OPTICAL DESIGN OF MULTIFOCUS POLARIZATION MICROSCOPY FOR 3D TIME-LAPSE IMAGING, AND ITS APPLICATIONS IN PROTEIN ASSEMBLY DYNAMICS STUDIES AND EMBRYONAL IMAGING

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ABSTRACT: We have built a 3D time-lapse polarization microscopy system (MF PolScope) and applied it in biological imaging. Using OpenPolScope technology1 we implement polarization-imaging modalities, such as fluorescence anisotropy and birefringence imaging (www.openpolscope.org). Our polarization image sequence takes less than one second, which is enough to image slowly moving samples in 2D. To enable live imaging also in 3D, we integrated the polarization system with multifocus (MFM) optics. MFM is a specialized diffractive Fourier optics technique that provides instant 3D images2. We design the MFM optical parts computationally3 and fabricate them from fused silica using laser writing, deep-UV lithography and wet or reactive ion etching.

We have recently applied the MF PolScope to probe both the position and the orientational alignment of protein structures in a fluorescence anisotropy study of septin in budding yeast during cytokinesis (Fig. 1). We show high-throughput imaging of multiple cells in 3D over extended time periods. In another study, we employ extended volume MFM with 25 simultaneous focal-planes covering the entire C. elegans embryo at high sampling rate. Using a non-invasive transmission polarization-imaging mode, we visualize spindle and chromatin rearrangement during embryonal development without staining.

Figure 1. Fluorescence anisotropy imaging of septin rings in budding yeast. Septin molecular orientation is indicated by color, according to the aster color-wheel in the upper left corner.