Random Illumination Microscopy
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Fluorescence microscopy is the most widespread tool for getting real time images of specific protein distribution in live specimen over large volumes of observation (hundreds of thousands of microns cube). Unfortunately, its resolution, about 300 nm transversally and 1000 nm axially at best, is not sufficient for an accurate study of proteins interactions. On the other hand, super-resolution fluorescence microscopes using saturated fluorescence, STED, pointillist approach, like STORM, or intrinsic fluorescence fluctuations, such as SOFI or HAWK, yield images with a resolution below 100 nm but their toxicity, the time required for the data acquisition and processing restrict their use to small volumes of observation and slow temporal dynamics.

Presently, Structured Illumination Microscopy (SIM) is the best compromise between resolution and practical implementation on living samples. It consists in recording several low-resolution images of the sample under different positions and orientations of a known periodic illumination. The super-resolved reconstruction is obtained by demodulating the data using the illumination pattern as a 'carrier wave'. The resolution can reach 100 nm transversally and 300 nm axially for the best periodic SIM [Shao11]. Yet, this achievement requires a precise knowledge of the illumination [Wicker13] and thus a complex experimental implementation to control it [Demmerle2017]. In addition, if the pattern is deformed by aberrations or scattering, the numerical process leading to the super-resolved image fails[Ayuk13]. As a consequence, SIM is limited to the observation of non-distorting, weakly scattering samples and cannot be applied to thick biological tissues or over large fields of view.

Recently, we have proposed a novel microscopy technique, Random Illumination Microscopy, which combines the resolution, low toxicity and high temporal resolution of periodic SIM with the ability to probe thick distorting samples over large fields of view and the ease of use of widefield fluorescence microscopy. RIM consists in recording hundreds images of a sample under different (unknown) speckle illumination. Its implementation is much simpler than SIM as it does not require the knowledge of the illuminations. It has been shown mathematically [Idier18] that a super-resolved estimation of the fluorophore density could be obtained by demodulating the auto-correlation of the speckle images using the speckle auto-correlation as a carrier wave. The latter being insensitive to scattering, distortions and aberrations, RIM is expected to succeed in configurations where SIM fails. Preliminary studies conducted on small 2D samples have demonstrated the gain in resolution, the robustness to aberrations and the small toxicity of this approach [Mudry12, Ayuk13, Labouesse17, Idier18, Negash16].

The objective of this thesis is to adapt RIM to large fields of view (about 500 x 500 μm^2) and 3D imaging (over 50 μm of axial extent). Yet, RIM extension to large samples raises major theoretical and numerical challenges as manipulating the autocorrelation of 3D speckle images comprising tens of millions of pixels is not an option. We propose to develop a computationally tractable 3D RIM by considering only the speckle image variance or its auto-correlation restricted to the acquisition of several focal planes. 3D-RIM addresses mathematical questions in the field of super-resolution imaging *without sparsity constraint* and requires the development of innovative inversion algorithms able to process large stacks of data. We are looking for a student having knowledge in signal processing or in imaging. He, she will develop the reconstruction algorithms and test it on experimental data provided by the set-up already built at the CBI Toulouse by T. Mangeat or by a home made RIM developed at Fresnel Institute in collaboration with Loic Le Goff. The project will take advantage of a long term collaboration with J. Idier of the LS2N at Nantes who is a specialist of signal processing.

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