Recommendations for accurate concentration measurements on the Attune NxT Flow Cytometer

Introduction

The Invitrogen™ Attune™ NxT Flow Cytometer uses a unique volumetric sample and sheath fluid delivery system. Samples are introduced into the Attune NxT cytometer with syringes, producing accurate measurements of the volumes of acquired samples, and thus accurate calculation of cell concentrations. Recommendations for optimizing experiments to measure the most accurate concentration are summarized in Figure 1.

	Sample type	
	0.2–3 µm	>3 µm
Example sample type	Bacteria Microspheres	Jurkat cells Ramos cells Leukocytes Microspheres Cardiomyocytes
Flow rate	12.5–1,000 µL/min	100–1,000 µL/min
Sample concentration	500–10^6 particles/mL	
Event rate	<8,000 events/sec	
Sample volume	50–4,000 µL	

Figure 1. Recommended parameters for accurate counting. The values listed are guidelines only. Accuracy and precision of the measurement should be verified per individual protocol.

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Key considerations for accurate concentration measurements:

- Ensure that the system is properly maintained. Follow all recommendations detailed in the **Attune NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide** (Pub. No. 100024234).
- Maintain the event rate at <8,000 events/second for the most accurate concentration measurement. Accuracy decreases at higher event rates based on Poisson statistics of coincident particle arrivals. It is recommended that the event rate be kept as low as possible for any given experiment.
- Acquire larger cells or particles at flow rates ≥100 µL/min due to sample settling of large particles and increased variation at low flow rates (see Figure 2).
- Collect enough events for accurate counting statistics (>400 events for cells or particles of interest)—see "How many events must be acquired?" in **BioProbes 71** (p. 14), available at **thermofisher.com/bioprobes**
- Round-bottom plates are recommended for the most accurate measurements.
- Proper sample preparation and pipetting technique are critical:
  - Ensure samples are thoroughly mixed during each stage of sample preparation that involves transfer from one container to another.
  - Minimize transfer steps where possible; pipetting and mixing errors compound with the number of steps.
  - Be sure to account for all sources of dilution when tracing to the original sample concentration.
  - Mix samples well before acquisition.
  - Use calibrated pipettors and rigorous pipetting techniques. See our **Good Laboratory Pipetting Guide** (Pub. No. BRHPGLPGuide0058) for more information on pipetting technique.

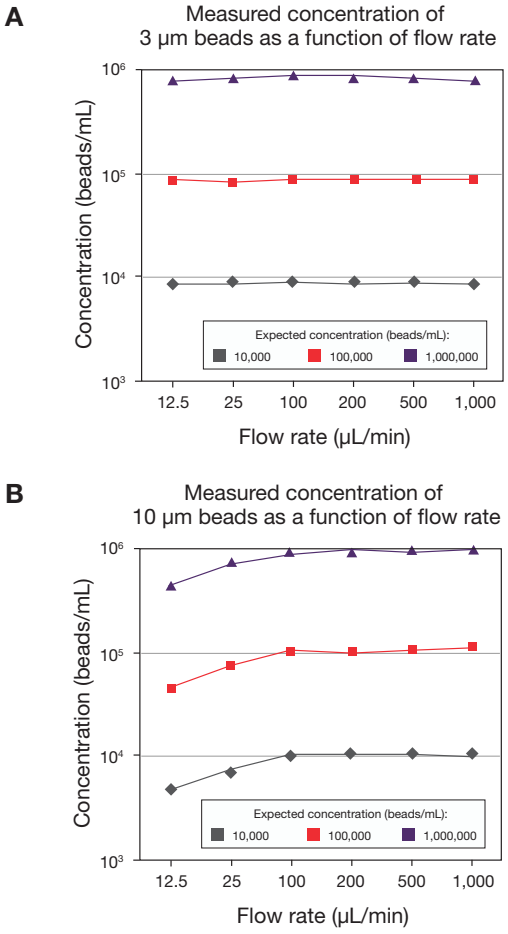

Figure 2. Data demonstrating the measured vs. expected concentration as a function of flow rate. (A) Smaller particles (e.g., 0.2–3 µm) show consistent concentration results across all flow rates for the three concentrations of beads tested. **(B)** Larger particles (e.g., 10 µm) show consistent results across the flow rate range 100–1,000 µL/min.

The sample concentration measurement may be affected by sample type, sample volume, sample processing rate, event rate, sample settling, and other factors. It is recommended that a comparison of serial dilutions of a sample be used to verify that the sample concentration measurement is accurate (e.g., twofold dilution = twofold change in concentration). In the event that the sample concentration measurement is not within the desired accuracy, the measured value may be calibrated to a known standard. If conducting a calibration, use the same instrument settings that will be used in the experiment (e.g., sample, sample processing rate, volume analyzed) during the calibration process.

Find out more at **thermofisher.com/attune**

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