PhD Thesis : 3D optical microscopy for quantifying T lymphocyte activation

Start date: by the end of 2018
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T lymphocyte activation is a key feature of the immune response, during which the adaptive immune system detects specific none-self antigens prior to induce targeted responses. Presently, no technique permits to perform a fast detection of T lymphocyte activation at an early stage, which is detrimental for the diagnosis of numerous diseases.

T lymphocyte activation is triggered by several signals exchanged between the lymphocyte and an antigen presenting cell. After the very early steps of activation, a highly organized interfacial structure appears between the two cells, called an immunological synapse. We propose a new method for quantification of T lymphocyte activation based on the detection of these immunological synapses.

The main objective of this project is to develop an optical microscope that is able to discriminate cell-cell interfaces with or without activation of the immunological synapse. It has to be a marker free (non fluorescent) microscopy technique to be compatible with fast medical diagnosis. A sketch of the two possible states of the monocyte and lymphocyte interaction is presented in Fig. 1. It is seen that the imaging tool should be able to provide a 3D image (the immune synapse being more likely to occur on the top-membrane of the monocyte) of deformed interfaces with a transverse resolution about 300 nm. This challenging imaging issue cannot be addressed with standard marker free optical microscopes.

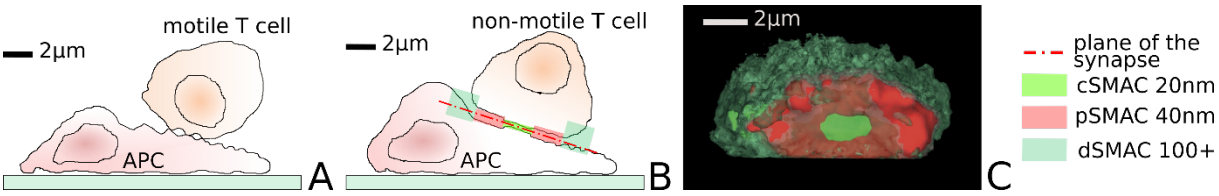

Figure 1: A, sectional representation of a T lymphocyte crawling on an antigen presenting cell (APC) or **B** with a fully established immune synapse. **C**, sectional view in the plane of the synapse (*i.e.*, along dotted red line in **B**) from fluorescence confocal microscopy showing the T lymphocyte side of the synapse.

Here, we will consider an original approach known as Tomographic Diffraction Microscopy (TDM). This computational microscopy technique was pioneered by the Fresnel Institute, and detects the field reflected by the sample using an interferometric measurement for various illumination angles. It has already been used to image thin (less than 1 micron) nanostructures on opaque and transparent substrates [1, 2] and its resolution was shown to be at least twice better than that of standard microscopes. However, it has never been applied to the visualization of thick (ten microns) samples over a large field of view. This novel application necessitates the development of numerical tools and the optimization of the experiment.

[1] C. Godavarthi *et al.*, "Super-resolution with full-polarized tomographic diffractive microscopy", J. Opt. Soc. Am. A., Vol. 32, p. 287, 2015.
[2] T. Zhang *et al.*, "Super-resolution with full-polarized tomographic diffractive microscopy", Optica., Vol. 3, p. 609, 2016.

PhD Thesis: Infrared tomographic microscopy for the characterization of silicium components

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Super-resolved contactless imaging is a critical area of research for component characterization and process control in micro and nanotechnology. The constantly decreasing dimensions of these components require new solutions for 3D metrology. No technique currently allows non-destructive and efficient imaging in the volume of semiconductors, including silicon.

Current optical techniques can only provide resolved information on surfaces: this is the domain of optical profilometry [1]. Indeed, they assume that the object probed does not disturb the illumination, which is false in the volume of semiconductors, especially because of the high index contrast with the external environment (usually air).

This subject proposes to explore the use of a volumic optical imaging technique, diffractive tomographic microscopy developed at Institut Fresnel to characterize nanostructures deposited on silicon [2] in the visible domain. This technique consists of detecting in phase and amplitude the diffracted field of the sample, illuminated from different angles, and reconstructing its permittivity map by a digital reconstruction procedure. The key point is that this reconstruction can take into account the disturbance made by the object on the illumination [3].

In this context, the doctoral student will aim to set up a tomography bench operating in the infrared, in order to initially characterize femto-second laser etched waveguides in the silicon sample volume. These guides will be produced in collaboration with the LP3 laboratory in Marseille.

In the longer term, the characterization of components in microelectronics will be studied.

[1] A. Bosseboeuf and S. Petitgrand, *Optical Inspection of Microsystems*, CRC Press (2006).

[2] T. Zhang, Y. Ruan, G. Maire, D. Sentenac et al., “Full-polarized tomographic diffraction microscopy achieves a resolution about one-fourth of the wavelength”, Phys. Rev. Lett. **111**, 243904 (2013).

[3] C. Godavarthi, T. Zhang, G. Maire, P. C. Chaumet, H. Giovannini, A. Talneau, K. Belkebir, and A. Sentenac, "Super-resolution with full-polarized tomographic diffractive microscopy", J. Opt. Soc. Am. **32**, p. 287 (2015).

PhD Thesis: Bimodal superresolved tomographic - fluorescence microscopy

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Total internal reflection structured fluorescence microscopy (TIRF-SIM) is the gold standard for surface imaging in dynamic and extended observation on biological samples [1]. However, it is unable to fulfill its promise of super-resolution because of the disturbance induced by the sample in the illumination figure, in particular because of scattering [2]. To overcome this limitation, this thesis aims to explore a bimodal approach of microscopy coupling fluorescence and diffraction measurements, in order to jointly estimate the intensity of actual excitation within the sample, as well as its density of fluorophores ρ and its refractive index map n. This subject uses experimental skills to develop the experimental setup, as well as numerical skills to work on inversion algorithms.

In the past, separate algorithms [3, 4] have been developed at Institut Fresnel to independently estimate ρ and n. It will be a question here of elaborating a bimodal algorithm able to estimate ρ and n more efficiently than with separated reconstructions.

The success of such an approach would be remarkable not only because of the improvement in resolution, but also by the joint estimation of two contrasts, ρ and n, which would make it possible to precisely locate the fluorophores within the sample. These aspects are sorely lacking in the characterization techniques currently used.

[1] H . Schenckenburger, "Total internal reflection fluorescence microscopy, technical innovations and novel applications", current opinion in biotechnology, 16, 13-18, 2005.

[2] Brunstein M, Teremetz M, Hérault K, Tourain C, Oheim M., "Eliminating unwanted far-field excitation in objective-type TIRF. Part I. identifying sources of nonevanescent excitation light", Biophys J 106(5):1020-32, 2014.

[3] R. Ayuk, H. Giovannini, A. Jost, E. Mudry, J. Girard *et al.*, "Structured illumination fluorescence microscopy with distorted excitations using a filtered blind-SIM algorithm", Opt. Lett. 38(22), 4723-4726, 2013.

[4] C. Godavarthi, T. Zhang, G. Maire, P. C. Chaumet, H. Giovannini, A. Talneau, K. Belkebir, and A. Sentenac, "Super-resolution with full-polarized tomographic diffractive microscopy", J. Opt. Soc. Am. 32, 287, 2015.