

Confocal Kit

AGS1927

Multichannel Confocal Calibration and Adjustment Kit

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One kit contains:

- 1 Coverglass mirror mounted upside down with Mowiol
- 1 slide with 0.22 μm beads in Mowiol (3.0 μg solids/ml)
- 1 slide with 0.22 μm beads in Mowiol (30 μg solids/ml)
- 1 slide with 0.54 μm beads in Mowiol (2.9 μg solids/ml)
- 1 slide with 0.54 μm beads in Mowiol (29 μg solids/ml)
- 1 slide with 1.05 μm beads in Mowiol (36 μg solids/ml)

All coverglasses used have a certified thickness of $0.17 \pm 0.005\text{mm}$.

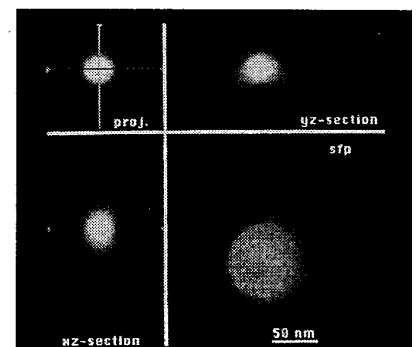
Mirror Resolution Test

The coverglass mirror is used to reveal the confocal resolving power in z-axis direction. The 9nm Aluminum layer on a coverglass is an infinitely thin, totally reflecting flat object for a confocal microscope. To perform the test, a series of images is taken with minimal stepsize. At the level of the mirror the confocal microscope gets a total reflection (100% intensity). The steps in z-direction are plotted against intensity (Fig. 1). In an ideal optical system, this peak should be infinitely narrow. For real systems, the peak has a finite, measurable width at 50% intensity (half height) that defines the confocal resolving power in z-direction. Secondary and higher order maxima give a hint about the quality of the optical system of the microscope (should be smaller than 20 % of the primary peak). As a rule of thumb: best resolving power is half the wavelength of illumination (also valid for lateral resolution). For double photon pulsing illumination the initial (long) wavelength applies to this rule.

Figure 1:

Lower right: 3D ray-tracing image of a 1 μm multi fluorescent latex bead, simultaneously imaged in three channels (FITC, TRITC, CY5) by a confocal laser scanning microscope (Leica).

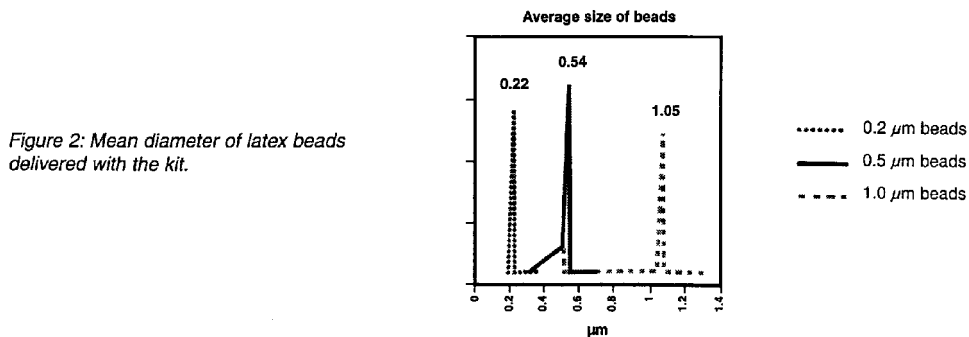
Upper left: Projection of a 0.5 μm multi-fluorescent latex bead, simultaneously recorded in three channels (FITC, TRITC, CY5) with the corresponding yz-section (upper right) and xz-section (lower left).



Size Calibration

Latex beads are the best approximation of an ideal spherical object. The kit contains three slides with nominal 0.2, 0.5, and 1.0 micron beads thus covering the whole resolution range of light microscopy. The precise diameter of these beads, was determined in the electron microscope by statistics of 1000 beads. The mean size is given in Fig. 2.

Warning for Fluorescence Application: Use a reasonable size of beads for a given optical set. Photons can always be detected, when originating from a bead with a diameter smaller than the real resolving power of the optical system applied! Therefore, if you record images with a 40X NA 0.75 air objective lens at 488nm, latex beads with a diameter of 0.2 μ m will be imaged with an apparent diameter identical to the real resolving power (about 0.5 μ m for this setup). By this method, the lateral resolution can be determined, but no correlative size calibration to a specimen can be done.



Multi Channel Adjustments

The beads are labelled for triple channel application in the visual range (460nm - 647nm). The emission spectra of the multifluorescent beads delivered in this kit have been recorded in a spectrometer and are shown in Fig. 3. Three typical excitation wavelengths were used : 488nm, 550nm, and 640nm. The beads should appear with the same diameter at the same lateral position in all confocal fluorescent channels. The same conditions must be fulfilled for the third dimension in x-z or y-z imaging. If not, mechanical adjustment of the beampath and/or digital size and position adjustments are recommended (adjust only with permission of your supplier of the microscope).

Deconvolution

The multifluorescent beads can be used for the determination of the optical transfer function of a confocal setup. The multifluorescent beads are ideal spheres with a diameter given in Fig.2. A stack of confocal images through several beads has to be recorded. Then individual beads from this stack are averaged by the deconvolution program. These averaged beads are compared with a calculated sphere with the given diameter (Fig. 2). The difference between the real image and the theoretical image is used to determine a transfer function of your real optical system. This precise transfer function is used by the deconvolution program to improve your data sets. It is obvious that for each change in your optical system (e.g. change of objective lens) an individual optical transfer function has to be determined. Channels must be treated separately, but the same stack of multichannel recorded multifluorescent beads can be used and leads to optimal results.

Emission Spectrum of the Beads

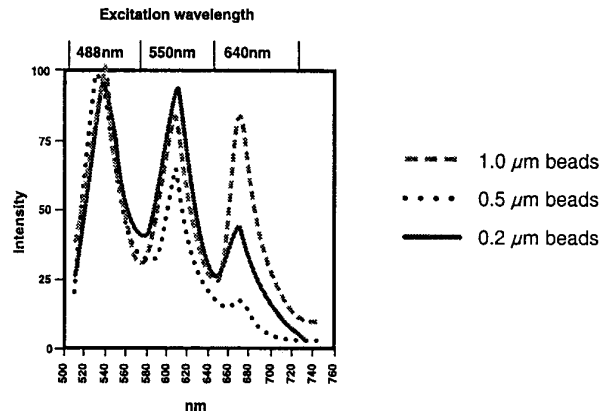


Figure 3: Emission spectra of multicolored beads with 488nm, 550nm, 640nm (all ± 1 nm) excitation. Photomultiplier was kept at constant high voltage for all spectra with a detection bandwidth of 8nm.

Storage of the Kit

Store this kit in darkness to avoid bleaching of the multicolored latex beads.