SCANNED APERTURE POLARIZING MICROSCOPE
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A traditional polarizing microscope can be used to measure the retardance and optic axis orientation of birefringent objects. However, the orientation is limited to the plane perpendicular to the microscope axis, while the inclination angle of the optic axis away from that plane is generally not known. Furthermore, the measured retardance of an object is modified by its inclination angle, without the ability to correct the measurement.

We have developed the Scanned Aperture PolScope which overcomes these limitations. The new technique builds on the LC-PolScope [1] and uses a universal compensator and aperture scanning device, both made from liquid crystal devices. We will report on the optical set-up, and image acquisition and processing algorithms for measuring the 3 dimensional birefringence distribution in biological specimens such as an aster.

**Fig. 1** shows a schematic of astral microtubules (MTs) radiating in all directions from a centrosome, a microtubule organizing center (aster diameter ~15 µm). Highlighted MTs illustrate the specimen slice that is within the depth of field (~0.5 µm) of a polarizing microscope. The position of the slice is displaced along the microscope axis by 5 µm from the center of the aster. In the middle of the slice, MTs are oriented perpendicular to the slice, having an inclination angle of 90°. Towards the periphery, the inclination angle decreases to 45° and less.

**Fig. 2** shows the astral MTs in a traditional polarizing microscope (100x/1.3NA) equipped with crossed polarizers and a compensator. The image reveals the birefringence and optic axis orientation of MTs in the imaged slice. However, in the middle of the pattern no birefringence is detected and in the rest of the pattern brightness is modulated by the orientation and inclination of the MT arrays.

**Fig. 3** was recorded with the LC-PolScope. The image brightness is directly proportional to the measured retardance (white=3nm) of the microtubule arrays and independent of the orientation of their optic axis. The orientation is also measured (image not shown). However, the inclination of the optic axis cannot be discerned and the measured retardance vanishes in the center of the radial pattern.

**Fig. 4** was recorded with the new Scanned Aperture PolScope [2]. The image brightness is proportional to the principal retardance (white=3nm) that is independent of orientation AND inclination angle. The azimuth and inclination angles are also measured (images not shown). Fig. 4 reveals the birefringence of all microtubules, even those near the center of the image that have an inclination of 90°.