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Thesis topic :
Full-field multimodal microscopy by Spectrally Structured Illumination

The objective is the development of a new optical microscope technology based on spectrally structured illumination using several fast switchable light-emitting diodes (LEDs), capable of imaging and quantifying very small variations in polarization anisotropy (birefringence and dichroism), but also phase shift within biological samples, and this at the video rate.

The phase-contrast or polarization microscopes commonly used in biology are full-field microscopes, i.e. the image is formed at once (all the points of the sample are imaged in parallel), unlike scanning microscopes. These microscopes are generally used to image without staining and with better contrast fine and transparent media (e.g. cell walls, oriented assemblies of proteins: collagen, myosin, microtubules, amylopectin) in cell cultures, biological tissues or organisms marine (algae). However, phase contrast or polarization microscopes are currently limited because if we need to quantify with good sensitivity the variations of phase and/or polarization anisotropy, it is necessary to insert electrically controllable optical elements into the microscope (ex: crystals liquids) and record several successive images. The switching speed of the optical elements as well as the number of images required do not currently make it possible to image or quantify the anisotropy and the phase at the video rate, which reduces the field of application of these microscopes.

The tool that we propose is of considerable interest because it will make it possible to quantify in real time (> 25 images/s) and with a very high sensitivity the weak variations of phase and anisotropy involved for example in the field intracellular mechanical stresses during mitosis, intracellular trafficking or, in the longer-term perspective, neuronal activity. In addition, the technical solution we are considering is compatible with the usual optical microscopes that use a camera as a detector (full-field microscopes), which can be an asset for industrial transfe. Indeed, it will enough to change the lighting system of the microscope by

our LED illumination and to add passive optical elements in the path of the light to transform a standard microscope into a microscope capable of quantifying the variations of phase or anisotropies at the video frame rate. This new technology therefore has strong industrial potential, which has aroused the interest of SATT Ouest Valorisation that wants to support the OPTIMAG laboratory in the patenting of the technology once the proof of concept has been demonstrated experimentally.

Finally, LED illumination could also be extended to fluorescence microscopy to determine the orientation of fluorophores in different biological structures (and dynamic measurements related to viscosity for example) or to locate them in depth (potential for 3D imaging), which is of considerable interest for understanding a large number of biological phenomena. Indeed, fluorescence will make the imaging of biological structures even more specific.

In summary, the challenge of this thesis project is to develop new devices in phase, polarization but also fluorescence microscopy, and to achieve a spatial and temporal resolution and a detection sensitivity allowing revealing, at the cellular scale, structures of biomedical interest. On the other hand, an essential advantage of this new imaging modality is its technical simplicity which allows it to be implemented on any commercial full-field microscope, thus with an industrial impact.

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