using a combination of these techniques or other techniques. In addition, the nucleation and growth phases may be carried out in discrete steps through merging plugs, as described herein.

Screening for protein crystallization can involve varying a number of parameters. During crystallization screening, a large number of chemical compounds

may be employed. These compounds include salts, small and large molecular weight organic compounds, buffers, ligands, small-molecule agents, detergents, peptides, crosslinking agents, and derivatizing agents. Together, these chemicals can be used to vary the ionic strength, pH, solute concentration, and target concentration in the plug, and can even be used to modify the target. The desired concentration of these chemicals to achieve crystallization is variable, and can range from nanomolar to molar concentrations.

A typical crystallization mix may contain a set of fixed, but empirically-determined, types and concentrations of precipitation agent, buffers, salts, and other chemical additives (e.g., metal ions, salts, small molecular chemical additives, cryoprotectants, etc.). Water is a key solvent in many crystallization trials of biological targets, as many of these molecules may require hydration to stay active and folded. Precipitation agents act to push targets from a soluble to insoluble state, and may work by volume exclusion, changing the dielectric constant of the solvent, charge shielding, and molecular crowding. Precipitation agents compatible with the PDMS material of certain embodiments according to the invention include, but are not limited to, nonvolatile salts, high molecular weight polymers, polar solvents, aqueous solutions, high molecular weight alcohols, divalent metals.

Precipitation agents, which include large and small molecular weight organics, as well as certain salts, may be used from under 1% to upwards of 40% concentration, or from < 0.5M to greater than 4M concentration. Water itself can act in a precipitating manner for samples that require a certain level of ionic strength to stay soluble. Many precipitation agents may also be mixed with one another to increase the chemical diversity of the crystallization screen. Devices according to the invention are readily compatible with a broad range of such compounds.

A nonexclusive list of salts that may be used as precipitation agents is as follows: tartrates (Li, Na, K, Na/K, NH_4); phosphates (Li, Na, K, Na/K, NH_4); acetates (Li, Na, K, Na/K, Mg, Ca, Zn, NH_4); formates (Li, Na, K, Na/K, Mg, NH_4); citrates (Li, Na, K, Na/K, NH_4); chlorides (Li, Na, K, Na/K, Mg, Ca, Zn, Mn, Cs, Rb, NH_4); sulfates (Li, Na, K, Na/K, NH_4); maleates (Li, Na, K, Na/K, NH_4); glutamates (Li, Na, K, Na/K, NH_4).

A nonexclusive list of organic materials that may be used as precipitation agents is as follows: PEG 400; PEG 1000; PEG 1500; PEG 2K; PEG 3350; PEG 4K; PEG 6K; PEG 8K; PEG 10K; PEG 20K; PEG-MME 550; PEG-MME 750; PEG-

MME 2K; PEGMME 5K; PEG-DME 2K; dioxane; methanol; ethanol; 2-butanol; *n*-butanol; *t*-butanol; jeffamine m-600; isopropanol; 2-methyl-2,4-pentanediol; 1,6 hexanediol.

5 Solution pH can be varied by the inclusion of buffering agents; typical pH ranges for biological materials lie anywhere between values of 3 and 10.5 and the concentration of buffer generally lies between 0.01 and 0.25 M. The microfluidics devices described in this document are readily compatible with a broad range of pH values, particularly those suited to biological targets.

10 A nonexclusive list of possible buffers that may be used according to the invention is as follows: Na-acetate; HEPES; Na-cacodylate; Na-citrate; Na-succinate; Na-K-phosphate; TRIS; TRIS-maleate; imidazole-maleate; bistrispropane; CAPSO, CHAPS, MES, and imidazole.

15 Additives are small molecules that affect the solubility and/or activity behavior of the target. Such compounds can speed up crystallization screening or produce alternate crystal forms or polymorphs of the target. Additives can take nearly any conceivable form of chemical, but are typically mono and polyvalent salts (inorganic or organic), enzyme ligands (substrates, products, allosteric effectors), chemical crosslinking agents, detergents and/or lipids, heavy metals, organometallic compounds, trace amounts of precipitating agents, and small molecular weight
20 organics.

The following is a nonexclusive list of additives that may be used in accordance with the invention: 2-butanol; DMSO; hexanediol; ethanol; methanol; isopropanol; sodium fluoride; potassium fluoride; ammonium fluoride; lithium chloride anhydrous; magnesium chloride hexahydrate; sodium chloride; calcium
25 chloride dihydrate; potassium chloride; ammonium chloride; sodium iodide; potassium iodide; ammonium iodide; sodium thiocyanate; potassium thiocyanate; lithium nitrate; magnesium nitrate hexahydrate; sodium nitrate; potassium nitrate; ammonium nitrate; magnesium formate; sodium formate; potassium formate; ammonium formate; lithium acetate dihydrate; magnesium acetate tetrahydrate; zinc
30 acetate dihydrate; sodium acetate trihydrate; calcium acetate hydrate; potassium acetate; ammonium acetate; lithium sulfate monohydrate; magnesium sulfate heptahydrate; sodium sulfate decahydrate; potassium sulfate; ammonium sulfate; di-sodium tartrate dihydrate; potassium sodium tartrate tetrahydrate; di-ammonium tartrate; sodium dihydrogen phosphate monohydrate ; di-sodium hydrogen phosphate

dihydrate; potassium dihydrogen phosphate; di-potassium hydrogen phosphate;
 ammonium dihydrogen phosphate; di-ammonium hydrogen phosphate; tri-lithium
 citrate tetrahydrate; tri-sodium citrate dihydrate; tri-potassium citrate monohydrate;
 diammonium hydrogen citrate, barium chloride; cadmium chloride dihydrate;
 5 cobaltous chloride dihydrate; cupric chloride dihydrate; strontium chloride
 hexahydrate; yttrium chloride hexahydrate; ethylene glycol; Glycerol anhydrous; 1,6
 hexanediol; MPD; polyethylene glycol 400; trimethylamine HCl; guanidine HCl;
 urea; 1,2,3-heptanetriol; benzamidine HCl; dioxane; ethanol; iso-propanol; methanol;
 sodium iodide; L-cysteine; EDTA sodium salt; NAD; ATP disodium salt; D(+)-
 10 glucose monohydrate; D(+)-sucrose; xylitol; spermidine; spermine tetra-HCl; 6-
 aminocaproic acid; 1,5-diaminopentane diHCl; 1,6-diaminohexane; 1,8-
 diaminooctane; glycine; glycyl-glycyl-glycine; hexaminecobalt trichloride; taurine;
 betaine monohydrate; polyvinylpyrrolidone K15; non-detergent sulfo-betaine 195;
 non-detergent sulfo-betaine 201; phenol; DMSO; dextran sulfate sodium salt;
 15 Jeffamine M-600; 2,5 Hexanediol; (+/-)- 1,3 butanediol; polypropylene glycol P400;
 1,4 butanediol; tert-butanol; 1,3 propanediol; acetonitrile; gamma butyrolactone;
 propanol; ethyl acetate; acetone; dichloromethane; *n*-butanol; 2,2,2 trifluoroethanol;
 DTT; TCEP; nonaethylene glycol monododecyl ether, nonaethylene glycol
 monolauryl ether; polyoxyethylene (9) ether; octaethylene glycol monododecyl ether;
 20 octaethylene glycol monolauryl ether; polyoxyethylene (8) lauryl ether; Dodecyl- β -
 D-maltopyranoside; Lauric acid sucrose ester; Cyclohexyl-pentyl- β -D-maltoside;
 Nonaethylene glycol octylphenol ether, Cetyltrimethylammonium bromide; *N,N*-
 bis(3-D-gluconamidopropyl)-deoxycholine; Decyl- β -D-maltopyranoside;
 Lauryldimethylamine oxide; Cyclohexyl-pentyl- β -D-maltoside; *n*-
 25 Dodecylsulfobetaine, 3-(Dodecyldimethylanionio)propane-1-sulfonate; Nonyl- β -
 D-glucopyranoside; Octyl- β -D-thioglucopyranoside, OSG; *N,N*-
 Dimethyldecylamine- β -oxide; Methyl 0-(*N*-heptylcarbamoyl)- α -D-
 glucopyranoside; Sucrose monocaprylate; *n*-Octanoyl- β -D-fructofuranosyl- α -D-
 glucopyranoside; Heptyl- β -D-thioglucopyranoside; Octyl- β -D-glucopyranoside,
 30 OG; Cyclohexyl-propyl- β -D-maltoside; Cyclohexylbutanoyl-*N*-
 hydroxyethylglucamide; *n*-decylsulfobetaine, 3-(Decyldimethylammonio)propane-
 1-sulfonate; Octanoyl-*N*-methylglucamide, OMEGA; Hexyl- β -D-glucopyranoside;
 Brij 35; Brij 58; Triton X-114; Triton X-305; Triton X-405; Tween 20; Tween 80;

polyoxyethylene(6)decyl ether; polyoxyethylene(9)decyl ether;
polyoxyethylene(10)dodecyl ether; polyoxyethylene(8)tridecyl ether;; Decanoyl-*N*-
hydroxyethylglucamide; Pentaethylene glycol mono-octyl ether; 3-[(3-
cholamidopropyl)-dimethylammonio]-1-propane sulfonate; 3-[(3-Cholamidopropyl)-
5 dimethylammonio] hydroxy-1-propane sulfonate; Cyclohexylpentanoyl-*N*-
hydroxyethylglucamide; Nonanoyl-*N*-hydroxyethylglucamide; Cyclohexylpropanol-
N-hydroxyethylglucamide; Octanoyl-*N*-hydroxyethylglucamide; Cyclohexylethanoyl-
N-hydroxyethylglucamide; Benzyl dimethyldodecyl ammonium bromide; *n*-
Hexadecyl- β -D-maltopyranoside; *n*-Tetradecyl- β -D-maltopyranoside; *n*-Tridecyl-
10 β -D-maltopyranoside; Dodecylpoly(ethyleneglycoether); *n*-Tetradecyl-*N,N*-dimethyl
ammonio-1-propanesulfonate; *n*-Undecyl- β -D-maltopyranoside; *n*-Decyl D-
thiomaltopyranoside; *n*-dodecylphosphocholine; α -D-glucopyranoside, β -D-
fructofuranosyl monodecanoate, sucrose mono-caprate; 1-*s*-Nonyl- β -D-
thioglucofuranoside; *n*-Nonyl- β -D-thiomaltopyranoside; *N*-Dodecyl-*N,N*-
15 (dimethylammonio)butyrate; *n*-Nonyl- β -D-maltopyranoside; Cyclohexyl-butyl D-
maltoside; *n*-Octyl- β -D-thiomaltopyranoside; *n*-Decylphosphocholine, *n*-
Nonylphosphocholine; Nonanoyl-*N*-methylglucamide; 1-*s*-Heptyl- β -D-
thioglucofuranoside; *n*-Octylphosphocholine; Cyclohexyl-ethyl D-maltoside; *n*-
Octyl-*N,N*-dimethyl ammonio-1-propanesulfonate; Cyclohexyl-methyl- β -D-
20 maltoside

Cryosolvents are agents that stabilize a target crystal to flash-cooling in a
cryogen such as liquid nitrogen, liquid propane, liquid ethane, or gaseous nitrogen or
helium (all at approximately 100- 120 °K) such that crystal becomes embedded in a
vitreous glass rather than ice. Any number of salts or small molecular weight organic
25 compounds can be used as a cryoprotectant, and typical ones include but are not
limited to: MPD, PEG-400 (as well as both PEG derivatives and higher molecular-
weight PEG compounds), glycerol, sugars (xylitol, sorbitol, erythritol, sucrose,
glucose, etc.), ethylene glycol, alcohols (both short- and long chain, both volatile and
nonvolatile), LiOAc, LiCl, LiCH₃O₂, LiNO₃, Li₂SO₄, Mg(OAc)₂, NaCl, NaCH₃O₂,
30 NaNO₃, etc. Again, materials from which microfluidics devices in accordance with
the present invention are fabricated may be compatible with a range of such
compounds.

Many of these chemicals can be obtained in predefined screening kits from a variety of vendors, including but not limited to Hampton Research of Laguna Niguel, CA, Emerald Biostructures of Bainbridge Island, WA, and Jena BioScience of Jena, Germany, that allow the researcher to perform both sparse matrix and grid screening experiments. Sparse matrix screens attempt to randomly sample as much of precipitant, buffer, and additive chemical space as possible with as few conditions as possible. Grid screens typically consist of systematic variations of two or three parameters against one another (e.g., precipitant concentration vs. pH). Both types of screens have been employed with success in crystallization trials, and the majority of chemicals and chemical combinations used in these screens are compatible with the chip design and matrices in accordance with embodiments of the present invention. Moreover, current and future designs of microfluidic devices may enable flexible combinatorial screening of an array of different chemicals against a particular target or set of targets, a process that is difficult with either robotic or hand screening. This latter aspect is particularly important for optimizing initial successes generated by first-pass screens.

In addition to chemical variability, a host of other parameters can be varied during crystallization screening. Such parameters include but are not limited to: (1) volume of crystallization trial; (2) ratio of target solution to crystallization solution; (3) target concentration; (4) cocrystallization of the target with a secondary small or macromolecule; (5) hydration; (6) incubation time; (7) temperature; (8) pressure; (9) contact surfaces; (10) modifications to target molecules; and (11) gravity.

Although the discussion below refers to proteins, the particular devices or methods described can also be used or suitably adapted for the crystallization of other types of samples such as those mentioned above (e.g., small molecules, other macromolecules, nanoparticles, colloidal particles, etc.). In one aspect of the present invention, protein crystallization is conducted using miniaturized microbatch conditions. The process consists of two steps. First, plugs are preferably formed wherein the concentrations of the protein, precipitant, and additive are adjusted by varying the relative flow rates of these solutions. This step corresponds to a screening step. Once the optimal concentrations have been found, the flow rates can then be kept constant at the optimal conditions. In this step, plugs are preferably transported through the channel as they form. Second, the flow is preferably stopped once the desired number of plugs are formed. The plugs are then preferably allowed to

incubate. In some embodiments according to the invention the flow may be continued, rather than stopped. In those embodiments, the flow is maintained sufficiently slow and the channels are made sufficiently long that plugs spend sufficient time in the channels for crystallization to occur (from tens of minutes to weeks, but may be faster or slower).

In one aspect, upon formation of the plugs, they are trapped using expansions in the channels. The expansions act as dead volume elements while the plugs are being formed in the presence of flow. Thus, the expansions do not interfere with the flow of the plugs through the channel. Once the flow is stopped, surface tension drives plugs into the expansions where surface tension is minimized. The expansions may be, but are not limited to, oval, round, square, rectangular, or star-shaped. In particular, a star-shaped expansion may prevent adherence of the plug or of a crystal to the walls of the expansion. The ratio of the size of the expansion opening to the width of the channel may be varied based on empirical results for a particular set of conditions. FIG. 16 is a schematic illustration of a microfluidic device according to the invention that illustrates the trapping of plugs. In experiments, plugs were sustained in perfluorodecaline inside a channel for one day, and did not appear to change during that time (a refractive index mismatch between the fluorinated and aqueous phase was introduced to aid in visualization of plugs).

The method described above allows a high degree of control over protein and precipitant concentrations. It also allows a high degree of control over a range of time scales through the control of plug size and composition. FIG. 17 shows a schematic of a microfluidic method for forming plugs with variable compositions for protein crystallization. Continuously varied flow rates of the incoming streams are preferably used to form plugs with various concentrations of the protein, precipitation agents, and additives. In FIG. 17, for example, the following can be introduced into the various inlets: buffers into inlets 171, 172; PEG into inlet 173; salt into inlet 174; solvent into inlet 175; and protein into inlet 176. These various solutions can enter a channel 177 through which a carrier fluid such as perfluorodecaline flows. For example, a 1-meter long channel with a $200 \times 80 \mu\text{m}^2$ cross section can be used to form approximately two hundred 6 nL (nanoliter) plugs. If each plug contains enough protein to form a $40\text{-}\mu\text{m}^3$ crystal, 200 trials will consume only about 1.2 μL of approximately 10 mg/mL protein solution (12 μg of protein). About one minute may be sufficient to form plugs in these trials.

In another aspect according to the invention, after plugs are formed as described above for the microbatch system, slow evaporation through a very thin PDMS membrane (or another membrane with slight water permeability) is preferably used for added control over the crystallization process. A slow decrease in the volume of the plug during evaporation is expected to produce a trajectory of the solution through the crystallization phase space similar to that in a vapor diffusion experiment. Hence, this method, in addition to microbatch methods, can be used to miniaturize and optimize vapor diffusion methods.

In the vapor diffusion method, a drop containing protein, stabilizing buffers, precipitants, and/or crystallization agents is allowed to equilibrate in a closed system with a much larger reservoir. The reservoir usually contains the same chemicals minus the protein but at an overall higher concentration so that water preferentially evaporates from the drop. If conditions are right, this will produce a gradual increase in protein concentration such that a few crystals may form.

Vapor diffusion can be performed in several ways. The one most often used is called Hanging Drop Technique. The drop is placed on a glass coverslip, which is then inverted and used to seal a small reservoir in a Linbro Plate. After a period of several hours to weeks, microscopic crystals may form and continue to grow. The other set up is known as Sitting Drop. In this method a drop (usually >10uL) is placed in a depression in either a Micro Bridge in a Linbro Plate or a glass plate and again placed in a closed system to equilibrate with a much larger reservoir. One usually uses the sitting drop technique if the drop has very low surface tension, making it hard to turn upside down or if the drops need to be larger than 20uL. Also, in some cases, crystals will grow better using one technique or the other.

In another embodiment, the plugs are preferably formed and transported such that excessive mixing of the protein with the precipitation agent is minimized or prevented. For example, gentle mixing using spiral channels may be used to achieve this and also to create interfaces between the protein and the precipitation agent. Alternatively, combining two streams of plugs in a T-junction without merging may be used to create plugs that diffuse and combine without significant mixing to establish a free interface after the flow is stopped. Diffusion of the proteins and precipitates through the interface induces crystallization. This is an analogue of the Free-Interface Diffusion method. It may be performed under either the microbatch or vapor diffusion conditions as described above.

Preferably, the spacing between plugs can be increased or the oil composition changed to reduce plug-plug diffusion. For example, a spacing of about 2.5 mm in paraffin oil can be used, which has been shown to be an effective barrier to aqueous diffusion in crystallization trials.

5 Visually identifying small crystals inside plugs with curved surfaces can be a challenge when performing microbatch experiments. In an aspect according to the invention, a method based on matching the refractive indices of carrier-fluid with that of the plug fluid to enhance visualization is used. Microscopic detection is preferably performed by using shallow channels and by matching the refractive indices of
10 carrier-fluid mixtures to those of the aqueous solutions.

In addition, at least three other novel methods of controlling protein crystallization are described below: (1) using surface chemistry to effect nucleation of protein crystals; (2) using different mixing methods to effect crystallization; and (3)
15 performing protein crystals seeding by separating nucleation and growth phases in space.

Control of nucleation is one of the difficult steps in protein crystallization. Heterogeneous nucleation is statistically a more favorable process than its solution-phase counterpart. Ideal surfaces for heterogeneous nucleation have complementary electrostatic maps with respect to their macromolecular counterparts. Critical nuclei
20 are more stable on such surfaces than in solution. Further, the degree of supersaturation required for heterogeneous nucleation is much less than that required for the formation of solution-phase nuclei. Surfaces such as silicon, crystalline minerals, epoxide surfaces, polystyrene beads, and hair are known to influence the efficiency of protein crystallization. Few studies have been done, but promising
25 results have been shown for protein crystallization at the methyl, imidazole, hydroxyl, and carboxylic acid termini of self-assembled monolayers on gold. Using self-assembled monolayers, proteins were crystallized over a broader range of crystallization conditions and at faster rates than when using the traditional silanized glass.

30 FIG. 18 is a schematic illustration of a method for controlling heterogeneous nucleation by varying the surface chemistry at the interface of an aqueous plug-fluid and a carrier-fluid. In FIG. 18, plugs are formed in the presence of several solutions of surfactants that possess different functional groups (left side of the diagram). The right side of FIG. 18 shows the aqueous phase region in which a precipitant, solvent,

and protein may be introduced into inlets 180, 181, and 182, respectively. The composition of the surfactant monolayer is preferably controlled by varying the flow rates. In another application of the method illustrated in FIG. 18, the surface chemistry can be varied continuously. The manipulation and control of the surface chemistry can be used for screening, assays, crystallizations, and other applications where surface chemistry is important.

In one aspect of the invention, heterogeneous nucleation of proteins is controlled by forming aqueous plugs in a carrier-fluid, preferably containing fluoro-soluble surfactants if the carrier-fluid is a fluorocarbon. Varying the relative flow rates of the surfactant solutions may generate a wide variety of liquid-liquid interface conditions that can lead to the formation of mixed monolayers or mixed phase-separated monolayers. Preferably, several surfactants are used to control the heterogeneous nucleation of protein crystals. Ethylene-glycol monolayers are preferably used to reduce heterogeneous nucleation, and monolayers with electrostatic properties complementary to those of the protein are preferably used to enhance heterogeneous nucleation. These methods for controlling heterogeneous nucleation are designed to induce or enhance the formation of crystals that are normally difficult to obtain. These methods may also be used to induce or enhance the formation of different crystal polymorphs that are relatively more stable or better ordered.

As mentioned above, control of nucleation is highly desired in an advanced crystallization screen. One method that can be used to achieve control of nucleation involves the transfer of nucleating crystals from one concentration to another via dilution. This method, which has been applied in macroscopic systems primarily to vapor diffusion, was intended to allow decoupling of the nucleation and growth phases. This method is difficult to perform using traditional methods of crystallization because nucleation occurs long before the appearance of microcrystals.

FIG. 19 illustrates a method of separating nucleation and growth using a microfluidic network according to the present invention using proteins as a non-limiting example. The left side of FIG. 19 shows plugs that are formed preferably using high concentrations of protein and precipitant. In FIG. 19, the following can be introduced into the various inlets shown: buffer into inlets 191, 196; PEG into inlets 192, 197; precipitant into inlets 193, 198; solvent into inlets 194, 199; and protein into inlets 195, 200. Oil flows through the channels 201, 202 from left to right. The portions 203, 204, and 205 of the channel correspond to regions where fast nucleation

occurs (203), no nucleation occurs (204), and where crystal growth occurs (205). The concentrations used are those that correspond to the nucleating region in the phase diagram. Nucleation occurs as the plugs move through the channel to the junction over a certain period. Preferably, these plugs are then merged with plugs containing a protein solution at a point corresponding to a metastable (growth, rather than
5 nucleation) region (right side of FIG. 19). This step ends nucleation and promotes crystal growth. When the combined channel has been filled with merged plugs, the flow is preferably stopped and the nuclei allowed to grow to produce crystals.

Nucleation time can be varied by varying the flow rate along the nucleation
10 channel. The nucleus is preferably used as a seed crystal for a larger plug with solution concentrations that correspond to a metastable region. Existing data indicate the formation of nuclei within less than about 5 minutes.

Fluid mixing is believed to exert an important effect in crystal nucleation and growth. Methods according to the invention are provided that allow a precise and
15 reproducible degree of control over mixing. FIG. 20 illustrates two of these methods. A method of mixing preferably places the solution into a nucleation zone of the phase diagram without causing precipitation. Preferably, gentle mixing (FIG. 20, left side) is used to achieve this by preventing, reducing, or minimizing contact between concentrated solutions of the protein and precipitant. Alternatively, rapid mixing
20 (FIG. 20, right side) is used to achieve this by allowing passage through the precipitation zone sufficiently quickly to cause nucleation but not precipitation. The two methods used as examples involve the use of spiraling channels for gentle mixing and serpentine channels for rapid mixing.

The two methods in accordance with the invention depicted in FIG. 20 can be
25 used to determine the effect of mixing on protein crystallization. In addition, the various methods for controlling mixing described previously (e.g., slow mixing in straight channels, chaotic mixing in non-straight channels, or mixing in which twirling may or may not occur) can be applied to crystallization, among other things.

After obtaining the crystals using any of the above described techniques, the
30 crystals may be removed from the microfluidic device for structure determination. In other systems, the fragile and gelatinous nature of protein crystals makes crystal collection difficult. For example, removing protein crystals from solid surfaces can damage them to the point of uselessness. The present invention offers a solution to this problem by nucleating and growing crystals in liquid environments. In an aspect

according to the invention, a thin wetting layer of a carrier-fluid covered with a surfactant is used to enable or facilitate the separation of a growing crystal from a solid surface. When the crystals form, they may be separated from the PDMS layer by using a thin layer of a carrier-fluid.

5 In one aspect, a microfluidic device of the present system can include further include capillary tubing suitable for collecting plugs ("the capillary device"; FIG. 46). The tubing is preferably composed of a material that prevents uncontrolled evaporation of solutions (such as water) through its wall. Further, use of the capillary tubing can enable direct screening of crystals by x-ray diffraction analysis or other
10 spectrophotometric detection/analysis means employing *e.g.*, optical or infrared detection. Plugs in the capillary tubing have been found to be stable and did not show signs of evaporation over several months, even in the absence of humidity control. Therefore, the capillary device can be incubated for a much longer time than all-PDMS microfluidic chips. Water diffusion can be controlled by varying the starting
15 salt concentration differences as well the distance between plugs. Production of crystals directly inside the capillary tubes can facilitate on-chip diffraction without having to move the crystal around.

 Upon formation of plugs in the PDMS portion and their transfer into capillary tubing, the flow rates are stopped, the capillary tubing is disconnected from the
20 PDMS portion and the ends are sealed by capillary wax. The capillary tubing may be incubated under suitable crystallization conditions (*e.g.*, temperature etc.) until crystals form inside the plugs. Formation of crystals can be monitored using optical detection and/or x-ray diffraction methods. Crystals grown at the fluid-fluid interface can be easily removed from the capillary by gentle flow, or by breaking the capillary and
25 wicking the liquid out. Upon formation of suitable crystals, the capillaries are frozen and structures are directly determined from inside the capillary using *e.g.*, synchrotron radiation. Because this method obviates the problem of handling and mounting crystals and because it can facilitate the determination of structure directly from within the capillary, it may be especially suitable for high-throughput, fully automated
30 crystallization.

 The plugs in the capillary tubing can be stable in both hydrophilic (*e.g.*, treated with by chromic acid) or hydrophobic (*e.g.*, silanized) capillaries for over a month, even if the capillary is placed vertically for over three days.

The use of x-ray capillary tubing for protein crystallization can also be applied to a controlled vapor diffusion process which lends itself to direct monitoring and structural determination of protein crystals in the capillary tubing (FIG. 49). In this modified vapor-diffusion process an array of plugs is generated in the channel portion of a capillary device (as described above) where the protein and precipitant plugs alternate with plugs containing a high concentration of precipitant. Syringe pumps attached to the capillary device cause the plugs to flow into suitable x-ray capillary tubing. At the conclusion of the experiment, the flow is stopped, the capillary is disconnected from the PDMS portion and the ends are sealed with capillary wax. The x-ray capillary is incubated under optimal conditions until crystals form inside the plugs.

The use of carrier fluid (oil) permeable to water causes the water from the plugs to diffuse through from the oil from the plugs that are low in osmolarity into plugs that are higher in osmolarity, thereby increasing the concentration of the protein and precipitants in the plugs for crystallization. The rate of water transfer from the plugs and the amount of water transferred between the two types of plugs may be controlled by using oils having different water permeabilities, by changing the size or distance between plugs or by altering the precipitant concentrations between the different types of plugs (*i.e.*, changing the difference in osmolarity between the different plug types). All of these parameters can be conveniently altered by changing the relative flow rates of the aqueous and carrier-fluid (oil) solutions. Poly-3,3,3-trifluoropropylmethylsiloxane (FMS-121) can be a suitable carrier-oil fluid for this procedure.

One scheme for generating alternating plugs by vapor diffusion involves attaching four different syringes to a PDMS device, each syringe associated with a syringe pump for introducing each of aqueous solutions A, B into respective aqueous inlet channels and for introducing each of carrier oil fluids C, D into respective oil inlet channels. The aqueous solutions can be the same or different. Multiple, distinct aqueous solutions can also be co-introduced together in one or both of the two aqueous channels. In principle, the same oil or different oils may be used in the two oil inlets. In either case, one oil inlet channel is parallel to the main channel; the other oil inlet channel is vertical to the main channel and is

positioned between the two aqueous inlet channels to separate the two aqueous streams into alternating plugs.

Importantly, the flow rates of solutions A and B may be changed in a correlated fashion. Thus, when the flow rate of solution A_1 is increased and solution A_2 is decreased, the flow rate of solutions B_1 is also increased and solution B_2 is also decreased. This can allow one to maintain a constant difference in osmolarity between the plugs of stream A and stream B to ensure that transfer from all plugs A to all plugs B occurs at a constant rate. Moreover, if the flow rates of the corresponding A and B streams are changed in a correlated fashion, the composition of plugs B will reflect the composition of plugs A thereby allowing one to incorporate markers into the B stream plugs to serve as a code for the plugs in the A stream. Thus, if the two types of plugs are made in a correlated way, one type of droplet may be used for crystallization, while the other type of droplet is used for indexing provided it contains a label conferring a read out with respect to crystallization. In other words, absorption/fluorescent dyes or x-ray scattering/absorbing materials can be incorporated in markers in the B streams to facilitate optical density quantification or x-ray diffraction analysis to provide a read out of relative protein and precipitant concentrations in the A streams. This approach can provide a powerful means for optimizing crystallization conditions for subsequent scale-up experiments.

The use of markers may be performed using an oil that is impermeable to water (as in a microbatch procedure) to prevent transfer of water or any other material between the A plugs and B plugs. Alternatively, the B plugs may additionally incorporate a high concentration of dehydration agents (salt, other precipitants) in conjunction with a water-permeable oil as described above. In this way, the B plugs can serve both as markers for the A plugs and as sinks for excess water. Oils that are selectively permeable to materials other than water may also be used to induce transfer of other materials between the plugs and through the oil.

Alternating plugs may be generated using a range of channel geometries. The plugs may also alternate in patterns other than A:B:A:B. For example, other patterns (such as A:A:A:B:A:A:A:B, etc) may be obtained where transfer of water from A plugs adjacent to B plugs is faster than transfer of water from the middle A

plug. This can create conditions favorable for creating multiple, different sets of crystallization conditions. The alternating droplet systems may be extended to more than two types of plugs alternating in the same channel or capillary (for example, A plugs with the crystallization solutions, B plugs with the dehydrating agents, and C plugs with markers or with a cryoprotectant).

The above described capillary systems are not limited to protein crystallization -- other types of crystallizations and experiments may be performed. For example, the vapor diffusion/alternating droplet approach can be extended to e.g., a process for concentrating materials (such as protein). Such a process would be effected through diffusion of water plugs that are relatively low in osmolarity into plugs having a higher osmolarity. It should be noted, however, that solution materials in the different plug types do not have to be aqueous in nature, but can be in the form of solvents also. Alternatively, the A and B plugs do not have to be in solution at all, but can instead be in the form of emulsions or suspensions.

It will be clear to one skilled in the art that while the above techniques are described in detail for the crystallization of proteins, techniques similar to the ones described above may also be used for the crystallization of other substances, including other biomolecules or synthetic chemicals. In addition, the devices and methods according to the invention may be used to perform co-crystallization. For example, a crystal comprising more than one chemical may be obtained, for example, through the use of at least one stream of protein, a stream of precipitant, and optionally, a stream comprising a third chemical such as an inhibitor, another protein, DNA, etc. One may then vary the conditions to determine those that are optimal for forming a co-crystal.

PARTICLE SEPARATION/SORTING USING PLUGS

The flow within the moving plugs can be used for separation of polymers and particles. Plugs can be used for separation by first using flow within a moving plug to establish a distribution of the polymers or particles inside the plug (for example, an excess of the polymer inside the front, back, right or left side of the plug) and then using splitting to separate and isolate the part of the plug containing higher concentration of the polymers or particles. When two polymers or particles are

present inside the plug and establish different distributions, splitting can be used to separate the polymers or particles.

The invention is further described below, by way of the following examples. It will be appreciated by persons of ordinary skill in the art that this example is one of many embodiments and is merely illustrative. In particular, the device and method described in this example (including the channel architectures, valves, switching and flow control devices and methods) may be readily adapted, *e.g.*, used in conjunction with one or more devices or methods, so that plugs may be analyzed, characterized, monitored, and/or sorted as desired by a user.

EXAMPLE

Example 1 - Fabrication of Microfluidic Devices and a General Experimental Procedure

Microfluidic devices with hydrophilic channel surfaces were fabricated using rapid prototyping in polydimethylsiloxane. The channel surfaces were rendered hydrophobic either by silanization or heat treatment. To silanize the surfaces of channels, (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies, Inc.) vapor was applied to the inlets of a device with dry nitrogen as a carrier gas at around 40-60 mm Hg above about 1 atm pressure. Vacuum was simultaneously applied to the outlet of the device at about 650 mm Hg below atmospheric pressure. The silane vapor was applied for a period of between about 1-3 hours. To treat the channels using heat, a device was placed in an oven at approximately 120°C for about three hours. Alternatively, a device can be heated in a Panasonic "The Genius" 1300 Watt microwave oven at power set to "10" for about ten minutes.

Oils and aqueous solutions were pumped through devices using a kdScientific syringe pump (Model 200) or Harvard Apparatus PhD 2000 pump. Hamilton Company GASTIGHT syringes were used (10-250 µl) and Hamilton Company 30 gauge Teflon® needles were used to attach the syringes to the devices. Oils and aqueous solutions were pumped through devices at volumetric flow rates ranging from about 0.10 µL/min to about 10.0 µL/min.

Aqueous solutions were colored using Crayola Original Formula Markers or Ferroin Indicator (0.025 M, Fisher Scientific). Oils that were used included perfluorodecaline (mixture of cis and trans, 95%, Acros Organics),

perfluoroperhydrophenanthrene (tech., Alfa-Aesar), or 1H,1H,2H,2H-perfluorooctanol (98%, Alfa-Aesar). The experiments were typically performed using 10:1 mixtures of perfluorodecaline and 1H,1H,2H,2H-perfluorooctanol.

5 The experiments were monitored using a Lica MZFLIII stereoscope with Fostec (Schott-Fostec, LLC) Modulamps. Photographs of the experiments were taken with a Spot Insight Color Camera, Model # 3.2.0 (Diagnostic Instruments, Inc.). Spot Application version 3.4.0.0 was used to take the photographs with the camera.

Example 2 - Varying the Concentration of Aqueous Solutions in Plugs

10 The left side of each of FIGS. 25A-C shows a schematic diagram of the microfluidic network and the experimental conditions. The right side of each of FIGS. 25A-C shows microphotographs illustrating the formation of plugs using different concentrations of the aqueous streams. Aqueous solutions of food dyes (red/dark and green/light) and water constituted the three streams. The volumetric
15 flow rates of the three solutions (given in $\mu\text{L}/\text{min}$) are indicated. The dark stream is more viscous than the light stream. Therefore, the dark (more viscous) stream moves (measured in mm/s) more slowly and occupies a larger fraction of the channel at a given volumetric flow rate.

FIG. 45a) shows a schematic of the microfluidic network used to demonstrate
20 that on-chip dilutions can be accomplished by varying the flow rates of the reagents. In FIG. 45a), the reagents are introduced through inlets 451, 453 while the dilution buffer is introduced through inlet 452. An oil stream flows through channel 454. The blue rectangle outlines the field of view for images shown in FIG. 45c)-d). FIG. 45b) shows a graph quantifying this dilution method by measuring fluorescence of a
25 solution of fluorescein diluted in plugs in the microchannel. Data are shown for 80 experiments in which fluorescein was flowed through one of the three inlets, where C_{measured} and $C_{\text{theoretical}}$ [μM] are measured and expected fluorescein concentration. FIG. 45(c) shows photographs illustrating this dilution method with streams of food dyes 455, 456, 457 having flow rates of 45 nL/s, 10 nL/s, and
30 10nL/s, respectively. FIG. 45(d) shows photographs illustrating this dilution method with streams of food dyes 458, 459, 460 having flow rates of 10 nL/s, 45 nL/s, and 10nL/s, respectively. Carrier fluid was flowed at 60 nL/s.

Example 3

Networks of microchannels with rectangular cross-sections were fabricated using rapid prototyping in PDMS. The PDMS used was Dow Corning Sylgard Brand 184 Silicone Elastomer, and devices were sealed using a Plasma Prep II (SPI
5 Supplies). The surfaces of the devices were rendered hydrophobic by baking the devices at 120°C for 2-4 hours.

In FIG. 26, the red aqueous streams were McCormick® red food coloring (water, propylene glycol, FD&C Red 40 and 3, propylparaben), the green aqueous streams were McCormick® green food coloring (water, propylene glycol, FD&C
10 yellow 5, FD&C blue 1, propylparaben) diluted 1:1 with water, and the colorless streams were water. PFD used was a 10:1 mixture of perfluorodecaline (mixture of cis and trans, 95%, Acros Organics): 1H, 1H, 2H, 2H-perfluorooctanol (Acros Organics). The red aqueous streams were introduced in inlet 260, 265 while the green aqueous streams were introduced in inlets 262, 263 in FIG. 26b). The colorless
15 aqueous stream was introduced in inlets 261, 264. The dark shadings of the streams and plug are due mainly from the red dye while the lighter shadings are due mainly from the green dye.

Aqueous solutions were pumped using 100 µL Hamilton Gastight syringes (1700 series, TLL) or 50 µL SGE gastight syringes. PFD was pumped using 1mL
20 Hamilton Gastight syringes (1700 series, TLL). The syringes were attached to microfluidic devices by means of Hamilton Teflon needles (30 gauge, 1 hub). Syringe pumps from Harvard Apparatus (PHD 2000 Infusion pumps, specially-ordered bronze bushings were attached to the driving mechanism to stabilize pumping) were used to infuse the aqueous solutions and PFD.

25 Microphotographs were taken with a Leica MZ12.5 stereomicroscope and a SPOT Insight Color digital camera (Model #3.2.0, Diagnostic Instruments, Inc.). SPOT Advanced software (version 3.4.0 for Windows, Diagnostic Instruments, Inc.) was used to collect the images. Lighting was provided from a Machine Vision Strobe X-Strobe X1200 (20Hz, 12µF, 600V, Perkin Elmer Optoelectronics). To obtain an
30 image, the shutter of the camera was opened for 1 second and the strobe light was flashed once with the duration of the flash being about 10µs.

Images were analyzed using NIH Image software, Image J. Image J was used to measure periods and lengths of plugs from microphotographs such as shown in Fig. 27b). Periods corresponded to the distance from the center of one plug to the center

of an adjacent plug, and the length of a plug was the distance from the extreme front to the extreme back of the plug (see FIG. 28 for the definitions of front and back). Measurements were initially made in pixels, but could be converted to absolute measurements by comparing them to a measurement in pixels of the 50 μ m width of the channel.

To make measurements of the optical intensity of $\text{Fe}(\text{SCN})_x^{(3-x)+}$ complexes in plugs, microphotographs were converted from RGB to CMYK color mode in Adobe Photoshop 6.0. Using the same program, the yellow color channels of the microphotographs were then isolated and converted to grayscale images, and the intensities of the grayscale images were inverted. The yellow color channel was chosen to reduce the intensity of bright reflections at the extremities of the plugs and at the interface between the plugs and the channel. Following the work done in Photoshop, regions of plugs containing high concentrations of $\text{Fe}(\text{SCN})_x^{(3-x)+}$ complexes appeared white while regions of low concentration appeared black. Using Image J, the intensity was measured across a thin, rectangular region of the plug, located halfway between the front and back of the plug (white dashed lines in FIG. 27a1)). The camera used to take the microphotographs of the system was not capable of making linear measurements of optical density. Therefore, the measurements of intensity were not quantitative. Several of the plots of intensity versus relative position across the channel (FIG. 27c) were shifted vertically by less than 50 units of intensity to adjust for non-uniform illuminations of different parts of the images. These adjustments were justified because it was the shape of the distribution that was of interest, rather than the absolute concentration.

FIG. 29a)-b) shows plots of the sizes of periods and sizes of plugs as a function of total flow velocity (FIG. 29a)) and water fraction (w/f) (FIG. 29b)). Values of capillary number (C.n.) were 0.0014, 0.0036, 0.0072 and 0.011, while values of the Reynolds number (R_e) were 1.24, 3.10, 6.21, and 9.31, each of the C.n. and R_e value corresponding to a set of data points with water fractions (wf) 0.20, 0.52, 0.52, and 0.20 (the data points from top to bottom in FIG. 29A)). In turn, each of these sets of data points corresponds to a particular flow velocity as shown in FIG. 29a). Plugs in FIG. 29b) travel at about 50 millimeter/second (mm/s). All measurements of length and size are relative to the width of the channels (50 μ m).

FIG. 30 shows microphotographs illustrating weak dependence of periods, length of plugs, and flow patterns inside plugs on total flow velocity. The left side of

FIG. 30 shows a diagram of the microfluidic network. Here, the same solutions were used as in the experiment corresponding to FIG. 27. The $\text{Fe}(\text{SCN})_x^{(3-x)+}$ solution was introduced into inlet 301 while the colorless aqueous streams were introduced into inlets 302, 303. The same carrier fluid as used in the FIG. 27 experiment was flowed into channel 304. The right side of FIG. 30 shows microphotographs of plugs formed at the same water fraction (0.20), but at different total flow velocities (20, 50, 100, 150 mm/s from top to bottom). Capillary numbers were 0.0014, 0.0036, 0.0072, and 0.011, respectively, from top to bottom. Corresponding Reynolds numbers were 1.24, 3.10, 6.21, and 9.31.

FIG. 31A-C are plots showing the distribution of periods and lengths of plugs where the water fractions were 0.20, 0.40, and 0.73, respectively. The total flow velocity was about 50 mm/s, $C.n. = 0.0036$, $R_e = 3.10$ in all cases.

FIG. 27 shows the effects of initial conditions on mixing by recirculating flow inside plugs moving through straight microchannels. FIG. 27a1) shows that recirculating flow (shown by black arrows) efficiently mixed solutions of reagents that were initially localized in the front and back halves of the plug. Notations of front, back, left, and right are the same as that in FIG. 28. FIG. 27a2) shows that recirculating flow (shown by black arrows) did not efficiently mix solutions of reagents that were initially localized in the left and right halves of the plugs. The left side of FIG. 27b) shows a schematic diagram of the microfluidic network. The two colorless aqueous streams were introduced into inlets 271, 272 while a carrier fluid in the form of perfluorodecaline flowed through channel 273. These solutions did not perturb the flow patterns inside plugs.

The right side of FIG. 27b) shows microphotographs of plugs of various lengths near the plug-forming region of the microfluidic network for water fractions of from 0.14 up to 1.00. FIG. 27c1) shows a graph of the relative optical intensity of $\text{Fe}(\text{SCN})_x^{(3-x)+}$ complexes in plugs of varying lengths. The intensities were measured from left ($x = 1.0$) to right ($x = 0.0$) across the width of a plug (shown by white dashed lines in FIG. 27a1)-a2)) after the plug had traveled 4.4 times its length through the straight microchannel. The gray shaded areas indicate the walls of the microchannel. FIG. 27c2) is the same as FIG. 27c1) except that each plug had traversed a distance of 1.3 mm. The d/l of each water fraction (wf) were 15.2 (wf 0.14), 13.3 (wf 0.20), 11.7 (wf 0.30), 9.7 (wf 0.40), 6.8 (wf 0.60), 4.6 (wf 0.73), and

2.7 ($w/0.84$), where d is the distance traveled by the plug and l is the length of the plug.

Example 4 - Merging of Plugs

5 Experiments were conducted to investigate the merging of plugs using different channel junctions (T- or Y-shaped), cross-sections, and flow rates (see FIG. 33a-d). The figures on the left side of FIGS. 33a-d show top views of microfluidic networks that comprise channels having either uniform or nonuniform dimension (e.g., the same or different channel diameters). The corresponding figures on the right
10 are microphotographs that include a magnified view of two plug streams (from the two separate channels portions of which form the branches of the Y-shaped junction) that merges into a common channel.

In FIG. 33a, the oil-to-water volumetric ratio was 4:1 in each pair of oil and water inlets. The oil streams were introduced into inlets 330, 332, while the aqueous
15 streams were introduced into inlets 331, 333. The flow rates of the combined oil/water stream past the junction where the oil and water meet was 8.6 mm/s. The channels, which were rectangular, had dimensions of 50 (width) x 50 (height) μm^2 . As shown in FIG 33a, plugs that flow in uniform-sized channels typically merged only when they simultaneously arrived at the T-junction. Thus, plug merging in these
20 channels occur infrequently. In addition, lagging plugs were typically not able to catch up with leading plugs along the common channel.

FIG. 33b illustrates plug merging occurring between plugs arriving at different times at the Y-shaped junction (magnified view shown). The oil streams were introduced into inlets 334, 336, while the aqueous streams were introduced into inlets
25 335, 337. In FIG. 33b, the flow rates for the combined oil/water fluid past the junction where the oil and water meet were 6.9 mm/s for channel 346 (the 50 x 50 μm^2 channel) and 8.6 mm/s for channel 347 (the 25 x 50 μm^2 channel). The oil-to-water volumetric ratio was 4:1 in each pair of oil and water inlets. The two channels (the branch channels) merged into a common channel 348 that had a 100 x 50 μm^2
30 cross-section. As shown in the figure, the larger plugs from the bigger channel are able to merge with the smaller plugs from the narrower channel even when they do not arrive at the junction at the same time. This is because lagging larger plugs are able to catch up with the leading smaller plugs once the plugs are in the common channel.

FIG. 33c depicts in-phase merging (*i.e.*, plug merging upon simultaneous arrival of at least two plugs at a junction) of plugs of different sizes generated using different oil/water ratios at the two pairs of inlets. The oil streams were introduced into inlets 338, 340, while the aqueous streams were introduced into inlets 339, 341. The flow rate corresponding to the fluid stream through channel 349 resulting from a 1:1 oil-to-water volumetric ratio was 4.0 mm/s, while that through channel 350 corresponding to the 4:1 oil-to-water volumetric ratio was 6.9 mm/s. Each branch channel of the Y-shaped portion of the network (magnified view shown) had a dimension of $50 \times 50 \mu\text{m}^2$ while the common channel 351 (the channel to which the branch channels merge) was $125 \times 50 \mu\text{m}^2$.

FIG. 33d illustrates defects (*i.e.*, plugs that fail to undergo merging when they would normally merge under typical or ideal conditions) produced by fluctuations in the relative velocity of the two incoming streams of plugs. The oil streams were introduced into inlets 342, 344, while the aqueous streams were introduced into inlets 343, 345. In this experiment, the flow rate corresponding to the fluid stream through channel 352 resulting from a 1:1 oil-to-water volumetric ratio was 4.0 mm/s, while that through channel 353 corresponding to the 4:1 oil-to-water volumetric ratio was 6.9 mm/s. Each branch channel that formed one of the two branches of the Y-shaped intersection (magnified view shown) was $50 \times 50 \mu\text{m}^2$ while the common channel 354 (the channel to which the two branch channels merge) is $125 \times 50 \mu\text{m}^2$.

Example 5 - Splitting Plugs Using a Constricted Junction

The splitting of plugs was investigated using a channel network with a constricted junction. In this case, the plugs split and flowed past the junction into two separate branch channels (in this case, branch channels are the channels to which a junction branches out) that are at a 180° -angle to each other (see FIGS. 34a-c each of which show a channel network viewed from the top). In these experiments, the outlet pressures, P_1 and P_2 , past the constricted junction were varied such that either $P_1 \approx P_2$ (FIG. 34b) or $P_1 < P_2$ (FIG. 34c). Here, the relative pressures were varied by adjusting the relative heights of the channels that were under pressures P_1 and P_2 . Since longer plugs tend to split more reliably, this branching point (or junction) was made narrower than the channel to elongate the plugs. FIG. 34a shows a schematic diagram of the channel network used in the experiment. The oil and water were introduced into

inlets 3400 and 3401, respectively. The oil-to-water ratio was 4:1 while the flow rate past the junction where the oil and water meet was 4.3 mm/s.

FIG. 34b is a microphotograph showing the splitting of plugs into plugs of approximately one-half the size of the initial plugs. The channels 3404, which were rectangular, had a cross-section that measured $50 \times 50 \mu\text{m}^2$. The constricted section of the channel 3402 right next to the branching point measured $25 \times 50 \mu\text{m}^2$. The outlet pressures, P_1 and P_2 , were about the same in both branch channels. Here, the plugs split into plugs of approximately the same sizes.

FIG. 34c is a microphotograph showing the asymmetric splitting of plugs (*i.e.*, the splitting of plugs into plugs of different sizes or lengths) which occurred when $P_1 < P_2$. The microphotograph shows that larger plugs (somewhat rectangular in shape) flowed along the channel with the lower pressure P_1 , while smaller plugs (spherical in shape) flowed along the channel with the higher pressure P_2 . As in FIG. 34b, each of the channel 3405 cross-section measured $50 \times 50 \mu\text{m}^2$. The constricted section of the channel 3403 at the junction measured $25 \times 50 \mu\text{m}^2$.

Example 6 - Splitting Plugs Without Using a Constricted Junction

The splitting of plugs was investigated using a channel network without a constriction such as the one shown in FIGS. 35b-c. The channel network used was similar to that shown in FIG. 34(a) except that here the plugs split and flowed past the junction in two separate channels at a 90°-angle to each other (the plug flow being represented by arrows). The oil and aqueous streams (4:1 oil:aqueous stream ratio) were introduced into inlets 3500 and 3501, respectively. An oil-only stream flowed through channel 3502. All channels had a cross-section of $50 \times 50 \mu\text{m}^2$. The flow rate used was 4.3 mm/s. FIGS. 35a-c, which represent top views of a channel network, show that plugs behave differently compared to the plugs in Example 3 when they flow past a junction in the absence of a channel constriction, such as a constriction shown in FIGS. 35b-c. As FIG. 35c shows, when $P_1 < P_2$, the plugs remained intact after passing through the junction. Further, the plugs traveled along the channel that had the lower pressure (P_1 in FIG. 35c) while the intervening oil stream split at the junction. The splitting of the oil stream at the junction gives rise to a shorter separation between plugs flowing along the channel with pressure P_1 compared to the separation between plugs in the channel upstream of the branching point or junction.

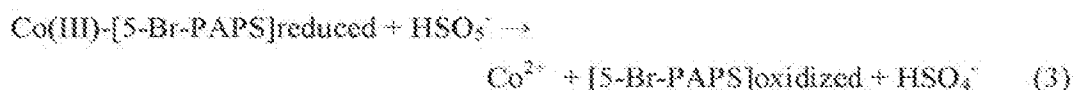
Example 7 - Monitoring Autocatalytic Reactions Using a Microfluidic System

FIG. 37 illustrates the design of an experiment involving chemical amplification in microfluidic devices according to the invention that involves an investigation of a stochastic autocatalytic reaction. This example illustrates how the devices of the present invention can be used to study the acid-sensitive autocatalytic reaction between NaClO_2 and NaS_2O_3 . On the left side of the microfluidic network, a three-channel inlet introduces an aqueous stream through channel 3702, an ester through channel 3701, and an esterase through channel 3703. Oil flowed through channels 3713, 3714. The reaction between ester and esterase yield plugs 3704 that contain a small amount of acid. On the right side of the microfluidic network, the five-channel inlet introduces NaClO_2 through inlet 3705, an aqueous stream through inlet 3706, a pH indicator through inlet 3707, a second aqueous stream through inlet 3708, and NaS_2O_3 through channel 3709. A carrier fluid flows through channels 3713, 3714. Unstirred mixtures of NaClO_2 and NaS_2O_3 are highly unstable and even a slight concentration fluctuation within that mixture leads to rapid decomposition. Thus, the plugs 3710 containing $\text{NaClO}_2/\text{NaS}_2\text{O}_3$ mixture must not only be quickly mixed but also promptly used after formation. In this proposed experiment, the curvy channels promote chaotic mixing. When a slightly acidic plug of the ester-esterase reaction is merged with a plug of an unstable $\text{NaClO}_2/\text{NaS}_2\text{O}_3$ mixture at the contact region 3712, an autocatalytic reaction will generally be triggered. Upon rapid mixing of these two plugs, the resulting plugs 3711 become strongly acidic. The pH indicator introduced in the five-channel inlet is used to visualize this entire amplification process.

Example 8 - Using Chemical Reactions as Highly Sensitive Autoamplifying Detection Elements in Microfluidic Devices

In one aspect according to the invention, a sequential amplification using controlled autocatalytic systems is used to amplify samples that contain single molecules of autocatalysts into samples containing a sufficiently high concentration of an autocatalyst such that the amplified autocatalyst can be detected with the naked eye can be detected with the naked eye. Although systems displaying stochastic behavior are expected to display high sensitivity and amplification, various autocatalytic systems can be used in accordance with the invention. A sequential amplification

using the microfluidic devices according to the invention can be illustrated using a reaction that has been characterized analytically: the autocatalytic decomposition of violet bis[2-(5-bromo-pyridylazo)-5-(*N*-propyl-*N*-sulfopropyl-amino-phenolato] cobaltate, (Co(III)-5-Br-PAPS), upon oxidation with potassium peroxomonosulfate to produce colorless Co²⁺ ions. Here, the Co²⁺ ions serve as the autocatalyst (the order of autocatalysis, *m*, has not been established for this reaction).



Addition of small amounts of Co²⁺ to the violet mixture of (Co(III)-5-Br-PAPS and peroxomonosulfate produces an abrupt loss of color to give a colorless solution. The time delay before this decomposition depends on the amount of the Co²⁺ added to the solution. This reaction has been used to detect concentrations of Co²⁺ as low as about 1×10^{-10} mole/L. The reaction shows good selectivity in the presence of other ions (V(V), Cr(III), Cr(VI), Mn(II), Fe(II), Ni(II), Cu(II) and Zn(II)).

To use this reaction for amplification, a microfluidic network as shown in FIG. 38 is preferably used. An unstable solution of Co(III)-[5-Br-PAPS]_{reduced} and peroxomonosulfate at pH=7 buffer in large plugs are preferably formed in a channel. These large plugs are preferably split in accordance with the invention into three different sizes of plugs. Preferably, the plug sizes are $(1 \mu\text{m})^3 = 10^{-15}$ L in the first channel; $(10 \mu\text{m})^3 = 10^{-12}$ L in the second channel; and $(100 \mu\text{m})^3 = 10^{-9}$ L in the third channel. A three-step photolithography is preferably used in the fabrication of masters for these microfluidic channels.

Example 9 - Multi-Stage Chemical Amplification In Microfluidic Devices For Single Molecule Detection

FIG. 38 illustrates a method for a multi-stage chemical amplification for single molecule detection using microfluidic devices according to the invention. This example illustrates the use of an autocatalytic reaction between Co(III)-5-Br-PAPS (introduced through inlet 3803) and KHSO₅ (introduced through inlet 3801) in a pH =7 buffer (introduced through inlet 3802) that is autocatalyzed by Co²⁺ ions. Oil streams are allowed to flow through channels 3804, 3805. This reaction mixture

(contained in plugs 3811) is unstable and decomposes rapidly (shown in red) when small amounts of Co^{2+} 3810 are added. Thus, this reaction mixture is preferably mixed quickly and used immediately. The reaction mixture is preferably transported through the network in $(1\text{ }\mu\text{m})^3$, $(10\text{ }\mu\text{m})^3$, $(100\text{ }\mu\text{m})^3$ size plugs. On the left side of the microfluidic network, the approximately $1\text{ }\mu\text{m}^3$ plugs of the sample to be analyzed form at a junction of two channels (shown in green). The merging of plugs containing Co^{2+} ions and plugs containing the reaction mixture results in a rapid autocatalytic reaction. By using an amplification cascade in which larger and larger plugs of the reaction mixture are used for amplification, each Co^{2+} ion in a plug can be amplified to about 10^{10} Co^{2+} ions per plug. The result of amplification is visually detectable.

The $(10\text{ }\mu\text{m})^3$ plugs are preferably merged with larger $(100\text{ }\mu\text{m})^3$ plugs in the third channel to give approximately 4×10^{-8} mole/L solution of Co^{2+} ions. Autocatalytic decomposition in the approximately 10^{-9} L plugs will produce plugs 3809 with about 2.4×10^{10} Co^{2+} ions (4×10^{-5} mole/L). The flow rates in this system are preferably controlled carefully to control the time that plugs spend in each branch. The time provided for amplification is preferably long enough to allow amplification to substantially reach completion, but short enough to prevent or minimize slow decomposition.

Using different plug sizes is advantageous when merging plugs. Plugs with a size of about $(1\text{ }\mu\text{m})^3$ are preferably formed by flowing a sample containing about 3×10^{-9} mole/L Co^{2+} through channel 3806. This reaction can be used to detect Co^{2+} at this, or lower, concentration (Endo *et al.*, "Kinetic determination of trace cobalt(II) by visual autocatalytic indication," *Talanta*, 1998, vol. 47, pp. 349-353; Endo *et al.*, "Autocatalytic decomposition of cobalt complexes as an indicator system for the determination of trace amounts of cobalt and effectors," *Analyst*, 1996, vol. 121, pp. 391-394.). These plugs have a corresponding volume of about 10^{-15} L and carry just a few cobalt ions, on average about 1.8 ions per plug (corresponding to a Poisson distribution). These plugs 3810 are preferably merged with the $(1\text{ }\mu\text{m})^3$ plugs 3811 containing the $\text{Co(III)-5-Br-PAPS/peroxomonosulfate}$ mixture (about 4×10^{-5} mole/L).

Upon autocatalytic decomposition of the complex, the number of Co^{2+} ions in the merged plug 3807 will increase by a factor of between about 10^4 to 1.2×10^4 Co^{2+} ions (2×10^{-5} mole/L in $2\text{ }\mu\text{m}^3$). These plugs 3807 are preferably merged with the $(10\text{ }\mu\text{m})^3$ plugs 3811 containing the unstable mixture (about 4×10^{-5} mole/L). The

concentration of Co^{2+} ions in these approximately 10^{-12} L plugs is preferably about 2×10^{-8} mole/L, which is sufficient to induce autocatalytic decomposition. The number of Co^{2+} ions will increase by a factor of between about 10^3 to about 2.4×10^7 ions/plug in plugs 3808. The starting solution is dark violet ($\epsilon = 9.8 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ for Co(III)-5-Br-PAPS). Channels are preferably designed to create an optical path through at least ten consecutive 100 μm plugs. These plugs will provide an approximately 1-mm long optical path, with absorbance of the starting 4×10^{-5} mole/L solution of about 0.4. This absorbance can be detected by an on-chip photodetector or with the naked eye. If Co^{2+} is present in the sample solution, an autocatalytic cascade will result in the disappearance of the color of the reaction mixture.

At low concentrations of Co^{2+} in the sample, the system may show stochastic behavior, that is, not every Co^{2+} ion would give rise to a decomposition cascade. However, the attractive feature of this system is that thousands of tests can be carried out in a matter of seconds, and statistics and averaging can be performed. Preferably, a sequence of controlled autocatalytic amplification reactions leads to a visual detection of single ions.

Example 10 - Enzyme Kinetics

A microfluidic chip according to the invention was used to measure millisecond single-turnover kinetics of ribonuclease A (RNase A; EC 3.1.27.5), a well-studied enzyme. Sub-microliter sample consumption makes the microfluidic chip especially attractive for performing such measurements because they require high concentrations of both the enzyme and the substrate, with the enzyme used in large excess.

The kinetic measurements were performed by monitoring the steady-state fluorescence arising from the cleavage of a fluorogenic substrate by RNase A as the reaction mixture flowed down the channel (see FIG. 40(a)). In FIG. 40, a substrate, buffer, and RNase A were introduced into inlets 401, 401, and 403, respectively. A carrier fluid flowed through channel 404. The amount of the product at a given reaction time t [s] was calculated from the intensity of fluorescence at the corresponding distance point d [m] ($t = d/U$ where $U = 0.43 \text{ m/s}$ is the velocity of the flow). The channels were designed to wind so that rapid chaotic mixing was induced, and were designed to fit within the field of view of the microscope so that the entire reaction profile could be measured in one spatially resolved image. Selwyn's test

(Duggleby, R. G., *Enzyme Kinetics and Mechanisms*, Pt D; Academic Press: San Diego, 1995, vol. 249, pp. 61-90; Selwyn, M. J. *Biochim. Biophys. Acta*, 1965, vol. 105, pp. 193-195) was successfully performed in this system to establish that there were no factors leading to product inhibition or RNase A denaturation.

5 The flow rate of the stock solution of 150 μM of RNase A was kept constant to maintain 50 μM of RNase A within the plugs. By varying the flow rates of the buffer and substrate (see FIG. 45), progress curves were obtained for eight different substrate concentrations. For $[E]_0 \gg [S]_0$, the simple reaction equation is $[P]_t = [S]_0(1 - \text{Exp}(-kt))$, where $[E]_0$ is the initial enzyme concentration, $[S]_0$ is the initial substrate
10 concentration, $[P]_t$ is the time-dependent product concentration and $k [\text{s}^{-1}]$ is the single-turnover rate constant. To obtain a more accurate fit to the data, the time delay Δt_n required to mix a fraction of the reaction mixture f_n was accounted for.

 An attractive feature of the microfluidic system used is that the reaction mixture can be observed at time $t = 0$ (there is no dead-time). This feature was used
15 to determine Δt_n and f_n in this device by obtaining a mixing curve using fluo-4 / Ca^{2+} system as previously described (Song *et al.*, *Angew. Chem. Int. Ed.* 2002, vol. 42, pp.

$$[P]_t = \frac{\sum_n f_n [S]_0 \text{Exp}(-k(t - \Delta t_n))}{\sum_n f_n} \quad [P]_t = \frac{\sum_n f_n [S]_0 (1 - \text{Exp}(-k(t - \Delta t_n)))}{\sum_n f_n}$$

768-772), and correcting for differences in diffusion constants (Stroock *et al.*, *Science*, 2002, vol. 295, pp. 647-651). All eight progress curves gave a good fit with the same rate constant of $1100 \pm 250 \text{ s}^{-1}$. The simpler theoretical fits gave
20 indistinguishable rate constants. These results are in agreement with previous studies, where cleavage rates of oligonucleotides by ribonucleases were shown to be $\sim 10^3 \text{ s}^{-1}$.

 Thus, this example demonstrates that millisecond kinetics with millisecond resolution can be performed rapidly and economically using a microchannel chip according to the invention. Each fluorescence image was acquired for 2 s, and
25 required less than 70 nL of the reagent solutions. These experiments with stopped-flow would require at least several hundreds of microliters of solutions. Volumes of about 2 μL are sufficient for ~ 25 kinetic experiments over a range of concentrations. Fabrication of these devices in PDMS is straightforward (McDonald, *et al.*, *Accounts Chem. Res.* 2002, vol. 35, pp. 491-499) and no specialized equipment except for a
30 standard microscope with a CCD camera is needed to run the experiments. This system could serve as an inexpensive and economical complement to stopped-flow methods for a broad range of kinetic experiments in chemistry and biochemistry.

Example 11 - Kinetics of RNA Folding

The systems and methods of the present invention are preferably used to conduct kinetic measurements of, for example, folding in the time range from tens of microseconds to hundreds of seconds. The systems and methods according to the invention allow kinetic measurements using only small amounts of sample so that the folding of hundreds of different RNA mutants can be measured and the effect of mutation on folding established. In one aspect according to the invention, the kinetics of RNA folding is preferably measured by adding Mg^{2+} to solutions of previously synthesized unfolded RNA labeled with FRET pairs in different positions. In accordance with the invention, the concentrations of Mg^{2+} are preferably varied in the 0.04 to 0.4 μM range by varying the flow rates (see, for example, FIGS. 25a)-c)) to rapidly determine the folding kinetics over a range of conditions. The ability to integrate the signal over many seconds using the steady-flow microfluidic devices according to the invention can further improve sensitivity.

As shown in FIGS. 25a)-c), the concentrations of aqueous solutions inside the plugs can be controlled by changing the flow rates of the aqueous streams. In FIGS. 25a)-c), aqueous streams were introduced into inlets 251-258 wherein flow rates of about 0.6 $\mu L/min$ for the two aqueous streams and 2.7 $\mu L/min$ was used for the third stream. The stream with the 2.7 $\mu L/min$ volumetric flow rate was introduced in the left, middle, and right inlet in FIGS. 25a)-c), respectively. A carrier fluid in the form of perfluorodecaline was introduced into channel 259, 260, 261. The corresponding photographs on each of the right side of FIGS. 25a)-c) illustrate the formation of plugs with different concentrations of the aqueous streams. The various shadings inside the streams and plugs arise from the use of aqueous solutions of food dyes (red/dark and green/light), which allowed visualization, and water were used as the three streams, the darker shading arising mainly from the red dye color while the lighter shading arising mainly from the green dye color. The dark stream is more viscous than the light stream, therefore it moves slower (in mm/s) and occupies a larger fraction of the channel at a given volumetric flow rate (in $\mu L/min$).

Example 12 - Nanoparticle Experiments With and Without Plugs

FIG. 15 illustrates a technique for the synthesis of CdS nanoparticles 155. In one experiment, nanoparticles were formed in a microfluidic network. The channels

of the microfluidic device had $50\ \mu\text{m} \times 50\ \mu\text{m}$ cross-sections. A fluorinated carrier-fluid (10:1 v/v mixture of perfluorohexane and 1H,1H,2H,2H-perfluorooctanol) was flowed through the main channel at $15\ \mu\text{L min}^{-1}$. An aqueous solution, pH = 11.4, of 0.80 mM CdCl_2 and 0.80 mM 3-mercaptopropionic acid was flowed through the left-most inlet channel 151 at $8\ \mu\text{L min}^{-1}$. An aqueous solution of 0.80 mM polyphosphates $\text{Na}(\text{PO}_3)_n$ was flowed through the central inlet channel 152 at $8\ \mu\text{L min}^{-1}$, and an aqueous solution of 0.96 mM Na_2S was flowed through the right-most inlet channel 153 at $8\ \mu\text{L min}^{-1}$. To terminate the growth of nanoparticles, an aqueous solution of 26.2 mM 3-mercaptopropionic acid, pH = 12.1, was flowed through the bottom inlet of the device 157 at $24\ \mu\text{L min}^{-1}$. FIG. 15 shows various regions or points along the channel corresponding to regions or points where nucleation 154, growth 158, and termination 156 occurs. Based on the UV-VIS spectrum, substantially monodisperse nanoparticles formed in this experiment.

Nanoparticles were also formed without microfluidics. Solutions of CdCl_2 , polyphosphates, Na_2S , and 3-mercaptopropionic acid, identical to those used in the microfluidics experiment, were used. 0.5 mL of the solution of CdCl_2 and 3-mercaptopropionic acid, 0.5 mL of polyphosphates solution, and 0.5 mL of Na_2S solution were combined in a cuvette, and the cuvette was shaken by hand. Immediately after mixing, 1.5 mL of 26.2 mM 3-mercaptopropionic acid was added to the reaction mixture to terminate the reaction, and the cuvette was again shaken by hand. Based on the UV-VIS spectrum, substantially polydisperse nanoparticles formed in this experiment.

Example 13 - Crystallization

Networks of microchannels were fabricated using rapid prototyping in polydimethylsiloxane (PDMS). The PDMS was purchased from Dow Corning Sylgard Brand 184 Silicone Elastomer. The PDMS devices were sealed after plasma oxidation treatment in Plasma Prep II (SPI Supplies). The devices were rendered hydrophobic by baking the devices at 120°C for 2-4 hours. Microphotographs were taken with a Leica MZ12.5 stereomicroscope and a SPOT Insight color digital camera (Model#3.2.0, Diagnostic Instruments, Inc.). Lighting was provided from a Machine Vision Strobe X-strobe XI200 (20 Hz, 12 μF , 600V, Perkin Elmer Optoelectronics).

To obtain an image, the shutter of the camera was opened for 1 second and the strobe light was flashed once with the duration of approximately 10 μ s.

Aqueous solutions were pumped using 10 μ l or 50 μ l Hamilton Gastight syringes (1700 series). Carrier-fluid was pumped using 50 μ l Hamilton Gastight syringes (1700 series). The syringes were attached to microfluidic devices by means of Teflon tubing (Weico Wire & Cable Inc., 30 gauge). Syringe pumps from Harvard Apparatus (PHD 2000) were used to inject the liquids into microchannels.

A. Microbatch Crystallization in a Microfluidic Channel

Microbatch crystallization conditions can be achieved. This experiment shows that size of plugs can be maintained and evaporation of water prevented. In this case, the PDMS device has been soaked in water overnight before the experiment in order to saturate PDMS with water. The device was kept under water during the experiment. During the experiment, the flow rates of carrier-fluid and NaCl solution were 2.7 μ L/min and 1.0 μ L/min, respectively. The flow was stopped by cutting off the Teflon tubing of both carrier-fluid and NaCl solution.

FIG. 16 shows a schematic illustration of a microfluidic device according to the invention and a microphotograph of plugs of 1M aqueous NaCl sustained in oil. The carrier-fluid is perfluorodecaline with 2 % 1H,1H,2H,2H-perfluorooctanol. Inside a microchannel, plugs showed no appreciable change in size.

B. Vapor Diffusion Crystallization in Microchannels: Controlling Evaporation of Water From Plugs

This experiment shows that evaporation of water from plugs can be controlled by soaking devices in water for shorter amounts of time or not soaking at all. The rate of evaporation can be also controlled by the thickness of PDMS used in the fabrication of the device. Evaporation rate can be increased by keeping the device in a solution of salt or other substances instead of keeping the device in pure water.

The plug traps are separated by narrow regions that help force the plugs into the traps.

In this experiment, a composite glass/PDMS device was used. PDMS layer had microchannel and a microscopy slide (Fisher, 35x50-1) was used as the substrate. Both the glass slide and the PDMS were treated in plasma cleaner (Harrick) then sealed. The device was made hydrophobic by first baking the device at 120°C for 2-4

hours then silanizing it by (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane(United Chemical Technologies, Inc.)

During the experiment, a flow of carrier-fluid at 1.0 $\mu\text{L}/\text{min}$ was established, then flow of aqueous solution was established at a total rate of 0.9 $\mu\text{L}/\text{min}$. Plug
5 formation was observed inside the microchannel. The flow was stopped approximately 5-10 minutes afterwards by applying a pressure from the outlet and stopping the syringe pumps at the same time.

FIG. 41 shows a microphotograph (middle and right side) of the water plugs region of the microfluidic network. FIG. 41(b)-(c) show the plugs at time $t=0$ and $t=2$
10 hours, respectively. Red aqueous solution is 50 % waterman red ink in 0.5 M NaCl solution. Ink streams were then introduced into inlets 411, 412, 413. An oil stream flowed through channel 414. The carrier-fluid is FC-3283 (3M Fluorinert Liquid) with 2 % 1H,1H,2H,2H-perfluorodecanol. This photograph demonstrates that the evaporation of water through PDMS can be controlled, and thus the concentration of
15 the contents inside the drops can be increased (this is equivalent to microbatch crystallization). FIG. 41(a) shows a diagram of the microfluidic network.

C. Controlling Shape and Attachment of Water Plugs

During the experiment, a flow of carrier fluid at 1.0 $\mu\text{L}/\text{min}$ was established,
20 then flow of aqueous solution was established at a total rate of 2.1 $\mu\text{L}/\text{min}$. Plug formation was observed inside the microchannel. The flow was stopped approximately 5-10 minutes afterwards by applying a pressure from the outlet and stopping the syringe pumps at the same time.

FIG. 39 shows a diagram (left side) of a microfluidic network according to the
25 invention. Aqueous streams were introduced into inlets 3901, 3902, 3903 while an oil stream flowed through channel 3904. FIG. 39 also shows a microphotograph (right side) of the water plug region of the microfluidic network. This image shows water plugs attached to the PDMS wall. This attachment occurs when low concentrations of surfactant, or less-effective surfactants are used. In this case 1H,1H,2H,2H-perfluorooctanol is less effective than 1H,1H,2H,2H-perfluorodecanol. In this
30 experiment the oil is FC-3283 (3M Fluorinert Liquid) with 2% 1H,1H,2H,2H-perfluorooctanol as the surfactant.

D. Examples of Protein Crystallization

During the experiment, a flow of oil at 1.0 $\mu\text{L}/\text{min}$ was established. Then the flow of water was established at 0.1 $\mu\text{L}/\text{min}$. Finally flows of lysozyme and precipitant were established at 0.2 $\mu\text{L}/\text{min}$. Plug formation was observed inside the microchannel. The flow of water was reduced to zero after the flow inside the channel became stable. The flow was stopped approximately 5-10 minutes afterwards by applying a pressure from the outlet and stopping the syringe pumps at the same time.

Fig 36 depicts lysozyme crystals grown in water plugs in the wells of the microfluidic channel. Lysozyme crystals started to appear inside aqueous plugs both inside and outside plug traps in approximately 10 minutes. The image of the three crystals in FIG. 36 was taken 1 hour after the flow was stopped. Lysozyme crystals appear colored because they were observed under polarized light. This is common for protein crystals.

The left side of FIG. 36 is a diagram of a microfluidic network according to the invention while the right side is microphotograph of the crystals formed in plugs in the microfluidic network. A precipitant, lysozyme, and water were introduced into inlets 3601, 3602, and 3603, respectively. Oil was flowed through channel 3604. The lysozyme solution contains 100 mg/ml lysozyme in 0.05 M sodium acetate (pH 4.7); the precipitant solution contains 30% w/v PEG (M.W. 5000), 1.0 M NaCl and 0.05 M sodium acetate (pH 4.7); The carrier-fluid is FC-3283 (3M Fluorinert Liquid) with 10 % 1H,1H,2H,2H-perfluoro-octanol. The microchannel device was soaked in FC-3283/H₂O for one hour before experiment.

FIG. 32 shows that plug traps are not required for formation of crystals in a microfluidic network. FIG. 32 shows a diagram (left side) of the microfluidic network. A precipitant was introduced into inlet 321, lysozyme was introduced into inlet 322, and an aqueous stream was introduced into inlet 323. Oil was flowed through channel 324. FIG. 32 also shows microphotographs (middle and right side) of lysozyme crystals grown inside the microfluidic channel. The experimental condition is same as in FIG. 36.

Example 14 - Oil-soluble Surfactants for Charged Surfaces

In accordance with the invention, neutral surfactants that are soluble in perfluorinated phases are preferably used to create positively and negatively-charged interfaces. To create charged surfaces, neutral surfactants that can be charged by interactions with water, *e.g.*, by protonation of an amine or a guanidinium group (FIG.

24B), or deprotonation of a carboxylic acid group (FIG. 24C), are preferably used. Preferably, charged surfaces are used to repel, immobilize, or stabilize charged biomolecules. Negatively charged surfaces are useful for handling DNA and RNA without surface adsorption. Preferably, both negatively and positively-charged surfaces are used to control the nucleation of protein crystals. Many neutral fluorinated surfactants with acidic and basic groups (RfC(O)OH , $\text{Rf(CH}_2)_2\text{NH}_2$, $\text{Rf(CH}_2)_2\text{C(NH)NH}_2$) are available commercially (Lancaster, Fluorochem, Aldrich).

To synthesize oligoethylene-glycol terminated surfactants, a modification and improvement of a procedure based on the synthesis of perfluoro non-ionic surfactants is preferably used. In one aspect, the synthesis relies on the higher acidity of the fluorinated alcohol to prevent the polycondensation of the oligoethylene glycol. The modified synthesis uses a selective benzylation of one of the alcohol groups of oligoethylene glycol, followed by activation of the other alcohol group as a tosylate. A Williamson condensation is then performed under phase transfer conditions followed by a final deprotection step via catalytic hydrogenation using palladium on charcoal.

Example 15 - Formation of Plugs in the Presence of Fluorinated Surfactants and Surface Tension

The surface tension of the oil/water interface has to be sufficiently high in order to maintain a low value of capillary number, C_n . The fluorosurfactant/water interfaces for water-insoluble fluorosurfactants have not been characterized, but these surfactants are predicted to reduce surface tension similar to that observed in a system involving Span on hexane/water interface (about 20 mN/m). The surface tensions of the aqueous/fluorous interfaces are preferably measured in the presence of fluorosurfactants using the hanging drop method. A video microscopy apparatus specifically constructed for performing these measurements has been used to successfully characterize interfaces. FIG. 24 illustrates the synthesis of fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group.

Example 16 - Forming Gradients by Varying Flow Rates

FIG. 42 shows an experiment involving the formation of gradients by varying the flow rates. In this experiment, networks of microchannels were fabricated using rapid prototyping in polydimethylsiloxane (PDMS). The width and height of the

channel were both 50 μm . 10% 1H,1H,2H,2H-perfluorodecanol in perfluoroperhydrophenanthrene was used as oil. Red aqueous solution prepared from 50 % waterman red ink in 0.5 M NaCl solution was introduced into inlet 421. The oil flowed through channel 424 at 0.5 $\mu\text{l/min}$. Aqueous streams were introduced into inlets 422, 423. To generate the gradient of ink in the channel, the total water flow rate was gradually increased from 0.03 $\mu\text{l/min}$ to 0.23 $\mu\text{l/min}$ in 20 seconds at a ramp rate of 0.01 $\mu\text{l/min}$ per second. At the same time, ink flow rate was gradually decreased from 0.25 $\mu\text{l/min}$ to 0.05 $\mu\text{l/min}$ in 20 seconds at a ramp rate of -0.01 $\mu\text{l/min}$ per second. The total flow rate was constant at 0.28 $\mu\text{l/min}$. The established gradient of ink concentration inside the plugs can be clearly seen from FIG. 42: the plugs further from the inlet are darker since they were formed at a higher ink flow rate.

Example 17 - Lysozyme Crystallization Using Gradients

FIG. 43 illustrates an experiment involving the formation of lysozyme crystals using gradients. The channel regions 435, 437 correspond to channel regions with very low precipitant concentration while channel region 436 corresponds to optimal range of precipitant concentration. In this experiment, networks of microchannels were fabricated using rapid prototyping in polydimethylsiloxane (PDMS). The width of the channel was 150 μm and the height was 100 μm . 10% 1H,1H,2H,2H-perfluorodecanol in perfluoroperhydrophenanthrene was used as oil.

During the experiment, a flow of oil through channel 434 at 1.0 $\mu\text{l/min}$ was established. Then the flow of water introduced through inlet 432 was established at 0.2 $\mu\text{l/min}$. The flows of lysozyme introduced through inlet 431 and precipitant introduced through inlet 433 were established at 0.2 $\mu\text{l/min}$. Plugs formed inside the channel. To create the gradient, water flow rate was first gradually decreased from 0.35 $\mu\text{l/min}$ to 0.05 $\mu\text{l/min}$ over 45 seconds at a ramp rate of (-0.01 $\mu\text{l/min}$ per 1.5 seconds), then increased back to 0.35 $\mu\text{l/min}$ in 45 seconds at a ramp rate of (0.01 $\mu\text{l/min}$ per 1.5 seconds). At the same time, precipitant flow rate was gradually increased from 0.05 $\mu\text{l/min}$ to 0.35 $\mu\text{l/min}$ in 45 seconds at a ramp rate of (0.01 $\mu\text{l/min}$ per 1.5 seconds), then decreased to 0.05 $\mu\text{l/min}$ in 45 seconds at a ramp rate of (-0.01 $\mu\text{l/min}$ per 1.5 seconds). The flow was stopped by pulling out the inlet

tubing immediately after water and precipitant flow rates returned to the starting values. The plugs created in this way contained constant concentration of the protein, but variable concentration of the precipitant: the concentration of the precipitant was lowest in the beginning and the end of the channel, and it peaked in the middle of the channel (the center row). Only the plugs in the middle of the channel have the optimal concentration of precipitant for lysozyme crystallization, as confirmed by observing lysozyme crystals inside plugs in the center row. Visualization was performed under polarized light. Preferably, all flow rates would be varied, not just the precipitant and water.

Example 18 - Lysozyme crystallization in capillaries using the microbatch analogue method

To grow lysozyme crystal inside plugs within capillaries, a 10 μ l Hamilton syringe was filled with 100 mg/ml lysozyme in 0.05 M NaAc buffer (pH4.7) and another 10 μ l Hamilton syringe was filled with 30% (w/v) MPEG 5000 with 2.0 M NaCl in 0.05 M NaAc buffer (pH4.7) as precipitant. A 50 μ l Hamilton syringe filled with PFP (10% PFO) was the oil supply. All three syringes were attached to the PDMS/capillary device and driven by Harvard Apparatus syringe pumps (PHD2000). The capillary has an inner diameter of 0.18 mm and outer diameter of 0.20 mm. Oil flow rate was 1.0 μ l/min and both lysozyme and precipitant solution were at 0.3 μ l/min. The channel was filled with oil first. Protein and precipitant streams converged immediately before entering the channel to form plugs. After the capillary (Hampton Research) was filled with the plugs containing lysozyme, the flows were stopped. The capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator (18°C). A lysozyme crystal appeared within an hour and was stable for at least 14 days without change of size or shape (FIG. 47A).

Example 19 - Thaumatin crystallization in capillaries using the microbatch analogue method

Experiment 1. A 10 μ l Hamilton syringe was filled with 50 mg/ml thaumatin in 0.1 M ADA buffer (pH 6.5) and another 10 μ l Hamilton syringe was

filled with 1.5 M NaK Tartrate in 0.1 M HEPES (pH 7.0). A 50 μ l Hamilton syringe filled with PFP (10% PFO) was the oil supply. All three syringes were attached to the PDMS/capillary device and driven by Harvard Apparatus syringe pumps (PHD2000). The capillary has an inner diameter of 0.18 mm and outer diameter of 0.20 mm. Oil flow rate was 1.0 μ l/min and both thaumatin and precipitant solution were at 0.3 μ l/min. The channel was filled with oil first. Protein and precipitant streams were mixed immediately before entering the channel to form plugs. After the capillary (Hampton Research) was filled with protein plugs, the flows were stopped. The capillary was cut from the PDMS device, sealed by wax and stored in an incubator (18°C). The thaumatin crystal appeared in 2-3 days and was stable for at least 45 days without size or shape change (FIG. 47B). Some thaumatin crystals grew at the interface of protein solution and oil, while others appeared to attach to the capillary wall.

Experiment 2. Thaumatin crystals were grown inside a capillary tube using 50 mg/mL thaumatin in 0.1M pH 6.5 ADA buffer and a precipitant solution of 1M Na/K tartrate in a 0.1M pH 7.5 HEPES buffer. Protein and precipitant solutions were mixed in a 1.4:1 protein:precipitant ratio. A fluorinated carrier fluid was a saturated solution of FSN surfactant in FC3283. The capillary was incubated at 18 degrees C. Tetragonal crystals appeared within 5 days (FIG. 48A, B). X-ray diffraction was performed at BioCARS station 14BM-C at the Advanced Photon Source at Argonne National Laboratory. Beam wavelength was 0.9Å. The final length of a single crystal was estimated at 100-150 microns.

Capillaries were cut to the appropriate length without disturbing crystal-containing plugs, resealed using capillary wax, and mounted on clay-tipped cryoloop holders at a distance of 12 +/- 5 mm from base to crystal. The holder was placed on the x-ray goniometer. Crystals were centered on the beam. Snapshots were taken using 10 second (thaumatin) exposures. Distance from sample to detector was 150 mm. Diffraction to better than 2.2 Å was obtained.

Example 20 - Vapor Diffusion Protein Crystallization in Capillaries By An Alternating Droplet System

The principle of transferring water inside a capillary from one set of plugs to another set of plugs is illustrated in FIG. 50. Briefly, a 10 μ l Hamilton syringe was filled with 0.01 $\text{Fe}(\text{SCN})_3$ and another 10 μ l Hamilton syringe was filled with 0.1 M $\text{Fe}(\text{SCN})_3$ with 2.5 M KNO_3 . Two 50 μ l Hamilton syringes were filled with FMS-121 (Gelest, Inc) (saturated with PFO), which provided the oil supply. All four syringes were attached to the PDMS/capillary device and driven by Harvard Apparatus syringe pumps (PHD2000). The capillary has an inner diameter of 0.18 mm and outer diameter of 0.20 mm. One of the oil inlet channels was between the two aqueous inlets channels to separate the two aqueous streams when forming the alternating plugs. This oil inlet channel was vertical to the main channel and had a flow rate of 2.0 μ l/min. The other oil inlet channel had a flow rate of 1.0 μ l/min and was parallel to the main channel. Both of the aqueous solutions had a flow rate of 0.5 μ l/min. After establishing alternating aqueous droplet streams in the capillary, the flows were stopped, and the capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator at 18°C. The size and color change of the plugs were monitored with a Leica microscope (MZ125) having a color CCD camera (SPOT Insight, Diagnostic Instruments, Inc.).

Following the stoppage of flow and sealing of the capillary tube, plugs containing 0.01 M $\text{Fe}(\text{SCN})_3$ in water were yellow, while those containing 0.1 M $\text{Fe}(\text{SCN})_3$ and 2.5 M KNO_3 in water were red (FIG. 50A). However, FIG. 50B shows that after 5 days, the yellow plugs were reduced in size and were more concentrated, while the red plugs increased in size and were more diluted. This demonstration reflects vapor diffusion conditions in the capillary tube that are predicted to facilitate protein crystallization. This technique can be further adapted to other applications requiring concentration of reagents, such as proteins.

Alternating plugs from two different aqueous solutions may be generated in accordance with several representative geometries as set forth in FIG. 51. In principle, the same oil or different oils may be used in the two oil inlets. One scheme for generating alternating plugs from two different aqueous solutions is depicted in FIG. 51A. In this case, one 10 μ l Hamilton syringe was filled with 0.1 $\text{Fe}(\text{SCN})_3$, another with 1.5 M NaCl. Two 50 μ l Hamilton syringes filled with PFP (with 10% PFO) provided the oil supply. All four syringes were attached to the PDMS device and driven by Harvard Apparatus syringe pumps (PHD2000).

Alternatively, multiple solutions can be co-introduced together in each of the two aqueous channels as depicted in FIG. 51B. In each of these two cases one of the oil inlet channels was between the two aqueous inlet channels. This oil inlet channel was used to separate the two aqueous streams into alternating plugs and
5 was vertical to the main channel, having a flow rate of 2.0 $\mu\text{l}/\text{min}$. The other oil inlet channel was parallel to the main channel and had a flow rate of 1.0 $\mu\text{l}/\text{min}$. Each of the two aqueous solutions had flow rates of 0.5 $\mu\text{l}/\text{min}$. Alternating plugs were found to form in the channel (FIG. 51C).

FIG. 52 illustrates another example of generating alternating plugs from
10 two different aqueous solutions. In this case, one 10 μl Hamilton syringe was filled with 0.1 $\text{Fe}(\text{SCN})_3$, the other with 1.5 M NaCl. Two 50 μl Hamilton syringes filled with FMS-121 (saturated with PFO) provided the oil supply. All four syringes were attached to the device and driven by Harvard Apparatus syringe pumps (PHD2000). One of the oil inlet channels was between the two aqueous
15 inlet channels and was used to separate the two aqueous streams prior to formation of alternating plugs (FIG. 52A). This oil inlet channel was vertical to the main channel and had a flow rate of 1.5 $\mu\text{l}/\text{min}$. The other oil stream had a flow rate of 1.5 $\mu\text{l}/\text{min}$ and was parallel to the main channel. Each of the two aqueous solutions had flow rates of 0.5 $\mu\text{l}/\text{min}$. Alternating plugs were found to form in
20 the channel (FIG. 52B).

Other geometries that can support the formation of alternating plugs are depicted in FIG. 53. Importantly, the flow rates of solutions A and B may be changed in a correlated fashion (FIG. 54). Thus, when the flow rate of solution A_1 is increased and solution A_2 is decreased, the flow rate of solutions B_1 is also
25 increased and solution B_2 is also decreased. This principle, depicted in FIG. 54, is useful for maintaining a constant difference in salt concentration between the plugs of stream A and stream B to ensure that transfer from all plugs A to all plugs B occurs at a constant rate.

FIG. 54 provides a schematic illustration of a device for preparing plugs of
30 varying protein concentrations where the flow rates of the A and B streams change in a correlated fashion. In this example, A_1 through A_3 are for protein solution, buffer and precipitants, such as PEG or salts. Highly concentrated salt solutions

are injected through $B_1 \sim B_3$. The flow rate ratio of inlet A_i to that of B_i ($i=1\sim3$) is maintained constant. Therefore all of the protein plugs will shrink at a rate similar to the salt plugs.

FIG. 54 shows that if the flow rates of corresponding A and B streams are
5 changed in a correlative fashion, the composition of plugs B will reflect the
composition of plugs A. Therefore, one can incorporate markers into the B stream
plugs to serve as a code for the plugs in the A stream. In other words,
absorption/fluorescent dyes or x-ray scattering/absorbing materials can be
incorporated in markers in the B streams to facilitate optical or x-ray-mediated
10 quantification so as to provide a read out of relative protein and precipitant
concentrations in the A streams. This approach can provide a powerful means for
optimizing crystallization conditions for subsequent scale-up experiments.

Electronic Acknowledgement Receipt

EFS ID:	19605228
Application Number:	13563347
International Application Number:	
Confirmation Number:	3611
Title of Invention:	Method of performing PCR reaction in continuously flowing microfluidic plugs
First Named Inventor/Applicant Name:	Rustem F. Ismagilov
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	UCH-800/US12 31097/10
Receipt Date:	17-JUL-2014
Filing Date:	31-JUL-2012
Time Stamp:	12:38:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment		no			
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	UCH_800_US12_Response_to_NTFCAP.PDF	22263	no	3
			8c79db4b1713b18a8ff1a4b971a8b12297b7fca		
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/563,347	07/31/2012	Rustem F. Ismagilov	UCH-800/US12 31097/10	3611

21710 7590 08/06/2014
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER

GAKH, YELENA G

ART UNIT	PAPER NUMBER
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1797

NOTIFICATION DATE	DELIVERY MODE
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08/06/2014

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

Response to Rule 312 Communication	Application No. 13/583,347	Applicant(s)
	Examiner	Art Unit

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

1. ☒ The amendment filed on 17 July 2014 under 37 CFR 1.312 has been considered, and has been:
- a) ☒ entered.
 - b) ☐ entered as directed to matters of form not affecting the scope of the invention.
 - c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.
 - d) ☐ disapproved. See explanation below.
 - e) ☐ entered in part. See explanation below.

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/563,347	09/02/2014	8822148	UCH-800/US12 31097/10	3611

21710 7590 08/13/2014
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Rustem F. Ismagilov, Chicago, IL;
Joshua David Tice, Chicago, IL;
Cory John Gerdtz, Chicago, IL;
Bo Zheng, Chicago, IL;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

COMPLIANCE WITH THE DATA PROTECTION ACT 1998

In accordance with the Data Protection Act 1998, the personal data provided on this form will be processed by EPSRC, and may be held on computerised database and/or manual files. Further details may be found in the guidance notes

Document Status: Submitter Pool

EPSRC Reference:

RESEARCH PROPOSAL

1 DETAILS OF PROPOSAL

You should read the separate notes for guidance, the 'EPSRC Funding Guide' and any specific call documentation on the EPSRC Web site before completing any research proposal. Form Je-SRP1 (EPSRC) must be accompanied by a Case for Support. EPSRC will reject incomplete research proposals.

A. Organisation Where Grant Would Be Held

Organisation	University of Cambridge	Research Organisation Reference:	C521285
Division or Department	Chemistry		
Address Line 1	The Old Schools		
Address Line 2			
Address Line 3			
Town/City	Cambridge		
Admin Area/County	Cambridgeshire	Postal Code	CB2 1TT

B. Investigators

Please give details of each investigator below. Please provide the details of any additional investigators on a separate sheet using the same format as below.

Details	Principal Investigator (PI)	Co-Investigator 1
Title	Professor	Dr
Forename(s)	Chris	Wilhelm
Surname	Abell	Huck
Organisation	University of Cambridge	University of Cambridge
Division or Department	Chemistry	Chemistry
Post will outlast project (Y/N)	Y	Y
% time committed to project	20 %	20 %
Other commitments (description and average hours per week)		

Total number of co-investigators (ie. excluding the PI)

3

C. Recognised Researchers

Please give details of each Recognised Researcher below. Please provide the details of any additional Recognised Researchers on a separate sheet using the same format as below.

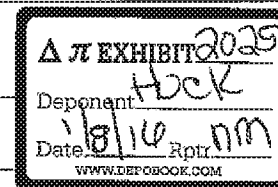
Details	Recognised Researcher 1	Recognised Researcher 2
Title		
Forename(s)		
Surname		
Organisation		
Division or Department		
% time committed to project		

Total number of Recognised Researchers

0

D. Title of Research Project [up to 150 chars]

Microfluidic microdroplet microreactors



(2025)

E. Start Date and Duration

a. Proposed start date 01/06/2005

b. Duration of the grant (months) 48

F. Type of Proposal

Scheme: Standard Research

Call: Basic Technology Call 4

G. Summary of EPSRC Resources Required for Project

a. Financial resources required

	Total £
Staff	1,912,484
Travel and Subsistence	66,000
Consumables	795,000
Exceptional Items	100,000
Equipment	698,403
Large Capital	0
PCTF	0
Sub-total	3,571,887
Indirect Costs	679,743
Total	4,451,630

b. Summary of staff effort requested

	Months
Research	624
Technician	120
Other	14.4
Project Students	0
Visiting Researchers	0
Total	758.4

c. Services

£0

H. Related Proposals

a. If this proposal is related to a previous proposal to EPSRC, please give the previous EPSRC research grant proposal reference number(s) and indicate the type of relationship.

EPSRC Reference Number	How related? (one of Continuation, Follow up to outline proposal, Invited resubmission, Uninvited resubmission)

b. If there is more than one organisation submitting a Je-SRP1 (EPSRC) proposal for this project, please give the number of proposals involved, the lead Research Organisation and the project common reference.

Total Number of Proposals being submitted	Name of Lead RO	Common Reference

I. Research Councils / MoD Joint Research Grants Scheme (JGS) If you have received a commitment of support from the Defence Science Technology Laboratory (DSTL), please give the following details:

Percentage funding indicated by DSTL	
DSTL contact (name and address)	
Title/Forename(s)	
Surname	
Address Line 1	
Address Line 2	
Address Line 3	
Town/City	
Administrative Area/County	
Postal Code	
Telephone	
Fax	
E-mail	
DSTL Reference (please ensure that the letter providing this reference is attached with the Case for Support)	

J. Objectives

List main objectives of the proposed research in order of priority [up to 4000 chars]

The Project has 2 overall objectives: to develop the basic technology (a) and to further refine the technology within three very ambitious sub-projects (b).

(a) Developing the basic technology. The objectives are to:

(i) Build devices for generating microdroplets within microfluidic systems, so that the droplets can be loaded with e.g. enzymes, substrates, inhibitors, DNA etc in a defined way.

(ii) To develop methods to sort and split droplets

(iii) To develop methods for assaying reactions in droplets.

(iv) To create integrated microfluidic devices incorporating these features

(b) Applying the basic technology

(i) To develop the microdroplet technology for massively parallel screening of potential enzyme inhibitors.

(ii) To develop the microdroplet technology for whole proteome analysis of protein-protein interactions.

(iii) To evolve superior catalysts for biofuel cells.

K. Summary

Describe the proposed research in a style that would be accessible to an interested 14 year old [up to 4000 chars]

Miniaturisation has been one of the hallmarks of technological progress. In the realm of electronics and computing the number of transistors per integrated circuit has roughly doubled every year since their invention in 1961 resulting in processors such as the Intel Pentium 4, which contain 42 million transistors. The Pentium 4 can also switch at 3.2Ghz (3.2 billion cycles per second) resulting in a quite astonishing computing power. Unfortunately, in no fields other than computing and electronics have miniaturisation produced such dramatic progress.

We now plan to develop a new technology, Microdroplet Technology, which will produce similar leaps in biology, chemistry and drug discovery. It is based on the idea of miniaturising the volume of biological and chemical reactions. Normally, each experiment in biology or chemistry is performed in a tube or multi-well plate. Using Microdroplet Technology, each experiment is performed in a separate microscopic droplet, with a volume one billion times smaller than a conventional assay. These droplets will be formed, manipulated and analysed in a microfluidic system, consisting of a network of microscopic channels through which the droplets will flow in a stream of oil. The droplets will be made and analysed at 10KHz (10,000 per second).

We will create integrated microfluidic chips, which can make, fuse, split, incubate and switch droplets and will have applications in many areas of science. In this Programme, we aim to demonstrate their use to search large numbers of compounds for new drugs, to investigate interactions between proteins in cells and to develop new catalysts for fuel cells.

L. Beneficiaries

Describe who will benefit from the research [up to 4000 chars]

Microdroplet Technology is applicable to a range of disciplines, which can exploit massively parallel experimental formats. It should prove useful to researchers in fields ranging through protein engineering, directed evolution, proteomics, organic chemistry and materials science.

It has the potential to supersede in vivo and in vitro selection and directed evolution techniques including SELEX and phage display, and to revolutionise high-throughput screening, a core technology in the pharmaceutical industry. Consequently the most obvious commercial beneficiaries will be in the pharmaceutical and biotech sectors.

One of the most important applications of Microdroplet Technology is likely to be in the generation of new protein and low molecular weight drugs. Hence, the technology is also likely to have a significant impact in healthcare.

Microdroplet Technology also has the potential to have a significant commercial impact in the generation of new molecules for non-medical applications. For example, enzymes generated using Microdroplet Technology have potential applications in many fields. Many of these applications also have the potential to have significant beneficial effects for the environment. Enzymes engineered using microdroplet technology can be used as catalysts for 'green' chemistry, for example, to catalyse the production of biofuels (such as ethanol), and as catalysts in biofuel cells, which could eventually function as environmentally friendly alternatives to conventional batteries.

Joint Negotiating Committee For Higher Education Staff (JNCHES - formerly UCEA) Posts

Section 1-4

Date Printed: 08/01/2016 06:24:09/09/2004 16:30
Date Saved: 09/09/2004 16:27
Je-SRP1(EPSRC) v1.1

[illegible]

Ma. Project Studentships

Name/Post Identifier	Start Date	London (Y/N)	Stipend (£)
Total			

Mb. Visiting Researchers Please provide the details of any additional visiting researchers on a separate sheet in the same format as below.

Details	Visiting Researcher 1	Visiting Researcher 2	Visiting Researcher 3
Title			
Forename(s)			
Surname			
Home Organisation			
Division or Department			
Address Line 1			
Address Line 2			
Address Line 3			
Town/City			
Administrative Area/County			
Postal Code			
Country			
Telephone			
Fax			
E-mail			
Post held			
Will Visiting Researcher be supporting dependants?			
What annual salary would host organisation pay staff of the Visiting Researcher's status? (£)			
If salary contribution required from EPSRC, state:			
(i) percentage of normal salary being received from any other source			
(ii) normal salary if less than given above (£)			

Total number of visiting researchers

Mc. Public Communication Training Funds (PCTF)Do you wish to apply for Public Communication Training Funds? YES ☐ NO ☒

N. Travel and Subsistence

Destination and purpose	Total £
(i) Within UK	
(ii) Outside UK	
Conference Travel (£500 per researcher per year x 52 person-years)	26,000
Visit to Prof. Weitz' lab at Harvard (16 visits of 4 weeks at £2000 each)	32,000
Visit to Prof. Bibette's lab (8 visits of 4 weeks at £1000 each)	8,000
Total £	66,000

O. Consumables

Description	Total £
Consumables for project "Microfluidics" (£15000 per year x 15 person-years)	225,000
Consumables for project "Small-molecule Screening" (£16500 per year x 11 person-years)	181,500
Consumables for project "Appl. Dir. Evolution" (£16500 per year x 11 person-years)	181,500
Consumables for project "Proteomics" (£17000 per year x 11 person-years)	187,000
Consumables for Informatics, computing (£5000 x 4 years)	20,000
Total £	795,000

P. Exceptional Items

Description	Total £
Refurbishment costs for dedicated new lab space for this project	100,000
Total £	100,000

Q. Equipment (single items between £3,000 and £99,999, including VAT)

Description	Country of Manufacture	Delivery Date	Basic price £	Import duty £	VAT £	Total £
AKTA purifier 10XT (including FPLC, Frac 950 fraction collector, A900 autosampler and support) / Amersham Biosciences	Sweden	01/06/2005	48,531	0	0	48,531
Gilson Nebula HPLC / Anachem	United States	01/06/2005	53,840	0	0	53,840
-86°C ULT Freezer / Thermo Life Sciences	United Kingdom	01/12/2005	17,780	0	0	17,780
2x MJ Research PTC220 Dyad PCR machine (with 96-well block and 48/48 block)	United States	01/06/2005	19,940	0	0	19,940
Eppendorf 5417R refrigerated benchtop centrifuge	Germany	01/06/2005	2,773	0	0	2,773
Thermo Savant SG210DR Integrated Gel Dryer	United States	01/06/2005	4,150	0	0	4,150
Eppendorf Concentrator (SpeedVac)	Germany	01/06/2005	4,000	0	0	4,000
Avestin EmulsiFlex-C5 High Pressure Homogeniser	Canada	01/06/2005	13,400	0	0	13,400
2 x High speed camera Phantom v4.2	United States	01/06/2005	41,618	0	0	41,618
2x Inverted microscope	United Kingdom	01/06/2005	40,240	0	0	40,240
5 syringe pumps	United Kingdom	01/06/2005	7,500	0	0	7,500
2 Genapta fibre optic probes and sensors for detection of drops and sorting	United Kingdom	01/06/2005	84,000	0	0	84,000
Thinky mixer/degasser	Japan	01/06/2005	8,141	0	0	8,141
Computer equipment for machines and researchers, software (ms office)	United States	01/06/2005	17,000	0	0	17,000
Microtitreplate reader (fluorescence) [Molecular Devices]	United States	01/07/2005	22,450	0	0	22,450
FlashScan 550 Diode array plate reader [Analytik Jena]	Germany	01/06/2005	46,800	0	0	46,800
Oven	Germany	01/06/2005	5,000	0	0	5,000
Vacuum pump [Edwards]	United States	01/06/2005	4,000	0	0	4,000
Plasma Cleaner	United Kingdom	01/06/2005	15,000	0	0	15,000
Mask aligner	United Kingdom	01/06/2005	68,500	0	0	68,500
Two programmable hot plates	United Kingdom	01/06/2005	8,000	0	0	8,000
Contingency Funds	United Kingdom	01/06/2005	95,000	0	0	95,000
Spin coater (x2)	United Kingdom	01/06/2005	6,740	0	0	6,740
Genapta fibre optic probes and sensors for detection of drops and sorting	United Kingdom	01/07/2005	42,000	0	0	42,000
3 Genapta alignment systems	United Kingdom	01/07/2005	24,000	0	0	24,000
Total £						698,403

R. Large Capital (single items £100,000 and over, including VAT)

Description	Country of Manufacture	Delivery Date	Basic price £	Import duty £	VAT £	Total £
Total £						0

Sa. Services

Service	Instrument(s)	Units	Cost £
Total		0	0

2 OTHER INFORMATION

Information in the section will be used for administrative purposes including the selection of peer reviewers.

A. Nominated Referees

Please give details of 3 referees whom the EPSRC may approach for assessment of this research proposal.

Details	Referee 1	Referee 2
Title	Dr	Professor
Forename(s)	Benjamin Guy	Chris A
Surname	Davis	Hunter
Organisation	University of Oxford	
Division or Department	Oxford Chemistry	
Address Line 1	Oxford Chemistry	Department of Chemistry
Address Line 2	University of Oxford	University of Sheffield
Address Line 3	Dyson Perrins Laboratory	
Town/City	Oxford	Sheffield
Administrative Area	Oxfordshire	South Yorkshire
Postal Code	OX1 3QT	S3 7HF
Country	United Kingdom	United Kingdom
Telephone	01865 275652	0114 22 29476
Fax	01865 275674	0114 222 9346
E-mail	Ben.Davis@chem.ox.ac.uk	c.hunter@sheffield.ac.uk

Details	Referee 3
Title	Professor
Forename(s)	Dan Saleh
Surname	Tawfik
Organisation	
Division or Department	
Address Line 1	Department of Biological Chemistry
Address Line 2	The Weizmann Institute of Science
Address Line 3	
Town/City	Rehovot
Administrative Area	
Postal Code	76100
Country	Israel
Telephone	0097289343637
Fax	0097289344118
Email	tawfik@weizmann.ac.il

4. ADDITIONAL DETAILS OF PROPOSAL

Presents details of additional investigators, recognised researchers and project partners.

B. Investigators (continued)

Details	Co-Investigator 2	Co-Investigator 3
Title	Dr	Dr
Forename(s)	Florian	Andrew David
Surname	Hollfelder	Griffiths
Organisation	University of Cambridge	Medical Research Council
Division or Department	Biochemistry	Laboratory of Molecular Biology
Post will oulast project (Y/N)	Y	Y
% time committed to project	30 %	50 %
Other commitments (description and average hours per week)		

C. Recognised Researchers (continued)

Details	Recognised Researcher 3	Recognised Researcher 4
Title		
Forename(s)		
Surname		
Organisation		
Division or Department		
% time committed to project		

Mb. Visiting Researchers (continued)

Details	Visiting Researcher 4	Visiting Researcher 5	Visiting Researcher 6
Title			
Forename(s)			
Surname			
Home Organisation			
Division or Department			
Address Line 1			
Address Line 2			
Address Line 3			
Town/City			
Administrative Area/County			
Postal Code			
Country			
Telephone			
Fax			
E-mail			
Post held			
Will Visiting Researcher be supporting dependants?			
What annual salary would host organisation pay staff of the Visiting Researcher's status? (£)			
If salary contribution required from EPSRC, state:			
(i) percentage of normal salary being received from any other source			
(ii) normal salary if less than given above (£)			

U. Project Partners (continued)

Details	Partner 3	Partner 4
Name of partner organisation		
Division/Department		
Address Line 1		
Address Line 2		
Address Line 3		
Town/City		
Administrative Area/County		
Postal Code		
Country		
Title/Forename(s) of Contact		
Surname of Contact		
Contact's Address (if different from organisation address)		
Address Line 1		
Address Line 2		
Address Line 3		
Town/City		
Administrative Area/County		
Postal Code		
Country		
Telephone		
Fax		
E-mail		
Direct contribution to project	Description	Value £
a. cash		
b. equipment/materials		
c. secondment of staff		
d. other		
Sub-Total		
Indirect contribution to project		
a. use of facilities/equipment		
b. staff time		
c. other		
Sub-Total		
Total Contribution		
Total Value (including contributions from additional project partners)		

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

CASE IPR 2015-01157

Patent 8,889,083

10X GENOMICS, INC.)
Petitioner)
vs.)
THE UNIVERSITY OF CHICAGO)
Patent Owner)

Videotaped Deposition of
Peter David Olmsted, Ph.D.
Washington, D.C.
May 13, 2016
9:00 a.m.

Veritext Legal Solutions
Mid-Atlantic Region
1250 Eye Street NW - Suite 350
Washington, D.C. 20005

1 Videotaped Deposition of Peter David Olmsted,
2 Ph.D. held at:

3
4
5
6 Sterne Kessler Goldstein Fox
7 1100 New York Avenue, N.W.
8 Washington, D.C.
9

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17
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20
21 Pursuant to Notice, when were present on behalf
22 of the respective parties:

1 APPEARANCES:

2 On behalf of the Petitioner:

3 ELDORA LYNN ELLISON, Ph.D., Esq.

4 DEBORAH STERLING, Ph.D., Esq.

5 DAVID H. HOLMAN, Ph.D., Esq.

6 STERNE KESSLER GOLDSTEIN FOX

7 1100 New York Avenue, N.W.

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9 202-371-2600

10 eellison@skgf.com

11 dsterling@skgf.com

12 dholman@skgf.com

13
14 On behalf of the Patent Owner:

15 EDWARD R. REINES, Esq.

16 WEIL, GOTSHAL & MANGES, LLP

17 201 Redwood Shores Parkway

18 Redwood Shores, California 94065

19 650-802-3022

20 edward.reines@weil.com

21 Also Present:

22 Maribeth Powers, Videographer

I N D E X

EXAMINATION OF PETER DAVID OLMSTED, Ph.D. PAGE
BY MR. REINES 6

EXHIBITS

PREVIOUSLY MARKED EXHIBITS

Exhibit 1001	U.S. Patent 8,889,083 B2
Exhibit 1004	U.S. Patent Application Publication US2002/0058332 A1
Exhibit 1006	U.S. Patent 6,524,456 B1
Exhibit 1085	Declaration of Peter David Olmsted, Ph.D.
Exhibit 2010	Article entitled "The origins and the future of microfluidics"

(Exhibits included with transcript.)

P R O C E E D I N G S

THE VIDEOGRAPHER: We are now on the record.

My name is Maribeth Powers representing Veritext.

The date today is May 13th, 2016. And the time is approximately 9:08 a.m.

This deposition is being held at Sterne Kessler Goldstein Fox.

The caption of this case is 10X Genomics, Incorporated, versus the University of Chicago. This case is filed in the United States Patent and Trademark Office, Case No. IPR 2015-01157.

The name of the witness is Peter David Olmsted.

At this time the attorneys present in the room will identify themselves and the parties they represent.

MR. REINES: Edward Reines from Weil Gotshall for Rain Dance.

1 MS. ELLISON: Eldora Ellison
2 together with Deborah Sterling and David Holman
3 for 10X Genomics.

4 THE VIDEOGRAPHER: Our court
5 reporter, Bonnie Russo, representing Veritext
6 will swear in the witness, and we can proceed.

7 PETER DAVID OLMSTED, Ph.D.,
8 being first duly sworn, to tell the truth, the
9 whole truth and nothing but the truth,
10 testified as follows:

11 EXAMINATION BY COUNSEL FOR THE PATENT OWNER

12 BY MR. REINES:

13 Q. Please state your name and address
14 for the record.

15 A. My name is Peter David Olmsted. I
16 reside at 3126 Dumbarton Street, Northwest, in
17 Washington, D.C.

18 Q. What is your current job position?

19 A. I am the Joseph Semmes Ives Chair in
20 physics at Georgetown University. So I'm a
21 professor of physics.

22 Q. You submitted a set of opinions in

1 this case; is that correct?

2 A. I did submit a set of opinions.

3 Q. And how long -- how much work have
4 you done in this case so far?

5 I understand you may need to
6 estimate, but as reasonably accurate as you can
7 be.

8 A. Of the order of 20 hours, 20 to 25
9 hours. Let me think a little bit. I may have
10 --

11 Q. Sure.

12 As I ask you questions today, I want
13 you to do what you need to do to answer it as
14 most -- as accurately as you can do it.

15 A. Sure. Sure. I understand.

16 It's between 20 and 30 hours.

17 Q. Have you ever had your deposition
18 taken before?

19 A. I have not had my deposition taken
20 before.

21 Q. Did you meet with counsel for 10X in
22 preparation for your deposition?

1 A. I did meet in preparation.

2 Q. When was your meeting?

3 A. I met yesterday. And I met briefly
4 earlier in the week on Monday.

5 Q. What was the duration of your
6 meeting yesterday?

7 A. We spend most of the day.

8 Q. Based on the fact that you met with
9 counsel in preparation for the deposition, I'll
10 assume that you have a general understanding of
11 the process. But let me highlight some of the
12 important points.

13 A. Thanks.

14 Q. First, because the court reporter's
15 taking down what's being said, it's important
16 for you to wait for me to complete my question
17 before you give your answer and for me to wait
18 for you to complete your answer before I ask my
19 next question and for your counsel to interpose
20 objections when we're not speaking and so
21 forth.

22 That's not typical conversation.

1 Just sometimes people run afoul of that. And
2 let's all do our best. And I may remind you of
3 that. That is just for purposes of the record.

4 Do you understand those things?

5 A. I understand.

6 Q. During the course of the deposition,
7 you may want to take a break to stretch your
8 legs, use the lavatory, whatever it is. Let me
9 know, and at an appropriate time we'll do that.

10 Is that understandable?

11 A. That's understood, yes.

12 Q. During the course of the deposition,
13 I'll be asking you questions. If you don't
14 understand a question or any aspect of it, let
15 me know. If you don't say anything, I'll
16 assume you understood my question.

17 Is that fair?

18 A. That's fair.

19 Q. Is there any reason you can't give
20 your best, most accurate answers today
21 concerning the subject matter of this case?

22 A. There's no reason.

1 Q. Okay. You're -- you're not taking
2 any medication, are you?

3 A. No.

4 Q. Okay. Do you understand that this
5 proceeding was -- is based on a petition filed
6 by 10X to invalidate the '083 patent?

7 A. I understand the petition involves
8 the patent.

9 Q. Do you know anything about what the
10 -- what's in the petition?

11 A. I haven't seen the petition.

12 Q. Okay. Do you have -- do you know
13 what the theory of the petition was in terms of
14 what the theory of invalidity specifically was?

15 A. I do not --

16 Q. Okay.

17 A. -- know the theory of invalidity.

18 Q. And you understand that originally
19 10X used a different expert, Professor Huck?

20 Did you --

21 A. I understand --

22 Q. -- know that?

1 A. -- that there was another expert.

2 Q. Did you read Professor Huck's
3 original report?

4 A. I have not read Professor Huck's
5 report.

6 Q. Do you have any idea what his
7 original theory was in there regarding
8 invalidity?

9 A. I have not read Professor Huck's
10 report.

11 Q. Right.

12 But do you have an understanding in
13 -- otherwise?

14 A. I have not read Professor Huck's
15 report.

16 Q. Okay. Let me start again.

17 I understand you haven't read
18 Professor Huck's report upon which the petition
19 in this matter is based, but do you have an
20 understanding of what the theory was pursued by
21 Professor Huck in that submission?

22 A. I have not read Professor Huck's

1 report, and I have not discussed the theory of
2 Professor Huck's report.

3 Q. With anybody.

4 A. No. No.

5 Q. I got to be careful about noes
6 because --

7 A. Okay. So let me -- let me --

8 Q. It may be the opposite of what I
9 said.

10 A. Okay. So -- so I have not read
11 Professor Huck's report.

12 Q. Okay.

13 A. So without having read Professor
14 Huck's report, I cannot actually say what
15 Professor Huck's theory is. So I have not had
16 the opportunity to read Professor Huck's report
17 and know what his theory is.

18 Q. Did you ever ask to see Professor
19 Huck's report or the original petition?

20 A. I did not.

21 Q. Have you ever written any paper
22 regarding droplets in microfluidics?

1 A. I wrote a paper in 1997 that
2 involved droplet breakup and formation in
3 microfluidic scales.

4 Q. In microfluidic --

5 A. At microfluidic length scales.

6 Q. What does that mean?

7 A. So what that means is, at the scale
8 of nanometers, which is the typical size at
9 which one finds droplets, I wrote a paper about
10 droplet forming and breaking up in a surfactant
11 solution.

12 Q. Have you ever worked hands-on in a
13 lab with microfluidic devices?

14 A. I have collaborated with
15 experimentalists who had worked with
16 microfluidic devices, and we worked together on
17 experiments involving microfluidic devices.

18 Q. When was that?

19 A. That was in -- so that was in 2008.

20 Q. Would you describe yourself as a
21 theoretician as compared to an experimentalist?

22 A. I am a theoretician.

1 Q. What is the first instance that
2 you're aware of a -- of a droplet approach
3 actually working in a microfluidic device?

4 MS. ELLISON: Objection. Scope.

5 BY MR. REINES:

6 Q. Would you like the question read
7 back?

8 A. No. The -- I think the question was
9 when was I first aware of -- well, please read
10 the question back.

11 Q. Sure.

12 What is the first instance that
13 you're aware of of a droplet approach actually
14 working in a microfluidic device?

15 A. I can't recall exactly when.

16 Q. Do you have any information on that
17 subject at all?

18 A. I started becoming aware of
19 microfluidics shortly after my Ph.D. in 1991.

20 Q. Can you identify any system that
21 you're aware of where droplets actually worked
22 in a microfluidic device?

1 A. I first become aware of
2 microfluidics in 1991. And then through the
3 '90s microfluidics were very prevalent, and
4 became more prevalent so, at conferences
5 starting in the early '90s.

6 And during that time I would have
7 seen a number of talks where people presented
8 results about microfluidic droplet formation.

9 Q. Can you identify any worker that you
10 believe ever made droplets work in a
11 microfluidic system?

12 A. I've seen Stephen Quake, who's the
13 subject of the patent, give talks in the late
14 '90s and early 2000s where he was presenting
15 work on droplet formation.

16 Q. Do you know if he ever actually had
17 a working droplet system, based on what he
18 reported?

19 MS. ELLISON: Objection. Scope.
20 Relevance.

21 THE WITNESS: In his talks he
22 presented work which showed droplet formation

1 in a number of ways, if I recall correctly.

2 BY MR. REINES:

3 Q. Do you have any idea when that was?

4 A. As I've said this was I think in
5 late 1990s or early 2000s.

6 Q. Can you be any more -- by early 2000
7 you mean --

8 A. Early -- '0 --

9 Q. -- the early --

10 A. 2000, 2001, 2 -- somewhere in --

11 Q. Okay.

12 A. -- somewhere in this range.

13 Q. Can you be any more specific than
14 that multirange period?

15 A. I cannot be more specific.

16 Q. Prior to your work in this case, had
17 your heard of Professor Quake?

18 A. I had heard of Professor Quake.

19 Q. Prior to your work in this case, had
20 you heard of Professor Huck?

21 A. I had not heard of Professor Huck.

22 Q. Prior to your work in this case, had

1 you heard of Professor Ismagolov?

2 A. I had not.

3 Q. Prior to your work in this case, had
4 you heard of Professor Whitesides?

5 A. I have heard of Professor
6 Whitesides.

7 Q. And who is Professor Whitesides, to
8 your understanding?

9 A. Professor Whitesides is at Harvard
10 University, and he has worked on microfluidics
11 and a number of other areas.

12 Q. And what is his reputation in the
13 field?

14 A. Professor Whitesides is well known
15 in the field.

16 Q. Have you ever heard of a paper -- of
17 a journal called Physics Today? Physics Today?

18 A. I have heard of Physics Today.

19 Q. And what- - can you describe what
20 that journal is?

21 A. Physics Today is the monthly journal
22 published by the American Physical Society.

1 Q. And have you heard of the journal
2 Nature?

3 A. I have heard of the journal Nature.

4 Q. Would you describe for the record
5 what the stature is of the journal Nature
6 relative to your -- this -- your scientific
7 studies?

8 Let me make it easier.

9 Would you agree that Nature is one
10 of the leading, if not the leading, journal in
11 the scientific field that you work in?

12 A. Nature is seen as a very prestigious
13 journal by many.

14 Q. Let me show you what's been marked
15 previously as Exhibit 2010. You have the nice
16 color version there.

17 Now, I -- I trust you subscribe to
18 Nature, have access to Nature on a regular
19 basis?

20 A. I do not subscribe to Nature, but I
21 do have access to Nature through the Georgetown
22 University library system.

1 Q. And you consider yourself an expert
2 in microfluidics; is that right?

3 A. I am an expert in fluid mechanics
4 and interfacial physics, which is the
5 underlying science behind microfluidic.

6 Q. Do you consider yourself an expert
7 in the field of microfluidics itself?

8 A. I'm an expert in the fluid mechanics
9 and interfacial science that underlies
10 microfluidics and, in particular, in the
11 properties of flow and confined geometries
12 involving surfactant fluidics.

13 Q. And why are you reluctant to say
14 that you're an expert in microfluidics beyond
15 that?

16 In other words, why don't you just
17 say, "Yes, I am an expert in microfluidics"?
18 What's the caveat there?

19 MS. ELLISON: Objection. Form.

20 THE WITNESS: I'm an expert in the
21 scientific areas that are necessary to both
22 understand and deploy microfluidic devices --

1 BY MR. REINES:

2 Q. Right.

3 A. -- that includes the physics.

4 Q. Right.

5 The -- the flow in confined spaces.

6 A. Exactly.

7 Q. That's your expertise, correct?

8 A. Yep.

9 Q. But you don't -- but you -- you
10 wouldn't call your -- you wouldn't hold
11 yourself out to the patent office as an expert
12 in other aspects of microfluidics, such as
13 the -- the type of injection equipment you use
14 to get the droplets in the system; is that
15 right?

16 MS. ELLISON: Objection. Form.

17 THE WITNESS: I can -- I -- excuse
18 me. My expertise in fluid mechanics allows me
19 to assess injection devices and to try to
20 provide design criteria for things such as
21 injection device. Microfluidics is a huge
22 field, so there are many areas in which I'm an

1 expert in.

2 BY MR. REINES:

3 Q. Now, in your Georgetown web site,
4 right, that -- you have a web site on
5 Georgetown that describes your work and your --

6 A. I --

7 Q. -- research that has --

8 A. I do have a web site.

9 Q. Right.

10 There's no mention of microfluidics;
11 is that right?

12 A. I don't recall what is on my web
13 site.

14 Q. Do you -- can you say that there is
15 any such reference to microfluidics in your web
16 site?

17 A. I cannot say because I -- I can't
18 recall what I've put on it.

19 Q. Okay. And is there any reference to
20 droplets as being an area of expertise for you
21 on your web site at Georgetown?

22 A. I don't think there is a specific

1 reference to droplets on my web site. The
2 reason I'm saying I don't think is I work in a
3 number of areas, as you have seen.

4 Q. Have you ever personally used a
5 microfluidic device?

6 A. I've worked with experimentalists
7 who have used microfluidic --

8 Q. Right.

9 A. -- device.

10 Q. But the question is have you ever
11 used a microfluidic device?

12 A. I've -- I've worked with the
13 experimentalists who've used it.

14 Q. Okay. But --

15 A. I have advised on the experiments
16 that they're doing. I have helped analyze
17 their data. I've provided calculations which
18 underlie the -- both design of the experiments
19 as well as the analysis of the data. So in
20 this respect, I've worked with the microfluidic
21 devices.

22 Q. Okay. So it's -- it's important --

1 I -- I understand what you're trying to do, but
2 at some point it's -- you need to be responsive
3 to the question. So I don't mind you -- if you
4 think you need to provide context to give an
5 accurate answer, if that's a fair thing, then
6 I'm not going to -- not -- as you can tell, I'm
7 letting you do that. I'm not contesting that.

8 But when you give me kind of a mixed
9 answer and then I ask a specific piece of it,
10 I'd appreciate if you just directly answered my
11 question. You can do it how you want, but I'm
12 going to make a record about it. So -- and we
13 may disagree with opposing counsel or whatever.

14 But I mean when I ask you something
15 very specifically like that, I -- I think I
16 deserve a straight answer. So you'll have to
17 decide how much you want to give straight
18 answers or not as we go through.

19 Have you ever personally fabricated
20 a microfluidic device?

21 Leave aside what other people might
22 have done.

1 A. I have advised on the construction
2 of devices.

3 Q. But you haven't --

4 A. And --

5 Q. -- personally fabricated them,
6 right?

7 MS. ELLISON: Ed, I think you
8 interrupted the witness.

9 MR. REINES: Oh, I'm sorry.

10 MS. ELLISON: Let him finish his
11 answer.

12 THE WITNESS: My -- my role has been
13 as a theoretician involved in the project.
14 My -- my role has been to advise on the
15 devices.

16 BY MR. REINES:

17 Q. Okay. So let me maybe do it this
18 way: Because you're a theoretician --

19 A. Theoretician.

20 Q. -- theoretician --

21 A. It's okay.

22 Q. -- you have not personally used,

1 fabricated or created microfluidic devices
2 yourself, but you've advised in that process.

3 Is that --

4 A. I have advised --

5 Q. -- a fair summary?

6 A. I have advised in the process of --

7 Q. Okay.

8 A. -- making microfluidic devices.

9 Q. And do you know whether any of those
10 systems were used for -- with a droplet
11 approach?

12 A. I do not know.

13 Q. Other than Stephen Quake, do you
14 know of anyone else that you believe has worked
15 with droplets in microfluidic devices?

16 A. Droplet formation is routinely used
17 and shown in conferences, which is where I
18 usually interact with scientists. So there are
19 many -- many scien -- many instances where I
20 have seen droplet formation used as part of a
21 research tool.

22 A collaborator of mine from -- who

1 is in France named Dr. Pascal Panizza has
2 worked on droplet formations. That's an
3 example.

4 Q. Other than Dr. -- I assume Dr.
5 Panizza --

6 A. Professor.

7 Q. -- Professor Panizza and Professor
8 Quake, can you identify anyone else that's
9 worked in the area of droplets in microfluidic
10 devices?

11 A. We've already mentioned Professor
12 Whitesides.

13 Q. Okay. Anyone else?

14 A. There's Professor Patrick Tablin at
15 ESPCI in France.

16 Q. Does he work with Professor Panizza?

17 A. No.

18 Q. Oh, it's a different branch group?

19 A. He's from a different group.

20 Q. What was the state of the art in May
21 2002 in the area of droplets in microfluidic
22 devices?

1 MS. ELLISON: Objection. Scope.

2 THE WITNESS: So in 2002, before
3 that time I had already seen, as I mentioned,
4 Stephen Quake give some quite impressive talks.
5 And there were others whose names I cannot
6 remember who were also working in the field.
7 So my impression at the time was that there was
8 already substantial progress made.

9 BY MR. REINES:

10 Q. What were the challenges for making
11 a working droplet system in microfluidic device
12 in May of 2002?

13 MS. ELLISON: Objection. Scope.

14 THE WITNESS: I cannot say with
15 certainty what the challenges were specifically
16 in making microfluidic drop -- droplet devices
17 in microfluidics because that's not an area in
18 which I was actively working with engineers at
19 that point.

20 BY MR. REINES:

21 Q. Based on your work in this case, can
22 you answer the question?

1 MS. ELLISON: Same objection.

2 Scope.

3 THE WITNESS: The work on this case
4 involves -- well, the work that I have been
5 asked to look at involves the properties of --
6 of the different fluids, the carrier fluid and
7 the aqueous fluid, and any additives and how
8 one uses these properties to be able to make
9 droplets. And --

10 BY MR. REINES:

11 Q. But -- but answer my question, if
12 you would.

13 A. And there are significant challenges
14 posed.

15 Q. What are the most significant
16 challenges -- what were the most significance
17 challenges in May 2002 in trying to make a
18 working droplet system in microfluidic devices,
19 if you know?

20 MS. ELLISON: Objection. Scope.

21 THE WITNESS: So the challenges
22 faced at that time are the kinds of challenges

1 that Quake, in fact, tries to address. And the
2 prime challenge would be to control the droplet
3 size and to control the uniformity of the
4 droplets.

5 BY MR. REINES:

6 Q. Anything else?

7 A. It is necessary, if you make a
8 device, to be able to do all of this in a
9 reproducible manner. The principles have been
10 long established before then.

11 Q. Now, if I understand you, the field
12 of microfluidics was very active in the 1990s;
13 is that correct?

14 A. (Witness nodding head.)

15 Q. You just need to answer --

16 A. Yes.

17 Q. -- audibly.

18 A. Sorry. That's correct.

19 Q. Starting from the early 1990s,
20 according to you, right?

21 A. Yes.

22 Q. How come it took until Professor

1 Quake's work that you referred to, the --
2 identified the patent publication, for droplets
3 to be addressed in terms of what the challenges
4 were?

5 MS. ELLISON: Objection. Scope.

6 THE WITNESS: I didn't say that it
7 took until 2002 until Professor Quake's work.

8 BY MR. REINES:

9 Q. Okay. Are you aware of any -- oh,
10 sorry. You go.

11 A. Go ahead.

12 Q. I was going to -- are you aware of
13 any working droplet system, other than -- that
14 occurred before the reports you've heard from
15 Professor Quake during this sort of four- or
16 five-year period in which, you know, you don't
17 know when it is, the end of the '90s and the
18 beginning of the 2000s?

19 A. Beginning in the early '90s, at
20 conferences, I started to see significant
21 amounts of microfluidics, including droplet
22 formation.

1 Q. Were any of those working droplet
2 systems?

3 A. I saw droplets being formed, and I
4 saw scientific research being conducted in
5 droplet formation in microfluidic geometry.

6 Q. Right.

7 But did you -- did you see a -- a
8 working microfluidic device with droplets being
9 reproducibly used?

10 A. I saw reproducible scientific data
11 which would have been produced from
12 microfluidic devices fabricated by -- I don't
13 know which methods.

14 Q. And do you know who's doing this
15 work?

16 A. I don't recall.

17 Q. So you were -- what were the issues
18 with the droplet systems that you learned about
19 in the 1990s that prevented, you know, industry
20 from being able to use them at the engineering
21 level?

22 A. I don't know what the issues were.

1 Q. How come you don't know?

2 A. So at that time most of the -- my
3 contact was at scientific conferences where the
4 emphasis was on the science of producing the
5 drops and not discussion at the scientific
6 conferences about applications to devices.

7 Q. Just so that the record's clear,
8 because there's been a little back and forth on
9 this, prior to what you gathered from the
10 reports from Professor Quake at some time that
11 you can't specify, but within a four- or
12 five-year period, are you aware of anyone else
13 reporting on successful experiments with
14 droplets on a microfluidic device; and if so,
15 who?

16 MS. ELLISON: Objection. Scope.

17 THE WITNESS: I cannot recall names
18 during that period before.

19 BY MR. REINES:

20 Q. And in terms of what challenges --

21 A. But I --

22 Q. Oh, sorry.

1 A. But I recall seeing successful
2 droplet formation being talked about at
3 conferences.

4 Q. What the problems were in the 1990s
5 that were faced by those trying to apply
6 droplets to microfluidic devices, that's not
7 something you know about because of your --
8 because of your status as a theoretician; is
9 that correct?

10 MS. ELLISON: Objection. Form.

11 THE WITNESS: Can you restate that?

12 BY MR. REINES:

13 Q. Yes.

14 What the problems were in the 19 --
15 let me start again.

16 You're not familiar with the
17 problems -- what the problems were in the 1990s
18 that were faced by those trying to apply
19 droplets to microfluidic devices, correct?

20 MS. ELLISON: Objection. Form.

21 THE WITNESS: I wasn't discussing
22 the problems with people at that time.

1 BY MR. REINES:

2 Q. So you don't know.

3 A. When -- when people gave scientific
4 talks, they talked about the science of making
5 droplets, which is a nontrivial science.

6 Q. That wasn't my question, was what
7 they were talking about.

8 My question is -- you're holding
9 yourself out as an expert for the patent office
10 on the subject, and my question is can you
11 reliably state what the problems were that were
12 faced by those trying to apply droplet science
13 to microfluidic devices in the 1990s?

14 MS. ELLISON: Objection. Form.
15 Foundation.

16 THE WITNESS: The problems that
17 would have been faced would have been exactly
18 the problems as I said before that Quake
19 discusses in his patent. And these are the
20 problems of how to form a droplet reproducibly
21 at a controlled size; and then, once you have
22 formed these droplets, how do you control it as

1 it flows to where you want it to go.

2 BY MR. REINES:

3 Q. And in terms of addressing those --
4 that problem set that you just described,
5 creating droplets that are reproducible at a
6 controlled size and with controlled flow, what
7 are the -- the variables that go into that?

8 MS. ELLISON: Objection. Scope.

9 THE WITNESS: The variables that go
10 into that include the properties of the two
11 fluids, the geometry within which the fluids
12 are flowing, the flow rates that are imposed on
13 the fluids in the geometries and the properties
14 at the interface between the two fluids.

15 BY MR. REINES:

16 Q. How complex a problem was that in
17 May 2002?

18 MS. ELLISON: Objection. Scope.

19 THE WITNESS: The basic principles
20 have been around for many, many years before.
21 And they had been employed already to make
22 successful drops in the decade before 2002.

1 The challenges posed, for example,
2 in the Quake patent involve trying to do it in
3 a -- to -- to make the drops in a -- in a
4 device which they could market.

5 My understanding is that there were
6 already devices before that. At the -- the
7 field is -- was reasonably mature.

8 BY MR. REINES:

9 Q. And when you say the field was
10 reasonably mature, you're saying in May 2002
11 the field of droplets in microfluidic devices
12 was relatively mature, correct?

13 That's your testimony?

14 MS. ELLISON: Objection. Form.

15 THE WITNESS: The understanding of
16 how one uses these variables to create droplets
17 is well established.

18 BY MR. REINES:

19 Q. Okay. But you can't name any worker
20 other than Quake that did this in the 1990s,
21 correct?

22 MS. ELLISON: Objection. Scope.

1 THE WITNESS: I was at a number of
2 conferences and saw a number of talks where
3 people were making microfluidic -- making
4 droplets before then.

5 BY MR. REINES:

6 Q. Right.

7 You can't identify anyone prior to
8 Professor Quake that demonstrated the use of
9 droplets in a microfluidic device, correct?

10 Can you name someone or not?

11 That seems like a straight question
12 that submits to a "yes" or "no" answer.

13 Are you still considering --

14 A. So --

15 Q. -- the question?

16 A. So as I've said, I -- I -- I went to
17 a number of conferences and heard a number of
18 talks in the 1990s, before I heard Quake, by
19 workers talking about droplet formation.

20 Q. But you can't identify anyone,
21 correct, by name?

22 A. Quake was the most flamboyant that I

1 saw, so that was the name that jumps out at the
2 time.

3 Q. Can you identify any other names?

4 MS. ELLISON: Objection. Scope.

5 BY MR. REINES:

6 Q. There's no shame in just saying you
7 can't. So -- but if you want to continue this
8 way, that's okay. I've seen worse.

9 A. I can't recall the names of the
10 people that I saw at the conferences.

11 Q. Have you ever had a publication that
12 was accepted by Nature magazine?

13 A. I have not had a publication in
14 Nature.

15 Q. Who's more prominent in the field of
16 microfluidics, you or Professor Whitesides?

17 A. I would have to say Professor
18 Whitesides is more prominent in the field of
19 microfluidics than myself.

20 Q. If you'd pick up Exhibit 2010,
21 please.

22 A. Uh-huh.

1 Q. Do you have that in front of you?

2 A. I have it.

3 Q. And what year is this article?

4 A. So this article is in 2006.

5 Q. Would you read the bolded abstract
6 of this paper in -- from Nature to yourself.

7 A. Okay.

8 Q. Do you see where Professor
9 Whitesides in 2006 states that the field is
10 still at an early stage of development?

11 A. I see that.

12 Q. Do you disagree with Professor
13 Whitesides regarding the stage of microfluidics
14 in 2006?

15 MS. ELLISON: Objection. Scope.

16 THE WITNESS: So microfluidics is
17 really a technology analogous to semiconductors
18 or lasers. So these technologies have
19 undergone evolution from basic science,
20 demonstrations, applications. And both those
21 industries continue to develop for over half a
22 century.

1 Now, microfluidics I believe will be
2 on a par with these industries for how they
3 influence society.

4 Whitesides points out many
5 applications and demonstrations that have
6 already existed prior to this time. And he
7 states the field's at an early stage of
8 development. And I believe what he means by
9 "development" is development of large-scale
10 commercial applications.

11 The demonstration applications
12 includes protein crystallization, separations
13 in spectroscopy, screening for drug development
14 and calculations of cells and proteins and
15 single molecules.

16 So there are many applications and
17 fabrication techniques that he already outlines
18 in the paper.

19 BY MR. REINES:

20 Q. One of the big challenges that
21 Professor Whitesides identifies in 2006 is
22 choosing and focusing on initial applications

1 for microfluidics; is that correct?

2 This is in the bolded three-sentence
3 abstract at the front. I guess fourth
4 sentence. Maybe five sentence.

5 MS. ELLISON: (Inaudible.)

6 MR. REINES: Felt like I took a
7 break at that last question and answer.

8 THE WITNESS: He -- he states
9 choosing and focusing on initial applications.

10 BY MR. REINES:

11 Q. Okay. So you'll agree with
12 Professor Whitesides that the -- the selection
13 and focusing on initial applications is
14 something that was in the era of 2006?

15 MS. ELLISON: Objection. Form.

16 THE WITNESS: He outlines many
17 applications, most of which occur -- I mean all
18 of them occurred before this paper, obviously.

19 So my reading of this choosing of
20 initial applications is to choose which of
21 these applications -- which already existed
22 before this paper -- which of these

1 applications he would -- are ripe for
2 commercial exploitation. That's my
3 understanding. The applications are noted --
4 many of them are already noted in the paper.

5 BY MR. REINES:

6 Q. And Professor Whitesides says the
7 solution to the problems in microfluidics will
8 require imagination and ingenuity in 2006.

9 Do you agree with that statement?

10 It's in that same bolded --

11 A. Sure.

12 Q. -- text.

13 A. To progress from initial
14 applications, demonstration applications, to
15 full-scale commercial development always
16 requires imagination and ingenuity.

17 Q. And turn to the conclusion, please.
18 And one of the things he states in the
19 conclusion is that he's helpful that
20 microfluidics can become more than an active
21 field of academic research.

22 Do you see that?

1 A. I see that.

2 Q. And in 2006 were you hopeful for it
3 to become something more than an active field
4 of academic research?

5 MS. ELLISON: Objection. Form.
6 Foundation.

7 THE WITNESS: It was clear to me in
8 2006 it was going to be more -- that it, in
9 fact, was already more than an academic field
10 of interest.

11 BY MR. REINES:

12 Q. Do you see where Professor
13 Whitesides says the field of microfluidics is
14 in its infancy?

15 It's in his conclusion.

16 A. Where he says: "It is also in its
17 infancy."

18 Q. Do you see that?

19 A. I see that.

20 Q. And he says a great deal of work
21 needs to be done before it can be claimed to be
22 a more than an -- a field of academic research.

1 Do you see that?

2 A. I see that.

3 Q. Do you agree or disagree with that
4 statement?

5 A. I disagree that it was only an
6 active field of academic research in 2006.

7 Q. Okay.

8 MS. ELLISON: Can we take a break?

9 MR. REINES: All right. Yes. I was
10 waiting for that answer. I didn't anticipate
11 the time.

12 MS. ELLISON: Thank you.

13 THE VIDEOGRAPHER: We are going off
14 the record at 10:42 a.m.

15 (A short recess was taken.)

16 THE VIDEOGRAPHER: We are back on
17 the record at 10:52 a.m.

18 This is the start of Media Unit No.
19 2 in the deposition of Peter Olmsted.

20 BY MR. REINES:

21 Q. If you'd turn to Page 372, if you're
22 not there already.

1 A. I am.

2 Q. And under "New Science and
3 Technology," do you see where Professor
4 Whitesides states in this science publication:
5 "The development of microfluidics has just
6 begun"?

7 Do you see that?

8 A. I see that.

9 Q. Okay. And is this journal peer
10 reviewed?

11 A. The articles and letters in Nature
12 are peer reviewed. I don't know whether an
13 overview is peer reviewed, but presumably it
14 is.

15 Q. And do you agree with Professor
16 Whitesides' statement that the development of
17 micro -- microfluidics has just begun as of the
18 2006 time period?

19 Can you answer the question?

20 A. Yes.

21 So in the paper he points out a
22 number of applications which go back as far

1 as -- from what I can see, as far as 1997. So
2 in that period up until 2006, he outlines a
3 number of developments. So for the 10 or 15
4 years so before this paper is when the
5 developments had really started to take off.

6 And he does make the point --

7 Q. Yes?

8 A. -- about -- sorry. I lost my --
9 well, he makes the point that there are many
10 applications still to come for commercial
11 applications. But he outlines a lot that's
12 already happened in terms of development of
13 applications. So --

14 Q. Was there demand for microfluidic
15 systems that would work using droplets such as
16 described in the '083 patent in the 1990s?

17 MS. ELLISON: Objection. Scope.

18 THE WITNESS: So in the 1990s there
19 was demand for many of the applications that
20 droplet technologies will provide.

21 BY MR. REINES:

22 Q. And --

1 A. Such as protein crystallization or
2 bioanalyses.

3 Q. And did that -- did that need begin
4 as -- I mean were you aware of that when you --
5 after you said you started microfluidics in the
6 early 1990s?

7 MS. ELLISON: Objection. Form.
8 Scope. Relevance.

9 THE WITNESS: Can you repeat the
10 question?

11 BY MR. REINES:

12 Q. Yes.

13 You -- you testified that in the
14 1990s there were demands for applications for
15 droplet technology in -- in the context of
16 microfluidics.

17 My question was was -- were those
18 existing when you entered the microfluidics
19 field in early 19 --

20 A. I believe I stated that there was a
21 demand for the applications that --

22 Q. Droplet --

1 A. -- droplet technologies can provide.

2 Q. Yeah.

3 A. Yeah.

4 Q. Exactly.

5 A. Okay. I -- I didn't state there was
6 a demand for droplet technologies, I believe.

7 Q. Okay.

8 A. Okay.

9 Q. Then let's be very clear of that
10 then.

11 A. So what -- what was my testimony?
12 Sorry.

13 Q. You have to -- you have to --

14 A. I don't have it written in front of
15 me, so...

16 Q. You have to be the source of your
17 testimony.

18 A. Okay.

19 Q. Let's just go back to you testified
20 that, in the 1990s, there was a need for the
21 performance that droplet technology could
22 provide, correct?

1 MS. ELLISON: Objection. Form.
2 Foundation.

3 BY MR. REINES:

4 Q. Is that right?

5 MS. ELLISON: And scope.

6 THE WITNESS: I believe I stated
7 that there was a demand for the -- some of the
8 applications that droplet technologies can
9 provide.

10 BY MR. REINES:

11 Q. Okay. And --

12 A. And I quoted protein crystallization
13 and biological analyses as examples.

14 Q. Okay. Was the need for the
15 applications that droplet technology can
16 provide existent in the early 1990s --

17 MS. ELLISON: Objection. Form.
18 Foundation.

19 BY MR. REINES:

20 Q. -- when you entered the
21 microfluidics field?

22 MS. ELLISON: Objection. Form.

1 Foundation.

2 THE WITNESS: In the 1990s there was
3 a need for the application that droplet
4 technologies provide, which includes protein
5 crystallization, bio and chemical analyses of
6 small examples. And this existed in the '90s.

7 BY MR. REINES:

8 Q. I understand it existed in the '90s.

9 I've been asking several times did
10 it exist when you entered the microfluidics
11 field in the early 1990s? Were you aware of it
12 then?

13 MS. ELLISON: Objection. Form.
14 Scope. Relevance.

15 THE WITNESS: It existed in the
16 1990s.

17 BY MR. REINES:

18 Q. Throughout the 1990s.

19 Is that what you're saying?

20 A. Yes.

21 Q. Okay. And was there also a need at
22 that time -- the same time frame for

1 reproducible droplet technology where you could
2 control the size, uniformity and flow of the
3 droplets?

4 Well, let's just make it -- let me
5 make it simpler.

6 Throughout the 1990s was there a
7 need for a droplet system that would -- could
8 be reproducible and allow control of the flow
9 in order to perform analyses such as on
10 biomolecules?

11 MS. ELLISON: Objection. Scope.
12 Foundation.

13 THE WITNESS: There was a need to
14 analyze to particular biological materials,
15 such as proteins for crystallization, or small
16 biological samples, such as cells.

17 Whether that need could be satisfied
18 only by droplet technologies is something I
19 cannot answer. A -- a need then can be
20 satisfied, and then there are of course many
21 different ways.

22 There was a need, and Quake's

1 patent, for example, outlines a claim for
2 satisfying this need.

3 BY MR. REINES:

4 Q. Can you say one way or the other --
5 do you know enough to say one way or the other
6 whether in the 1990s there was a need for a
7 droplet system that would be reproducible and
8 allow control of the flow sufficient to perform
9 analyses, for example, biomolecules?

10 If you don't know, that's fine. If
11 you do know, then say "yes" or "no" or
12 whatever.

13 A. There was a need to look at
14 biological samples at a small scale. And
15 droplet technologies which can control the flow
16 in ways to make reproducible small scale
17 samples that can be analyzed as biological
18 material, that is one technology that could
19 provide -- satisfy such a need.

20 Q. Now let's talk about Exhibit 1004,
21 which is the Quake patent application you
22 referred a few times. I know you've got --

1 A. Okay.

2 Q. -- your own set of materials you've
3 been --

4 MS. ELLISON: I'll look at this one.

5 BY MR. REINES:

6 Q. -- looking at. Well, you can look
7 at -- there's no magic to this one verse the
8 other one, but I'm giving it to you to make
9 sure you have it.

10 A. Uh-huh.

11 Q. So you have Exhibit 1004 in front of
12 you that's the Quake patent application?

13 A. I do.

14 Q. Do you know whether there's any
15 existing valid patent out of this patent
16 application?

17 A. Sorry. Can you repeat the question?

18 Q. Do you know whether there's an
19 existing valid patent out of the Quake patent
20 application that you're relying on?

21 A. I believe there is, but I'm not a
22 hundred percent sure.

1 Q. And what's your basis for believing
2 there is?

3 A. I'm not a legal expert, but my
4 understanding is that if this had not become a
5 full patent, there wouldn't be a dispute
6 happening now.

7 Q. What's your basis -- what's your --

8 A. I'm not --

9 Q. -- logic?

10 A. -- a legal expert.

11 Q. But what's your logic on that?

12 A. My logic on that is that, if it's a
13 patent, then it is -- let me see. That -- if
14 -- in the case of a successful patent, certain
15 rights are protected by the patent by law, and
16 that this dispute then relates to those rights.

17 Q. Okay. You understand that this case
18 relates to whether the '083 patent is valid?

19 Do you understand that?

20 A. I do understand that.

21 Q. Okay. And you understand that this
22 -- as a legal matter, the -- the right as to

1 whether Quake has patentable inventions in his
2 patent publication -- patent application
3 publication, that's not what's being challenged
4 directly in this litigation.

5 Do you understand that?

6 Do you understand that --

7 A. Say that again.

8 Q. The legal -- that there's no legal
9 question in this case as to whether Quake is
10 valid or not as a patent? Do you understand
11 that? Because it just -- you're just relying
12 on a patent application. Well, let me step
13 back.

14 You understand you're just relying
15 on a patent application as prior art, correct?

16 A. I understand this is --

17 Q. Okay.

18 A. -- the patent application.

19 Q. And you have no idea whether that is
20 currently valid -- a valid patent's been issued
21 off of that, right?

22 That's not something you've looked

1 at in your 20 and 30 hours' work on this?

2 Well, do you know?

3 A. I -- I've answered that question. I
4 think it is, but I don't know for sure.

5 Q. And the basis for thinking it is is
6 because you think this is a competition -- this
7 proceeding is a competition as to whether the
8 Quake patent application's valid or the '083
9 patent's valid; is that correct?

10 A. No, I don't think that's correct.

11 Q. Okay. What is your --

12 A. If -- if --

13 Q. Let me just ask a simple question.
14 What is your basis for stating that
15 you believe that the Quake has a -- currently
16 has a valid patent out of this application?

17 A. I stated that, if it -- I'm not a
18 legal expert. My understanding is that, if it
19 becomes a full patent, then there are certain
20 rights and protections afforded that patent
21 that have an influence on the '083 patent.

22 Q. Where did you gain that

1 understanding?

2 MS. ELLISON: I'm just going to
3 caution the witness not divulge the substance
4 of any communications with counsel.

5 THE WITNESS: Uh-huh.

6 MS. ELLISON: But other than that --

7 MR. REINES: Well --

8 MS. ELLISON: -- he can answer the
9 question.

10 MR. REINES: -- well, if he is
11 relying on it.

12 MS. ELLISON: We're not going to
13 waive privilege, so...

14 BY MR. REINES:

15 Q. Isn't it the truth that you have no
16 idea what patents, if any, have issued off of
17 the Quake application? Isn't that really true?

18 A. So I cannot say for certain whether
19 this has become a patent or not.

20 Q. Do you know whether the technology
21 in the Quake patent actually works -- in the
22 Quake patent application publication actually

1 works?

2 A. Yes, it does work.

3 Q. How do you know that?

4 A. So I've seen them demonstrated in
5 talks. And Whitesides actually points out
6 successful applications based upon it.

7 Q. When you say you've seen Quake
8 demonstrated at talks, how do you know that
9 those are the device that is described in this
10 patent application publication?

11 A. I can't say for sure that they use
12 the exact same device or devices.

13 Q. Do you have --

14 A. But the talks --

15 BY MR. REINES:

16 Q. -- any idea what surfactant --

17 MS. ELLISON: Sorry. I don't think
18 he was finished.

19 THE WITNESS: But the -- the talks
20 were about that same time, 2000, 2001, 2002,
21 around then, which is when this work would have
22 been done.

1 BY MR. REINES:

2 Q. Do you have any idea what
3 surfactants Professor Quake actually used?

4 A. In which situation?

5 Q. In any.

6 A. In the patent application, Quake
7 denotes a number of different surfactants that
8 can be used.

9 Q. Right.

10 My question is, in the real world,
11 do you know which ones he actually used?

12 A. In which particular case?

13 Q. Any case.

14 A. So in any case, no, I do not. It
15 would have been a number -- one of a number of
16 the -- surfactants that he or classes of
17 surfactants that he talks about in his patent.

18 Q. Can you identify at all even one
19 type of surfactant that you know Quake actually
20 used successfully?

21 MS. ELLISON: Objection. Scope.
22 Relevance.

1 THE WITNESS: An example would be
2 Tween.

3 BY MR. REINES:

4 Q. So you know he used that?

5 A. He mentions Tween.

6 Q. He mentions a lots of things.

7 A. Yes.

8 Q. Let me be clear.

9 There's a difference between someone
10 mentioning in a list options and actually using
11 a material.

12 Do you understand the distinction
13 between those two things?

14 A. I understand.

15 Q. Okay. My question's not focused on
16 lists of what he identifies as potential
17 options. I'm asking about what he actually
18 used.

19 Do you know what Professor Quake
20 actually uses as a surfactant? Can you
21 identify even one?

22 MS. ELLISON: Objection. Scope.

1 Relevance.

2 THE WITNESS: If I read a scientific
3 paper which states that you can use X, Y and Z,
4 which are named quite specifically, then as a
5 professional scientist my understanding is that
6 he will have used a surfactant from among that
7 list.

8 BY MR. REINES:

9 Q. When you say "from among that list,"
10 you mean one from among the list?

11 A. That's what I mean.

12 Q. Okay. And -- but you have no idea
13 which one Professor Quake actually used, right?

14 MS. ELLISON: Objection. Scope.
15 Relevance.

16 THE WITNESS: My assumption would be
17 that he would have used many.

18 BY MR. REINES:

19 Q. But you don't have any factual
20 knowledge of that, do you? Not just surmise?

21 A. So in Paragraph 200 of Quake on page
22 45, in Paragraph 209 of Quake on Page 46, he

1 specifically talks about example systems that
2 contain Tween.

3 Q. And so based on that, you concluded
4 that he's used that surfactant; is that
5 correct?

6 A. That's one example of a surfactant
7 that he will have used.

8 Q. Right.

9 And you're basing that just on this
10 application; is that correct?

11 A. This application states that he used
12 Tween as an example.

13 Q. Right. But --

14 A. This was in Example 7. So it
15 doesn't mean that he exclusively used Tween
16 because he specifically talks about a number of
17 different surfactants.

18 Q. Right.

19 And when a -- a scientist puts a
20 list of them, then you assume that they used at
21 least one of them; is that correct?

22 I believe that was your testimony

1 earlier.

2 A. That can often be the case.

3 Q. Did you use -- when you collaborated
4 on droplet technology, which is what you
5 testified you did --

6 A. I collaborated -- excuse me for
7 interrupting. I collaborated on microfluidic
8 devices.

9 Q. Did you ever --

10 A. Microfluidic --

11 Q. -- collaborate with anyone on
12 droplet technology in the context of a
13 microfluidic device?

14 A. My collaborations were involved in
15 developing devices to look at flow fields and
16 understand the flow fields in particular kinds
17 of fluids.

18 Q. Right.

19 But did you ever -- did you ever
20 develop a droplet system in the context of
21 microfluidics?

22 A. So my research was in using

1 microfluidic devices to understand the flow
2 fields. And there was a research project
3 involved in understanding the appearance of
4 droplets in a particular system.

5 Q. Okay. Whatever work you're saying
6 you did with droplets --

7 A. Okay.

8 Q. -- I -- I -- it's -- you know, it's
9 too much energy to try to get a straight
10 answer, to be honest.

11 Whatever work you say you did on
12 droplets, did it involve fluorinated oil as a
13 carrier?

14 Can you answer that?

15 A. My own work has not involved
16 fluorinated oils in my research.

17 Q. At any -- have you done any research
18 that's involved fluorinated oils at any time?

19 A. I have collaborated with a group who
20 used fluorinated materials at surfaces in
21 polymers.

22 Q. Was that droplet technology?

1 A. That was not related to droplet
2 technologies.

3 Q. Was it related to microfluidics?

4 Well, if I say "related," then that
5 opens I guess the world up.

6 With all the --

7 A. It was related to the development of
8 surface coatings.

9 Q. With all the droplet technology that
10 you've worked with over the years dating back
11 to the 1990s, according to you, did you -- how
12 come you never experimented with fluorinated
13 oils?

14 A. As I've explained, I'm a
15 theoretician. So I have not conducted
16 experiments myself.

17 Q. But you --

18 A. And I have worked with
19 experimentalists.

20 Q. Yeah. I think the impression you've
21 given here today is --

22 A. Uh-huh.

1 Q. -- that you're a theoretician, and
2 you collaborate with experimentalists --

3 A. That's correct.

4 Q. -- so that you're knowledgeable
5 about experimental work with --

6 A. That's correct.

7 Q. -- microfluidics, correct?

8 A. Uh-huh.

9 Q. That's how you're representing
10 yourself, right?

11 A. That's correct.

12 Q. Okay. So you can't -- when I'm
13 asking you about it, I -- we'll see if you can
14 deny it, but I don't think -- you can't have it
15 both ways, the way I look at it.

16 A. I understand.

17 Q. In the collaborative work you've
18 done in the droplet field -- and I understand
19 that you -- your testimony is you've worked
20 extensively in droplets dating back to the
21 early 1990s -- have you ever worked with
22 fluorinated oils?

1 A. I have not.

2 Q. How come you've avoided them?

3 A. I have --

4 MS. ELLISON: Objection.

5 Foundation.

6 THE WITNESS: I have not worked with
7 them.

8 BY MR. REINES:

9 Q. Why not?

10 A. There are many things I have not
11 worked with.

12 Q. Right.

13 And I'm asking about fluorinated
14 oils because they're the one that's important
15 to this case.

16 You understand that the use of
17 fluorinated oil as a carrier is an important
18 issue in this case?

19 A. I do.

20 Q. Okay. And you've never worked with
21 them, right? Fluorinated oils?

22 A. I have not worked with fluorinated

1 oils.

2 Q. Okay. And have you done any of your
3 theoretical work in the context of fluorinated
4 oils?

5 A. So the theoretical work that I've
6 done in fluids will encompass and have been
7 focused on areas such as hydrophobic and
8 hydrophilic fluids and how surfactants can
9 behave at the interface.

10 Now, all of that work is relevant
11 for different kinds of immiscible fluids,
12 whether they be aqueous in water -- sorry --
13 sorry -- immiscible fluids such as water and
14 oil or different kinds of oils.

15 So from a theoretical point of view,
16 whether the oil is a fluorinated oil or a
17 hydrocarbon oil is a change in a parameter in
18 the theory, but it doesn't change the
19 application of the theory.

20 So in that context, theorys that
21 I've worked on related to self-assembly and
22 interfacial structure in oil-water systems are

1 applicable to the general class of immiscible
2 fluids, which incorporates fluorinated oils.

3 Q. Is that as direct as you can be in
4 answering my question?

5 A. So the answer is yes, fluorinated
6 oils fall under class of materials that
7 theories will apply to that I would have worked
8 on, but not specifically fluorinated oils.

9 Q. Do you have any explanation for why
10 all of the droplet work you've use -- you've
11 work -- you've been involved with over the
12 decades you've never selected with your
13 collaborators fluorinated oil as the carrier?

14 A. So the fundamental work that I've
15 been work -- doing is related to behavior of
16 liquids with surfactants which involve oils and
17 waters. And when in -- doing experiments to
18 investigate the fundamental science, in many
19 cases fluorinated oils aren't necessary.
20 They're more expensive, and experimentalists
21 can use hydrocarbon oils to understand the same
22 phenomenon.

1 Q. Other than --

2 A. I under --

3 Q. I'm sorry.

4 A. I understand that in applications
5 fluorinated oils are important.

6 Q. Other than expense, is there any
7 other justification you can give for why, in
8 all your droplet work over the decades dating
9 back to the early 1990s, you've never selected
10 fluorinated oil?

11 A. I have nothing else to say for that,
12 no.

13 Q. Have -- have fluorinated oils been
14 a -- a consideration, you know, one of the
15 candidates for you, when selecting carrier
16 fluids in your droplet work?

17 A. In the research with my
18 collaborators, to my knowledge, fluorinated
19 oils were not a choice for their experimental
20 systems.

21 Q. Why not?

22 A. I don't know why. As I've said,

1 they are often more expensive. So maybe that's
2 part of it.

3 Q. Okay. That's just conjecture?

4 A. Yes.

5 Q. Are you aware of the biohazards that
6 were known to people of ordinary skill in the
7 art in May 2002 in using fluorinated oils?

8 MS. ELLISON: Objection. Form.
9 Foundation. Scope.

10 BY MR. REINES:

11 Q. Do you have an answer?

12 A. Can you restate the question?

13 Q. Are you aware of the biohazards that
14 were known to people of ordinary skill in the
15 art in May 2002 in using fluorinated oils?

16 MS. ELLISON: Objection. Form.
17 Foundation. Scope.

18 THE WITNESS: My understanding is
19 that fluorinated materials, as opposed -- well,
20 let me rephrase myself.

21 My understanding is that hydrocarbon
22 oils can incorporate into cell membranes --

1 hydrocarbon oils --

2 Q. Can?

3 A. -- incorporate into cell membranes,
4 which is a potential problem. But the
5 fluorinated oils may not face that problem.

6 Q. When you say "may not face that
7 problem," is that something within your
8 expertise?

9 A. It's -- I should say this is not
10 completely within my expertise, so I will stop.

11 Q. Do you know what your collaborators
12 considered in selecting the carrier fluid in --
13 in your droplet work that you've been
14 testifying about over the last couple of
15 decades?

16 MS. ELLISON: Objection. Form.

17 THE WITNESS: In the work that I've
18 done, the hydrophobic material that were used
19 were standard hydrocarbon oils, as I've
20 mentioned before.

21 BY MR. REINES:

22 Q. Do you know which ones?

1 A. Dodecane, pentanol, those kinds of
2 oils.

3 Q. Do you know why those --

4 A. Short chain --

5 Q. Oh, I'm sorry.

6 A. Short chain alcohol -- short chain
7 oils.

8 Q. Do you know why those were selected
9 over other hydrocarbon oils?

10 A. You can use varieties of different
11 lengths of oils depending on the viscosities
12 that you want.

13 Q. Do you know why your collaborators
14 selected the particular oils that were selected
15 for use in the droplet systems when you
16 collaborated with them?

17 A. The collaborators I worked with
18 chose materials for their own -- well, chose
19 materials that were working.

20 Q. What materials were used by your
21 collaborators when you worked on droplet and
22 microfluidic technology where they were the --

1 you were the theoretician, and they were
2 actually the experimentalist?

3 That was -- they were the ones that
4 close the materials, correct?

5 A. So when I was working on
6 microfluidic experiments, the materials were
7 chosen in the experiments by the
8 experimentalists.

9 Q. Okay. And that's true with respect
10 to droplets and other work.

11 A. It's true for the work that we did.

12 Q. Okay. And that's because you're a
13 theoretician, not an experimentalist, right?

14 MS. ELLISON: Objection. Form.

15 BY MR. REINES:

16 Q. Right?

17 MS. ELLISON: Objection. Form.

18 BY MR. REINES:

19 Q. The reason why the experimentalists
20 selected the materials when you did work in the
21 area of microfluidics and droplets was because
22 they're experimentalists and you're a

1 theoretician, right?

2 A. In this context they had been
3 working on experiments before, so they had
4 well-developed systems.

5 Q. I see.

6 So that, when you collaborated with
7 others -- the only work you did, whether it was
8 actual experiments performed and physical work
9 done, is with collaborators in the
10 microfluidics field, correct?

11 A. In the area of microfluidics.
12 That's right.

13 Q. And in all those instances, the
14 collaborators had already had working systems,
15 so you weren't involved in the setup of the
16 system; is that correct?

17 A. My role was not in choosing the
18 materials.

19 Q. In the work that you did in
20 microfluidics and specifically droplets, what
21 surfactant did you use?

22 A. So in the work that I did that

1 involved droplet formation, so that work was --
2 that work was not done in collaboration with
3 somebody.

4 Q. Okay.

5 A. So that work was done of -- with --
6 I was doing theory to try to understand the
7 experiments that had been done. And those are
8 experiments that have been done actually using
9 lipids.

10 Q. Okay. So in the --

11 A. Okay.

12 Q. -- droplet work that you've done in
13 your career that you've been testifying about,
14 there was no experiments involved in that that
15 you were involved with, correct. That was just
16 historical stuff that you were analyzing.

17 A. There were experiments that were
18 happening at the same time, just before. And
19 then that prompted us to do theory on it.

20 Q. Will you -- would you describe
21 yourself as having been a collaborator on the
22 experimental work in droplet technology that

1 you worked with?

2 A. I did not collaborate with the
3 experiments in that particular case.

4 Q. In any work that you've done related
5 to actual droplet experiments, what was the
6 surfactant that you -- that was used, if any?

7 Well, let me step back.

8 You were --

9 A. So --

10 Q. -- involved with some -- have you
11 ever been involved with droplet -- experiments
12 in droplets, something more than just theory?

13 A. So I had a experimental student who
14 was working on the formation of multilamellar
15 spherical drops which would appear out of a
16 surfactant solution, so surfactant oil and
17 water.

18 Q. Other than the work with the one
19 experimental student, have you ever done any
20 other work related to droplets -- have you ever
21 been involved in any other work related to
22 droplets that's not theoretical?

1 MS. ELLISON: Objection. Form.

2 THE WITNESS: So the work with the
3 student was a three-year project that involved
4 examining how the flow fields would induce a
5 lamellar layered surfactant solution, oil,
6 water and surfactant, to turn into drops.

7 Then I had a collaboration with an
8 experimentalist who was looking at the behavior
9 of these multilamellar drops, and I
10 collaborated theoretically with those
11 experiments.

12 BY MR. REINES:

13 Q. Anything else?

14 A. So those are the extent of the
15 collaborations or explicit supervision of work
16 on material involving drops.

17 Q. When were those experiments?

18 A. So the lipid tubule where the
19 cylindrical tubule broke up into drops, we did
20 the theory in 1996, and the experiments were
21 done 1993 and '94.

22 The work on lamellar surfactants

1 that rolled up into spherical drops under flow,
2 that work I think was done between 2003 and
3 2006. Let me just check to make sure. 2002
4 and 2006.

5 And the work done as a theorist
6 working with the experimentalists was done I
7 think in around 2000 -- between 2008 and 2009.
8 But I can't remember the date for sure.

9 Q. Okay. Starting with the work
10 relating to droplets in the 1990s, did -- did
11 that involve fluorinated surfactants?

12 A. That did not.

13 Q. Why not?

14 A. It was not chosen by the
15 experimental system -- by the experimentalists.

16 Q. Do you have any idea why or why not?

17 A. No.

18 Q. Have you ever been involved in the
19 selection of materials for a microfluidic
20 system?

21 Let me -- let me ask it this way:
22 Would you hold yourself out to the patent

1 office as being an expert in the selection of
2 materials for droplets in a microfluidic
3 system?

4 A. The droplet formation in
5 microfluidic systems involves the behavior of
6 surfactants at interfaces and the oils involved
7 as well as the water.

8 So the -- the choice of which kinds
9 of head groups or the lengths of the tails or
10 the properties of the surfactants is something
11 I can advise on based on the physical
12 properties of the use.

13 Q. Please answer my question directly
14 regarding whether you would hold yourself out
15 to the patent office as being an expert in the
16 selection of materials for droplets in a
17 microfluidic system.

18 I'm including the carrier fluid, the
19 fluid that you use for the actual droplet, the
20 materials you use for walls, surfactant.

21 MS. ELLISON: Objection. Form.

22 BY MR. REINES:

1 Q. Please answer it directly. That
2 should be capable of a "yes," "no" answer.

3 A. I can --

4 MS. ELLISON: Objection. Form.

5 THE WITNESS: I can provide advice
6 and expertise on how the choice of surfactant
7 and the choice of the oils and the water will
8 influence the properties of the droplet
9 formation.

10 BY MR. REINES:

11 Q. Can you say anything more than that?
12 Are you willing -- are you willing
13 --

14 A. So --

15 Q. Let me ask you this: Are you
16 willing to say that you're an expert in the
17 selection of materials for droplets in a
18 microfluidic system?

19 I know you can give some theoretical
20 advice in some aspects, but are you willing to
21 say that you're an expert on that subject?

22 MS. ELLISON: Objection. Form.

1 THE WITNESS: I can provide an
2 understanding of how the materials, such as the
3 oil and the water and the "defectant" can be
4 used to help make drops which could be used in
5 a microfluidic system.

6 BY MR. REINES:

7 Q. Can you answer "yes" or "no" whether
8 you are an expert in the selection of materials
9 for droplets in a microfluidic system?

10 A. I can provide an understanding of
11 how the surfactants chosen together with the
12 oils and the aqueous solution can help form the
13 drops during the action within microfluidic
14 devices.

15 Q. So I mean that's not my -- my
16 question is whether you can provide a "yes" or
17 "no" answer. Okay? So the following question
18 isn't asking for substance.

19 It's can you state "yes" or "no,"
20 one way or the other, are you capable of doing
21 that, to the question of whether you're an
22 expert in the selection of the carrier fluid,

1 the droplet material and the surfactants and
2 the channel walls in a microfluidic system?

3 MS. ELLISON: Objection. Form.

4 BY MR. REINES:

5 Q. If you can't answer "yes" or "no,"
6 say "no." If you can answer "yes" or "no," say
7 "yes."

8 MS. ELLISON: Objection. Form.

9 THE WITNESS: I can provide
10 expertise about how the choice of surfactant
11 together with the oil and the water can
12 influence and control the production of the
13 drops within a microfluidic device as well as
14 how they will flow through the device. And so
15 that involves the choice of the oils and the
16 choice of the surfactants.

17 MR. REINES: Move to strike as not
18 responsive.

19 BY MR. REINES:

20 Q. Outside of this litigation, have you
21 ever advised anyone to use a fluorinated
22 surfactant in a microfluidic system?

1 A. I have not advised anyone to use a
2 micro -- a fluorinated surfactant.

3 Q. Have you ever advised anyone to use
4 fluorinated oil as a carrier fluid?

5 A. I have not advised someone to use
6 fluorinated oils in an experimental system.

7 Q. Do you have any idea at all what
8 considerations a person of ordinary skill at --
9 in the art would have in selecting and
10 considering a fluorinated oil in May of 2002
11 for a droplet system?

12 MS. ELLISON: Objection. Form.

13 BY MR. REINES:

14 Q. Can you answer the question?

15 A. It's my understanding, as I stated
16 before, is that hydrocarbon oils have a greater
17 tendency to go inside living systems and get
18 incorporated in membranes.

19 Q. Is that an -- are you saying that
20 based on your expertise, or is that just
21 something you heard?

22 A. I also work in lipid membranes.

1 Q. Okay. Other than that one fact, do
2 you believe that you're a expert in what a
3 person of ordinary skill in the art in May of
4 2002 would consider in selecting -- in
5 considering fluorinated oil as a potential
6 carrier fluid?

7 A. Can you restate the question?

8 Q. Are you expert in what a person of
9 ordinary skill in the art in May 2002 would
10 consider in selecting among different options
11 for the carrier oil --

12 MS. ELLISON: Objection. Form.

13 BY MR. REINES:

14 Q. -- in the droplet system?

15 MS. ELLISON: Objection. Form.

16 BY MR. REINES:

17 Q. Please answer as directly as you
18 can.

19 A. So a person of ordinary skill is a
20 hypothetical person who is aware of all
21 pertinent art.

22 I would be able to advise on the

1 carrier fluid for a number of applications for
2 which I have expertise.

3 Q. What are those?

4 A. Which includes a number of
5 biological materials. I've work on protein
6 folding, protein unfolding. I've worked on the
7 properties of membranes in cells.

8 I've worked -- and so I can advise
9 on these types of applications. And for other
10 applications, we have to see. And the choice
11 of the carrier fluid would depend on the
12 applications.

13 MS. ELLISON: Can we break for lunch
14 soon?

15 MR. REINES: Yeah. Let me have
16 about ten more minutes.

17 BY MR. REINES:

18 Q. Would you be competent in -- were
19 you competent in May of 2002 of selecting
20 materials for a droplet system in terms of the
21 carrier fluid, the droplets and the
22 surfactants?

1 I'm not talking about advising
2 someone else on specific aspects.

3 Would you be basically competent on
4 that topic?

5 Can you answer the question?

6 A. So if I take myself back to 2002, I
7 had done some work at that point, and I would
8 be competent to advise on aspects of it. I'd
9 be more -- I'm competent now.

10 Q. Competent now to advise on aspects
11 of it, right?

12 A. In 2 -- no. In 2002 I was competent
13 to advise on aspects of it.

14 Q. But now you're --

15 A. Now I'm --

16 Q. -- fully competent?

17 A. -- more competent, yeah.

18 Q. So you wouldn't trust your own
19 knowledge in 2002 to select the materials for a
20 droplet system, but now you might; is that
21 correct?

22 A. In 2002 I could choose the materials

1 for a droplet system, not for all applications
2 and for many fewer applications than I could
3 choose now.

4 Q. Okay. So there you are in May of
5 2002.

6 Would you consider fluorinated oils
7 as the carrier fluid in May of 2002 as an --
8 for a droplet system?

9 A. In 2002 I would have chosen an
10 oil-water surfactant system of which
11 fluorinated oils could have been one of them.

12 Q. My question was, in May of 2002,
13 would fluorinated oil have been a candidate as
14 the carrier fluid in a droplet system.

15 Can you answer that?

16 A. It would have been a candidate.

17 MR. REINES: All right. Why don't
18 we take lunch.

19 THE VIDEOGRAPHER: We are going off
20 record at 12:23 p.m.

21 (A short recess was taken.)

22 THE VIDEOGRAPHER: We are back on

1 the record at 1:06 p.m.

2 This is the start of Media Unit No.
3 in the deposition of Peter Olmsted.

4 BY MR. REINES:

5 Q. Let me show you the Ramsey patent,
6 '456 patent. This is Exhibit 1006.

7 And are you generally familiar with
8 this kind of segmented continuous flow system?

9 MR. REINES: By the way, I think --
10 I do think this is going to be a seven-hour
11 deposition.

12 MS. ELLISON: Okay.

13 THE WITNESS: I know about -- excuse
14 me.

15 I know about segmented flows.

16 BY MR. REINES:

17 Q. And do you recognize that the Ramsey
18 patent is about segmented flows?

19 A. Yes.

20 Q. And Ramsey, this is not a droplet
21 system, correct?

22 A. This is not a droplet system.

1 Q. And from a flow dynamics
2 perspective, which is your expertise, what is
3 the difference between a segmented flow system
4 such as Ramsey and a droplet system such as the
5 '083 patent?

6 MS. ELLISON: Objection. Form.

7 THE WITNESS: The systems are
8 similar in that they both contain small
9 compartments of typically an aqueous material.
10 But the difference is that, in a droplet
11 system, the droplets have pinched off and are
12 in the center of the channel. And one
13 difference is that, in segmented flows, they
14 are not distinct drops that are away from the
15 channel wall typically.

16 BY MR. REINES:

17 Q. Have you had any experience with
18 segmented systems?

19 A. I have not.

20 Q. And I'm sorry if you answered this
21 before. I apologize.

22 But in terms of the droplet work

1 that you did in the '90s and in the 2000s, was
2 that in the context -- was that on a
3 microfluidic -- in a microfluidic system?

4 A. So the work that I did in the '90s
5 on confined flows and -- so one piece of work
6 was on droplets that formed on lipid bilayer
7 tubules. And the other pieces of work were on
8 the flow of complex fluids in confined
9 geometries, and then the other one that I spoke
10 about was on how lamellar systems break up into
11 droplets.

12 Q. Were any of those in microfluidic
13 systems?

14 A. One of the experiments was on
15 droplets that formed in lipid tubules induced
16 by lasers to induce the droplets. One of them
17 was in microfabricated devices specifically
18 made to look at the flow properties of complex
19 fluids. And the third that I spoke about was
20 looking at droplet formation due to shear in
21 solutions.

22 MR. REINES: Move to strike as

1 nonresponsive.

2 BY MR. REINES:

3 Q. I'm -- I think there's a problem
4 with the way you're answering. I don't -- I
5 think you're taking too long between the
6 questions and answers and evading the -- the
7 questions with often double-talk but certainly
8 nonresponsiveness.

9 And so I'm going to escalate my
10 attempting to force you to comply with what I
11 view as the proper conduct of a deposition.
12 Your counsel may disagree. That's certainly
13 here right. This is my right.

14 And, you know, we may need to seek,
15 you know, relief or -- or return. So that's --
16 that's kind of it.

17 I asked you a very simple question,
18 and I asked you multiple times. And I'd ask
19 you to respond directly, which is, you -- you
20 mentioned three different sets of work you did
21 in droplet systems.

22 Would you describe any of them as

1 having been in microfluidic -- actually in
2 microfluidic systems.

3 A. The work in particular on studying
4 flow fields as a function of confinement, those
5 were microfabricated devices specifically made
6 for the purposes of studying these particular
7 flow fields.

8 There was a microfabricated
9 microfluidic devices designed for studying
10 these scientific properties of the fluids that
11 were confined.

12 Q. How about the other two?

13 Please listen to my questions and
14 respond to them.

15 A. The first one was a case where it
16 was not encapsulated inside an externally
17 prepared device, but it was initiated by a
18 laser to induce the droplet formation.

19 And the second one, the droplets
20 were formed within a rheometer, which is a -- a
21 larger device to apply flow fields.

22 Q. Are segmented flows and droplet

1 systems interchangeable for purposes of the
2 applications described in the '083 patent?

3 MS. ELLISON: Objection. Form.
4 Foundation.

5 THE WITNESS: I am not aware of
6 whether they are interchangeable.

7 BY MR. REINES:

8 Q. Are -- are you aware whether
9 segmented flow systems and droplet systems are
10 fundamentally different?

11 MS. ELLISON: Objection. Form.
12 Foundation. Scope.

13 THE WITNESS: I've explained that,
14 in droplet systems, the droplets do not -- no.
15 They've -- they've come -- the droplets have
16 been -- been formed, and they don't necessarily
17 contact the walls of the container.

18 Whereas, in segmented flows, the
19 segmented flows are contained within the full
20 width of the container.

21 BY MR. REINES:

22 Q. And from a flow perspective, do you

1 consider that to be fundamentally different?

2 A. I do.

3 MS. ELLISON: Objection. Form.
4 Foundation. Scope.

5 BY MR. REINES:

6 Q. Let's turn to the Quake patent
7 application.

8 Now, you -- you knew the Quake --
9 Quake patent application was important, and you
10 prepared for this deposition understanding
11 you'd be questioned about it, the Quake --

12 A. I did --

13 Q. -- patent application?

14 A. -- prepare the Quake application.

15 Q. Okay. And do you have any
16 explanation at all why Quake used the carrier
17 fluids that he describes in this patent
18 application as compared to fluorinated oils,
19 based on all your work in this matter and your
20 expertise?

21 A. The main claim of Quake is that the
22 sample fluid is incompatible and admissible

1 with the extrusion fluid. And in 116 Quake
2 gives examples of extrusion fluids, which
3 include decane -- he quotes decane,
4 tetradecane, hexadecane or another oil.

5 And in my reading of Quake,
6 fluorinated oils classify as another oil. So
7 that's a -- certainly an example that Quake
8 would have used, based on my reading.

9 Q. So you believe Quake is proposing
10 the use of fluorinated oil as the carrier fluid
11 in his droplet system?

12 A. Quake proposes using a number of --
13 well, he proposes using a carrier fluid which
14 is admissible with the aqueous phase. And that
15 encapsulates oils such as decane, tetradecane,
16 hexadecane or other oils which encompass
17 fluorinated oils. So fluorinated oils are
18 among the candidates that he recommended for
19 the carrier fluid.

20 Q. Just because it could be any oil.

21 That's your position?

22 A. In 117 -- no. That's not my

1 position.

2 In 117 Quake is talking about agents
3 that could be added to the fluid to reduce
4 surface tensions. That is he talks about
5 adding surfactants.

6 And the surfactants that he names
7 that could be added include Tween, Span,
8 fluorinated oils and other agents. So as --

9 Q. Are you answering my question
10 concerning carrier fluids, or are you answering
11 something else?

12 A. I am answering a question about
13 carrier fluids. And The reason --

14 Q. Let me re-ask the question, and then
15 you can do what you're going to do.

16 A. Okay.

17 Q. Do you believe that Quake is
18 disclosing the use of fluorinated oil as the
19 carrier fluid in his droplet system?

20 A. Quake discloses the use of oils in
21 his droplet system.

22 Q. And so that could be --

1 MS. ELLISON: Were you finished?

2 THE WITNESS: Among -- among those
3 oils includes nonfluorinated oils as well as
4 fluorinated oils.

5 In 117 he recommends using an
6 exemplary surfactant, including Tween, Span,
7 fluorinated oils. And my reading is that the
8 fluorinated oils listed as surfactants include
9 fluorinated surfactants, which would be
10 appropriate if one was using a fluorinated oil.

11 So fluorinated oils are a candidate
12 that Quake would consider.

13 BY MR. REINES:

14 Q. Do you believe that Professor Quake
15 discloses all oils as potential carrier fluids
16 for his droplet system?

17 A. Quake discloses that the fluid
18 that's the carrier fluid would be a nonpolar
19 solvent in the case where one has aqueous
20 droplet.

21 And that droplet -- that nonpolar
22 solvent would include oils. A variety of

1 different oils could be known as polar
2 solvents.

3 Q. All kinds of oils, right?

4 A. There are different oils one could
5 choose from.

6 Q. Do you know whether even 10X is
7 taking the position that Quake discloses
8 fluorinated oil as a carrier fluid?

9 MS. ELLISON: Objection. Scope.

10 THE WITNESS: Can you repeat the
11 question?

12 BY MR. REINES:

13 Q. Yes.

14 Do you know whether 10X, in its
15 petition, is taking the position that Quake
16 discloses fluorinated oil as the carrier fluid?

17 MS. ELLISON: Objection. Scope.
18 Relevance.

19 THE WITNESS: I haven't read the
20 petition.

21 BY MR. REINES:

22 Q. You don't know one way --

1 A. So --

2 Q. -- or the other?

3 A. So I don't know what's stated in it.

4 Q. Okay. Now, are you contending that
5 Quake discloses fluorinated oil as the carrier
6 fluid for the specific examples he gives?

7 MS. ELLISON: Objection. Form.

8 THE WITNESS: I cannot tell whether
9 fluorinated oils are used in his examples. But
10 fluorinated oils are among -- among the
11 candidate oils for the carrier matrix that can
12 be used.

13 BY MR. REINES:

14 Q. Can you tell -- do you have any idea
15 from Quake's patent application what carrier
16 oil he actually uses?

17 A. Quake states that preferably the
18 fluid should be -- that acts as the carrier
19 fluid should be decane or another oil.

20 Q. Do you know whether he actually uses
21 decane in any of his examples?

22 A. I don't know without reading through

1 the examples in more detail.

2 Q. Read what you need to read.

3 Let me withdraw the question.

4 Will you agree that the Quake patent
5 application doesn't expressly disclose a
6 fluorinated oil as a carrier fluid?

7 MS. ELLISON: Objection. Form.

8 THE WITNESS: The Quake application
9 discloses the use of a nonpolar solvent as the
10 carrier fluid.

11 MR. REINES: Move to strike as
12 nonresponsive.

13 BY MR. REINES:

14 Q. Or you can continue.

15 A. Which includes decade and other
16 oils. And other oils could include fluorinated
17 oils.

18 MR. REINES: Okay. Move to strike
19 the whole thing as nonresponsive.

20 BY MR. REINES:

21 Q. Can you say one way or the other
22 whether -- can you answer "yes" or "no" as to

1 whether the Quake patent application expressly
2 discloses fluorinated oils as the carrier
3 fluid?

4 MS. ELLISON: Objection. Form. The
5 Quake application discloses use of oils as a
6 carrier fluid, which includes decane, which is
7 expressly noted, as well as other oils, which
8 could include fluorinated oils.

9 MR. REINES: Move to strike as
10 nonresponsive.

11 BY MR. REINES:

12 Q. I asked you whether you could answer
13 "yes" or "no." I didn't ask you for the
14 substance of your answer.

15 So because of your evasiveness, I'm
16 going to be asking you, for certain questions,
17 "Can you answer 'yes' or 'no'?"

18 I think you're astute enough to
19 understand that that means the -- the answer is
20 whether you can answer "yes" or "no," not to
21 just push out whatever you want to push out
22 onto the record. Okay? So that's what I'm

1 going to try to do.

2 Can you answer "yes" or "no" as to
3 whether the Quake patent application discloses
4 fluorinated oil expressly?

5 MS. ELLISON: Objection. Form.

6 BY MR. REINES:

7 Q. How long will that take you to
8 answer?

9 A. I believe I've already answered that
10 the Quake application discloses nonpolar
11 solvents which may include fluorinated oils.

12 Q. And that's as direct as you're
13 willing to be to the question I asked, correct?

14 Is that correct?

15 Does that -- how long will it take
16 you to answer that question as to whether
17 that's correct or not?

18 Do you need to read something in
19 order to answer my question?

20 A. That's the answer to the question.

21 Q. And you can't be any more direct
22 than that, correct?

1 A. I can't be any more direct than
2 that.

3 Q. Okay. You are currently a
4 professor, correct?

5 A. Correct.

6 Q. And you can answer that "yes" or
7 "no," correct?

8 A. Correct.

9 Q. You considered the Quake patent
10 application in forming your opinions in this
11 case; is that correct?

12 A. I did.

13 Q. Are you confident of that?

14 A. I did.

15 Q. The Quake application is assigned to
16 California Institute of Technology.

17 Is that true or not true in terms of
18 the patent application?

19 MS. ELLISON: Objection. Scope.

20 BY MR. REINES:

21 Q. How long will it take you to answer
22 that question?

1 A. The inventors are Stephen Quake and
2 Todd Thorsen, who are at Pasadena, California.
3 And Stephen Quake was at Cal Tech.

4 Q. Have you ever met Stephen Quake?

5 A. I've seen him give a talk.

6 Q. Have you ever --

7 A. More than one talk.

8 Q. -- met him in person?

9 A. I may have met him in person.

10 Q. Can you say one way or the other?

11 A. I don't think I met him, no.

12 Q. Do you have any explanation why
13 Professor Quake never expressly discloses
14 fluorinated oil as the carrier fluid in his
15 patent application?

16 MS. ELLISON: Objection.
17 Foundation. Form. Scope.

18 THE WITNESS: So Quake discloses
19 nonpolar solvents, which may include
20 fluorinated oils.

21 BY MR. REINES:

22 Q. Can you answer any more than that?

1 A. I don't know how he prepared the
2 application.

3 Q. Are there any specific fluorinated
4 oils disclosed in the Quake patent application
5 for -- as the carrier fluid?

6 How long will it take you to answer
7 that question?

8 A. So Quake discloses -- discloses
9 nonpolar solvents.

10 Q. Is that as responsive as you can be
11 to my question?

12 A. And these nonpolar may include
13 fluorinated oils.

14 Q. Did you understand what my question
15 was?

16 A. Yes.

17 Q. Why didn't you state what you --
18 perception of my question was when you gave
19 those two answers after I asked you to be as
20 direct as you could be?

21 I just want to understand if you --
22 I don't want an argument that you aren't

1 understanding my questions.

2 What was my --

3 A. I understand --

4 Q. What did you understand my question
5 to be when you answered that way?

6 A. Your question was about whether
7 Quake explicitly names fluorinated oils.

8 Q. Can you answer that question, the
9 one you just posed, as directly as you can?

10 How long will it take you to answer
11 that question?

12 A. Quake discloses oils, which include
13 fluorinated oils.

14 Q. Does Quake disclose any specific
15 fluorinated oils as the carrier fluid?

16 A. Quake discloses that oils can be
17 used as the carrier fluid.

18 Q. Does Quake disclose any specific
19 fluorinated oils as carrier fluid?

20 A. The examples that Quake discloses
21 specifically are decane, hexadecane,
22 tetradecane. But he also discloses other oils,

1 which would include fluorinated oils.

2 Q. Is that as responsive as you can be?

3 A. The statement of other oils
4 encompasses fluorinated oils.

5 Q. Does Quake disclose specific
6 fluorinated oils as the carrier fluid in his
7 patent application?

8 A. Quake discloses nonpolar solvents,
9 which include fluorinated oils.

10 Q. Are nonpolar solvents specific oils,
11 or is that a category of oils?

12 A. That's a category of oils that
13 includes oils such as decane and fluorinated
14 oils.

15 Q. Describe all the -- all the
16 different types of oils that fall in that same
17 category, as many as you can name.

18 MS. ELLISON: Objection. Scope.

19 THE WITNESS: There are lots of
20 different oils.

21 BY MR. REINES:

22 Q. Please name them all as individually

1 as you can.

2 MS. ELLISON: Objection. Scope.

3 THE WITNESS: So these could be
4 hydrocarbons of different lengths,
5 fluorocarbons of different lengths, degrees of
6 fluorination. You can have hydrocarbons, which
7 have methyl groups off the side which make them
8 a little more bulkier. There are many
9 different kinds of oils that one can use.

10 BY MR. REINES:

11 Q. Name others.

12 MS. ELLISON: Objection. Scope.

13 BY MR. REINES:

14 Q. Are you unable to identify any other
15 oil that fits in the category referenced by
16 Professor Quake?

17 A. So hydrocarbons of different lengths
18 encompasses things like pentane, decane,
19 dodecane, octadecane. So you can change the
20 length as you like. And the same with the
21 fluorocarbons. So there are a vast set of oils
22 under those categories.

1 Q. Are there others other than
2 hydrocarbons and fluorocarbons?

3 A. There are other molecules with
4 nonpolar groups.

5 Q. Please list them by category.

6 MS. ELLISON: Objection. Scope.

7 THE WITNESS: I don't think at this
8 stage I can list them in any more specificity
9 than I have.

10 BY MR. REINES:

11 Q. Are there other types of oils that
12 would fit within what you believe Quake is
13 disclosing for carrier fluid other than
14 hydrocarbons and fluorocarbons?

15 A. I believe there are. Most oils -- a
16 lot of oils -- many oils fall into this
17 category -- these categories.

18 Q. Can you identify them by category
19 the way you use the terms "fluorocarbon" and
20 "hydrocarbon"? Can you name others?

21 Are you being --

22 A. I cannot --

1 Q. -- evasive --

2 A. I cannot --

3 Q. -- intentionally?

4 A. I cannot at this time.

5 Q. Why? Because you just don't know
6 what they are?

7 Why can't you?

8 A. Because hydrocarbons encompasses a
9 large amount of the kinds of oils.

10 Q. Right. And so do fluorocarbons.

11 A. And so do fluorocarbons.

12 Q. Okay. What other categories fall
13 within the scope?

14 I'm asking you at the category
15 level. It can't be -- or whatever. I'll just
16 keep going.

17 Can you identify any other classes
18 of carrier oils that you believe are described
19 as options by Professor Quake beyond your
20 argument that it's fluorocarbons and
21 hydrocarbons?

22 A. At this point I cannot.

1 Q. Do you believe there are others?

2 A. Uh-huh. I do.

3 Q. Do you believe there are thousands?

4 A. I don't believe there are thousands.

5 Q. Do you believe there are hundreds?

6 A. Of -- I don't believe there are
7 hundreds of categories.

8 Q. How many categories do you believe
9 there are?

10 A. I'm not prepared to make an
11 estimate.

12 Q. Is that -- why -- why can't you make
13 an estimate?

14 What would you need to do to make an
15 estimate?

16 Let me ask you this: Within the
17 category of hydrocarbon and fluorocarbon, how
18 many different oils are candidates for carrier
19 fluid to one of ordinary skill in the art
20 reading the Quake patent application?

21 MS. ELLISON: Objection. Scope.

22 BY MR. REINES:

1 Q. I'm not talking about category.
2 Specific oils.

3 MS. ELLISON: Objection. Scope.

4 THE WITNESS: Within those
5 categories, there will be tens to a hundred
6 that one could consider.

7 BY MR. REINES:

8 Q. And are you contending that Quake
9 discloses actually examples of using
10 fluorinated oil as the carrier fluid in his
11 example section?

12 So the PTAB knows what your
13 contention is?

14 A. So the P --

15 Q. Yep.

16 A. I didn't understand the word. The P
17 temp?

18 Q. Do you contend that Quake discloses
19 using a fluorinated carrier oil for his
20 specific examples in the example section of his
21 patent application?

22 A. So in Example 7, for example, where

1 he's talking about cell sorting -- about
2 demonstrating sorting with beads and he goes on
3 for cells, he doesn't state the oils that are
4 used in this section.

5 Q. So you have no idea what oil he's
6 using, right?

7 A. So he may have used a fluorinated,
8 or he may have used a hydrogenated.

9 Q. You just have no idea, right?

10 A. It's not stated here.

11 Q. Right.

12 So, therefore, you don't know --

13 A. Right.

14 Q. -- correct.

15 A. It's not stated there, so I don't
16 know.

17 Q. So you can't state with a reasonable
18 degree of confidence that Quake uses
19 fluorinated oils for any of the examples in his
20 application.

21 Will you admit that much?

22 A. I don't know what oil Quake used in

1 his examples. He states that he could use
2 decane or other oils.

3 Q. Do you understand from the Quake
4 patent application which he considers to be the
5 preferred oil for use as a carrier fluid?

6 A. It says: Preferably the main
7 channels are nonpolar solvents. The fluid
8 through the main -- the main channel is a
9 nonpolar solvent, most preferably decane or
10 another oil.

11 Q. Do you understand decane to be his
12 most preferable oil for his carrier fluid?

13 MS. ELLISON: Objection. Form.

14 THE WITNESS: I understand that
15 there -- he's suggesting that there are choices
16 that one can make.

17 Now, based on the fact that 117
18 talks about surfactants that include
19 fluorinated surfactants, my reading is that in
20 some cases one would choose a fluorinated oil,
21 and in some cases one would not choose a
22 fluorinated oil.

1 BY MR. REINES:

2 Q. Do you know why Quake describes
3 decane as most -- most preferable or more
4 preferable?

5 MS. ELLISON: Objection.
6 Foundation.

7 THE WITNESS: Quake says it should
8 be most preferably decane or another oil.

9 BY MR. REINES:

10 Q. Are you denying that he includes
11 decane among what's -- considers most
12 preferable?

13 A. I'm not denying that.

14 Q. Are you sure?

15 A. There's decane or another oil.

16 Q. Right.

17 Are you denying that decane is
18 identified as among the most preferable by
19 Quake?

20 A. As well as another oil.

21 Q. Are you arguing, or are you giving
22 testimony here?

1 A. The first one he chooses is decane,
2 so I agree with that.

3 MR. REINES: All right. I'm going
4 to take a break.

5 THE VIDEOGRAPHER: We are going off
6 the record at 1:57 p.m.

7 (A short recess was taken.)

8 THE VIDEOGRAPHER: We are back on
9 the record at 2:09 p.m.

10 BY MR. REINES:

11 Q. If you could -- let me get you the
12 '083 patent. You probably have it right there,
13 but let me get it.

14 If you'd turn to Claim 1. This is
15 Exhibit 1001.

16 If you look at Claim 1, what is the
17 difference between the system of Claim 1 and
18 what Quake proposes in his application which
19 has been marked as Exhibit 1004?

20 MS. ELLISON: Objection. Scope.

21 THE WITNESS: So Claim 1 talks about
22 a microfluidic system with a channel, a carrier

1 fluid and then an aqueous plug. And those are
2 the same elements that are in the Quake patent.

3 The difference is that in here there
4 are some specific things noted; whereas, in the
5 Quake patent the different oils are described
6 more generally --

7 BY MR. REINES:

8 Q. And what are the --

9 A. As surfactants.

10 Q. -- specifics in Claim 1 that
11 differentiate the '083 patent from the Quake
12 disclosure?

13 MS. ELLISON: Objection. Scope.

14 THE WITNESS: So in the '083 patent,
15 it specifically talk about which oils and
16 surfactants are to be used.

17 Those oils and surfactants are also
18 encompassed within Quake's patent. They're not
19 explicitly named in the claims of the Quake
20 patent. But a POSA reading 116, 117, would
21 have understood that those could be among the
22 categories.

1 And this is a specific example of
2 the surfactant oil system that one could use.

3 BY MR. REINES:

4 Q. You understand the Quake's not a
5 patent; it's a patent application.

6 A. Patent application. Excuse me.

7 Q. Okay.

8 A. Excuse me.

9 Q. And you testified earlier that the
10 difference between Claim 1 of the '083 patent
11 and the Quake patent application was that there
12 were specific things noted in the Claim 1.

13 And my question to you is what were
14 those?

15 MS. ELLISON: Objection. Scope.

16 THE WITNESS: So in Claim 1 they
17 specify an example of the surfactants and oils
18 to be used in their claim.

19 Quake's patent application would be
20 read by a POSA as containing the same
21 materials, a fluorinated surfactant and
22 fluorinated oils.

1 BY MR. REINES:

2 Q. Where in Claim 1 of the '083 patent
3 do you see a specific oil?

4 MS. ELLISON: Objection. Scope.

5 THE WITNESS: Claim 1 talks about
6 the carrier fluid, and a specific oil is named.
7 A fluorinated oil is named.

8 BY MR. REINES:

9 Q. And you consider the term
10 "fluorinated oil" to be a specific oil.

11 A. It's a class of oils.

12 Q. And you're saying that -- that the
13 '083 patent is different from the Quake in that
14 it -- it identifies fluorinated oils as a class
15 of oils that could be a carrier fluid, whereas
16 Quake doesn't?

17 MS. ELLISON: Objection. Scope.

18 THE WITNESS: Quake encompass
19 fluorinated oils. So Quake's is actually a
20 broader class of materials which includes
21 fluorinated and unfluorinated oils. The '083
22 patent specifically focus on the -- on

1 fluorinated oils.

2 BY MR. REINES:

3 Q. So in your mind, Quake discloses a
4 genus, while the '083 patent discloses
5 species --

6 MS. ELLISON: Objection.

7 BY MR. REINES:

8 Q. -- of the oil?

9 A. I --

10 MS. ELLISON: Objection. Let me get
11 -- let me put my --

12 THE WITNESS: I'm not sure I
13 understand --

14 MS. ELLISON: -- objections in.
15 Objection. Scope.

16 THE WITNESS: I'm not sure I
17 understand the question.

18 BY MR. REINES:

19 Q. Does the Quake patent disclose the
20 concentration of surfactant that should be
21 introduced into the system?

22 MS. ELLISON: Objection. Form.

1 THE WITNESS: So in the examples,
2 Quake gives some recipes that include
3 concentrations of surfactants.

4 BY MR. REINES:

5 Q. And in those examples, you have --
6 you don't know what oil is the carrier fluid,
7 correct?

8 A. Quake does not specify the oil --

9 Q. Okay.

10 A. -- as the carrier fluid.

11 Q. Did he specify the surfactant?

12 A. I believe he does. So in Example 7
13 he gives an example that includes Tween as
14 surfactant.

15 Q. To meaningfully evaluate the surface
16 tension effect of the surfactant in the
17 examples, would you need to know what the
18 carrier fluid is?

19 MS. ELLISON: Objection. Form.

20 THE WITNESS: The surface tension
21 between the two fluids will depend on the
22 carrier fluid, the aqueous solution and the

1 surfactant at hand.

2 So those -- those -- that
3 information together will control the surface
4 tension.

5 BY MR. REINES:

6 Q. Right.

7 So in order to evaluate what the
8 surface tension is and the effect of the
9 concentration of the surfactant, you would need
10 to know what the -- what specific carrier fluid
11 it is, correct?

12 MS. ELLISON: Objection. Form.

13 THE WITNESS: The quantitative
14 determination does depends on those properties,
15 yes.

16 BY MR. REINES:

17 Q. Now, will you admit that the Quake
18 patent application at Paragraph 8 describes
19 problems relating -- including particles on the
20 tubing of the flow chamber?

21 MS. ELLISON: Object to form.

22 THE WITNESS: Yes. In Paragraph 8

1 Quake states the particle absorption may cause
2 turbulent flow, which could give rise to issues
3 in looking at it using optical devices.

4 BY MR. REINES:

5 Q. And if you turn to Paragraph 94,
6 please, do you see in the first sentence of
7 Paragraph 94 Quake in his patent application
8 states that he takes steps to prevent materials
9 from adhering to the channels -- channel walls?

10 Do you see that?

11 A. I see that.

12 Q. And do you agree that one of the
13 issues that Quake is attempting to address is
14 materials adhering to the sides of the
15 channels, such as particles and contaminants?

16 A. Yeah. It states -- Quake states
17 that if you want the keep stuff from attaching
18 to the wall, then you should coat it. And the
19 examples he gives include cell particles, other
20 molecules.

21 Q. And one way he states to address the
22 problem of contaminant adhesion to the

1 microfluidic walls is coating it with Teflon,
2 right?

3 A. That's what he states, yes.

4 Q. And in that case it would just be
5 coated with Teflon; you wouldn't have to put an
6 additive in the fluids because it would -- it
7 would --

8 A. It's made --

9 Q. -- be precoated, right? It's
10 intrinsic.

11 A. That's right.

12 Q. Yeah.

13 A. You could coat your material.

14 Q. And then an alternative way to
15 address this problem of contaminants on the
16 wall that's proposed by Quake is covering the
17 walls with a surfactant.

18 Do you see that?

19 A. I do see that.

20 Q. Okay. And that's in Paragraph 94?

21 A. I see it. That's in Paragraph 94,
22 yeah.

1 Q. Okay. And do you understand that
2 disclosure or a surfactant used to coat the
3 walls to be in the nature of Teflon where it's
4 coated as sort of part of what the structure
5 is, or do you understand that to be something
6 that's an additive to the reagents that flow
7 through the system?

8 MS. ELLISON: Objection. Form.
9 Scope.

10 THE WITNESS: I interpret that to
11 mean, in terms of surfactants, additives that
12 are added to the system together with other
13 surfactants, for example.

14 BY MR. REINES:

15 Q. You -- you say -- you testified
16 surfactants added to the system together with
17 other surfactants, for example.

18 What does that sentence mean?

19 MS. ELLISON: Objection. Form.
20 Foundation.

21 THE WITNESS: That's because
22 surfactants can be added as discussed in 117.

1 Surfactants can be added to reduce surface
2 tensions, including the surface tension at the
3 nonpolar aqueous interface.

4 So while there may be surfactants
5 added as mentioned in 094 and in 118,
6 surfactants can also be added which has a role
7 at the aqueous water -- aqueous nonpolar
8 interface.

9 MR. REINES: Move to strike all that
10 as unrelated to the question.

11 BY MR. REINES:

12 Q. The surfactants that you referred
13 to, you stated that they would be surfactants
14 added as additives coupled with other
15 surfactants. To me that's -- has an element of
16 doublespeak in it.

17 So let me step back at -- and try to
18 see if I can fillet this down.

19 In Paragraph 94 there's a reference
20 to surfactants that can be used to prevent
21 material contaminants from adhering to the
22 walls, correct?

1 A. So the surfactants in 94 would be
2 added to prevent material from adhering to the
3 wall.

4 Q. Okay. Let's --

5 A. That's correct.

6 Q. Let's talk about those. I
7 understand you want to make arguments about
8 other things, but I want to talk about the
9 surfactants in 94 that you just described.

10 Do you understand that?

11 A. I do.

12 Q. Okay. Those surfactants, as Quake
13 describes them, do you understand that they are
14 additives that go in the reagents or that they
15 are precoatings that are an intrinsic part of
16 the walls or both?

17 There's three options. If you can
18 come up with more or you can add -- I guess you
19 could answer a totally different question. But
20 maybe you could try to answer that one.

21 A. I would think of those as additives
22 added to the carrier fluid.

1 Q. Okay. My hands are high up in the
2 air, and I'm running around in circles.

3 All right. Now let's talk about 95
4 and its relationship to 94.

5 In 95 the reference to "preferred
6 surfactants that may be used include," that's a
7 reference to the surfactants from 94 and the
8 last sentence that says "Alternatively, the
9 channels may be coated with a surfactant"; is
10 that right?

11 A. So one could read preferred
12 surfactants, which -- after which the list
13 follows, as preferred surfactants following on
14 from '094.

15 Q. When you say "could," is that how
16 you read it, since we're talking about you,
17 since you're the one testifying today?

18 A. Although Quake goes on to talk about
19 other applications of the surfactants later, in
20 this case these surfactants are -- I would read
21 these as being first disclosed here in the
22 context of coating the channel walls.

1 Q. For purposes of preventing particles
2 -- contaminants from adhering to the walls.

3 A. This context, yes.

4 Q. Right.

5 And when you say "in this context,"
6 you mean Paragraphs 94 and 95.

7 A. Yes.

8 Q. Of the surfactants disclosed in
9 Paragraph 95, how many of those do you
10 recognize -- how many of those were you
11 familiar with prior to your involvement in this
12 matter?

13 A. Span, the sorbitols, carboxylic acid
14 esters.

15 Q. All right. Let me do it
16 differently.

17 A. SDS.

18 Q. Let me withdraw the question, do it
19 --

20 A. A number of them.

21 Q. -- do it the other way around.

22 A. Okay.

1 Q. Which of the surfactants disclosed
2 in Paragraph 95 of -- of Quake were you not
3 familiar with when you became involved with
4 this case?

5 A. So the diphenols and the mercaptans
6 I'm not so familiar with. The other ones I
7 will have some experience with and know about
8 through their use in a variety of different
9 systems.

10 Q. And the diphenols, where is that,
11 what line? We don't really have the lines in
12 here. That's all right.

13 A. Seven through eight, the phenols.

14 Q. Are you familiar with the ionic
15 surfactants referenced here?

16 A. The SDS?

17 Q. Yes.

18 A. Yeah. That's used quite a lot.

19 Q. In your view, are the surfactants in
20 Paragraph 95 suitable for controlling the
21 surface tension between the droplet and the
22 carrier fluid?

1 A. Yes, they would help control.

2 Q. All of them, according to you?

3 A. Depending on the carrier fluid.

4 Q. You would need to know what the
5 carrier fluid is?

6 A. Yeah. As we spoke about before, the
7 surface tension depends on the nature of the
8 carrier fluid and the encapsulant.

9 Q. Now, let me ask you this: Does your
10 expertise on surfactants provide a basis for
11 you to evaluate how these surfactants in
12 Paragraph 95 would work to prevent contaminants
13 from adhering to the walls?

14 MS. ELLISON: Object to form.

15 BY MR. REINES:

16 Q. Let me ask it this way: Do you
17 agree with Professor Quake that the use of the
18 surfactants in Paragraph 95 would successfully
19 prevent contaminants from adhering to the
20 walls?

21 MS. ELLISON: Objection. Form.
22 Foundation.

1 THE WITNESS: Yes. It depends on
2 the nature of the wall, the nature of the
3 contaminant. But generally, if the surfactant
4 goes to the wall, which it will because these
5 have a hydrophilic head and you're putting it
6 in a carrier fluid, the head will go away and
7 absorb onto the wall. That will reduce the
8 surface tension between the wall and the
9 carrier fluid, which then reduces some of the
10 attraction for particles to absorb at the wall.

11 And then -- then you can play, which
12 is what they've done, presumably, with
13 different surfactants.

14 BY MR. REINES:

15 Q. So the scientific principles
16 underlying the use of surfactants to prevent
17 contaminants from adhering to the walls is the
18 reduction of surface tension between the
19 carrier fluid and the wall itself; is that
20 correct?

21 A. That's one mechanism, yes.

22 Q. What other mechanisms at work in

1 Paragraphs 94 and 95?

2 A. Other mechanisms would be to adhere
3 onto the contaminants themselves, which would
4 have the same effect.

5 Q. How does the reduction of surface
6 tension between the carrier fluid and the walls
7 remove potential contaminants from the wall --
8 from adhering to the wall?

9 A. So if a contaminant adheres --

10 MS. ELLISON: Objection.

11 Foundation.

12 THE WITNESS: If something adheres
13 to a wall, it will adhere -- adhere to the wall
14 because, amongst other things, there's a lower
15 energy for it near the wall; or alternatively,
16 there's a high surface energy between wall and
17 the material, so it displace the fluid near
18 wall.

19 So if you lower the surface tension
20 between the fluid and the wall, then you reduce
21 the energy gain by going near wall so it won't
22 be as apt to go near the wall.

1 The same principle would apply if
2 you had -- if the surfactant went to the
3 contaminant or to the object that's flowing.
4 So it would --

5 BY MR. REINES:

6 Q. In Paragraph 96 Professor Quake has
7 described a particularly preferred embodiment
8 that uses the surfactant to coat the channel
9 walls to prevent contaminant attachment,
10 correct?

11 MS. ELLISON: Objection. Form.

12 THE WITNESS: So this is an example
13 of adding a surfactant to an oil in which it
14 would coat the channel wall. And in 94 he
15 talks about coating the channel walls to
16 prevent adhesion.

17 BY MR. REINES:

18 Q. And so Paragraph 96 is exemplary
19 parameters to use to coat the walls to prevent
20 contaminant adhesion, correct, via -- via a
21 surfactant?

22 A. And so, upon consideration of the

1 carrier fluid in the wall only, not any other
2 interfaces, 96 discloses that -- discloses
3 surfactant concentrations which will lead to
4 coating the wall of the carrier fluid.

5 Q. When you -- when you say not other
6 interfaces, you're referring here to the
7 interface between -- the interface that's being
8 addressed in Paragraph 96, according to you, is
9 solely the carrier-fluid wall interface; is
10 that correct?

11 MS. ELLISON: Objection. Form.

12 THE WITNESS: So in 96 he's -- he's
13 talking about -- the Quake, et al., they are
14 talk about the case of only having an extrusion
15 fluid -- nothing else is mentioned -- and a
16 wall. And in that case, upon adding the
17 surfactant, he will end up coating the wall.

18 Q. Right.

19 But when you said the only interface
20 that's being addressed in Paragraph 96 and not
21 others, you were referring to the carrier wall
22 and the carrier -- the carrier fluid and the

1 wall. Excuse me.

2 A. Yeah. So the -- in this case, the
3 -- the channel walls are the channel walls
4 between the carrier fluid and the -- the
5 channel walls butt up against the carrier
6 fluid. So it's that interface, the carrier
7 fluid and the channel -- the -- the carrier
8 fluid and the channel wall.

9 Q. Okay. Why don't you start that
10 again.

11 A. Okay. The end of 96 -- at the end
12 of 96, Quake states: "So the surfactant in the
13 extrusion fluid coats the channel walls."

14 Q. What does that tell you about
15 Paragraph 96 generally?

16 A. So that tells me that the -- that,
17 in this context, Paragraph 96, that the
18 surfactant will go to the -- well, some
19 surfactant will be absorbed onto the interface
20 between the carrier fluid and the channel wall.

21 Q. And the purpose of the -- the use of
22 the surfactants in Paragraph 96 is to prevent

1 the adherence of contaminants to the wall by
2 coating it, correct?

3 MS. ELLISON: Objection. Form.

4 THE WITNESS: Yes. Surfactant
5 that's added in 96 adheres to the carrier wall
6 -- carrier fluid-channel wall interface. And
7 that will aid in keeping particles and
8 contaminants from adhering to the wall. That's
9 one use of the surfactants.

10 BY MR. REINES:

11 Q. And --

12 MS. ELLISON: Can we take a break
13 soon?

14 MR. REINES: Yeah, sure.

15 BY MR. REINES:

16 Q. And the 2 percent preferred number
17 in Paragraph 96 by Quake, you understand that
18 to be what would be the right amount of
19 surfactant needed to coat the wall to avoid the
20 problem of contaminated adhesion on the walls?

21 MS. ELLISON: Objection. Form.
22 Foundation.

1 THE WITNESS: My reading is that, in
2 a particular preferred embodiment, there is a
3 certain concentration of surfactant in the
4 fluid -- in -- in the carrier fluid.

5 With regards to the properties of
6 the channel wall, this will lead to coating of
7 the channel wall in a way that's beneficial.

8 BY MR. REINES:

9 Q. And then let me just ask there --

10 A. But -- but --

11 Q. -- in terms of -- if I understand
12 you, the disclosure of Paragraph 96 is intended
13 to coat the channel walls.

14 Is there something that you can do
15 to ensure that the surfactant goes to the
16 channel walls rather than, for example, washing
17 out the bottom of the system without being
18 coated on the walls?

19 MS. ELLISON: Objection. Form.

20 BY MR. REINES:

21 Q. In other words -- let me ask the
22 question again.

1 Is there a way to preferentially
2 have the surfactant that's described in
3 Paragraph 96 go to the wall-carrier fluid
4 interface?

5 A. It will go to the wall because of
6 the chemical differences between the carrier
7 fluid and the wall. It's the chemical
8 differences between the carrier fluid and the
9 wall that lead the particles to stick there.

10 BY MR. REINES:

11 Q. Okay.

12 A. And because there -- I'll stop.

13 MR. REINES: All right. Well, why
14 don't -- I think there's a request for a break,
15 so why don't we do that. Thank you.

16 THE VIDEOGRAPHER: We are going off
17 the record at 3:12 p.m.

18 (A short recess was taken.)

19 THE VIDEOGRAPHER: We are back on
20 the record at 3:25 p.m.

21 This is the start of Media Unit No.
22 4 in the deposition of Peter Olmsted.

1 BY MR. REINES:

2 Q. If you turn to Paragraph 116, we're
3 getting closer to where you want to be. So
4 we're getting there.

5 A. Okeydoke.

6 Q. Do you have Paragraph 116 of Quake
7 in front of you?

8 A. I do have it in front of me.

9 Q. Thank you.

10 Paragraph 116 is about the droplet
11 formation process, correct?

12 A. Paragraph 116 describes the droplet
13 forming liquid.

14 Q. Okay.

15 A. And it describes the fluid passing
16 through the channel in which the drops are
17 formed. It doesn't actually describe the
18 process of forming the droplets.

19 Q. Fair enough.

20 So would it be fair to say Paragraph
21 116 is describing the fluids used at the point
22 of droplet formation?

1 A. Well, it describes the fluids -- the
2 two fluids in the problem, which eventually end
3 up as droplets encased in the carrier fluid.
4 So those two fluids would have to be present
5 when the droplets are formed, yes.

6 Q. Now the -- Paragraph 117 states that
7 the fluids in the invention may contain
8 additives, correct?

9 Do you see that?

10 A. It does say that.

11 Q. Yeah.

12 A. Fluids may contain additives.

13 Q. And then there's a list of exemplary
14 surfactants.

15 Do you see that?

16 A. I see that.

17 Q. Are those the same surfactants that
18 are referred to in Paragraph 95?

19 MS. ELLISON: Objection. Form.

20 THE WITNESS: So the exemplary
21 surfactants are introduced here and referred to
22 to control or optimize droplet size, flow and

1 uniformity. And Span, for example, is
2 explicitly noted in Paragraph 95 --

3 BY MR. REINES:

4 Q. Okay.

5 A. -- as well as in this place.

6 Q. Let me attempt again. I'm not
7 talking about potential uses or your arguments
8 about what 117 means or any of that.

9 My question is just, in terms of the
10 physical surfactant itself, just the noun
11 surfactant -- forget what application you're
12 using it for -- are the exemplary surfactants
13 described in Paragraph 117 all described
14 previously in Paragraph 95?

15 Do you understand my question?

16 MS. ELLISON: Objection. Form.

17 THE WITNESS: Paragraph "195" states
18 that "Preferred surfactants that may be used
19 include but are not limited to," and then they
20 go and give a long list.

21 Paragraph 117 has a list of
22 surfactants, some of which are explicitly noted

1 in 95, others of which are not but would fall
2 under the "but are not limited to."

3 BY MR. REINES:

4 Q. Okay.

5 A. So...

6 Q. But, in general, a person of
7 ordinary skill in the art reading the Quake
8 disclosure in May of 2002 would understand that
9 the exemplary surfactants from Paragraph 117
10 are all encompassed within the description of
11 surfactants on in Paragraph 95?

12 A. That's not how I would read it.

13 Q. Okay.

14 A. Because 95 follows -- where is 95?

15 Q. And again, to be clear, I'm not
16 referring to the functionality. I understand
17 you have arguments on the functionality.

18 I'm just saying just the -- the list
19 of surfactants as -- as chemicals -- and I know
20 "chemicals" is the wrong word because they're
21 chemicals compounds, but --

22 A. "Chemicals" is fine --

1 Q. -- you get --

2 A. -- with me.

3 Q. The -- the -- the chemicals of the
4 surfactants in Paragraph 95, do they encompass
5 all of the exemplary surfactants disclosed in
6 117?

7 MS. ELLISON: Objection. Form.

8 THE WITNESS: A POSA -- I'm sorry,
9 but --

10 MR. REINES: Yeah.

11 THE WITNESS: -- I'm going come back
12 to the function. But a POSA reading 117 as a
13 paragraph would see that there is a function
14 implied in 117.

15 BY MR. REINES:

16 Q. Okay. What does that tell you about
17 whether the surfactants disclosed in Paragraph
18 95 are the same as this -- encompass the
19 surfactants -- looks like a super set to me, at
20 least, or at least arguably -- of what's in
21 117?

22 MS. ELLISON: Objection to form.

1 And please make sure you let the
2 witness finish his answer. I'm not sure he was
3 finished with the last one.

4 THE WITNESS: Well, 117 talks about
5 Tween, Span, fluorinated oils and other agents,
6 which encompasses a lot. And 95 gives a long
7 list which is not inclusive.

8 MR. REINES: Okay.

9 THE WITNESS: So both -- both sets
10 are fairly open. And then a -- a POSA would
11 think, well, what -- what are we going to use
12 them for?

13 BY MR. REINES:

14 Q. Let me do it this way: In Paragraph
15 117, the first exemplary surfactant referenced
16 is Tween.

17 Is that disclosed in Paragraph 95?

18 A. So Tween is not listed by the brand
19 name "Tween" but its chemical name, which I
20 have here -- poly -- polyethyloxylated ester --
21 is also not explicitly noted there, but I would
22 classify it as one of these surfactants.

1 Q. Which one would it fit in under --
2 in Paragraph 94?

3 A. Under the "carboxylacid" --
4 carboxylic acid esters and the et cetera.

5 Q. And then Span obviously is disclosed
6 in Paragraph 95. That's not --

7 A. Uh-huh.

8 Q. -- hard to -- you need to answer
9 with a word, not a noise.

10 A. It's disclosed in 95.

11 Q. And then you -- then the -- the
12 third item, surfactant 117, is fluorinated
13 oils.

14 Do you see that?

15 Just two words.

16 Do you see that?

17 A. The fluorinated materials are not
18 explicitly noted but would fall under the water
19 category. As I say, it's not limited to, but
20 they're not explicitly noted there.

21 Q. And which broader category?

22 A. Oh, they say "preferred surfactants"

1 may be -- "that may be used include but are not
2 limited to." So they've given a list in 95 of
3 surfactants that can be used, but it's not
4 limited to those.

5 Q. One of ordinary skill in the art,
6 according to you, would understand that
7 fluorinated oils can be used as the surfactants
8 introduced by Quake as useful for preventing
9 contaminant attachment to the walls?

10 MS. ELLISON: Objection. Form.

11 THE WITNESS: Upon reading 117, a
12 POSA would understand that fluorinated
13 surfactants, perhaps Tween, Span and the other
14 agents, can help control droplet size, flow and
15 uniformity.

16 MR. REINES: Okay. I move to strike
17 that as nonresponsive.

18 BY MR. REINES:

19 Q. I asked you if the fluorinated oil
20 surfactant that's disclosed in Paragraph 117 --
21 whether fluorinated oils are disclosed also in
22 Paragraph 95. You said they are because it

1 says "including but not limited to."

2 So my question to you is would a
3 person of ordinary skill in the art reading
4 Paragraph 95 understand that fluorinated oils
5 are a surfactant that can be used for the
6 purpose of preventing the particles from
7 attaching to the walls?

8 MS. ELLISON: Objection. Form.

9 BY MR. REINES:

10 Q. Do you understand that question?

11 A. I understand the question.

12 Q. Okay. What's the answer?

13 A. So Paragraph 95 gives the principles
14 by which you can use surfactants to help coat
15 the walls. And these are example surfactants
16 that are given, which in this context -- in
17 this case, fluorinated surfactants are not
18 explicitly listed.

19 But a POSA would need to know how to
20 build the whole device, which includes
21 information about stabilizing the droplets,
22 forming the droplets. That information is

1 specified together with information about the
2 carrier fluids in 116 and 117.

3 In 116 the carrier fluids can be
4 oils, which can include decane as well as
5 fluorinated oils. 117 discuss a -- a number of
6 surfactants that can aid in optimizing the
7 droplet size, which can incorporate fluorinated
8 surfactants.

9 So when taking the whole device into
10 consideration, a POSA would, upon choosing a
11 carrier fluid in the application at hand, then
12 turn to 95 for teachings about the surfactants'
13 properties to coat the walls and 117 for
14 teachings about surfactant properties to
15 control the flow and droplet size.

16 So a POSA would read 95 as
17 explaining and giving examples of surfactants
18 that can be used to coat the wall.

19 Q. Let's talk about paragraph 118.

20 In the second sentence of Paragraph
21 118 there's a reference to that the channels
22 may also be coated additives or agents such as

1 surfactants, Teflon or fluorinated oils.

2 Do you see that?

3 A. I see it.

4 Q. Now, do you understand additives or
5 agents to be essentially synonymous with each
6 other?

7 A. I understand both of them to mean
8 things that can be added to the -- to the
9 system.

10 Q. Do you understand there to be a
11 difference between additives or agents in the
12 context of that usage in Paragraph 118?

13 A. I would think of the surfactants as
14 being additives and Teflon, for example, as
15 being a coating that can be applied.

16 Q. Do you understand there to be a
17 difference between additives or agents in -- as
18 those terms are used in Paragraph 118?

19 Can you answer that question now?

20 A. I would think of agents as being
21 something different from the material in the
22 channel wall and the carrier fluid.

1 Q. The question is if --

2 A. And --

3 Q. -- agents or additives are
4 different.

5 A. Additives would be something that I
6 would add to the carrier fluid. Both cases you
7 have material with -- that's actually different
8 from the channel wall.

9 Q. Okay. And then do you understand
10 surfactants to be different from Teflon, as
11 those terms are used in Paragraph 118?

12 A. I understand Teflon to be material
13 that's essentially permanently applied to the
14 walls; whereas, surfactants are additives that
15 would absorb to the wall.

16 Q. And what is the difference between
17 surfactants and fluorinated oils as those terms
18 are used in Paragraph 118?

19 A. The fluorinated oils mentioned in
20 118 would act as surfactants between a
21 hydrocarbon carrier fluid and the carrier --
22 and the channel walls. And as it would coat

1 the channel walls if a choice had been made to
2 use hydrocarbon carrier fluids.

3 Q. Why does this sentence distinguish
4 between surfactants and fluorinated oils?

5 A. Because they're different additives
6 which could accomplish similar effects
7 depending on the nature of the -- of the
8 channel walls.

9 Q. So as fluorinated oils are described
10 in Paragraph 118, they are not surfactants for
11 coating the channel walls?

12 A. They are surfactant for coating the
13 channel walls because what I explained.

14 Q. Well, that's actually not what I
15 understood from your testimony. But that is
16 what it is.

17 A. Because if they go to the
18 hydrocarbon oil-carrier wall interface, that's
19 what a surfactant does, is it tends to lower
20 the surface tension between the interface.

21 Q. Why isn't fluorinated oil a type --
22 why -- why do they use the term "surfactant"

1 and "fluorinated oil" separately in that
2 sentence?

3 Is that question understandable?

4 MS. ELLISON: Objection. Scope.

5 BY MR. REINES:

6 Q. You're saying a -- a -- a
7 fluorinated oil, as that term is used in 118,
8 is a type of surfactant, correct?

9 And I'm referring to
10 octadecafluorooctane and fluronononane.

11 But you're saying those are
12 surfactants, right? Or not?

13 A. These are surfactants -- these
14 particular chemical versions of fluorinated
15 oils are surfactants for the hydrocarbon oil
16 and the carrier wall interface. There are
17 other fluorinated surfactants, which are the
18 ones implied in 117, to act at the air-water --
19 at the aqueous-oil interface.

20 MR. REINES: I move to strike the
21 portion about 117.

22 BY MR. REINES:

1 Q. Do you have any explanation why
2 fluorinated oils is separated from the word
3 "surfactants" in 118 if it's just a type of
4 surfactant?

5 Do you see where it says "such as
6 surfactants, Teflon or fluorinated oils"?

7 MS. ELLISON: Objection. Scope.

8 THE WITNESS: I don't know.

9 BY MR. REINES:

10 Q. Do you know whether a person of
11 ordinary skill in the art in May 2002 would
12 understand that fluorinated oils referred to in
13 118 were surfactants or not?

14 Professor Huck didn't understand
15 the -- the usage.

16 MS. ELLISON: Objection. Form.

17 THE WITNESS: Can you state the
18 question again?

19 BY MR. REINES:

20 Q. Do you have any explanation why
21 Paragraph 118 describes fluorinated oils using
22 the word "or" as an alternative to surfactants

1 if you're saying that you believe it's a
2 surfactant?

3 A. In Paragraph 19 of my declaration, I
4 explain that a surfactant reduces a surface
5 tension between different phases. And this can
6 be between liquid-liquid, liquid-solid or
7 liquid-gas.

8 So the choice of material used for a
9 surfactant depends on the interface whose
10 surface tension you would like to reduce. Or
11 equivalently it depends on the surface to which
12 you would like something to absorb.

13 So in the context of a hydrocarbon
14 carrier fluid and a channel wall, a fluorinated
15 oil would actually act as a surfactant and go
16 to that interface between the fluid and the
17 solid.

18 In the context of Paragraph 117,
19 fluorinated is affected -- oils with a
20 hydrophilic head group would then act to reduce
21 the surface tension and aid in controlling and
22 optimizing the droplet size.

1 So there are, in fact, two different
2 kinds of surfactants being discussed.
3 Surfactants that go to the fluid-solid
4 interface, and they go to the fluid-fluid
5 interface. They can be the same material, or
6 they could be different materials.

7 MR. REINES: Move to strike the
8 argument regarding 117.

9 BY MR. REINES:

10 Q. The term "surfactant" in Paragraph
11 118, that relates to coating the walls to
12 address the carrier fluid-wall interface,
13 correct?

14 A. Yes. 118 is concerned with adding
15 either agents or additives to coat the channel
16 wall.

17 Q. But the use of the term
18 "surfactant," right, in that sentence, in 118,
19 the -- the surfactants that are referenced
20 there are for the purpose of coating the walls,
21 correct?

22 That's what it says?

1 A. Yeah. It -- here it references
2 surfactants to coat the wall.

3 Q. And the reference to fluorinated
4 oils in Paragraph 118, that's also referring
5 to -- for wall coating, correct?

6 A. That's correct.

7 Q. And you don't have any explanation
8 for why surfactant -- why it says surfactants
9 or fluorinated oils if surfactant -- if
10 fluorinated oils are a type of surfactant, do
11 you, in Paragraph 118?

12 A. Surfactants can act at the
13 solid-liquid interface or at the liquid-aqueous
14 oil interface. In conventional -- in common
15 usage, people often use the word "surfactant"
16 to think about the oil-water interface.

17 Q. I don't under -- I mean I don't know
18 if it's just part of your generalized argument,
19 but I don't understand why you keep bringing
20 that up.

21 The surfactants that are referenced
22 in that sentence in Paragraph 118 are specific

1 to wall coating, correct?

2 A. That's correct.

3 Q. And the fluorinated oils referred to
4 in Paragraph 118 are specific to wall coating,
5 correct?

6 A. That's correct.

7 Q. So the fact that there's
8 theoretically other interfaces that might be
9 useful for surfactants doesn't answer the
10 question as to why it says surfactants or
11 fluorinated oils, right?

12 Might because I checked my logic in
13 at the hotel and didn't take it out?

14 A. Well, in Paragraph 95 Quake has
15 listed explicitly a number of surfactants. So
16 a POSA reading this might come along and think,
17 oh, here's -- here's some surfactants.

18 Q. Okay.

19 A. And then these surfactants, some of
20 these -- in fact, as they show -- and I forget
21 which paragraph it was when we were talking
22 about the -- the Span. So Span is an example

1 of one of these exemplary surfactants named in
2 95 that will go to the channel wall.

3 Fluorinated oils weren't listed in
4 95, so they wanted to make that explicit, I
5 guess.

6 Q. Now, when you introduce surfactants
7 in a system such as the Quake systems, you --
8 what do you do to tune it for the particular
9 interface that you want to address?

10 Is it concentration? Are there
11 other things?

12 MS. ELLISON: Objection. Form.

13 THE WITNESS: So the propensity of
14 it to sit at an interface or the relative
15 solubility of a surfactant in the solution
16 adjacent to the interface will depend on the
17 chemical differences between the -- here are
18 typically two more ATs in the surfactant, maybe
19 there are more.

20 Typically the -- if you're talking
21 about an aqueous-hydrocarbon interface, the
22 polarity and size of the head group compared

1 with the size of the tail group.

2 But playing with these features you
3 can change the solubility in the parent
4 solution, which could be oil or could be water
5 depending on how -- so Quake talks about making
6 it soluble in oil, which is the preferred case.

7 And by playing with the parameters
8 of the head group and the tail group, you can
9 change the degree to which it adheres to one
10 surface or the other.

11 And if you change the concentration,
12 then you change how much it adheres; and
13 therefore, you change the surface tension.

14 BY MR. REINES:

15 Q. Other than expense, is there -- what
16 is the downside of putting in too much
17 surfactant?

18 For example, Quake says, you know, 2
19 percent is preferred over 5 percent for his
20 example in -- in 90 -- Paragraph 96.

21 MS. ELLISON: Objection. Form.
22 Foundation.

1 THE WITNESS: So if you increase a
2 surfactant concentration, you increase the
3 amount absorbed at the respective interfaces.
4 Eventually that reaches a limit. And you could
5 also -- and maybe that limit's too high. Or
6 you could get microfluidic structures that
7 start to change the fluid properties in the
8 carrier fluid.

9 Essentially you would want to choose
10 your surfactant concentration to optimize the
11 surface tensions.

12 BY MR. REINES:

13 Q. So you can have too much or too
14 little.

15 A. You could have too much or too
16 little.

17 Q. Now let's talk about Paragraph 117.
18 Back to that.

19 In terms of the use of surfactants
20 for reducing the shear force needed to form
21 droplets in the intersecting channel, can you
22 explain how the surfactants would assist in

1 that process?

2 MS. ELLISON: Objection. Form.

3 THE WITNESS: So upon adding a
4 surfactant to the -- the carrier fluid, for
5 example, the aqueous fluid that's pushed out
6 can be -- you have to push it out with a force
7 or flow rate. That's going push against the
8 bulging droplet, and the surface tension fights
9 against that.

10 And so the point at which the
11 droplet breaks off is then determined by the
12 mechanical forces of flow fighting against the
13 mechanical forces of the surface tension.

14 BY MR. REINES:

15 Q. What is the interface?

16 A. The interface between -- so it's the
17 actual formation of the drop. It's the -- you
18 push your aqueous phase out, and it's the
19 interface between the drop and the carrier
20 fluid where it first starts to form the drop.
21 So that's where the initial formation of the
22 droplet occurs.

1 Q. Is the shear -- is it not the
2 interface between the -- the walls of the
3 formation apparatus and the -- and the liquid?

4 MS. ELLISON: Objection. Form.

5 BY MR. REINES:

6 Q. Isn't that the surface tension
7 that's being controlled here?

8 MS. ELLISON: Objection. Form.
9 Foundation.

10 THE WITNESS: If you turn to Figure
11 16-A, please, of the Quake patent.

12 BY MR. REINES:

13 Q. There.

14 A. So that the T junction shows an
15 aqueous solution being extruded out into the
16 oil carrier fluid. And you can see the curved
17 meniscus of the aqueous solution. So that
18 curved meniscus is providing a force acting
19 back against the fluid flow.

20 And the surface tension at the
21 aqueous oil interface reduces -- the
22 surfactant, whatever is absorbed there that

1 reduces the surface tension, will then reduce
2 the force that counteracts the shear forces.

3 So in that way adding a surfactant
4 lowers the surface tension and aids the initial
5 formation in addition to helping with the flow
6 and uniformity of it.

7 Q. So the -- the reference of the use
8 of surfactants in Paragraph 117 to the droplet
9 formation process, that's referring to the
10 control -- the -- the -- the influence on
11 surface tension before there's a droplet
12 substantially encased by the carrier fluid,
13 correct?

14 MS. ELLISON: Objection.

15 THE WITNESS: That's --

16 MS. ELLISON: Form. Foundation.

17 BY MR. REINES:

18 Q. Is that correct?

19 A. That's one way --

20 MS. ELLISON: Objection. Form.
21 Foundation.

22 Let me interpose my objections.

1 BY MR. REINES:

2 Q. Sorry. She cut you off.

3 What's the answer?

4 A. That's one of the uses.

5 MS. ELLISON: He cut me off.

6 BY MR. REINES:

7 Q. When you say --

8 THE WITNESS: Sorry.

9 BY MR. REINES:

10 Q. -- "one of the uses," the -- the --
11 there's a reference in 117 to droplet formation
12 where it says "by reducing the sheer force
13 needed to extrude or inject droplets into an
14 intersecting channel."

15 Do you see that?

16 A. I do.

17 Q. Okay. And just referring to that
18 clause, the surface tension that's being
19 influenced by the proposed introduction of a
20 surfactant is before there's a droplet that's
21 substantially encased by the carrier fluid,
22 correct, that clause?

1 A. The sentence says that the
2 surfactant can aid in controlling or optimizing
3 the size, flow and uniformity.

4 A POSA reading this and knowing
5 about the effects of surface tension would
6 realize that the droplet size refers to the
7 clause.

8 Surfactants will also influence how
9 the droplets flow and the uniformity of the
10 droplets once they've been formed.

11 Q. Okay. Let me do it this way: Do
12 you see where it refers in Paragraph 17 -- 117
13 to reducing the shear force needed to extrude
14 or inject droplets into an intersecting
15 channel?

16 Do you see that?

17 A. I do.

18 Q. That refers to the droplet formation
19 process, correct?

20 A. That refers to the droplet formation
21 process.

22 Q. And during the droplet formation

1 process that's being referenced in Paragraph
2 117, the surface tension that's being
3 controlled is not taking place while the
4 droplet is substantially encased in carrier
5 fluid, correct?

6 MS. ELLISON: Objection. Form.

7 THE WITNESS: Well, it -- it applies
8 all the way until it breaks off and then after
9 that as well. The surface tension at the
10 aqueous carrier interface is a property that
11 influences both what happens as it pinches
12 off -- so as it pinches off, it becomes totally
13 encased, and then off it goes -- and then it
14 influences what happens as it goes down.

15 BY MR. REINES:

16 Q. That's after the shear force is
17 reduced to extrude or inject droplets, the
18 latter part of that -- your argument that the
19 surface tension is influenced by the surfactant
20 after the droplet is created and surrounded by
21 the carrier fluid, that relates to events that
22 take place after the shear force is reduced to

1 extrude or inject droplets into an intersecting
2 channel, right?

3 And whether you admit it or not,
4 we're --

5 A. No. I don't -- I don't -- I'm not
6 sure I understand the question.

7 Q. Okay.

8 A. Because surfactants, they reduce --

9 Q. We'll just argue it?

10 A. As soon as it comes in -- okay.

11 Q. Now, let's take the droplet
12 formation process.

13 If you reduce the shear force needed
14 to inject droplets into an intersecting
15 channel, does that allow you to control droplet
16 size better?

17 A. It will help you to control the
18 droplet size better.

19 Q. Will it help you -- will it help
20 with the flow?

21 A. It will also help with the flow.

22 Q. And will it help uniformity?

1 A. It will also help with uniformity.

2 Q. Now, I want to ask you, your -- your
3 theory of invalidity here in terms of the use
4 of surfactants in the specific way that's
5 described in the '083 patent, that centers on
6 the language in Paragraph 117, droplet size --
7 the -- that surfactants may aid in controlling
8 or optimizing droplet size, flow and
9 uniformity, correct?

10 MS. ELLISON: Objection. Form.

11 THE WITNESS: I don't understand
12 what you mean by my theory of invalidity.

13 BY MR. REINES:

14 Q. Do you believe the '083 claims are
15 disclosed by the Quake patent application?

16 Do you know one way or the other?

17 A. I was not asked to read the '083
18 patent, and I have not read it in sufficient
19 detail --

20 Q. Okay.

21 A. -- to comment on that.

22 Q. Now, the Quake patent discloses a

1 number of ways to control droplet flow, size
2 and uniformity other than surfactants, correct?
3 Such as shape?

4 A. They may have. Yes. For example,
5 in Paragraph 115, Quake mentions that
6 periodicity and drop in volume can depend upon
7 channel diameter, viscosity of the fluids, the
8 pressure. So there are a number of quantities
9 that can be employed to control droplet size.

10 Q. Now, can the Quake system flow
11 successfully if the surface tension at the
12 droplet-wall interface is high -- let me start
13 again.

14 Can the -- would a person of
15 ordinary skill in the art in 2002 understand
16 that the Quake system can flow successfully
17 even if the surface tension at the droplet-wall
18 interface is not higher than the surface
19 tension at the droplet-carrier fluid interface?

20 MS. ELLISON: Objection. Form.
21 Scope.

22 THE WITNESS: Which surface

1 tensions? Can you state that again?

2 BY MR. REINES:

3 Q. Would a person of ordinary skill in
4 the art in 2002 understand that the Quake
5 system can flow successfully even if the
6 surface tension at the droplet-wall interface
7 is not higher than the surface tension at the
8 droplet-carrier fluid interface?

9 MS. ELLISON: Objection. Form.
10 Scope.

11 THE WITNESS: Sorry. I'm getting
12 confused by which -- which is a negative, which
13 is a positive in your -- your case.

14 BY MR. REINES:

15 Q. Okay. Let me -- let me try it this
16 way: Would a person of ordinary skill in the
17 art understand that the Quake system can flow
18 successfully even if the surface tension at the
19 droplet-wall interface is not higher than the
20 surface tension at the droplet-carrier fluid
21 interface?

22 MS. ELLISON: Objection. Form.

1 Scope.

2 THE WITNESS: So you want to
3 consider a case where the droplet-carrier
4 interfacial tension is higher or lower than the
5 droplet wall?

6 BY MR. REINES:

7 Q. Does Quake teach whether the --
8 anything about whether the surface tension at
9 the droplet-wall interface must be higher than
10 the surface tension at the droplet-carrier
11 fluid interface?

12 MS. ELLISON: Objection. Form.
13 Scope.

14 THE WITNESS: So if the surface
15 tension between the droplet and the wall is
16 high compared to the carrier droplet, then it
17 costs you a lot of energy to adsorb that
18 droplet on the surface. Therefore, it won't
19 stick to the surface.

20 If it's the other way around, and
21 the surface tension between the aqueous wall is
22 lower, then, in fact, it will stick onto the

1 surface.

2 And this is the property of droplets
3 on surfaces. And a POSA would be familiar with
4 these properties of droplets on surfaces and
5 know that you need to have the surface tensions
6 between the droplet, the carrier and the
7 surface in such a way that the droplet doesn't
8 wet the surface.

9 So the process of the dropping --
10 droplet sticking to the surface is called
11 wetting to the surface. And a POSA would have
12 been familiar with wetting problems. They go
13 back hundreds of years -- hundred years.

14 So if a POSA had discovered that the
15 drops were sticking to the surface, the POSA
16 would realize that the surface tensions were
17 wrong.

18 BY MR. REINES:

19 Q. So it's -- it's your --

20 MR. REINES: Let me -- I move to
21 strike that as nonresponsive because it wasn't
22 responsive to the question.

1 BY MR. REINES:

2 Q. But it's your view that, in order
3 for the Quake system to work, the surface
4 tension at the droplet-wall interface must be
5 higher or lower -- or must be higher -- let me
6 start again.

7 Do you believe is it a requirement
8 that a person of ordinary skill in the art
9 would read into the Quake reference that the
10 surface tension at the droplet-wall interface
11 must be higher than the surface tension at the
12 droplet-carrier fluid interface?

13 MS. ELLISON: Objection. Form.
14 Scope.

15 THE WITNESS: Quake would read 117
16 and learn Quake's teachings that surfactants
17 can control and optimize the flow.

18 If a POSA had problems with the
19 flow, one problem is that it sticks to the
20 wall. Surfactants would allow a POSA to tune
21 the surface tension to bring the tension
22 between the aqueous droplet and the carrier

1 down low enough that it will no longer stick to
2 the wall.

3 And so the POSA would then realize
4 that the POSA needs to tune the properties of
5 the channel surfactant and surfactant that goes
6 to the aqueous-water interface -- in fact,
7 those could be the same surfactant -- such that
8 the droplet will not stick to the wall.

9 And because a POSA would have been
10 familiar with wetting problems, this would be
11 in their normal vocabulary.

12 MR. REINES: Move to strike as
13 nonresponsive.

14 BY MR. REINES:

15 Q. Would one of ordinary skill in the
16 art understand that, in implementing the Quake
17 system, that the -- would they believe that the
18 surface tension at the droplet-wall interface
19 must be higher than the surface tension at the
20 droplet-carrier fluid interface?

21 MS. ELLISON: Objection. Form.
22 Foundation.

1 BY MR. REINES:

2 Q. Would they believe that would be a
3 requirement of the Quake system?

4 MS. ELLISON: Objection. Form.
5 Foundation.

6 THE WITNESS: I believe that a POSA
7 would very quickly realize that that is the
8 situation, that a lower surface tension is
9 required between the droplet carrier interface
10 and between the droplet channel wall.

11 MS. ELLISON: Can we take a break
12 soon? We've been going almost an hour and a
13 half.

14 MR. REINES: Sure. Okay. Now's
15 fine.

16 THE VIDEOGRAPHER: We are going off
17 the record at 4:49 p.m.

18 (A short recess was taken.)

19 THE VIDEOGRAPHER: We are back on
20 the record at 4:59 p.m.

21 BY MR. REINES:

22 Q. Do all of the surfactants in

1 paragraph -- referenced in Paragraph 118 use
2 hydrophilic head groups, in your opinion?

3 A. In Paragraph 118?

4 Q. That's correct.

5 A. So 118 lists additives or agents,
6 such as surfactants Teflons -- Teflon,
7 fluorinated oils, with two explicit fluorinated
8 oils named.

9 Those fluorinated oils do not have
10 hydrophilic head groups. But the other
11 surfactants that are encapsulated could include
12 hydrophilic head groups. That, in fact, the
13 example that Quake gives in the paragraph where
14 he adds Tween or Span was an example with
15 hydrophilic head groups.

16 So some of them do and some of them
17 don't.

18 Q. Some surfactants will have
19 hydrophilic head groups, and some won't, right,
20 in the -- that Quake -- Quake describes?

21 MS. ELLISON: Objection. Form.
22 Foundation.

1 BY MR. REINES:

2 Q. Is that not true?

3 MS. ELLISON: Objection. Form.

4 THE WITNESS: The fluorinated oils
5 that are named here, the octadecafluorooctane,
6 does not have a hydrophilic head group. The
7 other surfactants that would fall under the
8 word "surfactants" will include surfactants
9 that have hydrophilic head groups. That's how
10 I would read it.

11 BY MR. REINES:

12 Q. In -- and as one skilled in the art
13 reading Quake and selecting surfactants for the
14 channel walls may or may not use surfactants
15 that have hydrophilic head groups, correct?

16 Depending on what the wall is made
17 of, right?

18 A. The choice would depend on the wall
19 and the channel. And Quake gives an example.
20 So he quotes here octadecafluorooctane, which
21 is one that doesn't have a hydrophobic head --
22 hydrophilic head -- excuse me -- and then the

1 example -- I forget which paragraph it is --
2 where he plays with the concentration of Span
3 that has a hydrophilic head.

4 Q. Okay. Let's --

5 A. But they are both examples chosen.

6 Q. Let's take a situation for -- where
7 you want to prevent contaminants from attaching
8 to the wall, and you're using a hydrophobic
9 head group for that on your surfactant.

10 Do you have that situation in mind?

11 A. Hydrophobic or hydrophilic?

12 Q. Phobic. Reverse. In the situation
13 where the channel wall is hydrophilic.

14 The channel walls can be either
15 hydrophobic or hydrophilic, correct?

16 Do you know that?

17 A. They can be.

18 Q. Okay.

19 A. Yes.

20 Q. All right. So let's say you're in a
21 situation where you're following Quake's
22 disclosure, and you're using a hydrophobic head

1 group to prevent contaminants from attaching to
2 the walls.

3 Do you have that in mind?

4 Let me do it this way: Will you
5 admit there are situations where, if you were
6 following Quake's disclosure, you use a
7 hydrophobic head group on a surfactant to
8 prevent contaminants from attaching to the
9 walls?

10 A. A hydrophobic head group, not a
11 hydrophilic head group.

12 Q. I'm --

13 A. Are you --

14 Q. Hydrophobic.

15 A. And what would the tail be in the
16 surfactant that you're asking about?

17 Q. Hydrophilic or hydrophobic.

18 A. If the molecule has a hydrophilic
19 and a hydrophilic moiety, that's something one
20 can use.

21 Q. If the head group -- if you -- if --
22 if a person following the Quake disclosure is

1 using a hydrophobic head group on the
2 surfactant to prevent contaminants from
3 attaching to the wall -- do you have that
4 situation in mind?

5 Please don't throw elbows.

6 Do you have that situation in mind?

7 You've acknowledged that that
8 situation can exist.

9 Do you have it in mind?

10 No? Got to go around again?

11 A. Are you thinking of a surfactant
12 that is -- has a hydrophobic piece and a
13 hydrophilic piece or in which case?

14 Q. If you're following the Quake
15 reference, can you use -- are there situations,
16 depending on the materials of the channel wall,
17 where you use a fluorinated surfactant with a
18 hydrophobic head group?

19 MS. ELLISON: Objection. Form.
20 Foundation.

21 THE WITNESS: The most natural case
22 would be to use a fluorinated surfactant with a

1 hydrophilic head group. The reason being that
2 it was a molecule that's got a fluorinated tail
3 and a hydrophobic head group and both if head
4 and the tail are hydrophobic.

5 BY MR. REINES:

6 Q. Will you admit that there's -- if
7 you were following the Quake teachings, that
8 there's situation where you'd use hydrophobic
9 head groups on surfactants to prevent
10 contaminants from attaching to the walls?

11 It's okay -- if you want to deny
12 it --

13 A. I -- I -- I don't -- I don't see any
14 examples off the top of my head --

15 Q. Okay.

16 A. -- or thinking about it.

17 Q. And so you're just denying it,
18 right? Right?

19 Okay. I can move on.

20 And so no -- there's no way to use
21 the Quake disclosure at all with surfactants
22 with hydrophobic head groups according to you,

1 right?

2 The -- you'll go that far?

3 It's impossible, right?

4 MS. ELLISON: Objection. Form.

5 THE WITNESS: I don't think anything
6 is impossible. Okay. Some things are
7 impossible.

8 Quake talks about using fluorinated
9 oils such as "octadecafluoride." That's a
10 hydrophobic object.

11 BY MR. REINES:

12 Q. What is the head group on that
13 fluorinated oil surfactant?

14 Just clock me out.

15 A. So this molecule is a molecule
16 that's hydrophobic. It's also fluorinated. So
17 if a POSA was using a nonfluorinated carrier
18 fluid and used this molecule as an -- an
19 additive to the fluid, it would go -- being
20 fluorinated, it is not so happy in the
21 hydrocarbon carrier. So it would go to the
22 channel wall and, in that sense, act as a

1 surfactant between the carrier fluid and the
2 channel wall.

3 It's not a molecule that has a
4 separate head group and tail group moiety.
5 It's a molecule that is a fluorocarbon, is
6 hydrophobic and is oleophobic, meaning that it
7 would act as a surfactant on the interface
8 between the oil and the channel wall.

9 Q. The fluorinated oil surfactants
10 disclosed in the Quake disclosure, they don't
11 have to have head groups at all, right?

12 MS. ELLISON: Objection. Form.

13 THE WITNESS: In Paragraph 117 Quake
14 discloses exemplary surfactants that include
15 fluorinated oils. In 117 Quake discloses these
16 surfactants to aid in optimizing the properties
17 of the droplets.

18 Fluorinated oil surfactants that
19 will aid in this property will have a
20 hydrophilic head group, and a POSA would be
21 aware of that. There are examples in Taylor,
22 which I guess is referenced 1079.

1 MR. REINES: Move to strike as
2 nonresponsive.

3 BY MR. REINES:

4 Q. The question I had is are there
5 fluorinated oil surfactants in the Quake
6 disclosure that can function without having any
7 head group at all?

8 A. There are molecules that can --
9 fluorinated molecules without a head group
10 which would act at the carrier-wall interface.
11 They would not play a role at the
12 aqueous-carrier interface without having a head
13 group.

14 Q. Would the surfactants referenced in
15 Paragraph 118 of the Quake disclosure have to
16 have a head group?

17 A. The surfactants disclosed in 118
18 could have a head group, or they might not have
19 a head group. And Quake gives examples of both
20 in his -- in the patent application.

21 Q. Can the fluorinated oils referred to
22 in Paragraph 118 of the Quake disclosure have a

1 head group that is hydrophobic, according to
2 you?

3 A. The fluorinated oil that's listed as
4 octadecafluorooctane is a hydrophobic molecule.
5 So, therefore, it has hydrophobic character.
6 So it has a hydrophobic head and a hydrophobic
7 tail if one likes to characterize it that way.

8 Q. Will you -- where Quake states in
9 Paragraph 117 that surfactants may aid in
10 controlling or optimizing droplet size, flow
11 and uniformity, do you see that?

12 A. I see that.

13 Q. Would that be accomplished by -- is
14 one way of accomplishing that by having
15 surfactants prevent contaminants from attaching
16 to the walls?

17 MS. ELLISON: Objection. Form.

18 THE WITNESS: It's certainly true
19 that, if too many contaminants are attached to
20 the wall, then the -- the droplets won't flow
21 through because they will eventually clog it
22 up.

1 But even in the absence of -- of
2 contaminants on the wall, the important issue
3 of whether the droplets will actually flow
4 without getting themselves stuck to the wall
5 needs to be addressed.

6 And in my opinion, that's one of the
7 important roles of the surfactants in
8 controlling the flow and uniformity of the
9 droplets after they've been formed.

10 Q. If flow improvement is sought
11 pursuant to Paragraph 117 via the use of
12 surfactants, if the tactic that's being used is
13 to remove the wall adherence, then you wouldn't
14 have -- then you could use surfactants that do
15 not have a hydrophobic head group, correct?

16 MS. ELLISON: Objection. Form.

17 MR. REINES: I mean -- excuse me --
18 yeah, it's hydrophobic. I said the right
19 thing.

20 MS. ELLISON: Objection to form.

21 THE WITNESS: Can you repeat the
22 question?

1 BY MR. REINES:

2 Q. If flow improvement pursuant to
3 Paragraph 117 of the Quake disclosure is
4 attempted through the use of surfactants with
5 the tactic of removing wall contaminants, you
6 could use a surfactant with a hydrophobic head,
7 correct?

8 MS. ELLISON: Objection to form.
9 Foundation.

10 THE WITNESS: I don't read 117 as
11 the use of surfactants to remove contaminants
12 from the wall.

13 BY MR. REINES:

14 Q. It's -- that's -- that's not -- to
15 your reading of Quake Paragraph 117, that
16 doesn't include at all the use of surfactants
17 to prevent contaminants from adhering to the
18 wall?

19 A. No.

20 Q. No, it does not?

21 A. In my opinion, 117 does not address
22 the use of surfactants to avoid contaminants

1 sticking to the wall.

2 Q. Now, the sentence in the end of
3 Paragraph 117 that says this may affect droplet
4 volume and periodicity or the rate or frequency
5 at which droplets break off into an
6 intersecting channel, that refers to the use of
7 surfactants as part of the droplet formation
8 process, correct?

9 MS. ELLISON: Objection.
10 Foundation. Form.

11 THE WITNESS: So this sentence comes
12 immediately after the final clause of the
13 preceding sentence, which discusses the shear
14 force to extrude -- to inject the droplets into
15 the channel. And then this injection process
16 will -- can be used to influence droplet volume
17 and periodicity.

18 The rest of the preceding sentence
19 talks about the flow of the droplets and the
20 uniformity. And those are properties of what
21 happens after the droplets have started to go
22 downstream.

1 BY MR. REINES:

2 Q. I'm sure you have this, but this is
3 your declaration which has been marked as
4 Exhibit 1085.

5 Now, the second -- if you turn to
6 Page 5, see your summary of the opinions?

7 A. Okay.

8 Q. And the second opinion says: "Quake
9 teaches adding surfactants to the oil carrier
10 fluid to reduce the surface tension at the
11 droplet fluid-carrier fluid interface."

12 Do you see that?

13 A. I see it.

14 Q. And how did you come up with that as
15 an opinion? What made you choose that as an
16 opinion to render?

17 A. On Page 13 of my declaration,
18 Paragraph 24, I address this opinion. So I
19 will summarize it.

20 Q. No, I don't want you to summarize
21 it.

22 A. Okay.

1 Q. What -- how did you decide that was
2 an opinion you wanted to include in your
3 declaration?

4 Let me ask it this way: You agree
5 that Quake discloses in multiple paragraphs the
6 use of surfactants to coat the walls, correct?

7 A. For example, in Paragraph 118 he
8 talks about surfactants to coat the walls.

9 Q. Right.

10 A. That's in --

11 Q. And in 94 and 95 and 96, right?

12 A. There are other examples, yes.

13 Q. Right.

14 But you didn't focus on those in
15 this opinion. You focused on Paragraph 117 and
16 your argument related to that.

17 Do you see that, the second item on
18 page --

19 A. I see that.

20 Q. How did you make that decision as to
21 which of those two things to rely -- how did
22 you make the decision not to rely on the wall

1 coating surfactants to make this argument based
2 on Paragraph 117?

3 MS. ELLISON: Objection. Form.
4 Foundation.

5 THE WITNESS: The bullet point
6 following this one on Page 5 states that I was
7 asked to consider the opinion of Dr. Sia that
8 the only teaching of surfactants are those that
9 would not -- likely not include a hydrophilic
10 head group.

11 Paragraph 117 is an example which is
12 given by Quake of the use of surfactants that
13 include hydrophilic head groups.

14 In paragraph 117, he points out that
15 surfactants will reduce the shear force needed
16 to extrude droplets into the channel.

17 A POSA would know that adding a
18 surfactant with a hydrophilic head group will
19 reduce the surface tension between the water
20 phase and the oil -- and the carrier oil and
21 will be familiar with the fact that the surface
22 tension is something that must be overcome by

1 the shear forces to break it up.

2 BY MR. REINES:

3 Q. What does that have to do with the
4 question I asked you?

5 A. You asked me the question how -- you
6 asked me the question why did I ask about -- if
7 I paraphrase correctly, you asked about why I
8 considered the use of surfactants with a
9 hydrophilic head group.

10 Is that an accurate phrasing of your
11 question?

12 And my answer is that I was --

13 Q. No. I didn't ask that question at
14 all.

15 A. Can you repeat the question then?

16 Q. In your second bullet point, you set
17 forth an opinion about Paragraph 117 of the
18 Quake reference and the droplet fluid-carrier
19 fluid interface when you focused on
20 surfactants.

21 How come you didn't reference all
22 the other paragraphs about surfactants in Quake

1 which made clear that Quake focused on
2 preventing contaminants from attaching to -- to
3 the walls?

4 MS. ELLISON: Objection. Form.
5 Foundation.

6 THE WITNESS: I was asked to
7 consider the opinion of Dr. Sia that the only
8 teaching of surfactants are those that will
9 likely not include a hydrophilic head group.

10 And the bullet point that you've
11 pointed out just above refers to Paragraph 117
12 in which surfactants with a hydrophilic head
13 group have been taught by Quake to modify the
14 properties of the droplet size, flow and
15 uniformity.

16 My final bullet point mentions that
17 agents for coating the channel wall have been
18 disclosed, but this is distinct from the use of
19 the surfactants with a hydrophilic head group
20 to affect the droplet breakup and also
21 influence how well the droplets flow when they
22 go down the channel.

1 BY MR. REINES:

2 Q. Do you agree with -- did you agree
3 that the surfactants disclosed in Quake in
4 Paragraphs 94, 95 and 96 might very well not
5 have a hydrophilic head group?

6 A. Paragraphs 94, 95, 96 discloses that
7 agents should -- could be added to prevent
8 material from adhering to the sides of
9 channels. And 95 lists a number of these
10 agents which have hydrophilic head groups, but
11 it's not limited to those that have hydrophilic
12 head groups.

13 And, in fact, Paragraph 118, as
14 we've discussed, mentions examples that don't
15 have hydrophilic head groups.

16 Q. Quake's disclosure doesn't describe
17 anything about head groups at all, correct?

18 MS. ELLISON: Objection to form.

19 THE WITNESS: Quake has a number of
20 disclosures. And in the disclosures about
21 surfactants he explicitly names numerous
22 surfactants that have hydrophilic head groups.

1 And he discusses the use of surfactants at the
2 oil-aqueous interface.

3 A POSA would understand surfactants
4 in that context together with the use -- the
5 naming of surfactants with hydrophilic head
6 groups as to refer to surfactants with
7 hydrophilic head groups in the context of the
8 air-water interface -- sorry -- water-oil
9 interface.

10 MS. ELLISON: I think --

11 MR. REINES: Move to strike as
12 nonresponsive?

13 MS. ELLISON: I think we're at seven
14 hours.

15 MR. REINES: Okay. We reserve all
16 rights on this. And we -- we, after reviewing
17 the transcript, may seek more time.

18 MS. ELLISON: We don't concede that
19 you have any right to reserve, but so be it.

20 Let's go off the record.

21 THE VIDEOGRAPHER: We are going off
22 the record at 5:36 p.m.

1 (A short recess was taken.)

2 THE VIDEOGRAPHER: We are back on
3 the record at 5:51 p.m.

4 MS. ELLISON: Dr. Olmsted, we don't
5 have any questions for you.

6 Thank you very much for your time.
7 Have a good weekend, everyone.

8 THE VIDEOGRAPHER: Going off the
9 record at 5:51 p.m.

10 (Whereupon, the proceedings were
11 concluded at 5:51 p.m.)

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CERTIFICATE OF NOTARY PUBLIC

I, Bonnie L. Russo, the officer before whom the foregoing deposition was taken, do hereby certify that the witness whose testimony appears in the foregoing deposition was duly sworn by me; that the testimony of said witness was taken by me in shorthand and thereafter reduced to computerized transcription under my direction; that said deposition is a true record of the testimony given by said witness; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this deposition was taken; and further, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of

Bonnie L. Russo

Notary Public in and for
the District of Columbia

My Commission expires: June 30, 2020

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Federal Rules of Civil Procedure

Rule 30

(e) Review By the Witness; Changes.

(1) Review; Statement of Changes. On request by the deponent or a party before the deposition is completed, the deponent must be allowed 30 days after being notified by the officer that the transcript or recording is available in which:

(A) to review the transcript or recording; and

(B) if there are changes in form or substance, to sign a statement listing the changes and the reasons for making them.

(2) Changes Indicated in the Officer's Certificate. The officer must note in the certificate prescribed by Rule 30(f)(1) whether a review was requested and, if so, must attach any changes the deponent makes during the 30-day period.

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VERITEXT LEGAL SOLUTIONS
COMPANY CERTIFICATE AND DISCLOSURE STATEMENT

Veritext Legal Solutions represents that the foregoing transcript is a true, correct and complete transcript of the colloquies, questions and answers as submitted by the court reporter. Veritext Legal Solutions further represents that the attached exhibits, if any, are true, correct and complete documents as submitted by the court reporter and/or attorneys in relation to this deposition and that the documents were processed in accordance with our litigation support and production standards.

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Patent Issued: November 6, 2012

For: METHOD FOR CONDUCTING AN
AUTOCATALYTIC REACTION IN
PLUGS IN A MICROFLUIDIC SYSTEM

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Examiner: Elizabeth L. McKane

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June 12, 2018

Mail Stop Petition
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF SERVICE OF RESPONSE TO FIRST OFFICE ACTION IN
EX PARTE REEXAMINATION OF U.S. PATENT NO. 8,304,193

In compliance with 37 C.F.R. § 1.550(f), the undersigned, on behalf of the Patent Owner, hereby certifies that a copy of the Response To First Office Action In *Ex Parte* Reexamination of U.S. Patent No. 8,304,193, and Exhibits 2001-2032 thereto has been served on the Third Party Requester by first class U.S. mail on June 12, 2018. The name and address of the party is served as follows:

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June 12, 2018

Mail Stop Petition
Commissioner for Patents
P.O. Box 1450
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Re: Reexamination of U.S. Patent No. 8,304,193
Reexam Control No. 90/014,043
For: METHOD FOR CONDUCTING AN AUTOCATALYTIC REACTION IN PLUGS IN A
MICROFLUIDIC SYSTEM

Sir:

Transmitted herewith for appropriate action is the following document:

1. Response To First Office Action In *Ex Parte* Reexamination of U.S. Patent No. 8,304,193;
2. Certificate of Service of Response To First Office Action In *Ex Parte* Reexamination of U.S. Patent No. 8,304,193;
3. Declaration of Dr. Samuel Sia Pursuant to 37 C.F.R. § 1.132 (Exhibit 2001); and
4. Exhibits 2002-2032.

Respectfully submitted,
WEIL, GOTSHAL & MANGES LLP

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<p>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>					

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Search Notes

U.S. Patent No. 8,304,193

- 1) I performed a KeyCite Search in Westlaw, which retrieves all history on the patent including any litigation.
- 2) I performed a search on the patent in Lexis CourtLink for any open dockets or closed cases.
- 3) I performed a search in Lexis in the Federal Courts and Administrative Materials databases for any cases found.
- 4) I performed a search in Lexis in the IP Journal and Periodicals database for any articles on the patent.
- 5) I performed a search in Lexis in the news databases for any articles about the patent or any articles about litigation on this patent.

Litigation: Cases found

Status	Description	Court	Docket Number
Open	Bio-Rad Laboratories, Inc. Et Al v. 10x Genomics Inc.	US-DIS-DED	1:15cv152
Closed	10X Genomics, Inc. vs. The University of Chicago	US-PTO-ALE	IPR2015-01163

2017 WL 382235
United States District Court,
D. Delaware.

RAINDANCE TECHNOLOGIES, INC.,
and The University of Chicago, Plaintiffs,

v.

10X GENOMICS, INC. Defendant.

Civil Action No. 1:15-cv-00152-RGA

Signed 01/26/2017

West Headnotes (1)

[I] Patents

☞ In general; utility

US Patent 7,129,091, US Patent 8,304,193,
US Patent 8,329,407, US Patent 8,658,430,
US Patent 8,822,148, US Patent 8,889,083.
Construed.

Cases that cite this headnote

Go to Markman Construed Terms

Attorneys and Law Firms

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MEMORANDUM OPINION

ANDREWS, U.S. DISTRICT JUDGE:

*1 Presently before the Court is the issue of claim construction of multiple terms in U.S. Patent Nos. 7,129,091 (“the ‘091 patent”), 8,822,148 (“the ‘148 patent”), 8,329,407 (“the ‘407 patent”), 8,304,193 (“the ‘193 patent”), 8,658,430 (“the ‘430 patent”), and 8,889,083 (“the ‘083 patent”). The Court has considered the Parties’ Joint Claim Construction Brief. (Civ. Act. No. 15–152–RGA, D.I. 93). The Court heard oral argument on December 16, 2016. (D.I. 105) (Hr’g Tr.).

I. BACKGROUND

Plaintiffs filed this action on February 12, 2015, alleging infringement of six patents on behalf of both Plaintiffs. (D.I. 1). Plaintiffs refer to these patents as the Ismagilov patents. On April 23, 2015, Plaintiffs filed an amended complaint asserting an additional patent, the ‘430 patent, on behalf of Plaintiff RainDance only. (D.I. 12). On March 25, 2016, Plaintiffs filed a second amended complaint in which they asserted only five of the Ismagilov patents, leaving six patents currently in suit as listed above. (D.I. 32).

II. LEGAL STANDARD

“It is a bedrock principle of patent law that the claims of a patent define the invention to which the patentee is entitled the right to exclude.” *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312 (Fed. Cir. 2005) (en banc) (internal quotation marks omitted). “‘[T]here is no magic formula or catechism for conducting claim construction.’ Instead, the court is free to attach the appropriate weight to appropriate sources ‘in light of the statutes and policies that inform patent law.’” *SoftView LLC v. Apple Inc.*, 2013 WL 4758195, at *1 (D. Del. Sept. 4, 2013) (quoting *Phillips*, 415 F.3d at 1324) (alteration in original). When construing patent claims, a court considers the literal language of the claim, the patent specification, and the prosecution history. *Markman v. Westview Instruments*,

Inc., 52 F.3d 967, 977–80 (Fed. Cir. 1995) (en banc), *aff'd*, 517 U.S. 370 (1996). Of these sources, “the specification is always highly relevant to the claim construction analysis. Usually, it is dispositive; it is the single best guide to the meaning of a disputed term.” *Phillips*, 415 F.3d at 1315 (internal quotation marks omitted).

“[T]he words of a claim are generally given their ordinary and customary meaning.... [Which is] the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention, i.e., as of the effective filing date of the patent application.” *Id.* at 1312–13 (citations and internal quotation marks omitted). “[T]he ordinary meaning of a claim term is its meaning to [an] ordinary artisan after reading the entire patent.” *Id.* at 1321 (internal quotation marks omitted). “In some cases, the ordinary meaning of claim language as understood by a person of skill in the art may be readily apparent even to lay judges, and claim construction in such cases involves little more than the application of the widely accepted meaning of commonly understood words.” *Id.* at 1314.

When a court relies solely upon the intrinsic evidence—the patent claims, the specification, and the prosecution history—the court’s construction is a determination of law. *See Teva Pharm. USA, Inc. v. Sandoz, Inc.*, 135 S. Ct. 831, 841 (2015). The court may also make factual findings based upon consideration of extrinsic evidence, which “consists of all evidence external to the patent and prosecution history, including expert and inventor testimony, dictionaries, and learned treatises.” *Phillips*, 415 F.3d at 1317–19 (internal quotation marks omitted). Extrinsic evidence may assist the court in understanding the underlying technology, the meaning of terms to one skilled in the art, and how the invention works. *Id.* Extrinsic evidence, however, is less reliable and less useful in claim construction than the patent and its prosecution history. *Id.*

*2 “A claim construction is persuasive, not because it follows a certain rule, but because it defines terms in the context of the whole patent.” *Renishaw PLC v. Marposs Societa' per Azioni*, 158 F.3d 1243, 1250 (Fed. Cir. 1998). It follows that “a claim interpretation that would exclude the inventor’s device is rarely the correct interpretation.” *Osram GMBH v. Int’l Trade Comm’n*, 505 F.3d 1351, 1358 (Fed. Cir. 2007) (citation and internal quotation marks omitted).

III. CONSTRUCTION OF DISPUTED TERMS

A. The Patents–In–Suit

The ‘091 patent is directed to a device and method for pressure-driven plug transport and reaction. Claim 1 is representative and reads as follows:

1. A method of conducting a *reaction* within at least one plug comprising the steps of:

introducing a carrier-fluid into a first microchannel of a device;

simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein:

a first plug-fluid comprises a first *reagent*;

a second plug-fluid comprises a second *reagent*;

each plug-fluid is immiscible with the carrier-fluid; and

each plug comprises both the first and second plug-fluids so that the *reaction* of the *reagents* substantially occurs in the plug;

each plug is substantially surrounded by carrier.

(‘091 patent, claim 1) (disputed terms italicized).

The ‘193 patent is directed to a method for conducting an autocatalytic reaction in plugs in a microfluidic system. Claim 1 is representative and reads as follows:

1. *A method for conducting an autocatalytic reaction in plugs in a microfluidic system*, comprising the steps of:

providing the *microfluidic system* comprising at least two channels having at least one junction;

flowing an aqueous fluid containing at least one substrate molecule and *reagents* for conducting an autocatalytic *reaction* through a first channel of the at least two channels;

flowing an oil through the second channel of the at least two channels;

forming at least one plug of the aqueous fluid containing the at least one substrate molecule and

reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and *reagents* for conducting an autocatalytic *reaction* with the at least one substrate molecule; and

providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

('193 patent, claim 1) (disputed terms italicized).

The '407 patent is directed to a method for conducting reactions involving biological molecules in plugs in a microfluidic system. Claim 1 is representative and reads as follows:

1. *A method for conducting a reaction in plugs in a microfluidic system*, comprising the steps of:

providing the *microfluidic system* comprising at least two channels having at least one junction;

continuously flowing an aqueous fluid containing at least one *biological molecule* and at least one *reagent* for conducting the *reaction* between the *biological molecule* and the at least one *reagent* through a first channel of the at least two channels;

*3 continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels;

forming at least one plug of the aqueous fluid containing the at least one *biological molecule* and the at least one *reagent* by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels, the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel, wherein the at least one plug comprises at least one *biological molecule* and the at least one *reagent* for conducting the *reaction* with the at least one *biological molecule*; and

providing conditions suitable for the reaction in the at least one plug involving the at least one *biological molecule* and the at least one *reagent* to form a *reaction product*.

('407 patent, claim 1) (disputed terms italicized).

The '148 patent is directed to a method of performing PCR reactions in continuously flowing microfluidic plugs. Claim 1 is representative and reads as follows:

1. A method comprising the steps of:

providing a *microfluidic system* comprising one or more channels;

providing within the one or more channels a continuously flowing carrier fluid comprising an oil and a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecules under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other;

controlling flow rates of said aqueous fluid and said carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid, each having a substantially uniform size of about 200 μm or less, wherein the target DNA or RNA in said plurality of plugs represents a *Poisson distribution*, and at least one member of said plurality comprises a *single target DNA or RNA molecule* and at least one of the other molecules that can react with the target DNA or RNA molecule; and

providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.

('148 patent, claim 1) (disputed terms italicized).

The '083 patent is directed to a device and method for pressure-driven plug transport and reactions. Claim 1 is representative and reads as follows:

1. *A microfluidic system* comprising:

a *non-fluorinated microchannel*;

a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel;

at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid, wherein the fluorinated surfactant is

present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.

('083 patent, claim 1) (disputed terms italicized).

The '430 patent is directed to manipulating droplet size. Claim 1 is representative and reads as follows:

1. A method for droplet formation, the method comprising the steps of:

*4 providing a plurality of aqueous fluids each in its own aqueous fluid channel in fluid communication with one or more immiscible carrier fluid channels;

forming droplets of aqueous fluid surrounded by an immiscible carrier fluid in the aqueous fluid channels;

applying a same constant pressure to the carrier fluid in each of the immiscible carrier fluid channels; and

adjusting pressure in one or more of the aqueous fluid channels, *thereby* to produce droplets of aqueous fluid in one or more outlet fluid channels.

('430 patent, claim 1) (disputed terms italicized).

B. Disputed Terms

1. "microfluidic system"

a. *Plaintiffs' proposed construction*: " 'microfluidics' refers to a field that involves the transport of fluids through networks of channels, typically having micrometer dimensions"

b. *Defendant's proposed construction*: "a substrate involving the transport of fluids through a network of channels having micrometer dimensions, *i.e.*, lab-on-a-chip"

c. *Court's construction*: "system comprised of at least one substrate having a network of channels of micrometer dimension through which fluid may be transported"

This term appears in asserted claims in the '193, '148, '083, and '407 patents. Defendant wishes to limit a "microfluidic system" to the substrate. Defendant contends that the patent describes the microfluidic system as a "lab-on-a-chip" which is a "physical object" that is referred to as

a "substrate" throughout the specification. (D.I. 93 at 27). As support, Defendant points to the abstract, which states, "The present invention provides microfabricated substrates and methods of conducting reactions within these substrates." (*Id.*; '014 patent at Abstract). Plaintiffs counter that the systems are only "sometimes called labs-on-a-chip" and reference descriptions of the system in the specification that include components that are not on the chip. (D.I. 93 at 15).

As an initial matter, I find Defendant's reliance on the abstract to be misplaced. The words "microfluidic system" do not appear in the abstract. ('148 patent at Abstract). Furthermore, the fact that the invention is said to "provide" substrates does not imply that the inventor intended "substrate" to be a synonym for "microfluidic system."

It seems clear to me that the inventor used the word "system" to mean something different from the words "device" and "substrate." Substrate is defined in the specification as "a layer or piece of material from which devices or chips are prepared or manufactured." (*Id.* at 11:13–14). The specification further provides that substrate "refers either to an entire device or chip or to a portion, area, or section of a device or chip." (*Id.* at 11:17–19). In addition, "[a] device according to the invention preferably comprises at least one substrate." (*Id.* at 14:28–29). The specification, in describing one use of the invention, provides that "these devices and methods may be used to build microfluidic systems according to the invention." (*Id.* at 51:16–17). In other words, devices are manufactured from one or more substrates and devices are used to build microfluidic systems. This indicates that the term "microfluidic system" is intended to mean something broader than the term "substrate." There are numerous other examples throughout the specification demonstrating that the inventor intended "system" to be broader than "substrate." For example, the specification describes synthesis of certain compounds in reference to the substrate (*Id.* at 47:57–58), but references the system when describing crystallization. (*Id.* at 50:24–26).

*5 Given this abundant evidence in the specification, the fact that the inventor chose to claim a "microfluidic system" rather than a "substrate," a term the inventor chose to expressly define, indicates that the invention as claimed is broader than what Defendant proposes. Therefore, I will reject Defendant's proposal. Plaintiffs'

proposed construction, however, is useless, as it does not define “microfluidic system”; rather, Plaintiffs have proposed a definition of the field of microfluidics in general. Therefore, I will reject Plaintiffs’ proposal as well. Instead, I will construe “microfluidic system” to mean “system comprised of at least one substrate having a network of channels of micrometer dimension through which fluid may be transported.” Defendant is prohibited from arguing that a “microfluidic system” is limited to or the equivalent of a “substrate.”

2. “reagent”

a. *Plaintiffs’ proposed construction*: “A component of a plug-fluid that undergoes or participates in at least one type of reaction.”

b. *Defendant’s proposed construction*: “component of a plug-fluid that undergoes or participates in at least one type of reaction in the substrate to produce one or more reaction products or intermediates which may undergo a further reaction or series of reactions”

c. *Court’s construction*: “component of a plug-fluid that undergoes or participates in at least one type of reaction to produce one or more reaction products or intermediates which may undergo a further reaction or series of reactions”

This term appears in asserted claims in the ‘193, ‘091, ‘083, and ‘407 patents. This term is expressly defined in the specification of the various patents. (‘091 patent at 10:60–11:7). The only point of dispute is whether the reaction the reagent undergoes must occur in the substrate. Defendant insists that the definition from the specification requires the reaction occur in the substrate. (D.I. 93 at 18). The specification states:

The term “reagent” refers to a component of a plug-fluid that undergoes or participates (e.g., by influencing the rate of a reaction or position of equilibrium) in at least one type of 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.

(‘091 patent at 10:60–66) (emphasis added). Defendant argues that the phrase “in the substrate” modifies both of the italicized phrases in the quote above; in other words, Defendant believes “in the substrate” modifies, and provides the location for, the “reaction.” (D.I. 93 at 18). Defendant contends that it has merely “simplif[ed] some of the wording, while maintaining its core language.” (*Id.*). I disagree. A more natural reading of this sentence is one in which “in the substrate” modifies only “a reagent-containing carrier-fluid.” Had the intent been to limit the location of all reactions in the way Defendant’s propose, the sentence would more logically be written as, “at least one type of reaction in the substrate with ...” This, in fact, is precisely the effect of Defendant’s “simplification.”

Furthermore, as Plaintiffs point out, “The carrier fluid is the medium that carries the plug down a microfluidic channel.” (*Id.* at 33). It makes sense, then, that a reaction involving the carrier fluid would necessarily take place in the substrate. In contrast, I find nothing in the specification or claims that limits the location of reactions involving plug-fluids to the substrate. For these reasons, I will reject Defendant’s proposed construction to the extent that it limits the location of the reaction. I construe “reagent” to mean “component of a plug-fluid that undergoes or participates in at least one type of reaction to produce one or more reaction products or intermediates which may undergo a further reaction or series of reactions.”

3. “reaction”

*6 a. *Plaintiffs’ proposed construction*: “No construction necessary, but if construed ‘a physical, chemical, biochemical or biological transformation’”

b. *Defendant’s proposed construction*: “a physical, chemical, biochemical or biological transformation that substantially occurs on a substrate”

c. *Court’s construction*: “physical, chemical, biochemical or biological transformation”

This term appears in asserted claims in the ‘193, ‘148, ‘083, ‘091, and ‘407 patents. As with the previous term, this term is expressly defined in the specifications of the patents

and the only dispute is again about whether to impose a limitation on the location of the reaction.

As an initial matter, I note that the definition in the specification does not mention the substrate or otherwise limit the location of the reaction. Defendant cites extensively to the descriptions of certain embodiments to argue that the only place a reaction may occur in this invention as claimed is on a substrate. (D.I. 93 at 19–23). Plaintiffs counter that the specification discloses at least one type of reaction, crystallization, which, in certain embodiments, occurs off-chip. (*Id.* at 16–17). Defendant attempts to rebut this argument by pointing to a restriction requirement imposed by the examiner during prosecution of the patent (D.I. 95–3 at 268) directing the inventor to elect either the “method of conducting a reaction” or the “method of crystallizing a substance.” (D.I. 93 at 44). Defendant argues that because crystallization and conducting a reaction are distinct inventions, Plaintiffs’ reliance on crystallization to show that a reaction may occur off-chip is misplaced. (D.I. 93 at 44).

I do not find the restriction requirement to be a persuasive reason to narrow the construction of this term. “[A] patent applicant’s response to a restriction requirement may be used to interpret patent claim terms or as a source of disclaimer.” *Uship Intellectual Properties, LLC v. United States*, 714 F.3d 1311, 1315 (Fed. Cir. 2013). The applicant’s response must, however, “constitute[] a clear and unmistakable disclaimer of claim scope.” *Id.* Here, the applicant’s response was to elect one of the inventions, “[f]or examination purposes only.” (D.I. 95–3 at 272). Neither the examiner nor the applicant addressed whether crystallization is a reaction, which is the point of dispute between the parties. Furthermore, even accepting that crystallization is sufficiently distinct from other types of reactions so as to justify the restriction requirement, this does not mean that crystallization is not itself a reaction. In fact, the specification clearly defines “reaction” to include crystallization. (’091 patent at 10:49–59). Therefore, I find no support for limiting the location where “reactions” occur to the substrate and I will adopt Plaintiffs’ proposed construction.

4. “providing conditions suitable for [the autocatalytic reaction/the reaction/a polymerasechain reaction]”

a. *Plaintiffs’ proposed construction*: “Providing a set of physical and chemical conditions that allow the [autocatalytic reaction/reaction/ polymerase-chain reaction] to occur”

*7 b. *Defendant’s proposed construction*: “requiring a global set of physical and chemical conditions that allow the [autocatalytic reaction/reaction/polymerase-chain reaction] to occur in plugs on a substrate”

c. *Court’s construction*: “Providing a set of physical and chemical conditions that allow the [autocatalytic reaction/reaction/polymerase-chain reaction] to occur.”

These terms appears in asserted claims in the ’193, ’148, and ’407 patents. As an initial matter, Defendant has made no argument regarding the phrase “requiring a global set” of conditions. It is unclear to me what Defendant intends to convey by “global set.” Since this only adds unnecessary confusion to the meaning of this term, I will reject Defendant’s proposed construction. Aside from this, the only dispute between the parties (and the only thing the parties argued) is whether the reactions must occur on a substrate. For the reasons stated above in my construction of “reaction,” I reject Defendant’s proposed limitation and adopt Plaintiffs’ construction.

5. “detecting”

a. *Plaintiffs’ proposed construction*: “No construction necessary.”

b. *Defendant’s proposed construction*: “ ‘detecting’ refers to detecting in the detection region”

c. *Court’s construction*: “No construction necessary.”

This term appears in asserted dependent claims of the ’091 patent. Defendant argues that the term must be construed with reference to a detector or detection region, terms that are defined within the specification. (D.I. 93 at 48–49). In other words, Defendant does not actually offer a construction, but seeks to limit the meaning of the term to restrict where “detecting” may occur. This limitation is not supported by the intrinsic evidence. While the specification offers an express definition for “detection region,” there is no express definition for “detecting,” nor is “detecting” used in the definition of “detection

region.” (’091 patent at 7:61–64). Furthermore, the claims at issue do not mention a detection region. Claim 22, for example, calls for “detecting the presence of at least one plug.” The specification indicates that, at least sometimes, detection occurs after the plugs leave the substrate. (’091 patent at 33:64–66). It seems to me that while “detecting” may occur in the detection region, there is nothing in the patent that limits “detecting” in this way. Therefore, I reject Defendant’s proposed construction and find that no construction is necessary for this term. Defendant is prohibited from arguing that detecting must occur in a detection region.

6. “A method for conducting an autocatalytic reaction in plugs in a microfluidic system”/“A method for conducting a reaction in plugs in a microfluidic system”

a. *Plaintiffs’ proposed construction*: “Preamble is not limiting.”

b. *Defendant’s proposed construction*: “Preamble is limiting.”

c. *Court’s construction*: The entire preamble is not limiting. The terms “reaction” and “microfluidic systems” are limiting as previously construed.

These terms appear in asserted claims in the ’407 and ’193 patents. The only dispute with respect to this term is whether the preamble is limiting. Defendant wishes to construe the entire preamble so as to limit the method such that the reaction takes place “in the substrate.” Defendant argues that the preamble provides an antecedent basis for the claim terms “microfluidic system” and “reaction.” (D.I. 93 at 52–53). Defendant further argues that “the preambles specify where ‘the reaction’ must occur” and, therefore, the preamble is necessarily limiting. (*Id.* at 53) (emphasis omitted). While portions of a preamble may be limiting where those portions provide an antecedent basis for terms appearing in the body of the claim, it is inappropriate to construe an entire preamble as limiting if the rest of the preamble language is not limiting. *TomTom, Inc. v. Adolph*, 790 F.3d 1315, 1323 (Fed. Cir. 2015). Here, the preamble language states an intended use for the invention, “followed by the body of the claim, in which the claim limitations describing the invention are recited.” *Id.* at 1324. Furthermore, the invention as claimed is “structurally complete” without the remaining preamble language. *Id.* The claim elements are duplicative of the

preamble in that it is clear that the reaction in question takes place “in the at least one plug.” (’193 patent, claim 1; ’407 patent, claim 1). Nothing in the body of the claims further limits the location of the reaction. Therefore, I decline to construe the entire preamble or find that the entire preamble is limiting. I find that the preamble is limiting only to the extent that it provides an antecedent basis for the terms “microfluidic system” and “reaction,” both of which I have already construed.

7. “a single target DNA or RNA molecule”

*8 a. *Plaintiffs’ proposed construction*: “No construction necessary, but if construed ‘a single DNA or RNA molecule of interest’ ”

b. *Defendant’s proposed construction*: “one target DNA or RNA molecule”

c. *Court’s construction*: “one and only one target DNA or RNA molecule”

This term appears in asserted claims in the ’148 patent. With respect to Defendant’s proposed construction, it is not clear to me how “one” is materially different from “a single.” That being said, it seems to me that what Defendant is actually arguing is that the plugs may contain one and only one DNA or RNA molecule. (D.I. 93 at 70; Hr’g Tr. at 106:7–107:17). Defendant points to both the specification and prosecution history to support its argument. (D.I. 93 at 69–70, 72). Plaintiffs object that this construction effectively reads out the word “target.” (*Id.* at 71).

I agree with Defendant. The specification makes clear in describing the possible concentrations of molecules in plugs that “the reagent concentration may be adjusted to be dilute enough that most of the plugs contain no more than a single molecule or particle.” (’148 patent at 20:15–17). The specification also indicates that, “The plug includes reagents sufficient for an autocatalytic reaction including a first species of molecule in a concentration such that the plug contains no more than a single molecule of the first species.” (*Id.* art 2:58–61). The molecule “may be DNA or RNA.” (*Id.* at 2:65–66). This, it seems to me, indicates that there may only be one DNA or RNA molecule in the plug. This reading is supported by the prosecution history. In fact, an earlier version of claim 1 of the ’148 patent specified that, “said subset of the plurality of plugs comprises a single biological molecule.” (D.I. 95–

3 at 287). The examiner rejected this claim under § 112, first paragraph, asserting that this portion of the claim was not adequately described in the specification. (*Id.* at 289). The applicant subsequently amended the phrase to read, “said plurality of plugs represents a Poisson distribution, and at least one member of said plurality comprises a single target DNA or RNA molecule.” (*Id.* at 287). There is no evidence in the prosecution history as to why the applicant inserted the word “target” into the claim, but the applicant argued for allowance on the basis that the new language specifying that plugs “include [] a single DNA or RNA” is supported by the portion of the disclosure cited above. (*Id.*; see also '148 patent at 20: 15–17).¹ The word “target” also appears earlier in the claim in reference to the composition of the fluid: “a continuously flowing aqueous fluid comprising target DNA or RNA molecules.” (*Id.* at claim 1). I think that this use of the word “target” is not inconsistent with plugs having a single DNA or RNA molecule. It seems clear from both the specification and prosecution history that the inventor intended “a single target DNA or RNA molecule” to mean that the plugs in question contained one and only one DNA or RNA molecule.

*9 While I agree in principle with Defendant's interpretation of this claim term, Defendant's proposed construction does not provide any clarity on the issue of whether the plugs contain one and only one DNA or RNA molecule or in any way add anything useful to the existing claim language. Furthermore, I decline to adopt Plaintiffs' position on the meaning of “target” not only because it is unclear what is meant by “of interest,” but also because allowing for the possibility of more than one DNA or RNA molecule in the plug is inconsistent with the intrinsic evidence. I will retain the word “target” in my construction, however, as it is used consistently throughout the claim to describe the DNA or RNA molecules that undergo reactions in the claimed method. Therefore, I construe this term to mean “one and only one target DNA or RNA molecule.”

8. “Poisson distribution”

- a. *Plaintiffs' proposed construction*: “A distribution of objects where there is an equal and independent probability for each object to be distributed into any one of a number of partitions”

- b. *Defendant's proposed construction*: “a distribution of target DNA or RNA molecules in plugs where there is an equal and independent probability for each target DNA or RNA molecule to be distributed into any one of a number of plugs”

- c. *Court's construction*: “distribution of target DNA or RNA molecules in plugs where there is an equal and independent probability for each target DNA or RNA molecule to be distributed into any one of a number of plugs”

This term appears in asserted claims in the '148 patent. Defendant would construe this term using references to the claim language, which Plaintiffs complain introduces unnecessary redundancy. (D.I. 93 at 72). As I noted at oral argument (Hr'g Tr. at 98:22–99:17), there does not seem to be an actual dispute as to the meaning of this term. Although Defendant's proposed construction does introduce redundancy, it is also less abstract than Plaintiffs' proposal. Therefore, I will adopt Defendant's construction.

9. “non-fluorinated microchannel”

- a. *Plaintiffs' proposed construction*: “A channel of a microfluidic device that has not been treated to include fluorine atoms at its surface”

- b. *Defendant's proposed construction*: “a microchannel that does not include one or more fluorine atoms”

- c. *Court's construction*: “microchannel that is not composed of a material that includes fluorine atoms or that is treated to include fluorine atoms at its surface (excluding the possible inclusion of impurities or contaminants)”

This term appears in asserted claims in the '083 patent. Plaintiffs argue that Defendant's proposed construction would encompass a microchannel that had an impurity or contaminant of even a single fluorine atom. (D.I. 93 at 73). Defendant complains that Plaintiffs' proposed construction would lead to the “nonsensical” result that a microchannel fabricated from a fluorinated polymer would be a non-fluorinated microchannel if it had not been treated. (*Id.* at 75). At oral argument, the parties agreed in principle that the presence of impurities would not create a fluorinated microchannel from an

otherwise non-fluorinated microchannel, but the parties did not agree upon exact language. (Hr'g Tr. at 111:24–113–16). Therefore, I will construe this term to mean: “microchannel that is not composed of a material that includes fluorine atoms or that is treated to include fluorine atoms at its surface.” Defendant is prohibited from arguing that fluorine impurities or contaminants render a non-fluorinated microchannel fluorinated.

10. “biological molecule”

a. *Plaintiffs' proposed construction*: “Molecules such as DNA, RNA, carbohydrates, and sugars and variants thereof.”

b. *Defendant's proposed construction*: “naturally occurring molecules such as proteins, DNA, RNA, carbohydrates, and sugars”

c. *Court's construction*: “molecules such as proteins, DNA, RNA, carbohydrates, and sugars”

*10 This term appears in asserted claims in the '407 patent. The parties dispute whether a molecule must be “naturally occurring” in order to be a biological molecule. Defendant argues that the patent “distinguishes between ‘biological’ and ‘synthetic small molecules’ that are not naturally occurring.” (D.I. 93 at 79). Defendant further urges that the patent “distinguishes between ‘biological molecules,’ which it defines as ‘proteins, DNA, RNA, carbohydrates, sugars, etc.’ and ‘biomolecules.’ ” (*Id.* at 81). Defendant contends that biomolecules are not biological molecules. (*Id.* at 79). Plaintiffs counter that the patent expressly contemplates the use of synthetically modified biological molecules. (*Id.* at 77). I agree with Plaintiffs. Nowhere in the specification or claims is there any mention of a requirement that biological molecules, as the term is used in the patent, be naturally occurring. As Plaintiffs point out, the express definition of protein, which Defendant recognizes as a biological molecule, specifies that a protein may be “naturally-occurring or man-made.” ('407 patent at 11:4–5). Defendant has not provided any citations to the intrinsic record or any extrinsic evidence to suggest that a person of ordinary skill would understand “biological molecule” as used in the patent to be restricted to only those biological molecules that happen to be naturally occurring.

Defendant further objects to Plaintiffs' “open-ended list” of molecules. (D.I. 93 at 78). While such an open-ended

list is not ideal, I doubt it would be possible to create a comprehensive list of biological molecules. Furthermore, the list Defendant objects to corresponds precisely with how the specification describes biological molecules. ('407 patent at 20:54–55). Therefore, I will construe “biological molecule” as “molecules such as proteins, DNA, RNA, carbohydrates, and sugars.” Defendant is prohibited from arguing that a biological molecule must be naturally occurring.

11. “thereby”

a. *Plaintiffs' proposed construction*: “No construction necessary.”

b. *Defendant's proposed construction*: “in order to”

c. *Court's construction*: “No construction necessary.”

This term appears in asserted claims in the '430 patent. Defendant contends that its construction is necessary to clarify “that pressure adjustment occurs during the forming step *in order to* produce droplets of a target size. (D.I. 93 at 87) (emphasis in original). Plaintiffs counter that Defendant's proposed construction suggests that the pressure is adjusted in order to form droplets (*i.e.*, that droplets are not formed prior to the pressure adjustment). (*Id.* at 86). Defendant claims this is not the intent of its proposed construction, while also arguing that “the pressure adjustment step occurs *in order to* produce droplets.” (*Id.* at 88). I agree with Plaintiffs that no construction of this term is necessary. The specification makes clear that the pressure adjustment step is undertaken in order to alter the size of the droplets being formed, not to produce the droplets in the first place. ('430 patent at 5:23–28). Therefore, I find that no construction is necessary for this term and Defendant is prohibited from arguing that “thereby” means “in order to.”

12. “each”

a. *Plaintiffs' proposed construction*: “No construction necessary, but if construed, ‘each and every ... of the method’”

b. *Defendant's proposed construction*: “each and every”

c. *Court's construction*: “no construction necessary”

This term appears in asserted claims in the '091 and '083 patents. Having considered the additional briefing submitted by the parties (D.I. 112), it seems to me that there is no real dispute as to the meaning of this term as it is used in the claims identified by the parties. Plaintiffs seek to introduce “of the method” into the construction of the terms, purportedly to prevent Defendant from arguing that “each” is construed to include plugs or plug-fluids other than those used in the claimed method. (D.I. 112 at 3). I think it is apparent from the claim language that the phrase “each plug-fluid” is not meant to include every plug-fluid in the Universe without expressly including such a limitation in the construction of this term. For example, claim 1 of the '091 patent describes the use of “a first plug-fluid” and “a second plug-fluid.” The claim then specifies that “each plug-fluid is immiscible with

the carrier-fluid.” I think it is clear from this language that “each plug-fluid” refers to the first and second plug-fluids listed earlier in the claim and not to every plug-fluid in existence. Therefore, I find that no construction is necessary, with the understanding that “each” refers only to the plugs and plug-fluids in the claim at issue and not to plugs and plug-fluids generally.

IV. CONCLUSION

*11 Within five days the parties shall submit a proposed order consistent with this Memorandum Opinion suitable for submission to the jury.

All Citations

Not Reported in F.Supp.3d, 2017 WL 382235, 2017 Markman 382235

Footnotes

- 1 The specification does not use the word “target” in connection with the method claimed in this patent. The word is used in a similar manner as in this claim, however, in the section on Crystallization. ('148 patent at 51:54–59; 51:64–52:3). I decline to opine on the meaning of “target” on its own at this time since the intrinsic evidence reveals that the meaning of “target” in this context is irrelevant to the construction of the phrase at issue.

2015 WL 7304663 (Patent Tr. & App. Bd.)
Only the Westlaw citation is currently available.

10X GENOMICS, INC., Petitioner,
v.
The UNIVERSITY OF CHICAGO, Patent Owner.

Patent Trial and Appeal Board.
IPR2015-01163 Patent 8,304,193 B2.
Nov. 16, 2015.

Attorneys and Law Firms

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Before DONNA M. PRAISS, CHRISTOPHER L. CRUMBLEY, and TINA E. HULSE, Administrative Patent Judges.

DECISION

Denying Institution of *Inter Partes* Review

37 C.F.R. § 42.108

HULSE, Administrative Patent Judge.

I. INTRODUCTION

10X Genomics, Inc. (“Petitioner”) filed a Petition requesting an *inter partes* review of claims 1–9 and 11 of U.S. Patent No. 8,304,193 B2 (Ex. 1001, “the #193 patent”). Paper 2 (“Pet.”). The University of Chicago (“Patent Owner”) filed a Preliminary Response to the Petition. Paper 9 (“Prelim.Resp.”).

We have jurisdiction under 35 U.S.C. § 314, which provides that an *inter partes* review may not be instituted “unless ... there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition.” 35 U.S.C. § 314(a). Upon considering the Petition and Preliminary Response, we determine that Petitioner has not established a reasonable likelihood that it would prevail in showing the

unpatentability of claims 1–9 and 11. Accordingly, we decline to institute an *inter partes* review of those claims.

A. Related Proceedings

Petitioner identifies as a related matter *RainDance Techs., Inc. v. 10X Genomics, Inc.*, No. 1:15-cv-00152-RGA (D.Del.), Pet. 55.

Petitioner has also concurrently filed petitions for *inter partes* review of related patents in IPR2015-01162 (U.S. 8,273,573), IPR2015-01158 (U.S. 8,329,407), IPR2015-01157 (U.S. 8,889,083), and IPR2015-01156 (U.S. 8,822,148).

B. The #193 Patent (Ex. 1001)

The #193 patent relates to a method for conducting an autocatalytic reaction in plugs in a microfluidic system. Ex. 1001, Title. Microfluidic systems transport fluids through networks of channels, typically having micrometer dimensions. *Id.* at 1:20–22. According to the Specification, the main advantages of microfluidic systems are high speed and low consumption of reagents. *Id.* at 1:25–26. The microfluidic systems may be used for various chemical and biochemical processes, including autocatalytic reactions, such as the polymerase chain reaction. *Id.* at 44:38–61.

Rather than rely on laminar flow of liquids through microfluidic channels, the #193 patent Specification describes using microfluidic droplets, or “plugs” in the system. Figure 2A–2, an embodiment of the system, is annotated and is reproduced below:

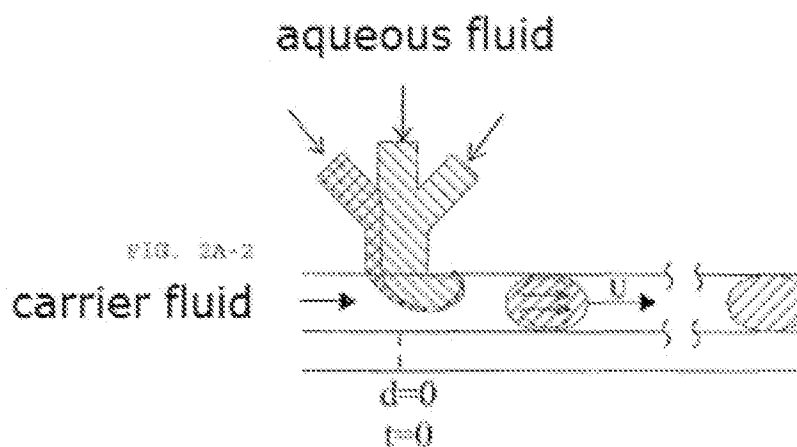


Figure 2A–2 depicts the formation of plugs, where the aqueous fluid containing a substrate and reagents enters

a channel with an immiscible carrier fluid, such as an oil, to form a series of plugs. *Id.* at 17:38–43. The plug volumes, which are dependent on channel size, can range from about 1 femtoliter to about 250 nanoliters. *Id.* at 19:45–54.

C. Illustrative Claim

Petitioner challenges claims 1–9 and 11 of the #193 patent. Claim 1, the only independent claim, is illustrative and is reproduced below:

1. A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

providing the microfluidic system comprising at least two channels having at least one junction;

flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

flowing an oil through the second channel of the at least two channels;

forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule; and

providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

D. The Asserted Grounds of Unpatentability

Petitioner challenges the patentability of claims 1–9 and 11 of the #193 patent on the following grounds:

References

Haff¹ and Quake²

Basis

§ 102

Claims challenged

1–5, 7–9, and 11

II. ANALYSIS

A. Person of Ordinary Skill in the Art

The parties dispute the proper definition of a person of ordinary skill in the art. Petitioner contends that a person of ordinary skill in the art would have had a Ph.D. in chemistry, biochemistry, mechanical engineering, or a related discipline, with two years of experience in using, designing or creating microfluidic devices. Pet. 3. Alternatively, Petitioner asserts that a skilled artisan would have had an M.S. or bachelor's degree in one of those disciplines with four or five years of additional relevant experience, respectively. *Id.* Patent Owner disagrees with Petitioner's definition, arguing that Petitioner overstates the level of ordinary skill in the art in the 2002 time frame. According to Patent Owner, a person of ordinary skill in the art would have been a researcher interested in microfluidics with at least a bachelor's degree in a “hard science,” such as chemistry, physics, chemical engineering, bioengineering, or mechanical engineering. Prelim. Resp. 34–35.

For purposes of this Decision, we do not discern an appreciable difference between Petitioner and Patent Owner's proposed levels of ordinary skill in the art that would impact our ultimate conclusions. Patent Owner does not indicate how many years of experience a “researcher interested in microfluidics” would have. Accordingly, we determine that a person of ordinary skill in the art would have at least a bachelor's degree in chemistry, physics, chemical engineering, bioengineering, or mechanical engineering, or a related discipline, with at least four or five years of relevant experience. We also consider the cited prior art as representative of the level of ordinary skill in the art. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed.Cir.2001) (finding the absence of specific findings on “level of skill in the art does not give rise to reversible error ‘where the prior art itself reflects an appropriate level and a need for testimony is not shown’”) (quoting *Litton Indus. Prods., Inc. v. Solid State Sys. Corp.*, 755 F.2d 158, 163 (Fed.Cir.1985)).

B. Claim Construction

In an *inter partes* review, the Board interprets claim terms in an unexpired patent according to the broadest

reasonable construction in light of the specification of the patent in which they appear. *See In re Cuozzo Speed Techs., LLC*, 793 F.3d 1268, 1276–80 (Fed.Cir.2015); 37 C.F.R. § 42.100(b). Under that standard, and absent any special definitions, we give claim terms their ordinary and customary meaning, as would be understood by one of ordinary skill in the art at the time of the invention. *See In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed.Cir.2007). Any special definitions for claim terms must be set forth with reasonable clarity, deliberateness, and precision. *See In re Paulsen*, 30 F.3d 1475, 1480 (Fed.Cir.1994).

1. “plug of the aqueous fluid”

Claim 1 requires forming at least one “plug of the aqueous fluid.” Petitioner asserts that the broadest reasonable interpretation of this term is “volumes of an aqueous fluid surrounded by a carrier fluid in which it is substantially immiscible, such as a non-polar or hydrophobic fluid, e.g., an oil, and the diameter of a plug is substantially similar to the cross-section of the channel in which it is formed and the aqueous fluid is separated from the channel by a thin film of carrier fluid” Pet. 13. Patent Owner disagrees, asserting that the term should be construed to mean “volumes of aqueous fluid formed when a stream of aqueous fluid is introduced into the flow of a substantially immiscible carrier-fluid.” Prelim. Resp. 19.

At this stage of the proceeding, we find Patent Owner's proposed construction more persuasive because it is more consistent with the Specification's definition of “plugs.” The Specification states: “‘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.” Ex. 1001, 9:27–30. To further limit the claim term, Petitioner relies on the Specification's disclosure of other optional characteristics of a plug. For example, the Specification states that the plug's “crosssection *should be* substantially similar to the cross-section of the channels in which they are formed.” *Id.* at 9:34–37 (emphasis added). The Specification also states that the carrier fluid “preferably” wets the walls of the channels and, “[i]f this condition is satisfied, the plug ... remains separated from the walls by a thin layer of the carrierfluid.” *Id.* at 20:59–63 (emphasis added). Because neither of these disclosures expressly limits the “plugs” of the claimed invention, we decline to include these limitations from the Specification in the broadest reasonable interpretation of the claim term.

Accordingly, on the current record, we determine the broadest reasonable interpretation of the term “plugs of the aqueous fluid” to be “volumes of aqueous fluid formed when a stream of aqueous fluid is introduced into the flow of a substantially immiscible carrier-fluid.”

2. “conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified”

Claim 1 recites “providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.” Petitioner asserts that this limitation “would encompass at least providing a stimulus such as radiation, heat, temperature or pressure change, ultrasonic wave, or a catalyst to induce a reaction that gives rise to the transformation of the reagent to another reagent, intermediate, or product.” Pet. 14. Patent Owner disagrees with Petitioner's construction to the extent that it simply requires a single stimulus to induce an autocatalytic reaction (regardless of whether that stimulus is sufficient to induce the reaction). Prelim. Resp. 21. According to Patent Owner, a skilled artisan would understand that the term refers “to a global set of physical and chemical conditions that allows one to conduct an autocatalytic reaction (including PCR) in droplets.” *Id.*

At this stage of the proceeding, we determine that Patent Owner's interpretation is more consistent with the plain and ordinary meaning of the claim term, which requires more than a single stimulus to induce a reaction. We are persuaded by Patent Owner's argument that the Specification sets forth certain conditions that must be addressed when conducting PCR in a microfluidic droplet, including unwanted adsorption of proteins on the surface of the droplet. Prelim. Resp. 20 (citing Ex. 1001, 35:59–66). Accordingly, we construe the claim term as requiring conditions that allow the substrate molecule to be amplified in a microfluidic system.

3. Remaining Claim Terms

Petitioner proposes constructions for other claim terms of the #193 patent. Based on the current record, however, we determine that the remaining claim terms need not be construed explicitly for purposes of this Decision.

C. Obviousness over Haff and Quake

Petitioner asserts that claims 1–5, 7–9, and 11 are unpatentable as having been obvious over Haff and Quake. Pet. 20–45. Petitioner relies on the testimony of its declarant, Wilhelm T.S. Huck, Ph.D. (Ex. 1002). Patent Owner opposes Petitioner's assertion. Prelim. Resp. 26–46. Based on the current record, we determine that Petitioner has not established a reasonable likelihood that it would prevail in showing the claims are unpatentable as having been obvious over the cited references.

1. Haff (Ex. 1021)

Haff relates to automated machines for performing amplification reactions for nucleic acids, such as a polymerase chain reaction (“PCR”). Ex. 1021, 1:12–15. Haff states that PCR has been performed in disposable reaction tubes such as small, plastic microcentrifuge tubes or test tubes that are placed in an instrument containing a thermally controlled heat exchanger. *Id.* at 1:50–53. Haff explains that rapid, small-scale PCR capillary tube instruments have appeared in which the reaction mixture is placed in capillary tubes. *Id.* at 2:7–12. The Specification describes various capillary tube PCR instruments that use different means to heat and cool the PCR reaction mixture and different tube and fluid-handling means to move the reaction mixture.

Figure 2 of Haff is one embodiment and is reproduced below:

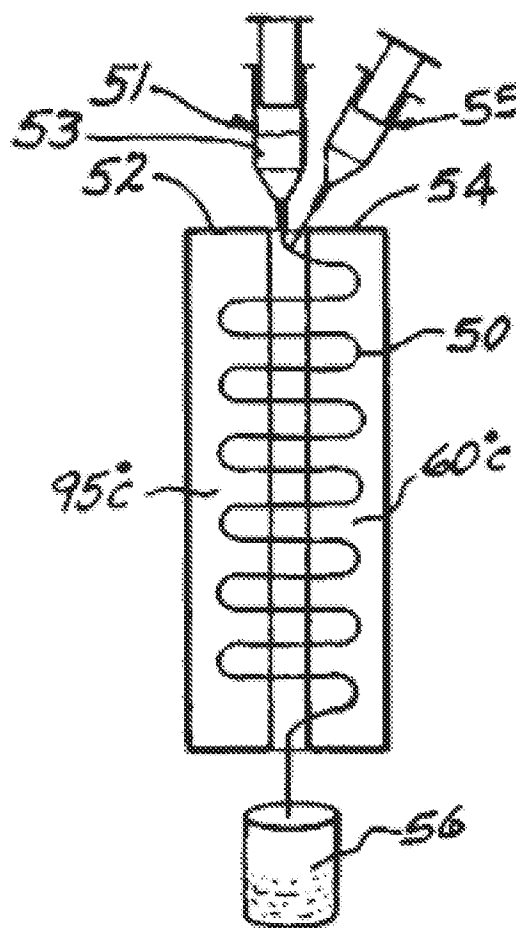


FIG-2

Figure 2 depicts a system that uses a length of capillary tubing 50 that is routed in and out of hot zone 52 and cool zone 54 a number of times. *Id.* at 9:58–65. Syringe 51, which contains PCR reaction mixture 53, and syringe 55, which contains air or an immiscible fluid, are connected in parallel with tubing 50. Ex. 1021, 10:20–32. Syringe 55 is operated intermittently to inject air bubbles or immiscible fluid bubbles into the flow stream of reagent mixture 53 in tubing 50. *Id.* at 10:32–35. The use of bubbles between samples results in “slug” flow where sample “slugs” are passed through the capillary tubes. *Id.* at 9:50–52. According to Haff, “[th]e length of the tubing 50, its internal diameter, the length of tubing in each of the hot and cool zones, and the flow rate of the reaction mixture inside the tube are all factors in the control of the amplification.” *Id.* at 10:15–19.

2. Quake (Ex. 1004)

Quake relates to microfluidic devices and methods for analyzing and/or sorting biological materials. Ex. 1004, Abstract. Quake states that its device and methods “offer several advantages over traditional flow cytometry devices

and methods.” *Id.* ¶ 14. Quake’s devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. *Id.* ¶ 3. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

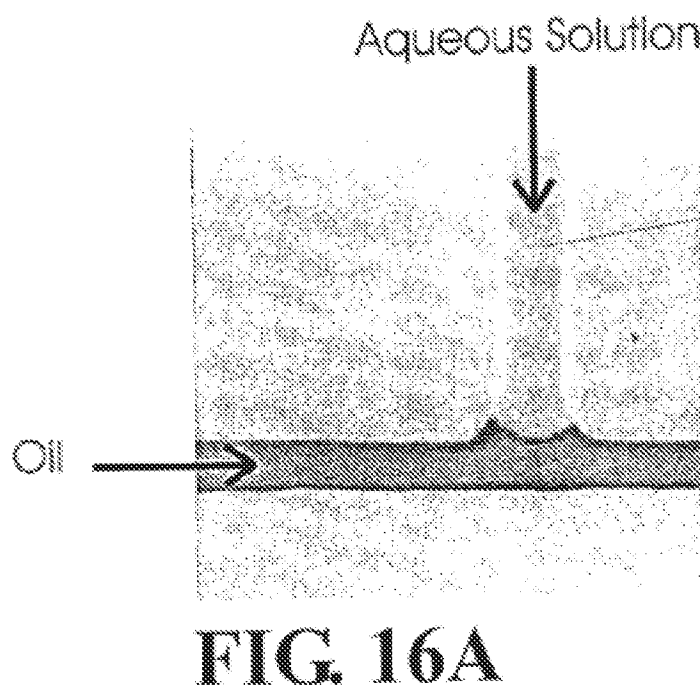


Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with the main channel 1602 containing oil. Ex. 1004 § 292. In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* § 80.

3. Analysis

Petitioner asserts that the combination of Haff and Quake teaches or suggests each limitation of claims 1–5, 7–9, and 11. Regarding claim 1, Petitioner provides a claim chart identifying the disclosures of Haff and Quake that purportedly teach or suggest each limitation.⁴ Pet. 21–

30. For example, Petitioner asserts that Figure 16A of Quake depicts a microfluidic system with two channels having one junction. Pet. 22–23 (citing Ex. 1004 §§ 3, 292, Fig. 16A). Petitioner also asserts that the combination of Haff’s PCR device and Quake’s microfluidics device suggests flowing the aqueous sample fluid and the oil through separate channels and forming a plug of aqueous sample fluid in the oil. Pet. 23–25 (citing Ex. 1021, 1:21–27, 10:20–35; Ex. 1004 ¶¶ 3, 14, 20, 120, 290, Fig. 16A). Quake teaches that the aqueous droplets are encapsulated, or substantially surrounded, by oil. Pet. 28 (citing Ex. 1004 ¶ 100). Finally, Petitioner asserts that the combination of Haff and Quake teach providing conditions suitable for the autocatalytic reaction in the plug such that at least one substrate molecule is amplified. Pet. 30 (citing Ex. 1021, 1:12–15, 9:55–10:2, 10:15–19; Ex. 1004 ¶ 80).

Petitioner then argues that a person of ordinary skill in the art would have had a reason to modify Haff’s method of PCR in capillary tube devices with the microfluidic device of Quake with a reasonable expectation of success. For example, Petitioner asserts that both references are directed to performing enzymatic reactions, such as PCR, in small volumes of aqueous fluids. *Id.* 31 (citing Ex. 1021, 9:56–63 and Fig. 2; Ex. 1004 ¶¶ 15, 80, 113, 290; Ex. 1002 ¶ 55). Petitioner also contends that a person of ordinary skill in the art would have had a reason to practice Haff’s PCR method in Quake’s microfluidic device to reduce the amount of reagents needed, which is beneficial when the availability of the reagents and substrate are limited. *Id.* at 33 (citing Ex. 1002 ¶ 65). According to Petitioner, the reason to combine Quake with a PCR method comes from Quake itself (*id.* at 35), which teaches that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1004 ¶ 80.

Petitioner argues that a person of ordinary skill in the art would have had a reasonable expectation of success in combining Haff and Quake. Pet. 35–36; Ex. 1002 ¶ 75. Dr. Huck explains that Quake teaches controlling the flow of oil and the flow of the aqueous sample fluid to generate an encapsulated stream of droplets. Ex. 1002 ¶ 75. Dr. Huck also states that Quake teaches adjusting the concentration of the biological molecule to ensure that the droplets contain no more than a single biological molecule and teaches that the device and method can be integrated with PCR. *Id.* Accordingly, Dr. Huck concludes:

[I]t would have required no more than routine skill and knowledge in the art for a [skilled artisan] to adapt Haff's device to form a stream of PCR reaction mixture droplets encapsulated, *i.e.*, substantially surrounded by an oil, which droplets comprise a single substrate DNA molecule, and to conduct PCR amplification, an autocatalytic reaction, in the droplets to amplify the single substrate molecule.

Id.

In response, Patent Owner first argues that Haff is not analogous art because the field of the #193 patent is microfluidic devices, and Haff involves larger volumes of fluids. Prelim. Resp. 26–28. Patent Owner also argues that Haff is not reasonably pertinent to the problems being addressed in the #193 patent. *Id.* at 28–30. We are not persuaded. Although Haff does not disclose the use of PCR in microfluidic devices, we do not believe such disclosure is necessary to consider Haff to be analogous art. “A reference is reasonably pertinent if, even though it may be in a different field from that of the inventor's endeavor, it is one which, because of the matter with which it deals, logically would have commended itself to an inventor's attention in considering his problem.” *In re ICON Health & Fitness, Inc.*, 496 F.3d 1374, 1380–81 (Fed.Cir.2007). On the current record, we find that Haff's disclosure of PCR in larger-volume capillary tube devices logically would have commended itself to the inventors' attention when considering methods of conducting PCR in smaller microfluidic devices. In other words, we find that the inventors logically would have considered how PCR is conducted in capillary tube devices to figure out how to scale down the reaction for microfluidic devices.

Patent Owner also argues that the Petitioner does not establish that one of ordinary skill in the art would have had a reasonable expectation of success combining Haff and Quake. Prelim. Resp. 37–46. In particular, Patent Owner challenges Petitioner's assertion that the combination of Quake and Haff would have been “routine.” Patent Owner states that Petitioner's assertion “is undermined by the lack of any reasoned explanation in the Petition for why this combination would have been successful.” *Id.* at 44–45.

We have considered Petitioner's arguments and evidence together with the Preliminary Response, and, on this record, we agree with Patent Owner that Petitioner has not met the threshold for instituting an *inter partes* review of the #193 patent. Petitioner and its declarant state that it “would have required no more than routine skill and knowledge in the art to adapt Haff's capillary PCR device to use Quake's method of creating encapsulated plugs ... to perform PCR in a microfluidic device.” Pet. 35–36. But Petitioner cites insufficient evidence to support this statement. For example, Petitioner and its declarant assert that a skilled artisan would have had a reasonable expectation of success in doing so because “Quake teaches that his microfluidic device designs can be used to perform PCR.” *Id.* at 31 (citing Ex. 1002 ¶ 75); *see also id.* at 30 (citing Ex. 1004 ¶ 80 in claim chart). We are not persuaded, however, that such a conclusory statement by itself would have given a person of ordinary skill in the art a reasonable expectation of success in combining Haff and Quake.

We acknowledge that the Petition and Dr. Huck provide an overview of the state of the art as of May 2002. Pet. 4–10; Ex. 1002 ¶¶ 16–26. For example, the Petition describes a microfluidic system for performing chemical reactions in droplets. Pet. 5–6 (citing Ex. 1011). And the Petition describes the development of PCR, including microfluidic devices that could perform PCR on a nanoliter scale. *Id.* at 7–8; *see, e.g.*, Ex. 1017 (describing continuous-flow PCR on a chip). According to Petitioner, those microfluidic devices “functioned by injecting a stream of a single PCR reaction mixture into the device, which was then flowed into a microfluidic PCR chamber, where the target sequence was amplified via thermocycling.” Pet. 8 (citing Ex. 1002 ¶ 22).

Despite that background information, we determine that Petitioner has not explained why a person of ordinary skill in the art would have had a reasonable expectation of success combining Haff's capillary tube PCR system with Quake's microfluidic system to amplify a substrate in droplets. Simply stating that it would have been “routine” is insufficient. To the extent Petitioner relies on the background information to support a reasonable expectation of success, Petitioner fails to explain any connection between the two. For example, if conducting PCR in a *stream* of reaction mixture in a microfluidic device was known, Petitioner has not explained why, with that knowledge, it would have been routine to

conduct PCR in *droplets* in a microfluidic device. Moreover, even though Haff describes conducting PCR in a capillary tube device, neither Petitioner nor its declarant explains whether the conditions for conducting PCR in microfluidic droplets would differ, or why a person of ordinary skill in the art would consider conducting PCR in microfluidic droplets to be “routine.”

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

The unexplained opinion of Dr. Huck that it would have required no more than routine skill and knowledge in the art “to adapt Haff’s device to form a stream of PCR reaction mixture droplets ... and to conduct PCR amplification, an autocatalytic reaction, in the droplets to amplify the single substrate molecule” (Ex. 1002 ¶ 75) also is not persuasive. See 37 C.F.R. § 42.65(a) (“Expert testimony that does not disclose the underlying facts or data on which the opinion is based is entitled to little or no weight.”); *Ashland Oil, Inc. v. Delta Resins & Refractories, Inc.*, 776 F.2d 281, 294 (Fed.Cir.1985) (stating a lack of objective support for an expert opinion “may render the testimony of little probative value in [a patentability] determination”).

Without a sufficient explanation regarding the combination of Haff and Quake, we determine that Petitioner has failed to establish a reasonable likelihood that it would prevail in its assertion that claim 1, or dependent claims 2–5, 7–9, and 11, are unpatentable as obvious over Haff and Quake.

D. Obviousness of Claim 6 over Haff Quake, and Ramsey

Claim 6 depends from claim 1 and further requires that the oil is fluorinated oil. Petitioner argues that claim 6 is unpatentable as obvious over Haff, Quake, and Ramsey. Pet. 46–48. For the same reasons stated above with respect to claim 1, we determine that Petitioner has failed to sufficiently show that claim 6 is unpatentable as obvious over the cited references.

III. CONCLUSION

We conclude that Petitioner has not established a reasonable likelihood of prevailing on its assertions that claims 1–9 and 11 of the #193 patent are unpatentable as obvious.

IV. ORDER

In consideration of the foregoing, it is hereby ordered that the Petition is *denied*.

Footnotes

1 Haff et al., U.S. 6,033,880, issued Mar. 7, 2000 (Ex. 1021).

2 Quake et al., U.S.2002/0058332 A1, published May 16, 2002 (Ex. 1004).

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3 Ramsey et al., U.S. 6,524,456 B1, issued Feb. 25, 2003 (Ex. 1006).

4 We note that the claim chart identifies the last limitation of claim 1 as “providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.” Pet. 30. The last limitation of claim 1, however, recites “providing conditions suitable for the *autocatalytic* reaction in the at least one plug *such that the at least one substrate molecule is amplified.*” Ex. 1001, claim 1 (emphasis added). Patent Owner did not object to the claim chart as incorrect. We, therefore, consider this to be a harmless typographical error.