

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; detecting a particle in the flow stream by illuminating the particle in the flow stream at the interrogation point; 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, wherein such disturbance further comprises a
25 change that is fanning of the flow stream or a change that is deflection of the flow stream; applying an electrical charge to a droplet forming at the break off point of the 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 the particle is detected by the flow cytometer at the interrogation point and the time the electrical
30 charge is applied to the droplet forming 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 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 a particle in the flow stream is detected by illuminating the particle in the flow stream at the interrogation point; wherein the images of the flow stream are captured in one or more detection fields; wherein an electrical charge is applied to a droplet forming at the break off point of the flow stream; 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, wherein such disturbance further comprises a change that is fanning of the flow stream or a change that is deflection of the 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 the particle is detected by the flow cytometer at the interrogation point and the time the electrical charge is applied to the droplet forming 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 a particle in the flow stream

is detected by illuminating the particle in the flow stream at the interrogation point;
wherein the images of the flow stream are captured in one or more detection fields;
wherein an electrical charge is applied to a droplet forming at the break off point of the
flow stream; algorithm for identifying a disturbance comprising a change in a break off
5 point in the imaged flow stream, wherein such disturbance further comprises a change
that is fanning of the flow stream or a change that is deflection of the flow stream; and
algorithm for calculating a drop delay of the flow stream based on the identified
disturbance, wherein the drop delay is the amount of time between the time the particle
is detected by the flow cytometer at the interrogation point and the time the electrical
10 charge is applied to the droplet forming 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
15 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,
20 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
25 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
30 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.

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 detecting a particle in the flow stream by illuminating the particle in the flow stream at the interrogation point;

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;

20 identifying a disturbance comprising a change in a break off point in the imaged flow stream, wherein such disturbance further comprises a change that is fanning of the flow stream or a change that is deflection of the flow stream;

applying an electrical charge to a droplet forming at the break off point of the flow stream;

25 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 the particle is detected by the flow cytometer at the interrogation point and the time the electrical charge is applied to the droplet forming at the break off point of the flow stream.

30 2. The method according to claim 1, wherein calculating the drop delay of the flow stream comprises:

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.

3. The method according to claim 2, wherein the predetermined drop equivalent is a one-half drop equivalent.

10 4. The method according to any one of claims 2-3, wherein calculating the drop delay at the maximal amplitude of the disturbance comprises applying a phase mask.

5. The method according to claim 4, wherein the phase mask comprises $1/32$ drop equivalents.

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6. The method according to claim 5, wherein determining the drop delay at the maximal amplitude of the disturbance comprises applying an $8/32$ drop equivalent phase mask.

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

calculating the drop delay corresponding to a maximal amplitude of the disturbance while applying a one-half drop equivalent offset phase mask; and

25 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.

8. The method according to claim 7, 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.

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9. The method according to any one of claims 1-8, 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.

10. The method according to any one of claims 1-9, wherein the flow stream is illuminated with a light source.

11. The method according to any one of claims 1-10, further comprising adjusting
5 one or more parameters of the flow cytometer in response to the calculated drop delay.

12. 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;

wherein the flow stream exits the nozzle as a continuous flow stream that
15 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 a particle in the flow stream is detected by illuminating the particle in the
20 flow stream at the interrogation point;

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

wherein an electrical charge is applied to a droplet forming at the break off point
of the flow stream; and

25 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, wherein such disturbance further comprises a change that is
fanning of the flow stream or a change that is deflection of the flow stream; and

30 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 the particle
is detected by the flow cytometer at the interrogation point and the time the electrical
charge is applied to the droplet forming at the break off point of the flow stream.

13. 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:

5 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;

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 wherein a particle in the flow stream is detected by illuminating the particle in the flow stream at the interrogation point;

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

20 wherein an electrical charge is applied to a droplet forming at the break off point of the flow stream;

algorithm for identifying a disturbance comprising a change in a break off point in the imaged flow stream, wherein such disturbance further comprises a change that is fanning of the flow stream or a change that is deflection of the flow stream; and

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