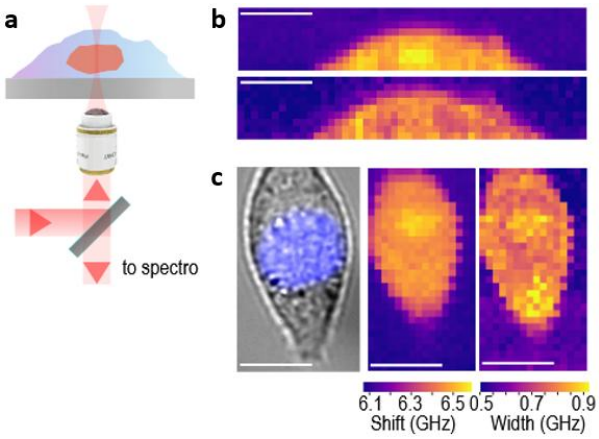
Master internship:

Quantitative imaging of phase thermodynamics in cells with Brillouin microscopy



a. BLS set-up. The laser light is focus inside the cell through a glass cover-slip. c. Brillouin frequency shift (top) and linewidth (bottom) images in the sagittal plane of a typical cell. **d**. Bright-field image of a cell with DNA labelled with Hoechst stain (left), and corresponding BLS images of the shift (center) and the linewidth (right). Scale bars: 10µm.

Biomolecular condensates (biocondensates) are membrane-less structures that contribute to essential biological functions thanks to their ability to selectively concentrate molecules, thus providing a fundamental mechanism for cellular organization and biomolecular processes. Most of the time the formation of biocondensates is reversible, but liquid-to-solid transitions resulting in irreversible aggregation that limits the availability of protein domains and induces toxicity can occur with aging or mutations as is the case of many neurodegenerative disorders. Yet, despite much efforts using in vitro assays, the mechanisms underlying intracellular phase transitions remain poorly understood in the cellular context due to the lack of techniques to assess the physical properties in cells.

In recent years, a new quantitative microscopy based on Brillouin light scattering (BLS) has been proposed. The BLS spectra, resulting from the interaction of laser light with density fluctuations, can be interpreted as the response of the sample to an infinitesimal uniaxial compression at picosecond timescales. The frequency shift and linewidth can be formally associated to the longitudinal sound velocity v_L and attenuation α_L, respectively, and hence probe the dynamic viscoelastic response of the material. This approach has been widely used since the 70's to characterize liquids and gels. In polymer solutions, as the network becomes more packed upon increase of the crowding, or more cross-linked upon polymerization, correlated intermolecular fluctuations increase and affect deeply how sound propagates. This usually manifests itself as an excess velocity and/or attenuation compared to thermodynamical equilibrium that has allowed identifying phase transitions occurring upon changing temperature, volume or volume fraction.

Based on our expertise, we have imaged live cells using BLS and have observed a clear contrast between condensates and the surrounding liquid in the nucleus. In this project, we want to push these preliminary findings further and probe the formation of condensates by controlling the aggregation parameters. To be able to observe such process in real time, it is key to speed up the acquisition. To do so, we will develop an innovative approach for stimulated BLS in cells. Based on this device, we will study the formation of condensates in pathogenic variants involved in several neurodegenerative diseases. We will use inducible systems and measure acoustic properties at different stages of the aggregation process.

The student will build the microscope for stimulated BLS, and demonstrate its operation in test samples. After this step, he/she will perform experiments on cells. He/she will develop the necessary tools to analyse the data using Python programming, and implement the corresponding theoretical modelling.

For these tasks, he/she will benefit from the supervision of T. Dehoux and an engineer recruited for this project. Cell culture and other biological tools will rely on the expertise of biology engineer in the host team at Institut Lumière Matière. The student will assist him in the basic tasks and cell maintenance.

The project will continue as a PhD program funded by the ANR.

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