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Flow Cytometry- Current Detection Limits and Future Prospects

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Flow Cytometry measures optical signals from particles, usually biological cells, flowing through intense illumination. Typical illumination time of each particle is 1 to 100 microseconds. Detection of dim fluorescence signals is limited by multiple factors including the properties of the fluorescent molecules used to stain the cells. Fluorescence intensity from a fluorophore bound to a probe molecule is affected by environment and rarely the same as from the fluorophore in solution [1]. For any particular fluorophore, there is a maximum optimal excitation intensity above which signal to noise will decrease [2]. Detection of the emitted fluorescence is limited by the overall detection efficiency of the optical system, background light and electronic noise in the data acquisition system. The ultimate limitation is due to the photon statistics of fluorescence from the stained and unstained populations of cells. A practical approach to quantitative assessment of fluorescence detection capability based on physical factors has been developed and implemented in routine testing of commercial flow cytometers [3]. When a photomultiplier is used to detect fluorescence, the overall detection efficiency, Q, is the effective number of photoelectrons per equivalent fluorescence emitter. Contributions to background light, B, include Raman scatter of water, unbound fluorescent probe and spectral overlap from different fluorophores in multicolor applications. Knowledge of Q and B and basic information about the sample allow prediction of the fluorescence population distributions of cells. New photon counting detectors, signal analysis methods and luminescent nanoparticles may provide increased detection sensitivity in the future.

[1] J Res Natl Inst Stand Technol. 107:83–91 (2002)
[2] Cytometry 29:204–214 (1997)
[3] Cytometry 33:267–279 (1998)