FULL-FIELD SURFACE TOMOGRAPHY OF CELL MEMBRANE

Thomas Barroca\textsuperscript{1}, Karla Balaa\textsuperscript{1}, Sandrine Lécart\textsuperscript{2}, Sandrine Lévêque-Fort\textsuperscript{2} & Emmanuel Fort\textsuperscript{1}

1. Centre d’Imageries Plasmoniques Appliquées, Institut Langevin, ESPCI ParisTech, CNRS UMR 7587, 10 rue Vauquelin, 75 231 Paris Cedex 05, France.
2. Institut des Sciences Moléculaires d’Orsay and Centre de photonique Biomédicale (CLUPS), Université Paris-Sud 11, CNRS UMR 8214, F91405 Orsay cedex, France.
E-mail: emmanuel.fort@espci.fr

KEY WORDS: Fluorescence, SAF, Membrane imaging

Understanding of cell membrane processes is of crucial importance in numerous biomedical applications. It explains the spectacular development of specific fluorescence imaging techniques like TIRFM [1].

We have developed an alternative full field imaging technique based on Supercritical Angle Fluorescence (SAF) [2]. When fluorescent emitters are placed in the vicinity of the glass slide, their near-field components become propagative at supercritical angles [3]. This supercritical emission sharply decays with the fluorophore/surface distance $d$ over a characteristic length of about 100 nm. Selecting the supercritical emission thus provides an efficient way to perform axial filtering. We have shown that SAF can be obtained simply with a modulation of the supercritical angles. There is no need for a scanning microscope with a specially designed objective [4].

The use of a simple mask to block the Undercritical Angles Fluorescence (UAF) results in a deterioration of the lateral resolution of the image [2]. We have developed an original method to maintain the lateral resolution. The surface image is obtained by the subtraction of two images: one with both the UAF and SAF, the other with the UAF only. This technique is similar to the use of a virtual mask blocking the UAF. With this new approach, the image originates in the interference term between UAF and SAF component. We have implemented a dual-channel optical system to project each image onto one half of the CCD camera in order to instantly perform the subtractions. It allows us to follow, in real time, dynamic events both at the surface and more in-depth phenomena.

The images obtained with the full-field SAF technique are comparable to those obtained using TIRFM. Moreover, we will show that a combination of these two techniques drastically not only reduces the depth of confinement (below 50 nm) but also gives access to the tomography of the cell membrane with a nanometer resolution.

\begin{itemize}
\end{itemize}

Figure1: HEK cells (a): Standard epifluorescence, (b): SAF, (c): TIRFM+SAF images