FocalCheck™ Fluorescence Microscope Test Slides

F36909 FocalCheck™ fluorescence microscope test slide #1
F36913 FocalCheck™ fluorescence microscope test slide #2
F36914 FocalCheck™ fluorescence microscope test slide #3

Table 1. Contents and storage information for FocalCheck™ fluorescence microscope test slide #1

	1	2	3	4	5	Storage and stability
Row A	15 µm	6 µm	4 µm	1 µm	0.5 µm	• Room temperature • Avoid prolonged exposure to light When stored as directed, the slides should be stable for at least 12 months.
	Green/orange/far red thin rings	Blue throughout/ green ring	Blue/green/red/ far red	Blue/green/red/ far red	Blue/green/red/ far red	
Row B (Intensity Series)	6 µm	6 µm	6 µm	6 µm	6 µm	
	Green	Green	Green	Green	Green	
	100% intensity	33% intensity	10% intensity	3% intensity	0.667% intensity	

Table 2. Contents and storage information for FocalCheck™ fluorescence microscope test slide #2

	1	2	3	4	5	Storage and stability
Row A	6 µm	6 µm	6 µm	6 µm	Mixture of all eight reference beads	• Room temperature • Avoid prolonged exposure to light When stored as directed, the slides should be stable for at least 12 months.
	Green	Orange	Red	Far red		
	Bead 1 503/511 nm Bead 2 511/524 nm	Bead 1 541/555 nm Bead 2 545/565 nm	Bead 1 578/605 nm Bead 2 589/613 nm	Bead 1 657/676 nm Bead 2 671/692 nm		
Row B	6 µm	6 µm	6 µm	6 µm	Mixture of all four double-stained beads	
	Green	Orange	Red	Far red		
	Ring 503/511 nm Core 511/524 nm	Ring 541/555 nm Core 545/565 nm	Ring 589/613 nm Core 578/605 nm	Ring 671/692 nm Core 657/676 nm		

Table 3. Contents and storage information for FocalCheck™ fluorescence microscope test slide #3

	1	2	3	4	5	Storage and stability
Row A	2.5 µm	2.5 µm	2.5 µm	2.5 µm	2.5 µm	• Room temperature • Avoid prolonged exposure to light When stored as directed, the slides should be stable for at least 12 months.
	Blue	Green	Orange	Red	Deep red	
	350/440 nm	505/515 nm	540/560 nm	580/605 nm	633/660 nm	
	Bright	Bright	Bright	Bright	Bright	
	100% intensity	100% intensity	100% intensity	100% intensity	100% intensity	
Row B	2.5 µm	2.5 µm	2.5 µm	2.5 µm	2.5 µm	
	Blue	Green	Orange	Red	Deep red	
	350/440 nm	505/515 nm	540/560 nm	580/605 nm	633/660 nm	
	Dim	Dim	Dim	Dim	Dim	
	1% intensity	1% intensity	1% intensity	1% intensity	1% intensity	

Introduction

FocalCheck™ fluorescence microscope test slides #1, #2, and #3 are specifically designed for calibrating fluorescence microscope systems and evaluating system and filter performance. The slides each contain 10 sample areas coated with proprietary fluorescent microspheres designed specifically for microscopy applications. The microspheres are mounted in optical cement (refractive index of ~1.52) for maximum stability and are intended for use on all fluorescence microscope systems.

- **FocalCheck™ fluorescence microscope test slide #1** is ideal for routine checking and calibration of confocal and widefield fluorescence microscopes.
- **FocalCheck™ fluorescence microscope test slide #2** provides a robust, reproducible method of evaluating the performance of spectral imaging systems, as well as the ability to discriminate closely overlapping spectra. The slide consists of 6-µm-diameter microspheres labeled with a series of spectrally overlapping dyes.
- **FocalCheck™ fluorescence microscope test slide #3** is useful for basic evaluation of filter performance and as a general practice slide for fluorescence microscopy and digital imaging.

Experimental Protocols

The basic procedure for using the FocalCheck™ fluorescence microscope test slides is to image the beads using standard techniques. Below are several general applications.

Color registration

For multicolor experiments, particularly on widefield microscopes, switching between different filter configurations can result in misregistration between the various color channels. Using the multicolor beads on FocalCheck™ fluorescence microscope test slide #1 (sample positions A1–5), the extent of the misregistration can be digitally corrected independently of the sample (Figure 1).

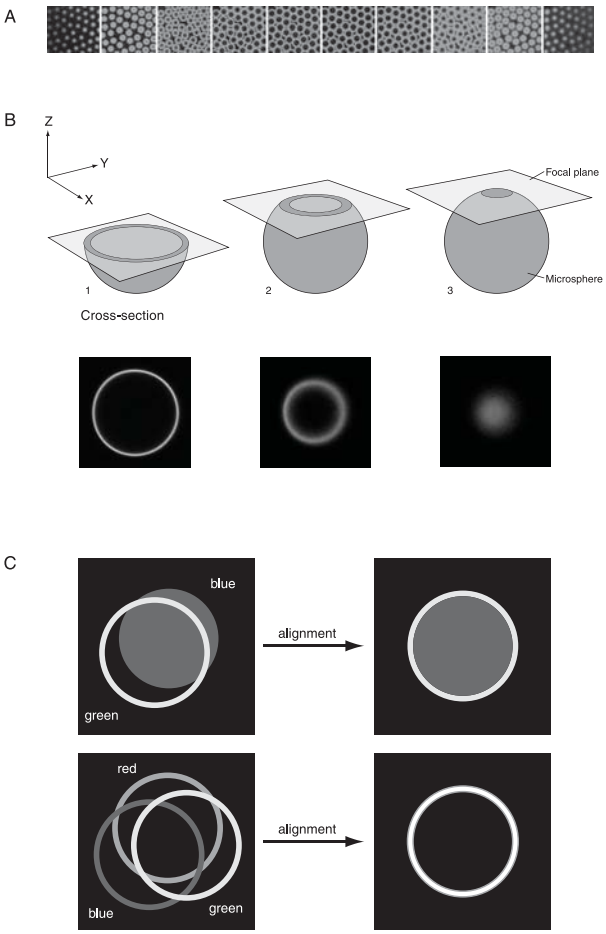


Figure 1. Confocal laser-scanning microscope optical cross-sectioning and alignment with FocalCheck™ microspheres. A) Serial optical sectioning from top-to-bottom along the Z-axis of ring-stained microspheres reveals a continuous pattern of disc-to-ring-to-disc images. B) The diameter of the fluorescent ring (or disc) 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. Also, emitted fluorescence is detected by different photomultipliers. Proper optical alignment may be obtained with either of two types of FocalCheck™ microspheres. For example, the microspheres with a green ring stain that are blue-fluorescent throughout the bead allow UV/visible wavelength alignment in three dimensions upon aligning the green ring with the blue disc. Focal alignment is also possible simultaneously in three colors by aligning the red, blue, and green rings of the FocalCheck™ microspheres containing fluorescent red, blue, and green ring stains.

Chromatic distortions

Modern microscopes use highly color-corrected (achromatic) optical components. However, it is possible that certain optical components may introduce wavelength-dependent image shifts that can affect the correct registration of multicolor labels. Such aberrations are critical to avoid in colocalization experiments. FocalCheck™ fluorescence microscope slide #1 is ideal for ensuring that the imaging system is free from chromatic distortions.

X- and Y-axis calibration

For distance and other morphometric measurements, microscope imaging should be calibrated for each objective and magnification setting using a stage micrometer. The larger 15-µm beads on FocalCheck™ fluorescence microscope test slide #1, sample positions A1 and A2, provide ideal targets for assessing the calibration of the microscope in three dimensions. When measured at the equator, the beads are nominally 15 µm in diameter; this should correspond with the measured X and Y dimensions by imaging.

Z-axis calibration

Positions A1 and A2 on FocalCheck™ fluorescence microscope test slide #1 can be used to assess the performance of the motorized focus control of an imaging system. To do this, acquire Z-series stacks from bottom-to-top and top-to-bottom. The refractive index of the slide mounting media is close to 1.515, which minimizes spherical aberration when using

an oil immersion lens. When viewed in orthogonal projections, the bead images should appear relatively circular. Any distortions could indicate problems in the Z-stepping of the microscope's focus system or a refractive index mismatch.

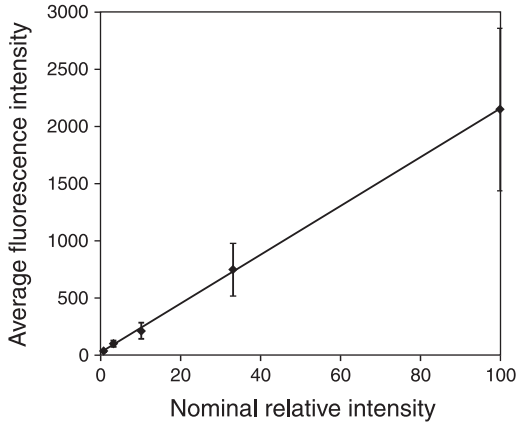
Lamp stability

Most standard mercury lamps are rated for 200–300 hours of use. While many can last longer, there is often little warning that the lamp is failing. Bead samples on any of the FocalCheck™ fluorescence microscope test slides can be used to monitor the stability of the excitation source on the microscope. Periodic imaging of the same sample, using the same acquisition parameters, can indicate changes in lamp output by plotting the intensity of the beads over time.

Intensity calibration

FocalCheck™ fluorescence microscope test slide #1 contains a series of intensity beads. By acquiring a series of images, you can create a relative intensity curve against which samples can be evaluated (Figure 2). The intensity of the beads can be used to generate curves for different instruments, thus providing a basis for assessing the relative fluorescence intensity of a sample in a system-independent manner.

FocalCheck™ fluorescence microscope test slide #1 can also be used to examine the linearity and dynamic range of the detection system on the microscope under various settings. The intensity range of the beads covers the approximate dynamic range of a typical 12-bit CCD camera (4096 gray levels) when imaged at a single exposure setting.



Nominal relative intensity	1.00%	5.00%	10.00%	40.00%	100%
Actual intensity	18.81	88.70	201.91	738.01	2138.48
Determined relative intensity	0.88%	4.15%	9.44%	34.51%	100%

Figure 2. Average fluorescence intensity of the intensity beads series on FocalCheck™ microscope test slide #1. The intensity bead series was imaged on a Nikon Eclipse 800 microscope using a 100-watt Hg-arc lamp and a 60×/1.4NA objective. The images were acquired using a Hamamatsu Orca-ER 12-bit CCD camera. The average fluorescence intensity and standard deviation of the bead targets were determined and plotted against nominal relative intensity as determined by flow cytometry. In this particular configuration, all five intensities were imaged using a single exposure setting. The measured average intensities shown in the table give relative intensities that correspond closely to the nominal values measured by flow cytometry.

Spectral scanning

FocalCheck™ fluorescence microscope test slide #2 can be used on spectral imaging instruments to help ensure that the wavelength detection system is working correctly. This slide can also be used to perform many of the general tests described above.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat no.	Product name	Unit size
F36909	FocalCheck™ fluorescence microscope test slide #1 *for alignment, intensity, and calibration*	each
F36913	FocalCheck™ fluorescence microscope test slide #2 *for spectral imaging systems*	each
F36914	FocalCheck™ fluorescence microscope test slide #3 *5 colors, high and low intensities*	each
P7220	PS-Speck™ Microscope Point Source Kit *blue, green, orange, and deep red fluorescent beads*	1 kit

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