Colour-coded nanoscale calibration and optical quantification of axial fluorophore position

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Abstract. Total internal reflection fluorescence (TIRF) has come of age, but a reliable and easy-to-use tool for calibrating evanescent-wave penetration depths is missing. We provide a test-sample for TIRF and other axial super-resolution microscopies for emitter axial calibration. Our originality is that nanometer (nm) distances along the microscope’s optical axis are color-encoded in the form of a multi-layered multi-colored transparent sandwich. Emitters are excited by the same laser but they emit in different colors. Layers are deposited in a controlled manner onto a glass substrate and protected with a non-fluorescent polymer. Decoding the penetration depth of the exciting evanescent field, by spectrally unmixing of multi-colored samples is presented as well. Our slide can serve as a test sample for quantifying TIRF, but also as an axial ruler for nm-axial distance measurements in single-molecule localization microscopies, super-critical-angle fluorescence, and related super-resolution.
contributes. 488 nm: the closer the dye to the interface, the more color mix upon evan... on a round microscope glass substrate (borosilicate arrangement of different pure and mixed color segments layers colour and capped with a transparent polymer having the nanometric transparent spacer layer (grey) of thickness calibration sample. A dye layer (red) is deposited on a... of the optical near field located at different axial distances, the relative contribution of the respective dye layers unmixing the measured color vector and indicating the need for spectral... field confinement of the evanescent wave. In the further studies will needed... of the optical near field to measure mixed color. One can clearly see that... emission, as expected from the near field... of Rubpy results in some spectral cross talk, the long... dominating the fluorescence emission, as... by the clear intensity differences: the excitation. Note the clear intensity differences: the... produced by the 488 nm evanescent radiation... protective layer. B, Backfocal plane (BFP), fluorescence... MC polymer as a color sandwiches featuring a yellow... measured mixed color. One can clearly see that... References

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