## CLAIMS

1. A method of calculating a drop delay in a stream of a flow cytometer, comprising:
   a) determining a widths plot by measuring the width of a stream based on an image of the stream,
   b) setting drop delay based on the widths plot.

2. A method of calculating a drop delay in a stream of a flow cytometer, comprising:
   (a) identifying a laser position relative to a frame of reference;
   (b) determining break-off point; and
   (c) calculating drop delay by
      1) setting a strobe phase,
      2) imaging the stream,
      3) determining a widths plot by measuring the widths of the stream based on the image of the stream,
      4. determining the peaks in the widths plot;
      5) calculating phase of laser intercept, and
      6) setting drop delay in response to the peaks in the widths plot, a phase of the laser intercept relative to a droplet generation wave and a charge phase.

3. The method of claim 2 further comprising the step of smoothing the widths plot.

4. The method of claim 2 wherein the step of determining of the peaks in the widths plot comprises the steps of calculating a derivative of the widths plot and recording a plurality of zero-crossings of the derivative, wherein each zero-crossing is indicative of a peak in the smoothed widths plot.

5. The method of claim 2 wherein the break-off point is the shortest break-off point.

6. The method of claim 2 wherein the step of imaging the stream comprises the steps of running a camera along a length of the stream, while taking a plurality of

images of the stream; and stitching the plurality of images together to form a single image.

7. The method of claim 2 wherein the strobe phase is set to the charge phase plus 180°.

8. The method of claim 2 wherein the drop delay is also set in response to a bias correction factor.

9. The method of claim 8, wherein the bias correction factor is equal to a linear function aX + b, where X is the time from nozzle to break-off and parameters "a" and "b" are determined from experimental measurement of system response.

10. The method of claim 2, wherein the smoothing of the widths plot is conducted using a discrete Fourier analysis.

11. The method of claim 6 wherein a drive voltage is increased from an operational value when the camera is at about the top of the stream, and the drive voltage is decreased to an operational value as the camera is driven down towards the break-off point.

12. The method of claim 2, wherein the calculation of drop delay is used to determine when an analyte of interest has reached the break-off point of the stream.

13. A flow cytometer system for automatically calculating drop delay, comprising:
means for determining a widths plot based on an image of the stream; and
means for calculating drop delay based on the widths plot.

14. A flow cytometer system for automatically calculating drop delay, comprising:
means for taking an image of a stream;
means for determining a widths plot based on an image of the stream;
means for determining peaks in the widths plot;; and

means for calculating drop delay based on the determined peaks in the widths plot.

15. The flow cytometer of claims 14 further comprising means for smoothing the widths plot.

16. The flow cytometer of claim 14 wherein the means for determining peaks in the widths plot comprises:
means for calculating a derivative on the widths plot; and
means for determining zero-crossings on the derivative of the widths plot.

17. The flow cytometer of claim 16 wherein the means for calculating drop delay based on the determined peaks in the widths plot does so in response zero-crossings of the derivative of the widths plot.

18. The flow cytometer system of claim 14 wherein the means for taking an image of the stream comprises taking a plurality of images of the stream and wherein the cytometer system further comprises means for stitching the plurality of images of the stream to form one entire image of the stream.

19. A flow cytometer system for automatically calculating drop delay, comprising:
a camera adapted to take an image of a stream; and
a processor adapted to determine a widths plot based on the image of the stream, and to calculate drop delay based on the widths plot.

20. A flow cytometer system for automatically calculating drop delay, comprising:
a camera mounted on a precision-calibrated, movable camera stage, which is adapted to take a plurality of images of a stream; and
a processor adapted to
stitch the plurality of images of the stream to form one entire image of the stream,
determine a widths plot based on the one entire image of the stream,
smooth the widths plot,

calculate a derivative on the smoothed widths plot,
determine zero-crossing on the smoothed widths plot, and
calculate drop delay based on the determined zero-crossings.

## CLAIMS

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

(a) identifying a laser position relative to a frame of reference;

(b) determining a break-off point; and

(c) calculating the drop delay by

1) setting a strobe phase,

2) imaging the stream,

3) determining a widths plot by measuring the widths of the stream based on the image of the stream,

4) determining the peaks in the widths plot;

5) calculating a phase of laser intercept relative to a droplet generation wave, and

6) calculating the drop delay in response to the peaks in the widths plot, the phase of laser intercept and a charge phase.

2. The method of claim 1, further comprising the step of smoothing the widths plot.

3. The method of claim 1, wherein the step of determining of the peaks in the widths plot comprises the steps of calculating a derivative of the widths plot and recording a plurality of zero-crossings of the derivative, wherein each zero-crossing is indicative of a peak in the smoothed widths plot.

4. The method of claim 1, wherein the break-off point is the shortest break-off point.

5. The method of claim 1, wherein the step of imaging the stream comprises the steps of running a camera along a length of the stream, while taking a plurality of images of the stream; and stitching the plurality of images together to form a single image.

6. The method of claim 1, wherein the strobe phase is set to the charge phase plus 180°.

7. The method of claim 1, wherein the drop delay is also calculated in response to a bias correction factor.

8. The method of claim 7, wherein the bias correction factor is equal to a linear function aX+b, where X is the time from nozzle to break-off and parameters "a" and "b" are determined from experimental measurement of system response.

9. The method of claim 2, wherein the smoothing of the widths plot is conducted using a discrete Fourier analysis.

10. The method of claim 5, wherein a drive voltage is increased from an operational value when the camera is at about the top of the stream, and the drive voltage is decreased to an operational value as the camera is driven down towards the break-off point.

11. The method of claim 1, wherein the calculation of drop delay is used to determine when an analyte of interest has reached the break-off point of the stream.

12. A flow cytometer system for automatically calculating a drop delay, comprising:

a camera adapted to take an image of a stream; and

a processor adapted to

determine a widths plot based on the image of the stream,

determine the peaks in the widths plot,

calculate a phase of laser intercept relative to a droplet generation wave, and

calculate the drop delay in response to the peaks in the widths plot and the phase of laser intercept.

13. A flow cytometer system for automatically calculating a drop delay, comprising:

a camera mounted on a precision calibrated, movable camera stage, which is adapted to take a plurality of images of a stream; and

a processor adapted to

stitch the plurality of images of the stream to form one entire image of the stream,

determine a widths plot based on the one entire image of the stream,

smooth the widths plot,

calculate a derivative on the smoothed widths plot,

determine zero-crossing on the smoothed widths plot,

calculate a phase of laser intercept; and

calculate the drop delay based on the determined zero-crossings and the calculated phase of laser intercept.

14. The flow cytometer system of claim 12, wherein the processor is further adapted to include a charge phase in the calculation of the drop delay.

15. The flow cytometer system of claim 12, wherein the processor is adapted to calculate the phase of laser intercept based on a position of a peak before a laser position and the position of a peak after the laser position in the widths plot.

16. The flow cytometer system of claim 15, wherein the processor is adapted to determine the number of peaks between the laser position and a break-off point in the widths plot.

17. The flow cytometer system of claim 16, wherein the processor is adapted to calculate the drop delay by adding the determined number of peaks between the laser position and the break-off point to the phase of laser intercept and subtracting a charge phase.

18. The flow cytometer system of claim 16, wherein the processor is adapted to calculate the break-off point from the image of the stream.

19. The flow cytometer system of claim 18, wherein the processor is adapted to calculate the break-off point by subtracting the length of the droplet generation wave from the position of the first detached droplet in the image.