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 to the Attune NxT Flow Cytometer with syringes, producing accurate measurements of the volumes of acquired samples, and thus accurate calculation of cell concentrations. Recommendations for optimizing experiments to obtain the most accurate concentration data are summarized here (Table 1).

Recommended conditions for accurate counting based on particle or cell size.*

	Particle or cell size range		
	0.5–3 µm	3–15 µm	>15 µm
Example sample type	Bacteria Microspheres	Jurkat cells Ramos cells Leukocytes Microspheres	Cardiomyocytes Microspheres
Flow rates	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		

* Accuracy and precision may vary with other conditions and should be verified per individual protocol.

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Key considerations for accurate concentration measurements:

1. 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).
2. Maintain the event rate at <8,000 events/second to keep coincidence <10%. The system supports acquisition rates up to 35,000 events/second, but the accuracy of the concentration measurement will decrease as the event rate increases above the 8,000 events/second limit.
3. Acquire cells or particles >3 µm in size at flow rates ≥100 µL/min (Figure 1).
4. Cells and particles from 0.5 to 3 µm in size can be acquired at all flow rates if the analysis is limited to parameters from a single laser source (e.g. blue laser). Otherwise, for analysis using multiple excitation sources, the flow rate should be limited to 12.5 and 25 µL/min.
5. Collect enough events to achieve statistically significant detection (>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. For further discussion on statistical considerations of rare-event detection, see Allan AL, and Keeney M (2010) *J Oncol* 426218.
6. Only use round-bottom plates when measuring concentration.
7. 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 Thermo Fisher's our Good Laboratory Pipetting Guide (Pub. No. BRHPGLPGuide0058) for more information on pipetting technique.

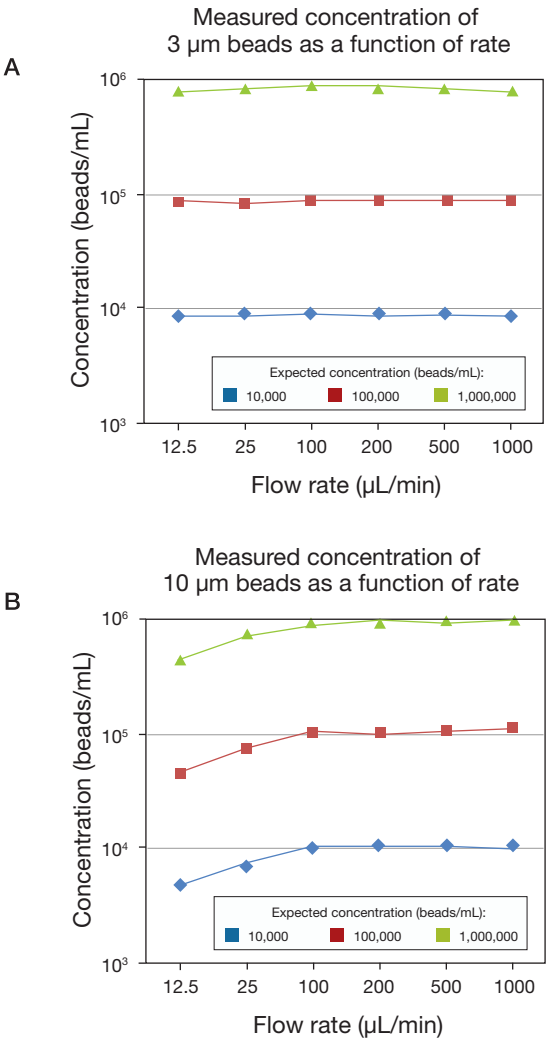

Figure 1. Measured vs. expected concentration as a function of flow rate. (A) Smaller particles (0.5–3 µm) show consistent concentration results at all flow rates for the three concentrations of beads tested. **(B)** Particles of larger size (>3 µm) show consistent results at the 100 to 1,000 µL/min flow rates.

To learn more about cell counting and accurate measurements of cell concentration using the Attune NxT Flow Cytometer, please also review "Accurately measure cell concentrations by flow cytometry" in *BioProbes* 73, available at **thermofisher.com/bioprobes**

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

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