SUPERCRITICAL ANGLE FLUORESCENCE EMISSION : AN ALTERNATIVE ACCESS TO AXIAL RESOLUTION IN MICROSCOPY

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Specific membrane imaging is commonly assessed using Total Internal Reflection Fluorescence microscopy (TIRF). This confinement at the excitation not only hampers an easy coupling with scanning based microscope but also prevents any simultaneous observation in a complementary deeper plane. Supercritical Angle Fluorescence (SAF) which takes advantage of evanescent waves at the detection rather than at the excitation, provide an alternative access to retrieve axial information [1]. As it only requires to modify the detection path, it can easily offer dual depth imaging in combination with scanning or wide field microscopy approaches.

The near field components of a fluorophore placed in the vicinity of the glass/cell interface becomes propagative and emitted at supercritical angles (*cf.* fig. 1A). This SAF emission appears as a ring beyond the critical angle in the objective back focal plane (BFP). This emission sharply decays with the fluorophore/surface distance *z* over a characteristic length of about 150 nm. Hence, selecting SAF provides an efficient way to perform axial filtering in densely labelled samples. Optical Fourier filtering/modulation in a conjugated plane of the BFP is commonly performed in super-resolution approaches to retrieve axial information, but can also be implemented for classical microscopy [2,3]. A dual imaging module (*cf.* fig. 1B) is inserted between the microscope and the detector (camera or hybrid/SPAD detectors), it permits the simultaneous acquisition of an unmodified epifluorescence image in one path, and to introduce a SAF-modulation in the second path to extract the SAF image. We will show that dual depth imaging in real time can be based on either amplitude or phase modulation depending on the excitation strategy (scanning vs wide field). It's worth noticing that phase modulation of the SAF emission permits dual depth imaging without inducing any loss, dynamics of CB1R receptors trafficking in HEK cells will be presented (*cf.* fig. 1C). We will illustrate that performing confinement at the detection implies several other benefits compared to TIRF, like a uniform sectioning depth, a homogenous excitation preserved or a straightforward dynamic following of multiple color coded proteins at the membrane and into the cytoplasm.

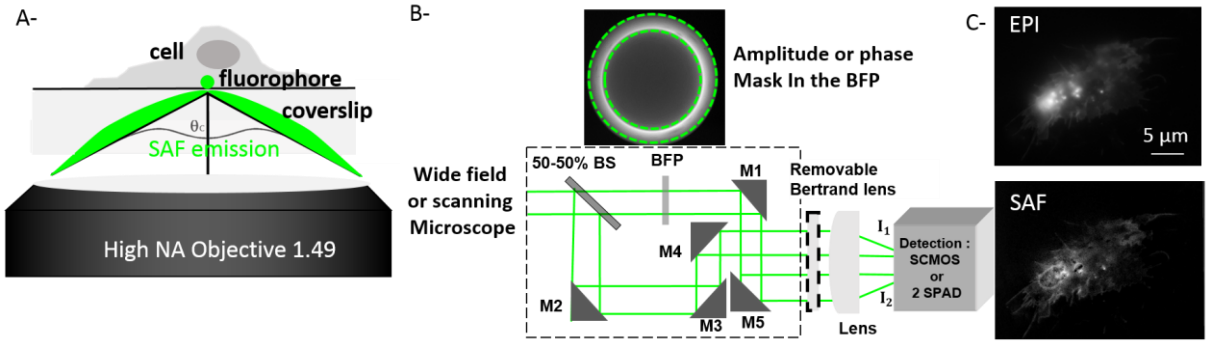

Fig. 1: A- Principle of SAF emission, B- dual imaging path for SAF detection C- Simultaneous images of CB1R receptor in HEK cells

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