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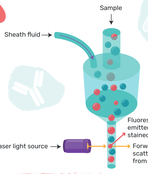
10 tips for flow cytometry data acquisition and analysis

1. Know your instrument!

There have never been more flow cytometry systems available than there are now. Choose the right system for your application.

If you need to excite specialized fluorescent probes and proteins, make sure your system has the correct laser and wavelengths.

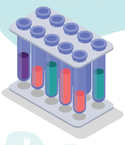


2. System maintenance and quality control

Follow the manufacturer's recommendations to monitor instrument performance. Use practices like detector 'voltration' to establish good ranges for your instrument detectors.

3. Sample preparation

Data acquisition and analysis are only as good as the quality of the sample. Flow cytometry requires single-cell suspensions.



4. Panel design

Choose compatible probe combinations, taking into account the spectral properties of the dyes, the density of a particular marker in the cell and the relationship between markers.

Mutually inclusive markers should be assigned to fluorescent probes with dissimilar spectral properties to minimize the effects of spectral overlap.

5. Prepare setup controls

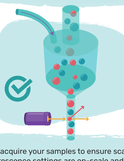
This includes single-color compensation or spectral deconvolution controls. The intrinsic autofluorescence in your system is important for all flow cytometry. Use deconvolution controls with similar properties to your intended cell type.

6. Prepare experiment controls

Experimental controls include isotype-matched antibodies and fluorescence-minus-one (FMO) or all-but-one (ABO) controls. Isotype-matched antibodies are used where non-specific antibody binding is a concern. FMO and ABO controls are very important in assessing the labeling of dim and modulated markers.

7. Data acquisition

While you can reanalyze your data many times, there will be only one acquisition! Make it count!



Make sure your instrument detectors are set correctly.

Pre-acquire your samples to ensure scatter and fluorescence settings are on-scale and correspond to the recommended ranges for your instrument.

Save a statistically valid number of events! Set a minimum collection number based on a gated region. Save experiment information, including marker names and sample information. The almost universal flow cytometry data file standard (FCS) makes this easy.

When in doubt, ask for help.

8. Data analysis

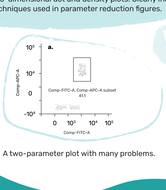
Cytometric data analysis is often done using third-party analysis software. Use unsupervised analysis tools for high-dimensional analysis but 'look under the hood' to monitor software-driven operations.

9. Data archiving

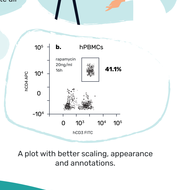
This can be done on physical media but should also be done on remote cloud-based systems. Most scientific publications also require your data to be deposited in a secure available location such as FlowRepository.

10. Data presentation

Use clear figures and axis labels on your data plots. Make your figures understandable without reference back to a figure legend. While sometimes necessary, single-parameter histograms are not as informative as two-dimensional dot and density plots. Clearly indicate all techniques used in parameter reduction figures.



A two-parameter plot with many problems.



A plot with better scaling, appearance and annotations.

This infographic has been recreated from William Telford's article **"10 tips for successful flow cytometry"** on **BioTechniques.com**

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