Technical Note:
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# Differences in Beads for Flow Cytometry QC and Quantitation

Key Words:

- Flow Cytometry
- Beads
- Hard dyed
- MESF
- Calibrate

## Introduction

Our strength is in offering you a complete particle technology. We give you simple protocols working with our particles. We provide you concrete data, backed by years of applications research in our own labs.

Microsphere or bead standards are used in a variety of applications in flow cytometry and their selection for specific applications based on physical characteristics has been discussed in the literature.

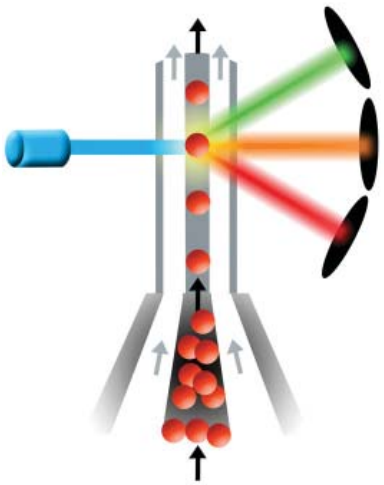
## Instrument Quality Control

Instrument quality control, which includes verification of the sensitivity and performance of the fluorescence detectors and PMTs, should be monitored frequently to ensure instrument consistency over time and to detect changes in performance that may compromise data. The ideal product for this application would feature dyes that are thermally and photolytically stable for at least one year.

Thermo Scientific Cyto-Cal Multifluor microspheres are hard-dyed polymer beads that utilize a proprietary process to incorporate the dye throughout the polymer matrix. These beads are stable for at least two years. The combination of a stable dye in a hard-dyed bead creates an ideal reference particle for those seeking long-term, inter/intra lab instrument performance monitoring. These beads will test and define the condition of the optics and flow stream and ensure the ability of the instrument to resolve different cell populations. The dyes in Cyto-Cal Multifluor beads have similar optical properties, but are not spectrally equivalent, to the common dyes used in flow cytometry. Therefore, Cyto-Cal beads should not be used to obtain absolute quantitation of fluorophores on cells.

## Quantitation of Fluorescent labeled Cells

Fluorescence quantitation is used to determine the amount of fluorphore bound per cell and ultimately the amount of antibody bound per cell. This requires spectrally equivalent microsphere standards that feature distinct populations of beads with the same dyes that are used to label the cells. These Surface-dyed microspheres simulate dye attachment to the



cell membrane. Users are then able to estimate the number of dye molecules bound per cell by comparing against a bead of known Molecules of Equivalent Soluble Fluorochrome (MESF) or Mean Equivalent Fluorochrome (MEFL) to describe the intensity level.

For years, bead calibration standards have been recommended and used to perform both fluorophore quantitation and to “calibrate” the response of the instrument (confirm linearity/quality control). Surface-dyed beads have often been used for both applications, however, users need to keep in mind that all surface-dyed beads are thermally and photolytically unstable and require refrigeration. They also suffer from a short shelf life. Both instrument manufacturers and bead vendors have perpetuated this single-bead concept. And while it may seem more convenient and economical from a user’s standpoint, the use of stable calibrators for instrument QC will provide better results and will be more economical.

We recommend the use of surface-dyed beads for fluorophore quantitation (featuring the common flow cytometry dyes) and hard-dyed beads, such as Cyto-Cal Multifluor, for instrument quality control.

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