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 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 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 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 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 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 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 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 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 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 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 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 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 embodiment of the present 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 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 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 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 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 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 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 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.

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.

9. Said method of claim 1, 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 said other plate, two plates with non-coplanar set of electrodes on one plate and no electrodes on said other plate, two plates with an array of electrodes on one plate and single electrode on 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.

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

11. Said method of claim 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.

13. The method of claim 12, wherein said one or more physical properties comprises droplet size, 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 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.

15. Said method of claim 14, wherein replenishing said droplet based on said replenishing volume comprises heating one or more fluids surrounding said droplet to reduce evaporation of said droplet.

16. Said method of claim 15, wherein said heating is performed by said actuation of a heater displaced below said array, said heating of a plate displaced above said array, 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.

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

20. Said method of claim 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.