High speed super-resolution microscopy to decipher membrane dynamics within living cells

Laboratoire Jean Perrin UMR 8237
Didier Chatenay
Responsable de stage : S. Bonneau (stephanie.bonneau@upmc.fr)

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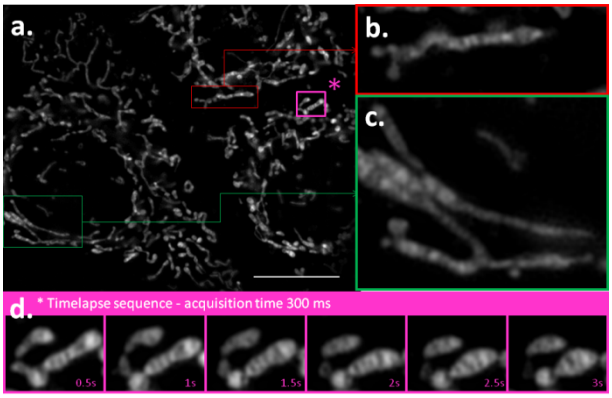
The cell is spatially highly structured at all levels: from molecular small assemblies to subcellular nano- and micro-compartments and to global micrometer scales at cells level. In recent years, this has led to a profound renewal of our vision of cellular behaviour, particularly through evidence that the architecture and shape of membranes modulate the regulatory network and functions of the cell. The system biology of cell membranes therefore converges with biophysics and the multi-scale spatio-temporal organization of cells also emerges as organizing signalling and coordinating cellular behaviour.

State of the art and objectives:

In this context, we address the issue of how modifications in membrane components imply a dynamics of its meso-scale organization, what impacts the mobility of protein complexes and can challenge both the thermodynamic state and the biological functions of cell compartments. We will focus on two key membranes of the cells, both affected and modulated in a controlled way in our pathophysiological cellular model: the plasma membrane, where the meso-scaled modulations are related to in-plane patterning called lipid raft[1], and the mitochondrial inner membrane, where the nano-scaled organization exhibits membrane 3D-invaginations called cristae[2]. Both of these membrane arrangements imply both physical and chemical hallmarks (such as enrichment in cholesterol or cardiolipin, respectively). They induce specific architectures of the membrane surface, thus causing particular modulations of the diffusion processes of molecules and proteins complexes (in-plane and 3D confinement effects) in the vicinity of the membrane, able of modulating their interactions and biological activity.

The plasma membrane presents different types of lipid and protein clusters, however the functional role of the clustering on the membrane surface is still not yet fully understood[3], even if super-resolution microscopy has been very helpful to apprehend both structure and function of these clusters[4,5]. Moreover, due to their small size, intracellular organelles are perfect targets for super-resolution imaging. Optical microscopy remained unsuitable to study the structures of mitochondria (1 µm), lysosomes (500 nm), endosomes (100 nm) or synaptic vesicles (50 nm) until recently. Today, super-resolution microscopy techniques give first access to the peculiar organization of mitochondrial membranes within cells.

In addition to spatial heterogeneity, cell membranes exhibit temporal heterogeneity. In a membrane, the components not only diffuse at different speeds, but also with different diffusion modes[6,7]. Most membrane compounds do not exhibit free diffusion. Some molecules undergo sub-diffusive behaviour, while most lipids and proteins undergo super-diffusion due to the active processes of the cells. This variety of diffusion characteristics enable to better understand the spatial and temporal organization of membranes at the nanoscale. For living cell dynamics studies, however, the leading-edge super-resolution techniques present some limitations, especially due to the time of acquisition (typically tens of second to minute). Moreover, a specific limitation for tracking the membrane dynamics is the limited repertoire of probes.



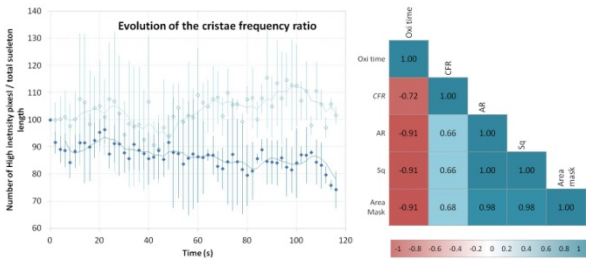


Fig. 1 - Left: Fast-SIM image of mitochondria within living HeLa cells labelled with MitoTracker Green. (a) Fast-SIM image. (b, c) Enlargements of image inserts (d) Enlargements of the inset indicated by* showing the time evolution of the mitochondrial topology under controlled oxidative stress. Scale bar 10 µm, Obj. x60, NA 1.2, $f_{mod} = 0.9xf_c$. Right: Evolution of the cristae frequency ratio and correlation matrix between quantitative parameters and oxidation state.

We wish to overcome these limitations and study living cells using a homemade SIM set-up, of which innovative approach increases the acquisition rate by a factor 2. Experiments on living HeLa cells given promising results (Fig. 1): we demonstrate a method, so-called Fast-SIM, providing a lateral resolution of ~100 nm at raw data acquisition rate of 15 frames per second for a wide field image of 85µmx85µm.

Approaches and methodology:

Leading-edge SIM microscopy on living cells: In SIM microscopy, high frequency features are encoded in the wide field image. Several images of the same sample are acquired for different illumination patterns and are digitally combined to reconstruct an extended-resolution image of the object. SIM is of particular interest for biological studies because of its rapidity, flexibility and low level of irradiation needed. In addition, it can be used with all conventional fluorescent probes, and thus offers a large range of applications. It provides a two-fold increase in spatial resolution compared to standard microscopy. Although seven images are typically needed, we have developed a reconstruction method based on only four images, improving the acquisition rate, essential for our study.

Cells models: Experiments will be conducted on two cell models. The HeLa cell line is a labile model and will be used for preliminary experiments, set-up development and tuning. We can control their physiological state by using a photoactivable molecule, which allows us to tune the level of cell stress[8]. Primary cells cultures of cortical neurons are a more realistic model, well-adapted to address our issue. Indeed, we have access to models of Huntington Disease (HD), a neurodegenerative disease. HD plasma membrane of neurons displayed an increase of cholesterol content within the lipid rafts and a dysregulation of NMDA (N-methyl-D-aspartate) ionotropic glutamate receptors (GluN2B). Mitochondrial properties and function are also drastically altered in HD. We developed biochemical tools enabling a fine control of these membrane compositions (methyl-β-cyclodextrin to induce over-expression of the CXP4AI25). Our strategy is to track, in controlled conditions, (1) the cholesterol-rich lipid rafts (Cholera-toxin B Alexa-488) and the GluN2B-Cherry subunit, and (2) the inner mitochondrial dynamics (MitoTracker Green) and activity (JC-1) and (3) the local dynamic viscosity of the cells compartments to obtain a nano-scaled mapping of viscoelastic properties (rotor-based fluorescent probes will be obtained from M. Kuimova, Imperial College, London[9]). First enhanced-resolution images of lipid rafts and mitochondria in primary neurons have been successfully obtained using the Fast-SIM set-up under development at LJP.

Statistical analysis and correlations: The cells will be characterized by conventional biochemical approaches (lipidomic, Western Blot, etc.) as well as imaging (both functional and Fast-SIM). Quantitative data will be derived from image analysis (see xx and Fig. 1) to correlate topology and dynamics with the functional state of the cells.

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