

Total Internal Reflection Fluorescence Lifetime Imaging Microscopy and FRET detection for neurobiological applications

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Förster Resonance Energy Transfer (FRET) imaging is nowadays widely used to monitor protein–protein interactions, biochemical reactions, and polymer organization within living cells. To quantify FRET efficiency and so relate it to interprotein distances, we choose to monitor fluorescence lifetime of the donor couple. This measurement is independent on concentration and intensity, and consequently suffers less from photobleaching. Furthermore, in some biomedical applications such as neurobiology, this dynamic imaging must be associated with a high spatial resolution. Indeed the spatial localization of membrane receptors gives crucial information about their trafficking and the neuronal structure. Total Internal Reflection Fluorescence Microscopy (TIRFM) is a very efficient and precise optical tool to observe the events on or just below the plasma membrane [1]. With TIRFM and by using transfected cells with FRET probes, one can also make functional imaging strictly localized at the membrane surface. For all these reasons, we combine these attractive techniques and so developed a Total Internal Reflection Fluorescence Lifetime Imaging Microscopy (TIRFLIM) setup, which is applied to dynamic imaging of membrane phenomena.

TIRFM combines the advantages of wide-field imaging, with a uniform excitation on a large area, reduced acquisition time (no scanning process), and subwavelength axial resolution. We perform TIRFM with “through the objective configuration”, using a high N.A. of 1.45 (Olympus TIRFM, 60x). To preserve this wide-field approach also for time-domain fluorescence lifetime measurements, we use a High Rate Imager (HRI, Kentech Ltd). With this FLIM technique, one can choose the optimal algorithm to measure the lifetime. The number of time-gated images can be reduced (until only two gates in the case of Rapid Lifetime Determination algorithm or RLD [2]) according to the lifetime expected and the signal level. This procedure considerably decreases the acquisition time which is a crucial parameter in the investigation of dynamic processes. To have a versatile setup compatible with a large range of fluorophores, we used a 15 MHz picosecond supercontinuum fibre laser (Fianium SC450) producing a wide excitation spectrum in the visible band [3]. Performances of the TIRFLIM microscope will be fully described.

Our setup is currently dedicated to neurobiological applications, with the following up of Amyloid Precursor Protein (APP), a membrane protein involved in Alzheimer’s disease. By transfecting HEK 293 cells with APP labeled with GFP or mCherry, we can monitor the homodimerisation of APP thanks to the subsequent FRET between GFP and mCherry. Heterodimerization of APP with its β -site cleavage enzyme (BACE) will also be study to fully understand the increase of APP dimerization in relation with the cellular cholesterol level. FRET-FLIM images in cells will be presented as well as first images on neurons.

References

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