Understanding the biophysical properties of cytoskeletal assemblies requires knowledge of not only their positional, but also their orientational organization. Polarization-dependent excitation and polarized emission of fluorescent dyes are key contrast mechanisms that provide access to orientation of molecules in cells. Measurement of rotational diffusion of dyes by exciting them with polarized light and analyzing their emission along orthogonal polarizations has been well established [1]. However, rotational diffusion measurements are biased when fluorophores are rotationally constrained. Measurement of fluorescence along at least three excitation or emission polarization orientations is needed for unbiased measurement of orientation of complex cytoskeletal assemblies.

Our group has reported use of LC universal compensator in a wide-field microscope to measure orientation of septin assemblies [2] by exciting the specimen along 4 polarization orientations. Recently, a confocal polarized excitation microscope has been reported [3] to measure orientation of membrane using > 30 measurements with varying polarization states. Large number of measurements was required to compensate for the bias due to polarization distortions introduced by components in the excitation light path, mainly by the dichroic mirrors.

We report a new method, named confocal LC-PolScope, which circumvents this limitation with precise compensation of polarization distortions. Our scheme allows depth-resolved measurement of the orientation and degree of order of an ensemble of fluorophores that decorate ordered structures such as membrane, cytoskeleton, and collagen. The method has the potential to provide new insights into structural changes that occur in such ordered assemblies during cell function.

References: