Obtain the highest-quality data your system can deliver

Fluorescent microspheres for calibrating microscopes and flow cytometers.

Advanced fluorescence techniques demand quality at every level, from specimen preparation and fluorophore choice to instrument set-up and calibration. Often the final step in a long experimental protocol, data collection is critically dependent on instrument performance. The R&D scientists in our labs have developed a variety of nonbiological calibration standards designed for reliably assessing the operation of conventional fluorescence microscopes, confocal laser-scanning microscopes, and flow cytometers. The dye molecules in these microsphere standards are localized within the polymeric matrix, making the beads brighter and more photostable than conventional surface-stained beads. The stability, uniformity, and reproducibility of Molecular Probes® fluorescent microspheres make them ideal reference standards for day-to-day calibrations as well as for sensitive image registration and quantitative measurements with multiply stained fluorescent cells and tissues.

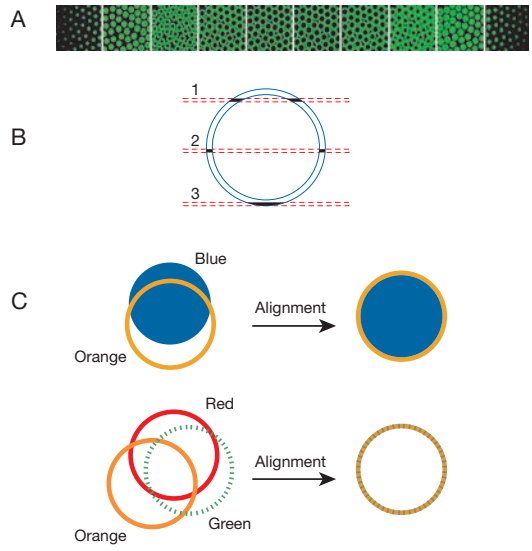

Figure 1. Confocal laser-scanning microscope optical cross-sectioning and alignment with FocalCheck™ microspheres. (A) Serial optical sectioning from top to bottom along the z-axis of ring-stained microspheres reveals a continuous pattern of disc-to-ring-to-disc images. **(B)** The diameter of the fluorescent ring (or disc) seen is dependent on the depth of the optical focal plane. **(C)** In the confocal laser-scanning microscope, separate light paths exist for UV and visible wavelengths, and emitted fluorescence is detected by different photomultipliers. Proper optical alignment in three dimensions may be obtained with either of two types of FocalCheck™ microspheres: 1) microspheres with a ring stain and a second fluorescent stain throughout the bead; or 2) microspheres containing three different fluorescent ring stains.

Confirm color alignment with FocalCheck™ Beads

High-resolution imaging of multiply stained specimens by confocal microscopy typically requires bracketed exposures, repetitive scans, and three-dimensional sectioning. Several factors such as optics, filters, and instrument alignment can affect the registration of different fluorescent colors, making it difficult to correctly interpret the positional relationships among stained structures. FocalCheck™ Fluorescent Microspheres are designed for examining the alignment and stability of confocal laser-scanning microscopes [1]. They are particularly useful for confirming the optical sectioning thickness ("z-resolution") in three-dimensional imaging applications [2].

The FocalCheck™ polystyrene beads—available with either 6 µm or 15 µm diameter—have been prepared by a proprietary method in which fluorescent dye is used to stain only the outermost portion of each microsphere. The resulting beads have a well-defined dye layer (ring stain) that, when viewed in cross section in the confocal laser-scanning microscope, appears as a fluorescent ring of varying dimensions depending on the focal plane (Figure 1). FocalCheck™ microspheres are provided in suspension or mounted on slides and in several different multicolored configurations of fluorescent ring stains with or without contrasting fluorescent dyes throughout the bead. The spectral properties of the fluorescent dyes are well matched to the laser sources and optical filters commonly used in imaging systems. We also offer three different FocalCheck™ test slides, including Test Slide #1 for alignment and intensity calibrations [3].

Adjust color registration with TetraSpeck™ Beads

TetraSpeck™ Fluorescent Microspheres provide a different approach for calibrating color registration with your imaging system. These microspheres are stained throughout the polymeric matrix with four different fluorescent dyes, yielding beads that display four well-separated excitation/emission peaks—365/430 nm (blue), 505/515 nm (green), 560/580 nm (orange), and 660/680 nm (dark red). They are available in five diameters (0.1 µm, 0.2 µm, 0.5 µm, 1 µm, and 4 µm), from subresolution- to nearly cell-size particles.

The 0.1 µm and 0.2 µm TetraSpeck™ beads are ideal as subresolution fluorescent sources for calibrating instrument optics, especially in three-dimensional applications. The 0.1 µm TetraSpeck™ microspheres

© 2014 Thermo Fisher Scientific Inc. All rights reserved. For Research Use Only. Not for use in diagnostic procedures.

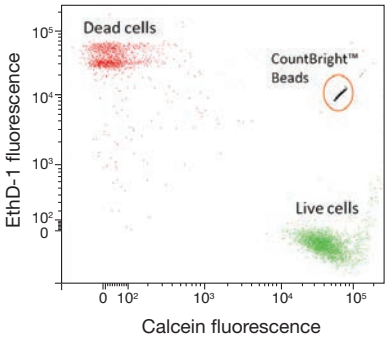

Figure 2. Using CountBright™ beads to count cells in viability experiments. A mixture of live and heat-killed Jurkat cells were treated with the reagents in the LIVE/DEAD® Viability/Cytotoxicity Kit (Cat. No. L3224). CountBright™ Absolute Counting Beads (Cat. No. C36950) were added, and the sample was analyzed by flow cytometry using 488 nm excitation. The plot of calcein fluorescence (using a 530/30 nm bandpass emission filter) vs. ethidium homodimer-1 (EthD-1) fluorescence (using a 610 nm longpass emission filter) shows clear separation of live and dead cells, as well as of the CountBright™ beads.

have been used as fiducial markers for super-resolution microscopy to adjust color registration and make lateral drifting corrections [4,5]. TetraSpeck™ microspheres have also been used to calibrate the spatial distribution of illumination for high-content screening (HCS) [6,7].

Calibrate daily with AlignFlow™ and Cell Sorting Set-Up Beads

Flow cytometers are designed to perform quantitative measurements on individual cells with speed, accuracy, and precision. As with all high-performance instrumentation, flow cytometers must be calibrated frequently to ensure accuracy and reliability.

AlignFlow™ alignment beads permit the calibration of a flow cytometer's laser(s), optics, and stream flow without wasting valuable experimental material [8,9]. The 2.5 µm–diameter AlignFlow™ beads are available for UV (350–370 nm), blue (488 nm), and red (630–640 nm) lasers; the 6 µm–diameter AlignFlow™ beads are available for the same three excitation-wavelength ranges. These fluorescently stained polystyrene microspheres are highly uniform in both size and fluorescence intensity and are designed to mimic the size and intensity of biological samples. AlignFlow™ beads have excellent photochemical and physical stability, providing reliable reference signals for aligning, focusing, and calibrating flow cytometers.

Whereas AlignFlow™ beads are used for the general alignment of your flow cytometer's optics and lasers, the Cell Sorting Set-Up Beads are reliable standards for cell-sorter instrument set-up and provide a means of adjusting settings, including drop delay and efficiency (cell loss during sorting). These fluorescent dye–infused microspheres have diameters of 6 µm and approximate the size, emission, and intensity of many biological samples. They have been optimized for use with UV, blue, green/yellow, and red lasers.

Count cells accurately with CountBright™ Beads

Absolute cell counts are widely used to follow disease progression. CountBright™ Absolute Counting Beads are designed to serve as an internal counting standard added to samples for simple concentration determination by flow cytometry [10] (Figure 2). CountBright™ beads are provided as a calibrated suspension of microspheres that are brightly fluorescent across a wide range of excitation (UV to 635 nm) and emission (385 to 800 nm) wavelengths; their fluorescence intensity has been adjusted to be 5–50 times brighter than that of typically stained cells. These microspheres are approximately 7 µm in diameter, have settling properties similar to lymphocytes, and fall above the scatter threshold. They can be used with any cell sample type (including no-wash/lysed whole blood), and with either a fluorescence or scatter threshold. Because CountBright™ beads are mixed in with the experimental sample, absolute cell counts using this single-platform method are more accurate and less complicated than cell concentration determined using multiple-platform testing.

Flow Cytometry Sub-Micron Particle Size References

The Flow Cytometry Sub-Micron Particle Size Reference Kit provides six suspensions of green-fluorescent microspheres, each with a known diameter (from 0.02 µm to 2 µm) determined by transmission electron microscopy. These microsphere suspensions provide reliable size references for flow cytometry applications (Figure 3) and can be used individually or together, as well as intermixed with the →

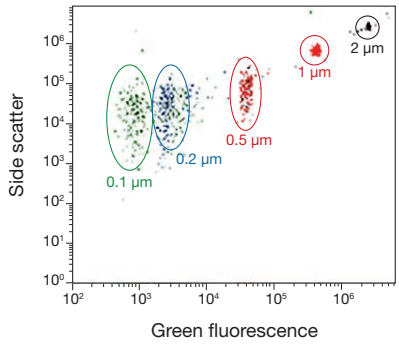

Figure 3. Separation of differently sized microspheres from the Flow Cytometry Sub-Micron Particle Size Reference Kit. Five microsphere suspensions from this reference kit (Cat. No. F13839) were analyzed using the Attune® Acoustic Focusing Cytometer (Blue/Violet). The diameters of the green-fluorescent microspheres are as marked.

© 2014 Thermo Fisher Scientific Inc. All rights reserved. For Research Use Only. Not for use in diagnostic procedures.

experimental sample or analyzed in parallel runs. This kit can also be used as a tool to verify instrument performance and to establish parameters that are suitable for analyzing sub-micron particles, including the particle-size resolution limits, dynamic range, and sensitivity of forward- and side-scatter PMTs.

The Flow Cytometry Sub-Micron Particle Size Reference Kit can help identify small particles such as exosomes, bacteria, and viruses in experimental samples. The size (or size range) of bioparticles in a sample can be estimated by comparing the forward-scatter (FSC) signals with those of the reference beads. Because FSC is dependent not only on particle size but also on factors such as the particle's refractive index, the size estimates obtained with this kit may not reflect the actual bioparticle sizes. However, the microspheres in this kit all have equivalent refractive indices (1.591 at 590 nm); therefore, differences in the FSC signals reflect their relative sizes.

Compensation made easy with the AbC™ and ArC™ Kits

For scientists performing multicolor flow cytometry assays, our microsphere tools can assist with compensation adjustments. The AbC™ Total Antibody Compensation Bead Kit (for all isotypes of mouse, rat, hamster, and rabbit antibodies) and the AbC™ Anti-Mouse and AbC™ Anti-Rat/Hamster Bead Kits provide an easy-to-use method for setting compensation when using fluorophore-conjugated antibodies. These kits contain two types of specially modified 6 µm–diameter microspheres: the AbC™ capture beads for binding antibodies and the negative-control beads, which have no antibody-binding capacity. After incubation with a fluorescent antibody, the two bead components provide distinct positive and negative bead populations that can be used to set compensation. Because of their consistent light scatter and high surface antibody–binding capacity, these beads allow more accurate compensation settings for any combination of fluorophore-labeled antibodies. See the online article at **lifetechnologies.com/microspheresbp70** for more information and data on the AbC™ Antibody Compensation Bead Kits.

The ArC™ Amine-Reactive Compensation Bead Kit is designed to provide a simple and accurate method for setting compensation when using any of the LIVE/DEAD® Fixable Dead Cell Stains [11]. Unlike methods that require compensation controls prepared from cellular material, the ArC™ Amine-Reactive Compensation Bead Kit is ready to use, with two bead suspensions provided in convenient dropper bottles: the ArC™ reactive beads, which have been optimized to react with the stains and provide a positive signal, and the ArC™ negative beads, which have no reactivity. This kit allows you to set compensation without the hassle of heat-treating cells as a control, saving both time and sample.

Find more calibration beads for imaging and flow cytometry

We have developed an assortment of fluorescent microspheres for calibrating your lab instruments; learn more about our calibration beads at **lifetechnologies.com/microspheresbp70**. ■

References

1. Zucker RM (2014) *Methods Mol Biol* 1075:321–374.
2. Talwar S, Kumar A, Rao M et al. (2013) *Biophys J* 104:553–564.
3. Pachoud B, Sharma P, Bergerot A (2014) *Neuroscience* 256:412–425.
4. Szymborska A, de Marco A, Daigle N et al. (2013) *Science* 341:655–658.
5. Dedecker P, Mo GC, Dertinger T et al. (2012) *Proc Natl Acad Sci U S A* 109:10909–10914.
6. Flottmann B, Gunkel M, Lisauskas T et al. (2013) *Biotechniques* 55:243–252.
7. French AP, Mills S, Swarup R et al. (2008) *Nat Protoc* 3:619–628.
8. Bortner CD, Sifre MI, Cidlowski JA (2008) *J Biol Chem* 283:7219–7229.
9. Dolezel J, Greilhuber J, Suda J (2007) *Nat Protoc* 2:2233–2244.
10. Trinidad EM, Ballesteros M, Zuloaga J et al. (2009) *Blood* 114:5081–5090.
11. Perfetto SP, Chattopadhyay PK, Lamoreaux L et al. (2006) *J Immunol Methods* 313:199–208.

Selected products*	Quantity	Cat. No.
Fluorescence Microscopy Calibration Standards		
FocalCheck™ Fluorescent Microspheres Kit (slides), 6 µm	1 kit	F24633
FocalCheck™ Microspheres, 15 µm, fluorescent green ring stain/dark red throughout	0.5 mL	F7238
FocalCheck™ Fluorescence Microscope Test Slide #1, for alignment, intensity, and calibration	1 each	F36909
TetraSpeck™ Microspheres, 0.1 µm, fluorescent blue/green/orange/dark red	0.5 mL	T7279
TetraSpeck™ Microspheres, 0.2 µm, fluorescent blue/green/orange/dark red	0.5 mL	T7280
Flow Cytometry and Cell Sorting Calibration Standards		
AbC™ Anti-Mouse Bead Kit	1 kit	A10344
AbC™ Anti-Rat/Hamster Bead Kit	1 kit	A10389
AbC™ Total Antibody Compensation Bead Kit	1 kit	A10497
ArC™ Amine-Reactive Compensation Bead Kit	1 kit	A10346
AlignFlow™ Flow Cytometry Alignment Beads, for UV lasers, 2.5 µm or 6 µm	3 mL	A16502 A16505
AlignFlow™ Flow Cytometry Alignment Beads, for blue lasers, 2.5 µm or 6 µm	3 mL	A16500 A16503
AlignFlow™ Flow Cytometry Alignment Beads, for red lasers, 2.5 µm or 6 µm	3 mL	A16501 A16504
Cell Sorting Set-Up Beads, for UV lasers, 6 µm	3 mL	C16506
Cell Sorting Set-Up Beads, for blue lasers, 6 µm	3 mL	C16508
Cell Sorting Set-Up Beads, for green-yellow lasers, 6 µm	3 mL	C16509
Cell Sorting Set-Up Beads, for red lasers, 6 µm	3 mL	C16507
CountBright™ Absolute Counting Beads, 7 µm	5 mL	C36950
Flow Cytometry Sub-Micron Particle Size Reference Kit	1 kit	F13839

* A complete list of microsphere calibration products can be found in Chapter 23, "Antifades and Other Tools for Fluorescence Applications", of *The Molecular Probes® Handbook*, available online at lifetechnologies.com/handbook.

© 2014 Thermo Fisher Scientific Inc. All rights reserved. For Research Use Only. Not for use in diagnostic procedures.