IMPLEMENTATION OF TIME RESOLVED ANISOTROPY IMAGING ON A TOTAL INTERNAL REFLECTION FLUORESCENCE LIFETIME IMAGING MICROSCOPE

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KEY WORDS: Time-resolved anisotropy, FLIM, depolarization, FRET.

Time resolved fluorescence anisotropy imaging (tr-FAIM) [1] is a powerful spectroscopic technique, which can evidence homo-dimerization of proteins using homo-FRET approach, or reveal difference of intracellular viscosity. We implemented a polarization-resolved imager (dualview, Cairn) on our versatile microscope which can precisely excite the sample with angle from epifluorescence to evanescent wave leading to a Total Internal Reflection Fluorescence Lifetime Imaging Microscope (TIRFLIM). This TIRFLIM set-up already allowed us to follow dynamic processes at the plasma membrane using FRET [2]. Thus, we can perform wide-field imaging with sub-wavelength axial resolution. To preserve the wide-field approach for time-domain fluorescence lifetime measurements, we used a High Rate Imager (Kentech Ltd) which allows us to smartly sample the fluorescence decay, we can excite a large range of fluorophores using a supercontinuum source and measure long fluorescence lifetimes or anisotropy decays (20 MHz repetition rate).

Using time resolved anisotropy measurements, we can have a dynamic follow-up of the interaction between two identical proteins, which often occurs in biological processes. The main asset of this technique is that it requires only one labeling (i.e. one fluorophore), thus obviating all classical-FRET problems regarding differential expression of donor and acceptor proteins.

First, in order to probe the environment’s viscosity, calibration measurements were performed on fluorescein solutions with different viscosities and various objectives, in epifluorescent excitation. Using a linearly polarized excitation light, we acquired simultaneously fluorescent lifetime imaging for parallel and perpendicular polarization components. It allows us to deduce the anisotropy decay which gives us information about the rotational diffusion of fluorophores and about the viscosity of their local environment. We evidenced that high NA objectives used in our wide field configuration induce a depolarization in the detection pathway [3], hence modifying the anisotropy decays as predicted by simulations. Then, experiments of homo-FRET were pursued on HEK-293 cells. We used GFP since its rotation correlation time is very long compared to its fluorescence lifetime, so the depolarization observed will only be due to energy transfer. HEK-293 cells expressed cytosolic GFP or a membrane anchored GFP-GPI fusion protein. We also used a constitutively dimerizing GFP tandem as a positive control. We will present and discuss results regarding anisotropy calibration using fluorescein solutions or cells. Besides, first results of time-resolved anisotropy in TIRF configuration will also be shown.