

SUPERCritical WIDE FIELD FLUORESCENCE MICROSCOPY FOR MEMBRANE IMAGING

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Numerous cell mechanisms involve membrane processes. The understanding of such processes is thus of crucial importance in biomedical applications. It explains the spectacular development of specific fluorescence imaging techniques like TIRFM [1].

Here, we present an alternative wide field imaging technique based on supercritical emission of particular interest for cell membrane studies. This technique is based on selecting the fluorescence emission at supercritical angles (also called forbidden light) [2]. When fluorescent emitters are placed in the vicinity of the glass slide, their near-field components become propagative. This supercritical emission decays sharply the fluorophore/surface distance d (as d^6) with a characteristic decay length of about 100 nm. Selecting the supercritical emission thus provides an efficient mean of spatial filtering. This can be obtained simply with a mask placed in the back focal plane of a high numerical microscope objective. There is no need for a scanning microscope with a dedicated specially designed objective [3]

This technique has numerous advantages over techniques based on excitation confinement like TIRFM. In particular, it avoids the major drawbacks of the loss of confinement due to light scattering. Standard light sources, with a homogeneous lighting, can be used instead of the usually required lasers.

We will show wide field real-time images of live cell membrane activity using this technique. We will give the measured high performances of this technique in terms of image quality, sensibility and resolution. Moreover, we will show that simultaneously standard epifluorescence images can be acquired to give precious complementary information on inner cell activity and membrane events.

[1] D. Axelrod, "Total Internal Reflection Fluorescence Microscopy in Cell Biology", *Traffic*, **2**, 764-774 (2001).

[2] J. D. Jackson, "Classical Electromagnetism", 3rd ed., Chap. 9, Wiley, New York (1998).

[3] T. Ruckstuhl and D. Verdes, Supercritical angle fluorescence (SAF) microscopy", *Opt. Express*, **12**, 4246 (2004).