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Specialized Flow Cytometry Solutions

Droplet Volume Estimations

1. Preface

Estimating the volume of a sorted droplet can be useful in anticipating the total volume generated during a bulk sort to derive the number of collection vessels needed.

The volume is instrument-specific and is dependent on various settings impacting fluid velocity such as flow cell geometry, nozzle tip diameter, and sheath pressure, as well as the droplet generation frequency employed by the piezoelectric transducer.

A wider nozzle tip will require a lower pressure and drop drive frequency generating a lower number of larger droplets. By contrast, a smaller nozzle tip will require a higher pressure and drop drive frequency generating a higher number of smaller droplets.

Three methods, described below, can be used to calculate the volume of a sorted droplet.

2. Volume Extrapolation Method (adapted from the work of Tobias Rubner)

- Hold a 20mL cylinder in the sheath stream off the nozzle tip for exactly 1 minute.
- Measure the volume collected.
- Repeat this procedure three times and average the flow rate results in nL/s.
- Divide the flow rate by the drop drive frequency in Hz or s^{-1}.

$$Volume\ Droplet\ (nL) = \frac{Flow\ Rate\ [\frac{nL}{s}]}{Drive\ Frequency\ [\frac{1}{s}]}$$

3. Mass Extrapolation Method

- Tare an empty collection vessel.
- Sort 100,000 microspheres into the collection vessel using the “Single” sort mode to ensure only droplets containing centered particles are deflected.
- Once the sort is complete, subtract the tare from the weight of the collection vessel and divide by 100,000 to obtain the weight of one droplet.

$$Volume\ Droplet\ (nL) \sim Weight\ Droplet\ (\mu g) = \frac{Collection\ Vessel\ Weight\ (\mu g) - Tare\ (\mu g)}{100{,}000}$$

assumes microsphere weight is negligible and sheath fluid density is ~1kg/L

4. Mathematical Method (adapted from the work of Dominic Vergata)

Controllable parameters

- D_0 Nozzle size: 100 microns
- U_0 Fluid velocity: to be measured via flow rate
- f^* Frequency: to be calculated and compared to typical values

Nozzle size:
Selected during instrument startup.

$$D_0 = 100\ \mu m = 100 * 10^{-6}\ m$$

Fluid velocity:
Once system fluidics are turned on, collect sheath in a tube as it passes through to waste. Measure volume (V, mL) and time (t, sec) during collection to calculate flow rate $\left(Q, \frac{m^3}{s}\right)$.

$$Q = \frac{V}{t}$$

Example of flow rate measurements (MoFlo XDP):

V, mL	t, sec	$Q, \frac{mL}{s}$
4	40	0.1000
2.5	25	0.1000
5.5	57	0.0965
6.5	64	0.1016
5	49	0.1020
9.5	93	0.1022
6	61	0.0984
6.25	64	0.0977
2.25	22	0.1023
9	91	0.0989

$$Q_{avg} = 0.0999\frac{mL}{s} = 9.99 * 10^{-8}\frac{m^3}{s}$$

Divide Q by the cross-sectional area of the nozzle (A_0, m^2) to calculate velocity $\left(U_0, \frac{m}{s}\right)$.

$$A_0 = \pi R_0^2 = \pi(50 * 10^{-6}\ m)^2 = 7.854 * 10^{-9}\ m^2$$

$$U_0 = \frac{Q_{avg}}{A_0} = \frac{\left(9.99 * 10^{-8}\,\frac{m^3}{s}\right)}{(7.854 * 10^{-9}\,m^2)} = 12.72\,\frac{m}{s}$$

Ideal Frequency:
Operating frequency for fastest droplet breakup is given by the formula

$$f^* = 0.222\,\frac{U_j}{D_j}$$

Use jet contraction formulas to obtain U_j from U_0 and D_j from D_0:

$$U_j = \frac{4}{3}U_0 = \frac{4}{3}\left(12.72\,\frac{m}{s}\right) = 16.96\,\frac{m}{s}$$

$$D_j = \frac{\sqrt{3}}{2}D_0 = \frac{\sqrt{3}}{2}(100\,\mu m) = 86.60\,\mu m = 86.60 * 10^{-6}\,m$$

Ideal frequency is

$$f^* = 0.222\,\frac{\left(16.96\,\frac{m}{s}\right)}{(86.60 * 10^{-6}\,m)} = 43{,}474.61\,Hz$$

Assume we are operating as near as possible to the ideal frequency while maintaining a good breakoff pattern on screen.

$$f = 43{,}500\,Hz$$

Droplet Sizing:
Droplet diameter is given by the formula

$$D_d = \left(\frac{3}{2}\frac{U_j D_j^2}{f}\right)^{\frac{1}{3}}$$

$$D_d = \left(\frac{3}{2}\frac{\left(16.96\,\frac{m}{s}\right)(86.60 * 10^{-6}\,m)^2}{(43{,}500\,Hz)}\right)^{\frac{1}{3}} = 163.7\,\mu m$$

Volume of a droplet is

$$V_d = \frac{4}{3}\pi R_d^3$$

$$V_d = \frac{4}{3}\pi\left(\frac{163.7}{2}\,\mu m\right)^3 = 2.296 * 10^{-7}\,\mu m^3 = 2.296\,nL.$$

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To calculate without using intermediate values use the following formula

$$V_d = \frac{\pi}{4}\frac{U_0 D_0^2}{f} = \frac{\pi}{4}\frac{\left(12.72\frac{m}{s}\right)(100 * 10^{-6}\, m)^2}{\left(43{,}500\frac{1}{s}\right)} = 2.297 * 10^{-12}\, m^3 = 2.297\, nL$$

$$V_d \approx 2.3\, nL$$