A Set of Candidate Spectral Calibration Beads for Fluorescence-based Imaging and High-Throughput Devices

K. Hoffmann, N. Nirmalananthan-Budau, U. Resch-Genger
Bundesanstalt für Materialforschung und -prüfung (BAM), Richard-Willstätter-Str. 11, 12489 Berlin, Germany; E-Mail: katrin.hoffmann@bam.de

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Intensity signals recorded with fluorescence-based techniques contain not only sample-related but also instrument-specific contributions. This limits the direct comparison of data obtained e.g. on different devices or at different times and often hamper quantification.[1] Accepted fluorescence standards for the control of instrument specifications and performance are needed to exclude instrumentation as major source of variability of emission data. For microscopy and flow cytometry (FCM), a large variety of fluorescence reference material is available. Commonly used are fluorophore-stained beads differing in emission wavelength and intensity for testing instrument alignment, sensitivity, and other performance parameters. These calibration tools facilitate the assessment of instrument performance to ensure reliable measurements and quantitative microscopic studies.
We developed a set of candidate spectral calibration beads loaded e.g. with luminophores from the BAM-Kit "Spectral fluorescent standards" (BAM-F001-F005), initially designed for calibration of fluorescence spectrometers.[1,2] The assembly of this set from highly fluorescent polystyrene beads represents an important step towards an improved comparability of fluorescence data for (micro)spectroscopic methods used for the characterization of nano- and micrometer-sized fluorescent objects.

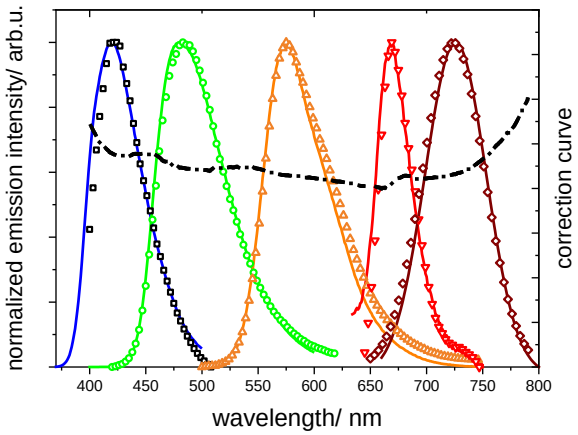

Figure 1.
A spectral sensitivity curve (black dash-dotted line) of a Laser Scanning Microscope (CLSM), suitable to correct for microscope-specific spectral distortions calculated from emission spectra of candidate calibration beads measured on bead ensembles (solid lines) and single particles (dotted lines), recorded with a CLSM.

Here, we present first results from spectroscopic studies of candidate calibration beads.[4] The final set of fluorophore-loaded polymer beads, covering the UV/VIS and NIR wavelength range, are designed for calibration of fluorescence imaging systems to meet the increasing demand for reliable and comparable fluorescence data especially in strongly regulated areas like e.g. medical diagnostics.[4]

References

1. U. Resch-Genger, K. Hoffmann, A. Hoffmann, Ann. N.Y. Acad. Sci. 2008, 1130, 35-43.
2. D. Pfeifer, K. Hoffmann, U. Resch-Genger et al., J. Fluoresc. 2006, 16, 581-587.
3. K. Hoffmann, N. Nirmalananthan, U. Resch-Genger, Anal. Bioanal. Chem, submitted.
4. Y. Han, Y. Gu, A.C. Zhang; Y.H. Lo, Lab Chip, 2016, 16, 4639–4647.