VIRTUAL SUPERCRITICAL ANGLE FLUORESCENCE MICROSCOPY FOR REAL TIME 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].

We present an alternative full field imaging technique based on Supercritical Angle Fluorescence (SAF). When fluorescent emitters are placed in the vicinity of the glass slide, their near-field components become propagative at supercritical angles. This supercritical emission decays sharply the fluorophore/surface distance $d$ with a characteristic decay length of about 100 nm. Selecting the supercritical emission thus provides an efficient way to realize spatial filtering. 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 have shown that SAF can be obtained simply with a simple mask placed in the back focal plane of a high numerical microscope objective to block the Undercritical Angles (UA). There is no need for a scanning microscope with a dedicated specially designed objective [3]. The main drawback of this method is that the lateral resolution is reduced. Here, we propose an original technique based on the modulation of the supercritical angles using an iris diaphragm. The sectioned image is obtained by subtraction of two successive images one with the UA and Supercritical Angles (SA) the other with the UA only. This technique is similar to the use of a virtual mask blocking the UA. Beyond the ease of implementation of this virtual SAF, we show that the origin of the signal to compose the image is quite different in the two configurations. It enables to recover the lateral resolution.

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.

Figure 1: Standard epifluorescence (left) and virtual-SAF (right) images of murine neuroblastoma cells tagged with mCherry