

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Fluorescence calibration standards made from broadband emitters encapsulated in polymer beads for fluorescence microscopy and flow cytometry

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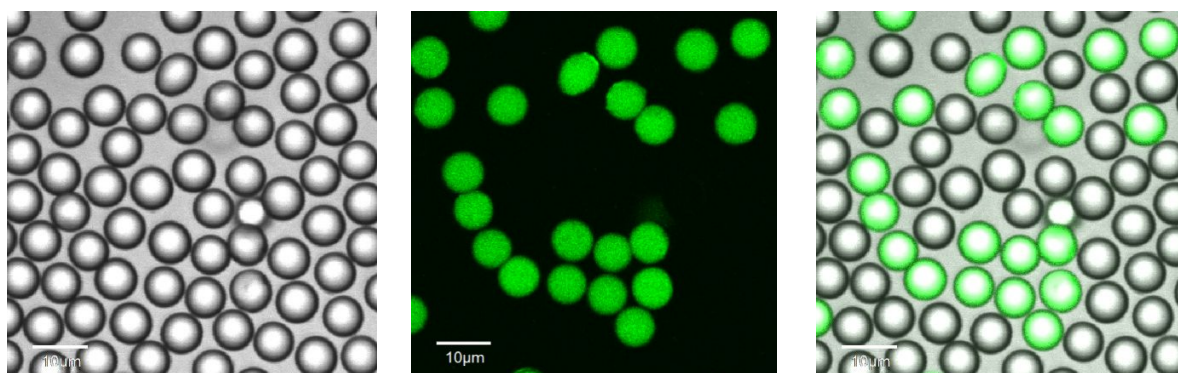


Fig. S1 Control images of an exemplary mixture of non-modified polystyrene (PS) beads (8 μm) and PS beads with encapsulated Dye D: transmission image (left), fluorescence image (middle) and their overlay (right). No emission signal can be detected from the non-modified PS beads

Table S1 Fluorescence data (emission wavelengths) of dyes measured in different environments, in ethanolic solution, and in 8 μm polystyrene (PS) beads in aqueous suspensions in the ensemble and at single particle level

fluorophore	EtOH	emission wavelength $\lambda_{\text{max}}/\text{nm}$	
		8 μm PS (ensemble)	8 μm PS (single bead level)
DyeA	329 \pm 2	330 \pm 0.5	n.d.
DyeB	390 \pm 2	370 \pm 0.5	n.d.
DyeC	456 \pm 2	420 \pm 0.5	422 \pm 0.8
DyeD	530 \pm 2	480 \pm 0.5	485 \pm 2
DyeE	636 \pm 2	575 \pm 0.5	576 \pm 0.8
DyeO ClO ₄	664 \pm 2	668 \pm 0.5	669 \pm 3.6
DyeO ClO ₄ US	-	654 \pm 0.5	-
DyeO PF6	-	668 \pm 0.5	-
DyeO BARF	-	673 \pm 0.5	-
DyeO BARF US	-	672 \pm 0.5	678 \pm 0.8
DyeI ClO ₄	766 \pm 2	726 \pm 0.5	719 \pm 0.5
DyeI ClO ₄	-	724 \pm 0.5	-
DyeI PF6	-	733 \pm 0.5	-
DyeI BARF	-	772 \pm 0.5	742 \pm 0.8

*) Standard deviations derived from the wavelength accuracy of the fluorescence instrument

**) Standard deviations derived from evaluation of single bead spectra (N \geq 5)

***) US: ultrasound

Table S2 Fluorescence quantum yields (QY) of dispersions of dye-encoded 8 μm polystyrene (PS) beads absolutely measured with a C11347 (Hamamatsu Photonics K.K.) integrating sphere setup (error represents the standard deviation (SD) of twelve independent measurements). The integral and average intensities of different bead-related regions of interest (ROI) of the dyes-stained bead batches were obtained to estimate bead staining homogeneity

fluorophore	Quantum yield/ %		Staining homogeneity (integral bead intensity)	
	$\lambda_{\text{exc}}/\text{nm}$	8 μm PS (ensemble)	ROI integration	ROI average
DyeC	355	87 \pm 3	5.40E+06 \pm 3.28E+05	2.64E+03 \pm 1.60E+02
DyeD	488	31 \pm 2	2.99E+06 \pm 1.61E+05	1.98E+03 \pm 1.02E+02
DyeE	515	66 \pm 2	5.29E+06 \pm 3.05E+05	2.87E+03 \pm 1.66E+02
DyeO*)	633	5 \pm 1	5.11E+06 \pm 1.08E+06	1.94E+03 \pm 4.08E+02
DyeI*)	633	12 \pm 1	4.17E+06 \pm 1.74E+06	2.33E+03 \pm 9.72E+02

*) counterion ClO₄

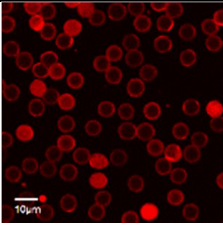
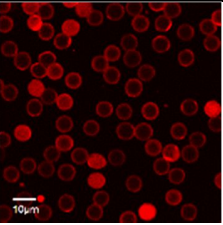
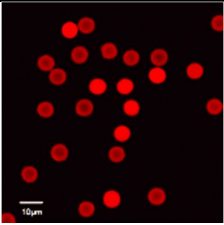
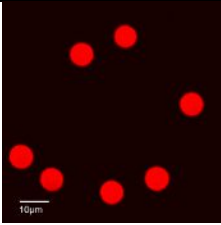
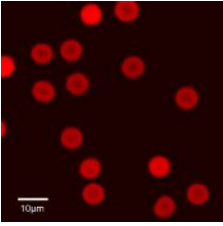
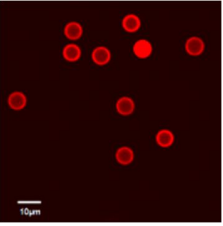
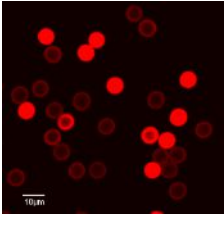
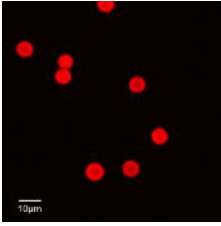

Counter anion	ClO_4^-	BF_4^-	PF_6^-	BARF
DyeO				
DyeI				
Hydrophobicity				

Fig. S2 Visual, microscopic investigation of candidate calibration beads during the course of counter anion exchange studies (confocal laser scanning Microscope OLYMPUS FV1000; UPLSAPO 40x/0.9; excitation wavelength 633 nm)