# METHODS AND SYSTEMS FOR DROPLET MANIPULATION

## CROSS-REFERENCE

**[0001]** This application claims benefit of U.S. Provisional Application No. 62/892,495 filed August 27, 2019, U.S. Provisional Application No. 62/980,013 filed February 21, 2020, U.S. Provisional Application No. 63/005,097 filed April 3, 2020, and U.S. Provisional Application No. 63/009,376 filed April 13, 2020, which are herein incorporated by reference in their entirety.

## BACKGROUND

**[0002]** Biological samples may be processed for various applications. For example, a deoxyribonucleic acid (DNA) molecule or a ribonucleic acid (RNA) molecule may be processed (e.g., sequenced) to identify genetic variants, which may be useful to identify a disease, such as cancer. Such biological samples may be processed in partitions, such as droplets. Sequences of DNA or RNA may be determined by sequence identification, such as nucleic acid sequencing.

**[0003]** Droplets containing biological samples may be manipulated by using electrowetting, which may employ electric fields from electrodes to move a droplet adjacent to a surface.

## SUMMARY

**[0003a]** In one aspect, the present disclosure provides a method of processing a droplet, comprising: monitoring a volume of said droplet on a surface of an array; determining, using an algorithm, one or more determined volumes or a range of determined volumes associated with one or more droplet actuations of said droplet, wherein said one or more determined volumes or said range of determined volumes comprises volumes within a 20% coefficient of variation of droplet volume relative to said monitored volume; utilizing said algorithm to replenish or partially evaporate said droplet as to prevent said droplet from exceeding said 20% coefficient of variation based on a differential between said one or more determined volumes or said range of determined volumes and said monitored volume.

**[0004]** In an ~~aspect~~ embodiment the present disclosure provides a method for processing a plurality of biological samples, comprising (i) receiving, adjacent to an array, a plurality of droplets comprising the plurality of biological samples, and (ii) using at least the array to process the plurality of biological samples in the plurality of droplets or derivatives thereof at a coefficient of variation (CV) of at least one parameter of the plurality of droplets or derivatives thereof, or the array, of less than 20% at cross-talk between the plurality of droplets at less than 5%, thereby processing the plurality of biological samples.

**[0005]** In another ~~aspect~~embodiment, the present disclosure provides a method for customizing an array system for processing a plurality of biological samples, comprising (i) receiving a request for a configured array system from a user, which request comprises one or more specifications, and (ii) using the one or more specifications to configure the array system to yield the configured array system, which configured array system is configured to receive a plurality of droplets comprising the plurality of biological samples and process the plurality of droplets or derivatives thereof at a coefficient of variation

(CV) of at least one parameter of the plurality of droplets or derivatives thereof, or the array, of less than 20% at cross-talk between the plurality of droplets at less than 5%.

**[0006]** In another ~~aspect~~embodiment, the present disclosure provides a method for processing a biological sample, comprising providing, adjacent to an open array, a droplet comprising the biological sample, and using the open array to process the biological sample in the droplet or derivative thereof, wherein, during processing, a position of the droplet varies by at most 5% over a time period of at least 10 seconds.

**[0007]** In another ~~aspect~~embodiment, the present disclosure provides a method for processing a biological sample, comprising (i) receiving, adjacent to an array, a droplet comprising the biological sample, and

least one surface. In some embodiments, gas, liquid, solid, or any combination thereof is transferred by the at least one hole. In some embodiments, the gas, liquid, solid, or any combination thereof is transferred in or out of the plurality of arrays. In some embodiments, the gas, liquid, solid, or any combination thereof is transferred between at least two surfaces of the plurality of arrays. In some embodiments, at least two droplets of the plurality of droplets are separated by at least one permeable membrane. In some embodiments, at least a portion of the components of the at least two droplets of the plurality of droplets are exchanged from one droplet of the at least two droplets of the plurality of droplets to another droplet of the at least two droplets of the plurality of droplets by the at least one permeable membrane. In some embodiments, the at least one permeable membrane is permanently or temporarily attached to the array.

**[0032]** In another ~~aspect~~embodiment, the present disclosure provides a system for processing a plurality of biological samples, comprising (i) receiving, adjacent to an array, a plurality of droplets comprising the plurality of biological samples, and (ii) using at least the array to process the plurality of biological samples in the plurality of droplets or derivatives thereof at a coefficient of variation (CV) of at least one parameter of the plurality of droplets or derivatives thereof, or the array, of less than 20% at cross-talk between the plurality of droplets at less than 5%, thereby processing the plurality of biological samples.

**[0033]** In another ~~aspect~~embodiment, the present disclosure provides a system for biological sample processing, comprising: a housing configured to contain a plurality of arrays, wherein an array of the plurality of arrays is configured to (i) receive, adjacent to the array, a plurality of droplets comprising the plurality of biological samples, and (ii) use at least the array to process the plurality of biological samples in the plurality of droplets or derivatives thereof at a coefficient of variation (CV) of at least one parameter of the plurality of droplets or derivatives thereof, or the array, of less than 20% at cross-talk between the plurality of droplets at less than 5%.

**[0034]** In some embodiments, the plurality of arrays is removable from the housing. In some embodiments, the housing is configured to couple to a nucleic acid sequencing platform. In some embodiments, the housing is a nucleic acid sequencing platform. In some embodiments, an environment of the array is controlled by the housing, thereby producing a controlled environment. In some embodiments, ambient humidity, droplet coating, temperature, pressure, droplet size, lighting condition, or any combination thereof are maintained by the controlled environment. In some embodiments, the housing comprises an enclosure. In some embodiments, the enclosure comprises a cover, a seal, a chamber, an immiscible high vapor-pressure fluid, a film, or any combination thereof.

**[0035]** Another ~~aspect~~ embodiment of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

**[0036]** Another ~~aspect~~ embodiment of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises

machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

**[0037]** Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

**[0038]** In another ~~aspect~~embodiment, the present disclosure provides a method for processing a droplet, comprising: providing said droplet on an array, wherein said droplet comprises one or more detectable labels, wherein a detectable label of said one or more detectable labels corresponds to a physical property of said droplet; using one or more light sources to illuminate said droplet on said array, wherein upon illumination by said one or more light sources, said detectable label generates a signal; using a detector to detect said signal; using said signal detected in to determine said physical property of said droplet; and manipulating said droplet if said physical property determined does not meet a threshold.

**[0039]** In some embodiments, said physical property is selected from the group consisting of droplet size, droplet volume, droplet position, droplet speed, droplet wetting, droplet temperature, droplet pH, beads in droplets, number of cells in droplets, droplet color, concentration of chemical material, concentration of biological substance, or any combination thereof. In some embodiments, said droplet comprises a plurality of detectable labels corresponding to different physical properties of said droplet, wherein said plurality of detectable labels comprises said detectable label.

**[0040]** In some embodiments, said detector comprises at least one camera. In some embodiments, manipulating said droplet comprises computer processing said physical property and a threshold value or value range. In some embodiment, said signal is detected from said droplet at multiple time points on said array. In some embodiments, wherein said detector comprises one or more optical filters, and wherein said one or more optical filters is used to detect said signal. In some embodiments, the method further comprises altering at least a subset of said one or more optical filters to detect an additional signal from said droplet.

**[0041]** In some embodiments, said parameter is droplet volume, and wherein said volume is determined to be below a threshold volume, and wherein said droplet is contacted with one or more replenishing droplets. In some embodiments, said replenishing droplets are contacted with said droplet through electrowetting motion. In some embodiments, said replenishing droplets replenish about 1% to about 50% of a volume of said droplet.

**[0042]** In some embodiments, said parameter is employed in generating a machine learning model for determining said parameter for one or more additional droplets to be introduced to said array.

**[0043]** In some embodiments, the method further comprises a step of heating one or more fluids surrounding said droplet to reduce evaporation of said droplet. In some embodiments, said heating is performed by the actuation of a heater displaced below said array, the heating of a plate displaced above said array, the heating of one or more sidewalls contacting said array, or a combination thereof. In some

embodiments, said one or more fluids comprises water, and the method further comprises contacting an area displaced on said array with said one or more fluids.

**[0044]** In some embodiments, a relative humidity of about 50% to about 100% is maintained in said area. In some embodiments, contacting said area with said one or more fluids comprises introducing one or more sacrificial droplets to said prior to or subsequent to introducing a droplet comprising a sample for analysis. In some embodiments, said contacting said area with said one or more fluids comprises displacing a water reservoir within said area. In some embodiments, the method further comprises encapsulating said area in a chamber. In some embodiments, the method further comprises uniformly heating said chamber.

**[0045]** In some embodiments, said plate displaced above said array comprises electrodes. In some embodiments, an electrode of said electrodes is individually encapsulated. In some embodiments, said electrodes are transparent. In some embodiments, said plate displaced above said array is transparent. In some embodiments, said sidewalls comprise a conductor, a circuit board, or both. In some embodiments, said circuit board comprises serpentine traces. In some embodiments, said array, said plate displaced above said array, said sidewalls, or a combination thereof further comprise a resistive film heater, thermal insulation, a temperature sensor, or any combination thereof. In some embodiments, said temperature senor is coupled to a side of said array, wherein said side is opposite of a side comprising said droplet. In some embodiments, said heater displaced below said array comprises an electrode. In some embodiments, said electrode is individually encapsulated. In some embodiments, said volume of said droplet is maintained within at least about 30%, 20%, 10%, 5%, 1%, 0.1%, or 0.01% of an original droplet volume.

**[0046]** Another ~~aspect~~ embodiment of the present disclosure provides a method of synthesizing a polynucleotide on an array, comprising providing a droplet on said array, wherein said droplet comprises a nucleic acid molecule, and using said nucleic acid molecule to synthesize said polynucleotide, wherein said has a volume from about 1 picoliter to about 2 microliters, and wherein during said synthesis, a volume of said droplet changes by at most 50%.

**[0047]** In some embodiments, during said synthesis, said volume changes by at most 10%. In some embodiments, during said synthesis, said volume changes by at most 1%. In some embodiments, said polynucleotide is synthesized at least in part by ligating an additional nucleic acid molecule to said nucleic acid molecule. In some embodiments, said polynucleotide is synthesized at least in part by hybridizing an additional nucleic acid molecule to said nucleic acid molecule. In some embodiments, said additional nucleic acid molecule is comprised in an additional droplet. In some embodiments, the method further comprises contacting said droplet with said additional droplet. In some embodiments, said biomolecule is synthesized at in least in part by addition of a single nucleotide to a 3'-overhang or 3' blunt end or 3' recessed end of a nucleic acid molecule.

**[0048]** Another ~~aspect~~ embodiment of the present disclosure provides system for manipulating a droplet, comprising: an array configured to support said droplet; a plurality of magnets; a shield disposed

between said array and said plurality of magnets, wherein said shield comprises one or more cutouts and wherein

said one or more cutouts are aligned to said plurality of magnets; and a controller coupled to said plurality of magnets, wherein said controller is configured to direct actuation of a magnet of said plurality of magnets to manipulate said droplet on said array.

**[0049]** In some embodiments, the system further comprises a stage supporting said plurality of magnets wherein said coupled to an actuator, wherein said actuator is configured to move said stage upon a linear axis. In some embodiments, said plurality of magnets comprises ferromagnet flux focusers, ferromagnetic back iron, or a combination thereof. In some embodiments, said shield comprises ferromagnetic material.

**[0050]** In some embodiments, said plurality of magnets comprises one or more rotary switchable magnets. In some embodiments, said one or more rotary switchable magnets is configured to rotate a magnet of said plurality of magnets.

**[0051]** Another ~~aspect~~ embodiment of the present disclosure provides a method for manipulating a droplet with a magnetic field, comprising: disposing said droplet on an array, wherein said array comprises a shield, wherein said shield comprises one or more cutouts; displacing said array within a proximity of a plurality of magnets, wherein a magnet of said plurality of magnets contacts said one or more cutouts; actuating at least one magnet of said plurality of magnets to manipulate said droplet on said array with said magnetic field.

**[0052]** In some embodiments, displacing said array comprises actuating a stage configured to support said plurality of magnets. In some embodiments, said plurality of magnets comprises ferromagnet flux focusers, ferromagnetic back iron, or a combination thereof. In some embodiments, said shield comprises ferromagnetic material. In some embodiments, said plurality of magnets comprises one or more rotary switchable magnets. In some embodiments, said one or more rotary switchable magnets is configured to rotate a magnet of said plurality of magnets.

**[0053]** Another ~~aspect~~ embodiment of the present ~~invention~~ disclosure provides a system for processing one or more droplets, comprising: a holder configured to support a cartridge, said cartridge comprising an array that is configured to process one or more droplets, wherein said array does not comprise an overlying electrowetting electrode; and a computer processor configured to direct processing of said one or more droplets when said cartridge is supported.

**[0054]** In some embodiments, the system further comprises a plurality of electrodes. In some embodiments, said plurality of electrodes is in electrical communication with said cartridge. In some embodiments, said cartridge further comprises a dielectric. In some embodiments, said dielectric is adjacent to said array. In some embodiments, said cartridge further comprises a plurality of electrodes. In some embodiments, said plurality of electrodes is adjacent to said array. In some embodiments, said cartridge further comprises an additional plurality of electrodes. In some embodiments, said plurality of electrodes and said additional plurality of electrodes are non-coplanar.

**[0055]** In some embodiments, said array comprises a polymeric film. In some embodiments, said array comprises a liquid layer. In some embodiments, said liquid layer forms a liquid to liquid interface with said one or more droplets. In some embodiments, said cartridge comprises a frame configured to

maintain or generate a tension of a surface of said array. In some embodiments, said frame generates vacuum pressure on said surface of said array. In some embodiments, said frame comprises a fluid dispensing unit. In some embodiments, said frame is configured to replenish said liquid layer. In some embodiments, said cartridge further comprises one or more additional arrays. In some embodiments, said cartridge is removable from said holder.

**[0056]** In some embodiments, said array is in communication with said device by fine-pitched elastomeric connectors, board-to-board connectors, pogo-pins, or any combination thereof. In some embodiments, said device further comprises a module configured to house said array. In some embodiments, said module comprises one or more electrical connectors, wherein said electrical connectors are in communication with said processor and said plurality of electrodes coupled to said processer.

**[0057]** In some embodiments, said module comprises a cover, wherein said cover is configured to contact said array with said electrical connectors. In some embodiments, said cover is transparent.

**[0058]** In some embodiments, said electrical connectors comprise fine-pitched elastomeric connectors, board-to-board connectors, pogo-pins, spring connectors, conductive paste, or any combination thereof. In some embodiments, said module is configured to house one or more additional arrays.

**[0059]** In some embodiments, the system further comprises a projector configured to emit a light onto one or more tiles of said array. In some embodiments, said light comprises positional information specific to a location on said array. In some embodiments, the system further comprises one or more scanning mirrors or galvanometers configured to direct said light onto said array.

**[0060]** In one ~~aspect~~ embodiment, the present disclosure provides device for processing a sample, comprising: an array comprising a surface configured to support said droplet, wherein said array comprises an electrode that is configured to subject droplet to motion adjacent said surface; and a manipulation feature disposed on or adjacent to said surface, wherein said manipulation feature is configured to split said droplet.

**[0061]** In some embodiments, said manipulation feature comprises a microstructure disposed on said surface. In some embodiments, said microstructure comprises a hydrophobic material. In some embodiments, said manipulation feature comprises a hydrophilic region displaced on said surface. In some embodiments, said hydrophilic region is configured to bind to a hydrophobic article comprised in said droplet. In some embodiments, said manipulation feature is conformed specific to a volume of said fraction droplet. In some embodiments, said manipulation feature is non-coplanar with said surface. In some embodiments, said droplet comprises a volume between 1 femtometer and 1 milliliter.

**[0062]** Another ~~aspect~~ embodiment of the present disclosure provides a method of synthesizing a polynucleotide on an array, comprising: providing a droplet on said array, wherein said droplet comprises a nucleic acid molecule; and using said nucleic acid molecule to synthesize said polynucleotide, wherein during said synthesis, said droplet or a derivative thereof has a volume from about 1 femtoliter to about 2 microliters; and a volume of said droplet or said derivative thereof changes by at most 50%.

**[0063]** In some embodiments, during said synthesis, said volume changes by at most 10%. In some embodiments, during said synthesis, said volume changes by at most 1%. In some embodiments, said polynucleotide is synthesized at least in part by ligating an additional nucleic acid molecule to said nucleic acid molecule. In some embodiments, said polynucleotide is synthesized at least in part by hybridizing an additional nucleic acid molecule to said nucleic acid molecule. In some embodiments, said additional nucleic acid molecule is comprised in an additional droplet or a derivative thereof.

**[0064]** In some embodiments, the method further comprises detecting said volume of said droplet or said derivative thereof with a detector. In some embodiments, said detector comprises at least one camera. In some embodiments, said volume is detected from said droplet or said derivative thereof at multiple time points on said array. In some embodiments, the method further comprises manipulating said droplet or derivative thereof if said volume does not meet a threshold. In some embodiments, said threshold comprises a value range. In some embodiments, said manipulating comprises contacting said droplet or derivative thereof with a replenishing droplet. In some embodiments, said replenishing droplet does not comprise a biological sample.

**[0065]** Another ~~aspect~~ embodiment of the present disclosure provides a method for processing a plurality of biological samples, comprising receiving , adjacent to an array, a plurality of droplets comprising said plurality of biological samples, and using at least said array to process said plurality of biological samples in said plurality of droplets or derivatives thereof at a coefficient of variation (CV) of at least one parameter of said plurality of droplets or derivatives thereof, or said array, of less than 20% at cross-talk between said plurality of droplets at less than 5%, thereby processing said plurality of biological samples.

**[0066]** In some embodiments, said at least one parameter comprises one or more members selected from the group consisting of droplet size, droplet volume, droplet position, droplet speed, droplet wetting, droplet temperature, droplet pH, beads in droplets, number of cells in droplets, droplet color, concentration of chemical material, concentration of biological substance, or any combination thereof.

**[0067]** In some embodiments, said plurality of biological samples is processed by combining a force field with an electric field. In some embodiments, said force field is generated by fluid flow, vibration, or a combination thereof, on said array. In some embodiments, said force field is selected from the group consisting of acoustic waves, vibrations, air pressure, light field, magnetic field, gravitational field, centrifugal force, hydrodynamic forces, electrophoretic forces, dielectrowetting force, and capillary forces.

**[0068]** In some embodiments, said array comprises a plurality of sensors, and wherein said plurality of sensors measure signals from said plurality of droplets or derivatives thereof before, during, or subsequent to said processing said plurality of biological samples. In some embodiments, said plurality of sensors comprises an impedance sensor, a pH sensor, a temperature sensor, an optical sensor, a camera, a current measurement sensor, an electronic sensor for biomolecular detection, an x-ray sensor, electrochemical sensors, electrochemiluminescent sensors, piezoelectric sensors, or any combination

thereof. In some embodiments, the method further comprises using said plurality of sensors in a feedback loop to regulate one or more parameters of said array while processing said plurality of biological

dielectrophoretic forces (DEP) for cell sorting, cell separation, manipulating at least one bead, or any combination thereof.

**[0078]** In some embodiments, said biological samples is deposited on a plurality of arrays. In some embodiments, said plurality of arrays comprises at least two arrays. In some embodiments, an array of said at least two arrays is adjacent to another array of said at least two arrays. In some embodiments, said array of said at least two arrays is horizontally adjacent to another array of said at least two arrays. In some embodiments, said array of said at least two arrays is vertically adjacent to another array of said at least two arrays. In some embodiments, said plurality of arrays comprises at least one channel, at least one hole, or any combination thereof. In some embodiments, said at least one channel traverse between at least one surface. In some embodiments, gas. liquid, solid, or any combination thereof is transferred by said at least one hole.

**[0079]** Another ~~aspect~~ embodiment of the present disclosure provides a system for biological sample processing, comprising: a housing configured to contain a plurality of arrays, wherein an array of said plurality of arrays is configured to receive, adjacent to said array, a plurality of droplets comprising said plurality of biological samples, and use at least said array to process said plurality of biological samples in said plurality of droplets or derivatives thereof at a coefficient of variation (CV) of at least one parameter of said plurality of droplets or derivatives thereof, or said array, of less than 20% at cross-talk between said plurality of droplets at less than 5%.

**[0080]** In some embodiments, said plurality of arrays is removable from said housing. In some embodiments, said housing is configured to couple to a nucleic acid sequencing platform. In some embodiments, said housing is a nucleic acid sequencing platform. In some embodiments, an environment of said array is controlled by said housing, thereby producing a controlled environment. In some embodiments, ambient humidity, droplet coating, temperature, pressure, droplet size, lighting condition, or any combination thereof are maintained by said controlled environment. In some embodiments, said housing comprises an enclosure. In some embodiments, said enclosure comprises a cover, a seal, a chamber, an immiscible high vapor-pressure fluid, a film, or any combination thereof. In some embodiments, said enclosure comprises an immiscible high vapor-pressure fluid.

**[0081]** One ~~aspect~~ embodiment of the present disclosure provides a method for customizing an array system for processing a plurality of biological samples, comprising receiving a request for a configured array system from a user, which request comprises one or more specifications, and using said one or more specifications to configure said array system to yield said configured array system, which configured array system is configured to receive a plurality of droplets comprising said plurality of biological samples and process said plurality of droplets or derivatives thereof at a coefficient of variation (CV) of at least one parameter of said plurality of droplets or derivatives thereof, or said array, of less than 20% at cross-talk between said plurality of droplets at less than 5%.

**[0082]** Another ~~aspect~~ embodiment of the present disclosure provides a system for processing one or more droplets, comprising: an array, wherein said array comprises an open configuration with an

electrode array, open configuration with no electrode array, open configuration with non-coplanar set of electrodes,

## CLAIMS

WHAT IS CLAIMED IS:

1. A method ~~for~~ of processing a droplet, comprising:

monitoring a volume of said droplet on a surface of an array;

determining, using an algorithm, one or more determined volumes or a range of determined volumes associated with one or more droplet actuations of said droplet, wherein said one or more determined volumes or said range of determined volumes comprises volumes within a 20% coefficient of variation of droplet volume relative to said monitored volume;

utilizing said algorithm to replenish or partially evaporate said droplet as to prevent said droplet from exceeding said 20% coefficient of variation based on a differential between said one or more determined volumes or said range of determined volumes and said monitored volume.

~~(a) providing said droplet on an array, wherein said droplet comprises one or more detectable labels, wherein a detectable label of said one or more detectable labels corresponds to a physical property of said droplet;~~

~~(b) using one or more light sources to illuminate said droplet on said array, wherein upon illumination by said one or more light sources, said detectable label generates a signal;~~

~~(c) using a detector to detect said signal;~~

~~(d) using said signal detected in (c) to determine said physical property of said droplet; and~~

~~(e) manipulating said droplet if said physical property determined in (d) does not meet a threshold.~~

~~2. A method for processing a droplet, comprising:~~

~~(a) providing said droplet on an array;~~

~~(b) using one or more sensors to detect a signal generated from said droplet on said array, said array, an area adjacent to said array or said droplet, or any combination thereof;~~

~~(c) using said signal generated from said droplet on said array, said array, an area adjacent to said array or said droplet, or any combination thereof detected in (b) to determine a physical property of said droplet on said array, said array, an area adjacent to said array or said droplet, or any combination thereof; and~~

~~(d) manipulating said droplet on said array, said array, an area adjacent to said array or said droplet, or any combination thereof if said physical property determined in (c) does not meet a threshold.~~

2. Said method of claim 1, wherein determining, using said algorithm, said one or more determined volumes or said range of determined volumes comprises determining said one or more determined volumes based on one or more images of said droplet.

3. Said method of claim 2, wherein said one or more images comprises:

a first image of said droplet during a droplet actuation; and

a second image of said droplet after said droplet actuation.

4. Said method of claim 3, wherein,

said first image of said droplet is associated with a first determined volume of said one or more determined volumes or said range of determined volumes, and

said second image of said droplet is associated with a second determined volume of said one or more determined volumes or said range of determined volumes.

5. Said method of claim 4, wherein said first determined volume and said second determined volume are different.

6. Said method of claim 4, wherein,

said at least one determined volume of said one or more determined volumes or said range of determined volumes comprises said second determined volume, and

determining said differential comprises comparing said second determined volume to said one or more determined volumes or said range of determined volumes.

7. Said method of claim 4, wherein utilizing said algorithm to replenish or partially evaporate said droplet comprises replenishing about 0.1% to about 50% of said second detected volume.

8. Said method of claim 1, wherein said droplet is illuminated.

~~3.~~9. ~~The~~ Said method of claim 1 ~~or 2~~, wherein said array comprises an open configuration with an electrode array, open configuration with no electrode array, open configuration with non-coplanar set of electrodes, two plates with an array of electrodes on one plate and no electrodes on ~~the~~ said other plate, two plates with non-coplanar set of electrodes on one plate and no electrodes on ~~the~~ said other plate, two plates with an array of electrodes on one plate and single electrode on ~~the~~ said other plate, two plates with an non-coplanar set of electrodes on one plate and single electrode on other plate, two plates with electrodes arrays on both plates, two plates with non-coplanar set of electrodes on both plates, or any combination thereof.

~~4.~~10. ~~The~~ Said method of claim ~~3~~9, wherein said array comprises one or more open configurations, and wherein said array is at least partially enclosed.

~~5. The method of claim 1, further comprising:~~

~~i. using one or more sensors to detect said array, an area adjacent to said array or said droplet, or any combination thereof;~~

~~ii. using said array, an area adjacent to said array or said droplet, or any combination thereof detected in (i) to determine a physical property of said array, an area adjacent to said array or said droplet, or any combination thereof; and~~

~~iii. manipulating said array, an area adjacent to said array or said droplet, or any combination thereof if said physical property determined in (ii) does not meet a threshold.~~

~~6.~~11. ~~The~~ Said method of claim ~~2 or 5~~1, further comprising using one or more sensors to detect a signal generated from said droplet on said surface of said array, wherein said one or more sensors comprise an impedance sensor, a pH sensor, a temperature sensor, an optical sensor, a humidity sensor, a camera, a current measurement sensor, an electronic sensor for biomolecular detection, an x-ray sensor, electrochemical sensors, electrochemiluminescent sensors, piezoelectric sensors, or any combination thereof.

12. Said method of claim 11, wherein said signal indicates one or more physical properties of said droplet.

~~7.~~13. The method of claim ~~1 or~~ 12, wherein said one or more physical properties~~y~~ comprises droplet size, ~~droplet volume,~~ droplet position, droplet speed, droplet wetting, droplet temperature, droplet pH, beads in droplets, cells in droplet, cell count in droplet, droplet color or optical properties, kinematics, droplet shape, color, contact angle, reaction state, absorbance, surface plasmon resonance, other detectable property-, concentration of chemical material, concentration of biological substance, type of biological or chemical substance in droplet, voltage across droplet, current through ~~the~~ said droplet or any combination thereof.

14. Said method of claim 1, wherein

determining, using said algorithm, said one or more determined volumes or said range of determined volumes associated with said one or more droplet actuations of said droplet comprises determining, using a machine learning model, said one or more determined volumes or said range of determined volumes based on one or more images of said droplet; and

utilizing said algorithm to replenish or partially evaporate said droplet as to prevent said droplet from exceeding said 20% coefficient of variation of droplet volume based on said differential comprises:

determining, using said machine learning model, a replenishing or evaporating volume based on said differential and said 20% coefficient of variation of droplet volume; and

replenishing or evaporating said droplet based on said replenishing or evaporating volume.

~~8. The method of claim 1, wherein in (a) said droplet comprises a plurality of detectable labels corresponding to different physical properties of said droplet, wherein said plurality of detectable labels comprises said detectable label.~~

~~9. The method of claim 1, wherein said detector comprises at least one camera.~~

~~10. The method of claim 1, wherein (e) comprises computer processing said physical property and a threshold value or value range.~~

~~11. The method of claim 1, wherein said signal is detected from said droplet at multiple time points on said array.~~

~~12. The method of claim 2 or 5, wherein said signal is detected from said droplet on said array, said array, an area adjacent to said array or said droplet, or any combination thereof, at multiple time points.~~

~~13. The method of claim 9, wherein said detector comprises one or more optical filters, and wherein said one or more optical filters is used to detect said signal.~~

~~14. The method of claim 13, further comprising altering at least a subset of said one or more optical filters to detect an additional signal from said droplet.~~

~~15. The method of claim 1 or 2, wherein said parameter is droplet volume, and wherein said volume is determined to be below a threshold volume, and wherein said droplet is contacted with one or more replenishing droplets.~~

~~16. The method of claim 15, further comprising, prior to contacting said one or more replenishing droplets with said droplet, modulating said one or more replenishing droplets to a known temperature.~~

~~17. The method of claim 15, wherein said replenishing droplets replenish about 0.1% to about 50% of a volume of said droplet.~~

~~18. The method of claim 1 or 2, wherein said parameter is employed in generating a machine learning model for determining said parameter for one or more additional droplets to be introduced to said array.~~

~~19.~~15. ~~The~~ Said method of claim 14~~1 or 2~~, wherein replenishing said droplet based on said replenishing volume ~~further~~ comprises~~ing~~ heating one or more fluids surrounding said droplet to reduce evaporation of said droplet.

~~20.~~16. ~~The~~ Said method of claim ~~19~~15, wherein said heating is performed by ~~the~~ said actuation of a heater displaced below said array, ~~the~~ said heating of a plate displaced above said array, ~~the~~ said heating of one or more sidewalls contacting said array, or a combination thereof.

17. Said method of claim 14, wherein replenishing said droplet based on said replenishing volume comprises actuating a dispenser to dispense one or more replenishing droplets onto said surface of said array.

18. Said method of claim 17, wherein said one or more replenishing droplets are dispensed directly into said droplet.

~~21.~~19. ~~The~~ Said method of claim 1~~ or 2~~, wherein a relative humidity of about 50% to about 100% is maintained in ~~said~~ an area adjacent to ~~said array or~~ said droplet.

~~22.~~20. ~~The~~ Said method of claim ~~21~~19, wherein said maintaining said relative humidity of about 50% to about 100% comprises introducing one or more sacrificial droplets to said array prior to or subsequent to introducing said droplet comprising a sample for analysis.

~~23. The method of claim 1 or 2, wherein said parameter is droplet position, wherein said array comprises a plurality of electrodes and one or more reference electrodes, and wherein said method further comprises activating an electrode of said plurality of electrodes, wherein a change in voltage on said one or more reference electrodes indicates droplet position of said droplet.~~

~~24. The method of claim 23, wherein said plurality of electrodes and said one or more reference electrodes are coplanar.~~

~~25. The method of claim 23, wherein said plurality of electrodes and said one or more reference electrodes are adjacent to a dielectric.~~

~~26. The method of claim 25, wherein said plurality of electrodes and said one or more reference electrodes are separated by said dielectric.~~

27. The method of claim 23, wherein said plurality of electrodes and said one or more reference electrodes are on opposite sides of said droplet.

28. A method of synthesizing a biomolecule on an array, comprising (a) providing a droplet on said array, wherein said droplet comprises one or more preliminary biomolecules, and (b) using said preliminary biomolecules to synthesize said biomolecule, wherein said droplet has a volume from about 1 femtoliter to about 2 microliters, and wherein during said synthesis, a volume of said droplet changes by at most 50%.

29. The method of claim 28, wherein during said synthesis, said volume changes by at most 10%.

30. The method of claim 29, wherein during said synthesis, said volume changes by at most 1%.

31. The method of claim 28, wherein said biomolecule is synthesized at least in part by ligating an additional preliminary biomolecule to said one or more preliminary biomolecules.

32. The method of claim 28, wherein said biomolecule is synthesized at least in part by hybridizing an additional preliminary biomolecule to said one or more preliminary biomolecules.

33. The method of claim 31 or 32, wherein said additional nucleic acid molecule is comprised in an additional droplet.

34. The method of claim 33, further comprising contacting said droplet with said additional droplet.

35. The method of claim 28, wherein said biomolecule is deoxyribonucleic acid or ribonucleic acid.

36. The method of claim 28, wherein said biomolecule is a polypeptide.

37. The method of claim 28, wherein said biomolecule is a small molecule.

38. The method of claim 28, wherein said one or more preliminary biomolecules comprise a monomer.

39. The method of claim 28, wherein said one or more preliminary biomolecules comprise a polymer.

40. The method of claim 28, wherein one or more reagents necessary for said synthesis are pre-fabricated on said array.

41. The method of claim 35, wherein said biomolecule is synthesized at in least in part by addition of a single nucleotide to a 3' overhang or 3' blunt end or 3' recessed end of a nucleic acid molecule.

42. A system for processing one or more droplets, comprising:

a holder configured to support a cartridge, said cartridge comprising an array that is configured to process one or more droplets, wherein said array does not comprise an overlying electrowetting electrode; and

a computer processor configured to direct processing of said one or more droplets when said cartridge is supported.

43. The system of claim 42, further comprising a plurality of electrodes.

44. The system of claim 43, further comprising a dielectric layer adjacent to said plurality of electrodes.

45. The system of claim 44, wherein said dielectric layer and said plurality of electrodes are non-coplanar.

46. The system of claim 43, wherein said plurality of electrodes is in electrical communication with said cartridge.

47. The system of claim 42, wherein said cartridge further comprises a dielectric adjacent to said array.

48. The system of claim 42, wherein said cartridge further comprises a plurality of electrodes adjacent to said array.

49. The system of claim 48, wherein said cartridge further comprises an additional plurality of electrodes.

50. The system of claim 49, wherein said plurality of electrodes and said additional plurality of electrodes are non-coplanar.

51. The system of claim 42, wherein said array comprises a polymeric film.

52. The system of claim 42, wherein said array comprises a liquid layer.

53. The system of claim 52, wherein said liquid layer is adjacent to said dielectric and said plurality of electrodes adjacent to said array.

54. The system of claim 53, wherein said liquid layer facilities contact between said dielectric and said plurality of electrodes adjacent to said array.

55. The system of claim 42, wherein said array comprises a surface liquid layer.

56. The system of claim 55, wherein said surface liquid layer forms a liquid to liquid interface with said one or more droplets.

57. The system of claim 42, wherein said cartridge comprises a frame configured to maintain or generate a tension of said array.

58. The system of claim 57, wherein said frame is configured to generate vacuum pressure on said array.

59. The system of claim 57, wherein said frame comprises a fluid dispensing unit, wherein said fluid dispensing unit is configured to replenish said liquid layer.

60. The system of claim 42, wherein said cartridge further comprises one or more additional arrays.

61. The system of claim 42, wherein said cartridge is removable from said holder.

62. The system of claim 42, wherein said array is in communication with said device by fine-pitched elastomeric connectors, board-to-board connectors, pogo-pins, or any combination thereof.

63. The system of claim 42, wherein said device further comprises a module configured to house said array.

64. The system of claim 63, wherein said module comprises fine-pitched elastomeric connectors, board-to-board connectors, pogo-pins, spring connectors, conductive paste, or any combination thereof.

65. The system of claim 42, further comprising a projector configured to emit a light onto one or more tiles of said array, wherein said light comprises positional information specific to a location on said array.

66. The system of claim 65, further comprising one or more scanning mirrors or galvanometers configured to direct said light onto said array.

67. The system of claim 42, wherein said array comprises a reagent, wherein said reagent is prefabricated onto at least a portion of said array.

68. A method for processing a plurality of biological samples, comprising (i) receiving, adjacent to an array, a plurality of droplets comprising said plurality of biological samples, and (ii) using at least said array to process said plurality of biological samples in said plurality of droplets or derivatives thereof at a coefficient of variation (CV) of at least one parameter of said plurality of droplets or derivatives thereof, or said array, of less than 20% at cross-talk between said plurality of droplets at less than 5%, thereby processing said plurality of biological samples.

69. The method of claim 68, wherein said at least one parameter comprises one or more members selected from the group consisting of droplet size, droplet volume, droplet position, droplet speed, droplet wetting, droplet temperature, droplet pH, beads in droplets, number of cells in droplets, droplet color, concentration of chemical material, concentration of biological substance, or any combination thereof.

70. The method of claim 68, wherein said plurality of biological samples is processed by combining a force field with an electric field.

71. The method of claim 70, wherein said force field is selected from the group consisting of acoustic waves, vibrations, air pressure, light field, magnetic field, gravitational field, centrifugal force, hydrodynamic forces, electrophoretic forces, dielectrowetting force, and capillary forces.

72. The method of claim 68, wherein said plurality of biological samples is processed with no more than four pipetting operations, no more than three pipetting operations, no more than two pipetting operations, or no more than one pipetting operation.

73. The method of claim 72, wherein said plurality of biological samples is processed with no pipetting operations.

74. The method of claim 68, further comprising using one or more sensors in a feedback loop to regulate one or more parameters of said array while processing said plurality of biological samples.

75. The method of claim 74, wherein said one or more sensors measure one or more signals from said plurality of droplets or derivatives thereof, said array, an area adjacent to said droplet or said array, or any combination thereof, before, during, or subsequent to said processing said plurality of biological samples.

76. The method of claim 74, wherein said one or more sensors comprise an impedance sensor, a pH sensor, a temperature sensor, an optical sensor, a camera, a current measurement sensor, an

electronic sensor for biomolecular detection, an x-ray sensor, electrochemical sensors, electrochemiluminescent sensors, piezoelectric sensors, or any combination thereof.

77. The method of claim 68, further comprising at least one reagent, wherein said at least one reagent is prefabricated into a component of said array.

78. The method of claim 68, wherein said processing said plurality of biological samples comprises an isothermal amplification of at least one selected nucleic acid, said isothermal amplification comprising:

i. providing at least one sample that comprises at least one nucleic acid by merging droplets containing a plurality of reagents effective to permit at least one isothermal amplification reaction of said sample without mechanical manipulation; and

ii. conducting at least one isothermal amplification reaction to amplify said nucleic acid.

79. The method of claim 68, wherein said array partitions at least one droplet into a plurality of droplets comprising using: electrowetting force, dielectrowetting force, dielectrophoretic (DEP) effect, acoustic force, hydrophobic knife, or any combination thereof, thereby producing at least one partitioned droplet.

80. The method of claim 68, said array uses dielectrophoretic forces (DEP) for cell sorting, cell separation, manipulating at least one bead, or any combination thereof.

81. The method of claim 68, wherein said plurality of droplets is deposited on a plurality of arrays.

82. The method of claim 81, wherein said plurality of arrays comprises at least one EWOD array, at least one DEW array, at least one DEP array, at least one microfluidic array, glass, plastic, or any combination thereof.

83. The method of claim 81, wherein said plurality of arrays comprises at least one channel, at least one hole, or any combination thereof.

84. The method of claim 83, wherein said at least one channel traverse between at least one surface.

85. The method of claim 83, wherein a gas, liquid, solid, or any combination thereof is transferred by said at least one hole.

86. A system for processing one or more droplets, comprising:

(a) an electrowetting array;

(b) an enclosure adjacent to said array; and

(c) an enclosed area within said enclosure and adjacent to said array, wherein said enclosed area does not comprise a filler liquid, and wherein said enclosure is configured to modulate a temperature, a relative humidity, a pressure, or any combination thereof, within said enclosed area.

87. The system of claim 86, wherein said electrowetting array does not comprise an overlying electrowetting electrode.

88. The system of claim 86, further comprising one or more electrowetting electrodes adjacent to said electrowetting array.

89. The system of claim 86, wherein said enclosure comprises a cover, a seal, a chamber, an immiscible fluid, a wax, a film, or any combination thereof.

90. The system of claim 86, further comprising a heater adjacent to said electrowetting array, wherein said heater is configured to heat said enclosed area.

91. The system of claim 90, wherein said heater is coupled to a sidewall, a top surface, a bottom surface, or any combination thereof, of said enclosure.

92. The system of claim 91, wherein said heater comprises serpentine tracks.

93. The system of claim 86, further comprising a dispenser configured to dispense one or more sacrificial droplets onto said electrowetting array and within said enclosed area.

94. The system of claim 86, further comprising a water reservoir adjacent to said electrowetting array and within said enclosure.

95. The system of claim 86, further comprising one or more sensors selected from the group consisting of a temperature sensor, a humidity sensor, and a camera, wherein said one or more sensors are coupled to said enclosure or said electrowetting array.

96. The system of claim 86, further comprising a dispenser configured to dispense one or more replenishing droplets onto said electrowetting array and within said enclosed area.

97. The system of claim 86, wherein said enclosure is configured to maintain said relative humidity at about 50% to about 100%.

98. The system of claim 95, further comprising a computer processor configured to process a signal detected by said one or more sensors and a threshold value or value range, wherein said threshold value or value range is specific to said signal.

99. The system of claim 98, wherein said computer processer is further configured to actuate a heater to heat said enclosed area.

100. The system of claim 98, wherein said computer processer is further configured to actuate a dispenser to dispense one or more sacrificial droplets onto said electrowetting array.

101. The system of claim 98, wherein said computer processer is further configured to actuate a dispenser to dispense one or more replenishing droplets onto said electrowetting array.

102. The system of claim 95, wherein said one or more sensors are configured to detect a signal from said one or more droplets or said enclosed area at multiple time points on said electrowetting array.

103. A method for electroporating a cell comprised in a droplet on an array, wherein said array comprises one or more electrowetting electrodes and one or more electroporation electrodes, and wherein said array does not comprise an overlying electrowetting electrode, the method comprising:

(a) disposing said droplet over said one or more electroporation electrodes;

(b) pulsing at least said one or more electroporation electrodes with a voltage; and

(c) actuating said one or more electrowetting electrodes to induce a motion of said droplet.

104. The method of claim 103, wherein said one or more electrowetting electrodes are located above said array.

105. The method of claim 103, wherein said one or more electrowetting electrodes comprise one or more reference electrodes.

106. The method of claim 103, wherein said array comprises a slippery surface.

107. The method of claim 106, wherein said slippery surface comprises a slippery coating.

108. The method of claim 107, wherein said slippery coating comprises a polymer film.

109. The method of claim 107, wherein said slippery coating is porous.

110. The method of claim 109, wherein said slippery coating is filled with a slippery material to achieve a uniform surface.

111. The method of claim 110, wherein said slippery material is oil.

112. The method of any one of claims 107 to 111, wherein said slippery coating is further filled with a conductive material.

113. A system for processing one or more droplets, comprising:

(a) an array, wherein said array comprises an open configuration with an electrode array, open configuration with no electrode array, open configuration with non-coplanar set of electrodes, two plates with an array of electrodes on one plate and no electrodes on the other plate, two plates with non-coplanar set of electrodes on one plate and no electrodes on the other plate, two plates with an array of electrodes on one plate and single electrode on the other plate, two plates with an non-coplanar set of electrodes on one plate and single electrode on other plate, two plates with electrodes arrays on both plates, two plates with non-coplanar set of electrodes on both plates, or any combination thereof, and wherein said array does not comprise a filler liquid adjacent to said array;

(b) one or more liquid handling units, wherein said one or more liquid handling units direct said one or more droplets adjacent to said array.

114. The system of claim 113, wherein said one or more liquid handling units comprise robotic liquid handling systems, acoustic liquid dispensers, syringe pumps, inkjet nozzles, microfluidic devices, needles, microdiaphragm based pump dispensers, piezoelectric pumps, piezo acoustic devices, or any combination thereof.

115. The system of claim 113, wherein said array is coupled to at least one reagent or sample storage unit, or a combination thereof.

116. The system of claim 113, further comprising one or more sensors, wherein said one or more sensors are configured to detect a signal generated from said droplet on said array, said array, an area adjacent to said array or said droplet, or any combination thereof.

117. The system of claim 116, wherein said one or more sensors comprise an impedance sensor, a pH sensor, a temperature sensor, an optical sensor, a humidity sensor, a camera, a current measurement sensor, an electronic sensor for biomolecular detection, an x-ray sensor,

~~electrochemical sensors, electrochemiluminescent sensors, piezoelectric sensors, or any combination thereof.~~

~~118. The system of claim 116, further comprising a computer processor configured to process a signal detected by said one or more sensors and a threshold value or value range, wherein said threshold value or value range is specific to said signal.~~

~~119. The system of claim 118, further comprising a feedback loop, wherein said feedback loop comprises communication between said array, said one or more liquid handling units, said one or more sensors, said computer processor, or any combination thereof.~~

~~120. The system of claim 119, wherein said feedback loop is configured to discover, optimize, or both, reaction conditions on said array autonomously.~~

~~121. The system of claim 113, wherein said plurality of arrays comprises at least two arrays.~~

~~122. The system of claim 121, wherein an array of said at least two arrays is adjacent to another array of said at least two arrays.~~

~~123. The system of claim 121, wherein said array of said at least two arrays is horizontally adjacent to another array of said at least two arrays.~~

~~124. The system of claim 121, wherein said array of said at least two arrays is vertically adjacent to another array of said at least two arrays.~~