Where Light Meets Life®

Delaware Flow NanoCytometer®

Tech Note: Absolute Concentration

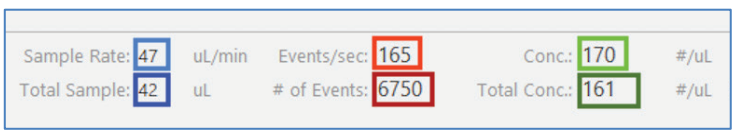
The vast majority of flow cytometers performs sample analysis on a relative basis: A sample is analyzed, a total number of events is detected, and based on certain characteristics (e.g., particle size, presence or absence of one or more surface antigens, membrane permeability, etc.) the corresponding relative fraction of events is displayed. For example, it is standard practice to display the results of an assay in terms of the percentage of events satisfying certain criteria (82% live, 28% CD3 positive, etc.)

While this allows the comparison of results across a series of controlled experiments with few variables, it does not provide a critical measurement–the absolute concentration, or "count," of particles of interest. Absolute concentration is essential in diagnostics: For example, in hematology, it makes a huge difference whether there are 10,000 white blood cells per µL or 100,000–the difference between a normal result and one that suggests leukemia–even if the relative percentages of, say, neutrophils are the same in each case. And absolute concentration provides important information in research, too, especially when a sample is one-of-a-kind; and where the "count" is used (e.g., in pharmaceutical development) to gauge effectiveness of synthesis or selection.

Until now, flow cytometry users generally had to resort to using counting beads to determine, indirectly, the concentration of detected events. This added costs (the counting beads themselves) and introduced additional measurement uncertainty (such as those due to operator error and variability among operators). Alternatively, users had to perform their own instrument calibrations on the fly, introducing yet other sources of measurement error, aside from the added labor burden.

The *Delaware* Flow NanoCytometer® provides the **absolute concentration** of detected events automatically, without the need for counting beads or operator-performed calibration. The Delaware performs **absolute volumetric measurements** of the samples being analyzed, delivering in real time (see screenshot below from the *Delaware's Panama* software):

- the instantaneous sample flow rate in µL/min (light blue box)
- the total analyzed sample volume for a given run in µL (dark blue)



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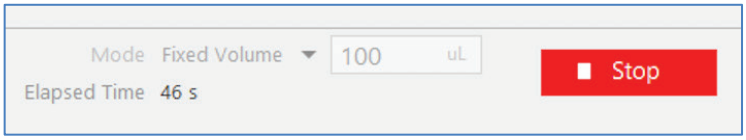
- the instantaneous event rate in events/sec (light red)
- the total number of events for a given run (dark red)
- the instantaneous absolute concentration in events/µL (light green)
- the **total absolute concentration** for a given run in events/µL (dark green)

This capability eliminates uncertainty, costs, and time from the workflow, delivering several critical parameters automatically. This makes it possible for users to perform absolute measurements and to compare results quantitatively from different instruments and different laboratories–without needing additional steps or control materials.

As in any laboratory measurement, it is important to identify and measure the proper background, and to account for it. In experiments involving cells and larger nanoparticles (greater than about 0.2 µm), proper selection of trigger threshold and gating boundaries can reduce the count of background events to negligible levels. In measurements involving exosomes and other extracellular vesicles (EVs) near the limit of detection, the measured absolute concentration needs to be corrected for the background concentration (e.g., from a background buffer run).

In addition to providing absolute particle concentration, the ***Delaware*** also allows the user to select one of four different acquisition modes:



- continuous
- fixed number of events
- fixed time
- **fixed volume** (see detail from *Panama* at right)

This last mode in particular, due to the ***Delaware***'s ability to perform **true volumetric analysis**, is of great utility when running EVs and other small particles. In combination with performing a background run using the same acquisition volume, it provides the most accurate way to determine the absolute concentration of particles in the sample, and to standardize measurement protocols across experiments or quality control batches.

The ***Delaware*** Flow NanoCytometer was designed from the ground up as a flow cytometer for analyzing nanoparticles, such as exosomes and other extracellular vesicles (EVs). It combines superior sensitivity (≤ 65 nm liposomes; ≤ 28 nm gold) with high throughput, ease of use, and the ability to measure fluorescence on up to six channels. It is not limited to EVs; it can just as easily measure whole cells and other particles, up to sizes of tens of microns, by simply loading a different settings file. As this Tech Note shows, the ***Delaware*** offers not only excellent sensitivity and resolution, but also the **absolute count**, or concentration, of particles in a sample–without the need for counting beads or calibration by the user. The ***Delaware*** is, to our knowledge, the only research analyzer capable of doing so.

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