Fast label-free imaging of architectural order with multi-wavelength instant PolScope (miPolScope)

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Quantitative label-free imaging reports on physical properties of biological specimens, such as phase[1] or birefringence[2], and can be used as a scalable and non-destructive tool for studying biological specimens with minimal photodamage. Recently, we reported joint imaging of phase and birefringence in live specimens (Figure 1) along with deep learning-based methods to identify specific components within label-free images[3], enabling segmentation and tracking of structures of interests.

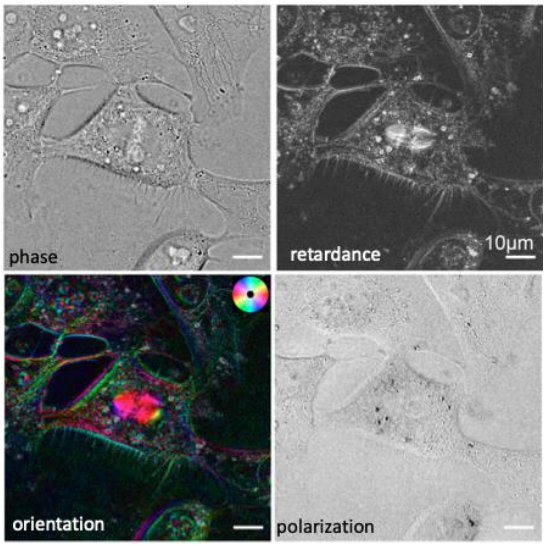

Figure 1. Phase, retardance, slow axis orientation (given in color), and degree of polarization measurements in a dividing U2OS cell. Condensed chromosomes are visible in the phase image, while the mitotic spindle displays high birefringence contrast. The measured orientation of microtubule bundles aligns with the axis of the structure, as expected. The polarization channel reports on structures causing multiple light scattering.

However, the throughput and temporal resolution of quantitative phase and birefringence measurement methods is limited by the need to acquire a set of images with different illumination angles and/or polarizations states of light. Furthermore, polarized microscopes are typically designed to work at a specific wavelength, constraining the ability detect wavelength-dependent properties or to multiplex with other microscopy techniques.

We developed miPolScope, a method for broadband measurement of birefringence from a single snapshot and of phase from 3D distribution of intensities. We designed a detector with four parallel polarization-resolved imaging modules with achromatic performance in the 400 – 800 nm range. We developed a robust calibration procedure and data analysis pipeline for quantitative reconstruction of the sample phase, retardance, slow axis orientation, and degree of polarization.

The ability to measure physical properties of biological samples at camera rates provides access to new timescales of biophysical measurements important for biology and pathology. Our microscopes and algorithms will be particularly useful in understanding how ordered architecture emerges in diverse biological systems across a range of spatial and temporal scales.

References:

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