Near-membrane refractometry using supercritical angle fluorescence

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Total internal reflection fluorescence (TIRF) microscopy and its variants are key technologies for visualizing the dynamics of single molecules or organelles in live cells. Yet, truly quantitative TIRF remains problematic. One unknown hampering the interpretation of evanescent-wave excited fluorescence intensities is the undetermined cell refractive index (RI). Here, we use a combination of TIRF excitation and supercritical angle fluorescence [1,2,3,4] emission detection to directly measure the average RI in the 'footprint' region of the cell, during imaging. Our RI measurement is based on the determination on a back-focal plane image of the critical angle separating supercritical and undercritial fluorescence emission components (**fig. 1a**). We validate our method by imaging mouse embryonic fibroblasts [5]. By targeting various dyes and fluorescent-protein chimerae to vesicles, the plasma membrane as well as mitochondria and the ER, we demonstrate local RI measurements with subcellular resolution (**fig. 1b**) on a standard TIRF microscope with a removable Bertrand lens as the only modification. Our technique has important applications for imaging axial vesicle dynamics, mitochondrial energy state or detecting cancer cells.

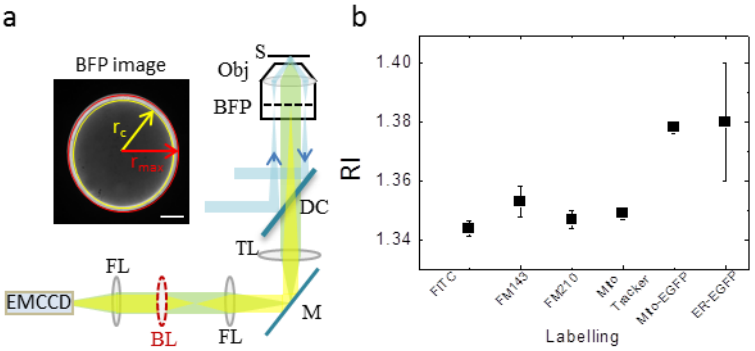

Fig. 1. (a) Simplified optical layout. Obj: objective, BFP: backfocal plane, TL: tube lens, BL: Bertrand lens, FL: focusing lens, EMCCD: electron-multiplying charge-coupled device, S: sample, DC: dichroic mirror, M: mirror. Excitation wavelength was 488nm. Inset shows BFP image of a thin layer of 488/515nm (100-nm diameter) beads in water. r_c identifies critical angle at a given RI. r_{max} corresponds to the objective maximum angle of collection. Scale bar: 1 mm. (b) SAF-based RI measurements in live MEFs for different subcellular stainings. Error bars correspond to the SD of the mean RI for 6-8 different cells each.

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