VISCOSITY AND MOTION IMAGING BY HETERODYNE HOLOGRAPHIC MICROSCOPY

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KEY WORDS: Holography, 3D imaging, Heterodyning, Viscosity imaging, Plasmonics

Viscosity-driven mechanisms are crucial in biology, and the field of bio-microrheology, i.e. the study of cells via their mechanical properties, has recently emerged [1]. With 2D microscopy, this is essentially achieved through the tracking of nanomarkers undergoing lateral diffusion [2], since the mean square displacement of a particle gives access to the viscosity of the medium. However, these methods have their own biases and limitations: at these scales, cells are clearly 3D systems, which cannot be fully investigated using 2D methods. Moreover, translational Brownian motion yields an integrated viscosity, along the path of the marker, and is difficult to distinguish from driven flow in the cell.

The presented technique relies on Brownian rotation [3] instead of translation. The anisotropy of the rotating objects creates a blinking at the rotation frequency, which can reveal the local viscosity since no translation is involved. Holography, associated to particle superlocalization, provides high 3D spatial resolution dark field images of the scattering either by components of the cell (organelles), or injected plasmonic gold nanorods. A heterodyne frequency beating ΔF between the object and reference arms reveals high frequency blinking on a dark background, even with a low frame rate camera.

This work aims at high resolution mapping of the viscosity of the medium by decomposing the imaged volume in macropixels, each containing a local viscosity probe, with the help of superlocalization. A first set of measurements was conducted in various concentrations of glycerol to model the viscosity values expected in a cell, revealing variation in the rotation frequency spectra. In live mouse oocytes, local structures associated to cell metabolism are revealed in the cell’s component movements.

Fig.1: Heterodyne holographic microscope. The camera records the hologram, an interference between the object and reference waves. Each beam is phase-modulated by Acousto-Optical Modulators (AOMs) to create a frequency beating. A classical camera (Hz-range frame rate) can then record high-frequency (up to the MHz) variations in the scattering of rotating probes.