

detecting the target particle with the interrogating laser. Variability in the size of particles present in the flow stream or drift in the flow cytometer components can impact the predictability of whether a drop in the flow stream contains a particle of interest.

5 Inaccurate predictions of droplets that contain particles (e.g., applying a charge to the wrong droplet) can be detrimental to qualitative analysis, resulting in imprecise cell sorting, contamination of sorted samples as well as quantitative loss of biological sample.

SUMMARY

10 In a first aspect, the present disclosure refers to a method of determining a drop delay of a flow stream in a flow cytometer, the method comprising: flowing a liquid sample containing particles through a detection region of the flow cytometer, wherein the detection region comprises a flow cell having: an input, into which the liquid sample is introduced, an interrogation point, and a nozzle from which a flow stream of the liquid
15 sample exits the flow cell; wherein the flow stream exits the nozzle as a continuous flow stream that transitions to a stream of liquid droplets at a break off point, wherein the break off point is a point at which, upstream from the break off point, the flow stream is a continuous and connected fluidic stream and, downstream from the break off point, the flow stream is composed of a plurality of droplets having no physical contact with each
20 other; capturing images of the flow stream to obtain an imaged flow stream, wherein the images of the flow stream are captured in one or more detection fields by one or more image sensors; identifying a disturbance comprising a change in a break off point in the imaged flow stream; calculating the drop delay of the flow stream based on the identified disturbance, wherein the drop delay is the amount of time between the time a particle is
25 detected by the flow cytometer at the interrogation point and the time a corresponding charge is applied to a droplet at the break off point of the flow stream.

In a second aspect, the present disclosure refers to a system comprising: an imaging sensor configured to capture images of a flow stream of a flow cytometer, wherein a liquid sample containing particles flows through a detection region of the flow
30 cytometer, wherein the detection region comprises a flow cell having: an input, into which the liquid sample is introduced, an interrogation point, and a nozzle from which a flow stream of the liquid sample exits the flow cell; wherein the flow stream exits the

nozzle as a continuous flow stream that transitions to a stream of liquid droplets at a break off point, wherein the break off point is a point at which, upstream from the break off point, the flow stream is a continuous and connected fluidic stream and, downstream from the break off point, the flow stream is composed of a plurality of droplets having no physical contact with each other; wherein the images of the flow stream are captured in one or more detection fields; and a processor comprising memory operably coupled to the processor, wherein the memory includes instructions stored thereon to: identify a disturbance comprising a change in a break off point in the imaged flow stream; and calculate a drop delay of the flow stream based on the identified disturbance, wherein the drop delay is the amount of time between the time a particle is detected by the flow cytometer at the interrogation point and the time a corresponding charge is applied to a droplet at the break off point of the flow stream.

In a third aspect, the present disclosure refers to a non-transitory computer readable storage medium comprising instructions stored thereon for determining drop delay of a flow stream in a flow cytometer, the instructions comprising: algorithm for capturing images of a flow stream with one or more image sensors to obtain an imaged flow stream, wherein a liquid sample containing particles flows through a detection region of the flow cytometer, wherein the detection region comprises a flow cell having: an input, into which the liquid sample is introduced, an interrogation point, and a nozzle from which a flow stream of the liquid sample exits the flow cell; wherein the flow stream exits the nozzle as a continuous flow stream that transitions to a stream of liquid droplets at a break off point, wherein the break off point is a point at which, upstream from the break off point, the flow stream is a continuous and connected fluidic stream and, downstream from the break off point, the flow stream is composed of a plurality of droplets having no physical contact with each other; wherein the images of the flow stream are captured in one or more detection fields; algorithm for identifying a disturbance comprising a change in a break off point in the imaged flow stream; and algorithm for calculating drop delay of the flow stream based on the identified disturbance, wherein the drop delay is the amount of time between the time a particle is detected by the flow cytometer at the interrogation point and the time a corresponding charge is applied to a droplet at the break off point of the flow stream.

Examples of the present disclosure include methods for determining a drop delay of a flow stream in a flow cytometer. Methods according to certain examples include capturing an image of the flow stream to obtain an imaged flow stream, identifying a

disturbance at a break off point in the imaged flow stream and calculating a drop delay of the flow stream based on the identified disturbance. In some examples, the disturbance is the fanning of the flow stream at the break off point. In other examples, the disturbance is a deflection of the flow stream at the break off point. In examples, methods include capturing one or more images of the flow stream in a detection field and identifying a disturbance in the flow stream in the detection field. In some examples, the detection field includes the flow stream upstream from the flow stream break-off point and downstream from the laser irradiation point.

Methods also include, according to some examples, determining the drop delay that produces the maximal amplitude of the identified disturbance. The drop delay of the flow cytometer may be set across a range of drop delays and the disturbance at the break off point is visualized at each drop delay to identify the drop delay that produces the maximal amplitude of the identified disturbance. In some examples, the disturbance is fanning of the flow stream at the break off point and the methods include setting the flow cytometer across a range of drop delays and identifying the drop delay which produces the greatest amount of fanning of the flow stream at the break off point. In other examples, the disturbance is a deflection (i.e., deviation in the trajectory) of the flow stream and the methods include setting the flow cytometer across a range of drop delays and identifying the drop delay which produces the greatest amount of deflection from the longitudinal axis of the flow stream. To determine the drop delay that produces the maximal amplitude of the identified disturbance, a phase mask may be also applied, such as a phase mask having $1/32$ drop equivalent increments.

Calculating a drop delay according to certain examples of the present disclosure includes determining the drop delay that produces the maximal amplitude of the identified disturbance and adding a predetermined drop equivalent to the determined drop delay that produces the maximal amplitude of the identified disturbance. The resultant drop delay will be the optimized drop delay for the particular stream conditions. For example, the drop equivalent may range from a 1/32 drop equivalent to 1 drop equivalent, such as a 1/2 drop equivalent. In certain instances, no drop equivalent is added to the drop delay that produces the maximal amplitude of the identified disturbance. In some examples the predetermined drop equivalent is half a drop. In other examples, calculating a drop delay of the flow stream includes determining the drop delay that produces the maximal amplitude of the identified disturbance while applying a predetermined offset. For example, a drop delay may be calculated by applying a one-half drop equivalent offset and then determining the drop delay that produces the maximal amplitude of the identified disturbance.

In certain examples, the subject methods reduce the need for user input or manual adjustment during setup of a flow cytometer or between analyses of different samples. In some examples, methods of interest may partially or fully automate a flow cytometer so that parameters, such as calculation of drop delay, of the flow cytometer are processor controlled. In certain examples, the methods include determining drop delay of the flow cytometer without any human input. For example, methods may include determining drop delay of the flow stream in the absence of calibration particles (e.g., fluorescent beads). Parameters of the flow cytometer may be adjusted in response to the determined drop delay, such as changing the timing of applying an electrical charge of the flow stream, adjusting the duration of the electrical charging pulse, the voltage of the electrical charge or changing the flow rate of the flow stream.

Examples of the present disclosure also include systems for determining drop delay of a flow stream in a flow cytometer. Systems according to certain examples include an imaging sensor configured to capture one or more images of a flow stream and a processor having memory operably coupled to the processor where the memory includes instructions to identify a disturbance at a break off

point in the imaged flow stream and to calculate a drop delay of the flow stream based on the identified disturbance. For example, the memory may include instructions to identify fanning of the flow stream at the break off point or a deflection of the flow stream at the break off point.

In examples, the subject systems are configured to capture one or more images of the flow stream in a detection field and identify a disturbance in the flow stream in the detection field. In some instances, the detection field includes the flow stream upstream from the flow stream break-off point and downstream from the laser irradiation point.

5 Examples of the present disclosure also include a non-transitory computer readable storage medium for determining a drop delay of a flow stream in a flow cytometer. Non-transitory computer readable storage mediums according to certain examples include instructions stored thereon having algorithm for capturing an image of a flow stream with an image sensor, algorithm for identifying a disturbance at a break off
10 point in the imaged flow stream and algorithm for calculating drop delay of the flow stream based on the identified disturbance. The non-transitory computer readable storage medium may also include algorithm for determining the drop delay that produces the maximal amplitude of the identified disturbance. In these examples, the computer readable storage medium includes algorithm for setting the flow cytometer across a
15 range of drop delays and algorithm for determining the drop delay that produces that maximal amplitude of the identified disturbance. For example, the computer readable storage medium may include algorithm that determines the drop delay that produces the greatest amount of fanning of the flow stream at the break off point or algorithm that determines the drop delay that produces the greatest amount of deflection from the
20 longitudinal axis of the flow stream.

BRIEF DESCRIPTION OF THE FIGURES

The invention may be best understood from the following detailed description when read in conjunction with the accompanying drawing. Included in the drawing is the following figure:

25 **FIG. 1** depicts a flow diagram for determining a drop delay of a flow stream in a flow cytometer according to certain embodiments.

DETAILED DESCRIPTION

Aspects of the present disclosure include methods and systems for determining a drop delay of a flow stream in a flow cytometer. Methods according to certain
30 embodiments include capturing an image of the flow stream to obtain an imaged flow stream, identifying a disturbance at a break off point in the imaged flow stream and

What is claimed is:

1. A method of determining a drop delay of a flow stream in a flow cytometer, the method comprising:

5 flowing a liquid sample containing particles through a detection region of the flow cytometer, wherein the detection region comprises a flow cell having: an input, into which the liquid sample is introduced, an interrogation point, and a nozzle from which a flow stream of the liquid sample exits the flow cell;

10 wherein the flow stream exits the nozzle as a continuous flow stream that transitions to a stream of liquid droplets at a break off point, wherein the break off point is a point at which, upstream from the break off point, the flow stream is a continuous and connected fluidic stream and, downstream from the break off point, the flow stream is composed of a plurality of droplets having no physical contact with each other;

15 capturing images of the flow stream to obtain an imaged flow stream, wherein the images of the flow stream are captured in one or more detection fields by one or more image sensors;

identifying a disturbance comprising a change in a break off point in the imaged flow stream;

20 calculating the drop delay of the flow stream based on the identified disturbance, wherein the drop delay is the amount of time between the time a particle is detected by the flow cytometer at the interrogation point and the time a corresponding charge is applied to a droplet at the break off point of the flow stream.

25 2. The method according to claim 1, wherein the disturbance comprises a change that is fanning of the flow stream or deflection of the flow stream.

3. The method according to any one of claims 1-2, wherein calculating the drop delay of the flow stream comprises:

30 varying the drop delay of the flow cytometer by increments of drop equivalents across a plurality of different drop delays, wherein one or more images of the flow stream is captured corresponding to each drop delay;

calculating the drop delay corresponding to a maximal amplitude of the disturbance; and

adding a predetermined drop equivalent to the drop delay at the maximal amplitude of the disturbance wherein the drop delay at the maximal amplitude of disturbance plus a predetermined drop equivalent provides for an optimized drop delay.

5 4. The method according to claim 3, wherein the predetermined drop equivalent is a one-half drop equivalent.

5. The method according to any one of claims 3-4, wherein calculating the drop delay at the maximal amplitude of the disturbance comprises applying a phase mask.

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6. The method according to claim 5, wherein the phase mask comprises 1/32 drop equivalents.

7. The method according to claim 6, wherein determining the drop delay at the maximal amplitude of the disturbance comprises applying an 8/32 drop equivalent phase mask.

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8. The method according to any one of claims 3-4, wherein calculating the drop delay of the flow stream comprises:

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calculating the drop delay corresponding to a maximal amplitude of the disturbance while applying a one-half drop equivalent offset phase mask; and adding a predetermined drop equivalent to the drop delay at the maximal amplitude of the disturbance to determine the drop delay of the flow stream.

25 9. The method according to claim 8, wherein the method comprises adding no drop equivalent to the drop delay at the maximal amplitude of disturbance while the one-half drop equivalent offset phase mask is applied.

10. The method according to any one of claims 1-9, wherein the detection field of the one or more detection fields comprises the flow stream upstream from the flow stream break-off point and downstream from the interrogation point.

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11. The method according to any one of claims 1-10, wherein the flow stream is illuminated with a light source.

12. The method according to any one of claims 1-11, further comprising adjusting one or more parameters of the flow cytometer in response to the calculated drop delay.

5 13. A system comprising:

an imaging sensor configured to capture images of a flow stream of a flow cytometer,

10 wherein a liquid sample containing particles flows through a detection region of the flow cytometer, wherein the detection region comprises a flow cell having: an input, into which the liquid sample is introduced, an interrogation point, and a nozzle from which a flow stream of the liquid sample exits the flow cell;

15 wherein the flow stream exits the nozzle as a continuous flow stream that transitions to a stream of liquid droplets at a break off point, wherein the break off point is a point at which, upstream from the break off point, the flow stream is a continuous and connected fluidic stream and, downstream from the break off point, the flow stream is composed of a plurality of droplets having no physical contact with each other;

wherein the images of the flow stream are captured in one or more detection fields; and

20 a processor comprising memory operably coupled to the processor, wherein the memory includes instructions stored thereon to:

identify a disturbance comprising a change in a break off point in the imaged flow stream; and

25 calculate a drop delay of the flow stream based on the identified disturbance, wherein the drop delay is the amount of time between the time a particle is detected by the flow cytometer at the interrogation point and the time a corresponding charge is applied to a droplet at the break off point of the flow stream.

14. A non-transitory computer readable storage medium comprising instructions stored thereon for determining drop delay of a flow stream in a flow cytometer, the instructions comprising:

30 algorithm for capturing images of a flow stream with one or more image sensors to obtain an imaged flow stream,

wherein a liquid sample containing particles flows through a detection region of the flow cytometer, wherein the detection region comprises a flow cell having: an input,

into which the liquid sample is introduced, an interrogation point, and a nozzle from which a flow stream of the liquid sample exits the flow cell;

wherein the flow stream exits the nozzle as a continuous flow stream that transitions to a stream of liquid droplets at a break off point, wherein the break off point is a point at which, upstream from the break off point, the flow stream is a continuous and connected fluidic stream and, downstream from the break off point, the flow stream is composed of a plurality of droplets having no physical contact with each other;

wherein the images of the flow stream are captured in one or more detection fields;

algorithm for identifying a disturbance comprising a change in a break off point in the imaged flow stream; and

algorithm for calculating drop delay of the flow stream based on the identified disturbance, wherein the drop delay is the amount of time between the time a particle is detected by the flow cytometer at the interrogation point and the time a corresponding charge is applied to a droplet at the break off point of the flow stream.