INCIDENT ANGLE CALIBRATION FOR TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

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Total Internal Reflection Fluorescence (TIRF) microscopy became more and more popular in biology for routine cellular observation, as well as for super-resolution imaging. TIRF is indeed the core imaging technique behind most of single molecule localization methods such as PALM and STORM. Ensuring that performance of the TIRF microscope remains excellent is therefore critical. The key parameter to perform reliable TIRF observation is the incident angle. A fine angular control of the laser beam ensures that a perfect evanescent field is generated in the sample. Thus, we propose a simple and robust calibration routine useful to evaluate the incident angle in TIRF microscopy [1]. This procedure is based on critical angle measurements conducted in the back focal plane (BFP) of the objective. Such BFP imaging can be easily implemented on any TIRF setup, that makes this technique very attractive. Calibration exactitude was demonstrated by comparing the theoretical angular dependence of the electric field intensity at glass/water interface with experimental observations.

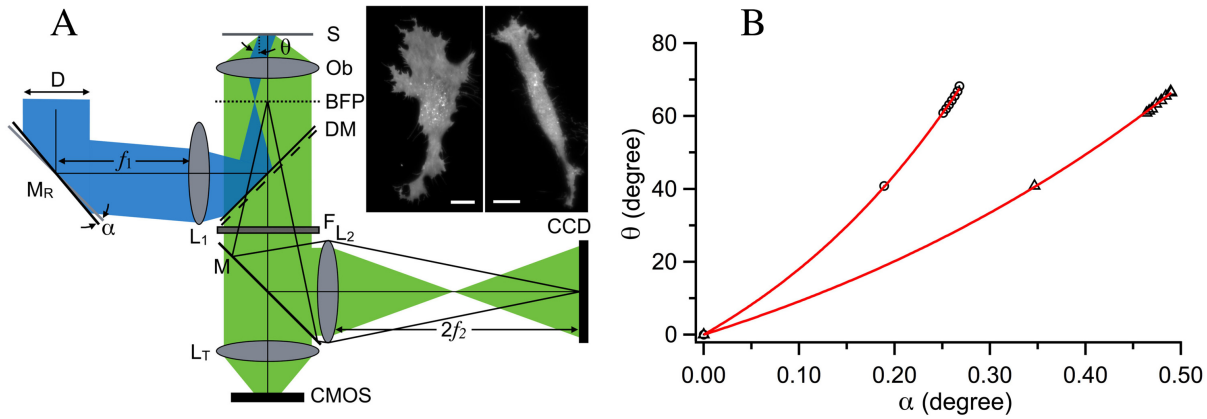

Figure 1: (A): Experimental setup. Inset: two typical TIRF image of living cell (bar = 10µm). (B): Calibration curves of the incident angle θ versus the tilting of the incoming laser beam α, for common Olympus (△) and Zeiss (□) TIRF objectives.

[1] D. El Arawi, M. Cardoso Dos Santos, C. Vezy and R. Jaffiol, "Incidence angle calibration for prismless Total Internal Reflection Fluorescence microscopy," submitted in *Optics Letters* (2018).