UNIFORM ILLUMINATION FOR WIDE FIELD MICROSCOPY FROM TIRF TO EPIFLUORESCENCE EXCITATION

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Total internal reflection fluorescence microscopy (TIRF) has proved to be an efficient way of achieving optical sectioning while enhancing the fluorescence signal to noise ratio. It is used in both standard Wide-Field and Single Molecule Localization Microscopy (SMLM), and usually achieved via focalisation of a gaussian laser beam in the back focal plane (BFP) of an objective. Unfortunately, coherence of the laser may form unwanted interference patterns such as speckle or fringes, while the gaussian shaped illumination at the sample plane hinders quantification of fluorescence processes. In SMLM, this results in field-dependant photon count, localization precision, on-time … Strategies have been developed to provide wide-field uniform illuminations. They involve fibres[1], micro-lens arrays[2] or refractive beam-shaping elements[3]… but their implementation tends to prevent their application to TIRF excitation and are often constrained to a fixed field of view (FOV) size. Furthermore for SMLM where 5 to 10kW/cm² excitation power is needed to ensure single molecule regime, it generally leads to the use of high 4-5W power to cover a wide FOV (200µm)² imaging.

We will present an illumination technique which is based on a classical 300mW, 647nm laser and benefits from the flexibility of a scanning-mirror system to achieve a uniform illumination over the (200µm)² FOV of a sCMOS camera. By focusing the laser on the scanning mirrors, we can control its position in the sample plane and uniformly spread laser power on any FOV. This hybrid scanning/wide field excitation can be applied from TIRF to epifluorescence excitation as can be seen on figure 1 on wide field imaging of COS7 cells with tubulin network labelled with Alex 647. In combination with SMLM, this approach allows us to control the sectioning and thus the background, furthermore the uniform excitation setup can also enhance photons counts and localization precision on cytoskeleton network SMLM imaging of COS7 cells (fig. 2)

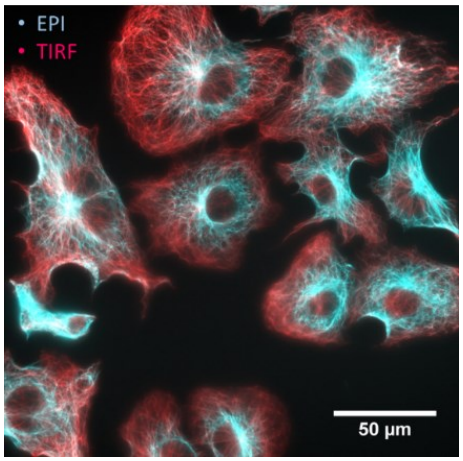

Figure 1 : Full-field EPI and TIRF images of COS7 cells tubulin labelled with AF647 antibodies.

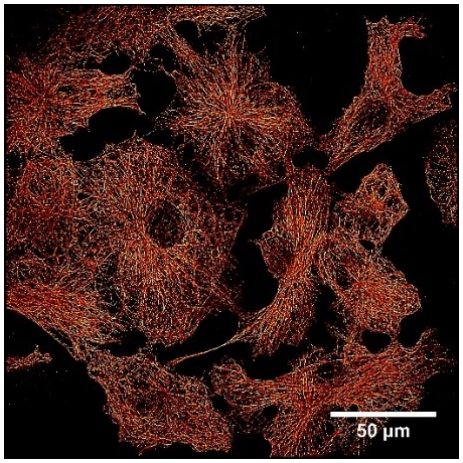

*Figure 2 : Full-field SMLM image of AF647-labelled COS7 cells, showing homogeneous resolution on 200*200µm²*

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[3] I. Khaw et al., *Opt. Express*, vol. 26, no. 12, p. 15276, 2018.