REAL TIME OBSERVATION OF LIPID ORGANIZATION DYNAMICS USING FAST POLARIZATION MODULATION IN NONLINEAR MICROSCOPY

Naveen K. Balla, Matthias Hofer, Sophie Brasselet
Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, F-13013 Marseille, France
Email: sophie.brasselet@fresnel.fr

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ABSTRACT
Polarization resolved nonlinear microscopy not only enables us to image samples in 3D using endogenous contrast, it also provides us with valuable information about the local organization of molecules in the samples [1,2]. The spatial resolution of this local structural information can extend beyond the diffraction limit in the case of plasmonic nanoparticles [3]. In biological samples, local organization of molecules is tightly related to essential functions such as the mechanical properties of cells and tissues, or to malfunction such as the formation of proteins aggregates at the origin of neurodegenerative diseases. The technique involves acquiring multiple images of a sample with different incident polarizations, and post processing this data to calculate local dipole orientation and angular disorder, averaged over the diffraction limit size (~200 nm) and the integration time. This methodology is however often too slow for observing dynamics which occur over time scale of a few seconds. To overcome this limitation, we have engineered a new technique to map local dipole organization with high precision without compromising the image acquisition speed. Our method involves modulating the excitation laser polarization at 100 KHz using a Pockels cell, and analyzing the modulated nonlinear signal per pixel simultaneously using a lock-in amplifier. We demonstrate the use of this technique with coherent Raman scattering microscopy to image dynamics of lipid order during the formation of multi-lamellar vesicles (MLVs). We emphasize the sensitivity of the technique by the real time detection of ghost red blood cells, which are single lipid bilayer structures. These results demonstrate the sensitivity and the speed of the technique, which are essential for observing dynamics in biological processes like mitosis, meiosis or cell migration.

REFERENCES