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Elevating Polarized Light Microscopy to the Third Dimension

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The polarized light microscope was Shinya Inoué's favorite tool for discovery. To many of us, polarized light microscopy remains a bit mysterious, because our eyes are not sensitive to polarization, which is a basic property of light. Whenever light interacts with matter, its polarization is likely to be affected, altered by the arrangement of molecular bonds and shapes in materials, biological tissues and cells. This sensitivity to molecular order attracted Shinya to using and improving the polarized light microscope for revealing the architectural dynamics in living cells and for analyzing the structural basis of cell function, specifically cell division, which was his life-long research interest.

Another reason why polarized light microscopy remains a bit mysterious is the fact that the visibility or contrast achieved in polarized light images depends on the orientation of the ordered structures with respect to the polarizing components in the optical path of the microscope. In **Figure 1**, we can recognize several mitotic spindles that were all recorded with a polarized light microscope. In **panel A**, a picture taken by Shinya Inoué with a traditional polarized light microscope, four mitotic spindles are visible. There is a stark difference in appearance, though, between horizontally and vertically oriented spindles, even though the spindle apparatus is nearly identical between all four spindles. In **panel B**, however, a similar spindle isolated from a fertilized sea urchin egg was imaged with the LC-PolScope, a new type of polarized light microscope that we developed with Shinya's support [1]. All astral and spindle microtubules are shown with equal contrast, regardless of their orientation in the object/image plane. Therefore, the LC-PolScope might be called an orientation-independent polarizing microscope.

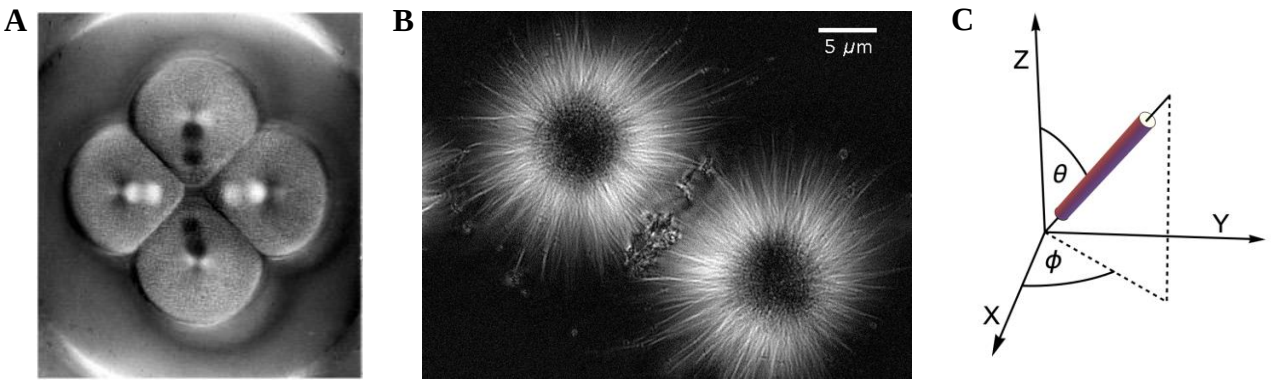

Figure 1. (A) Micromere-forming 4th mitosis in sand dollar egg, imaged with traditional polarized light microscope by Shinya Inoué. **(B)** Retardance image of a mitotic spindle isolated from a fertilized sea urchin egg and recorded with the LC-PolScope. **(C)** Schematic illustrating the orientation of a polymer bundle represented by a cylinder in object space. The orientation is given by two angles, the azimuth ϕ in the focus plane X-Y and the tilt θ away from the microscope axis Z.

However, calling it an orientation-independent polarizing microscope is still somewhat misleading, as there is yet another angle that affects the contrast of birefringent objects and is not yet accounted for. This angle is the tilt angle of the optic axis of a birefringent object with respect to the microscope axis. The tilt and azimuth angles are illustrated in **Figure 1C** that shows a slender cylinder representing a birefringent object like a microtubule or actin bundle placed in an object space coordinate system. While the LC-PolScope generates contrast independent of the azimuth angle, the contrast still depends on the tilt angle, with a tilt of 90° producing maximum contrast, while zero tilt abolishes the contrast, rendering the bundle invisible in both, the traditional polarized light microscope and the LC-PolScope.

Over the last two decades, we have introduced several schemes that can potentially overcome the contrast dependence on the tilt angle as well. It started with the experimental and theoretical exploration of the point spread function of the polarizing microscope [2]. This study made clear that for measuring the tilt and azimuth angle independently, it is necessary to image a birefringent sample along several viewing directions. Instead of rotating the sample under the microscope using a device like the universal stage [3], we first opted for tilting the chief ray of the illuminating light using a configurable mask in the aperture plane of the condenser lens [4]. This so-called Scanned Aperture LC-PolScope was able to recover the distribution of both, the azimuth and tilt angles independently in the focus plane of the sample. In addition, the same measurements revealed the principal retardance values that were independent of the two angles.

More recently, we have turned to the polarized light field microscope for comprehensive 3D birefringence imaging [5, 6]. For its realization, we added to the camera sensor a microlens array in the image plane of an otherwise unchanged microscope setup, as first proposed by Marc Levoy (**Fig. 2A**, [7]). After adding LC-PolScope components, we recorded light field images, such as the two retardance images of a small single calcite crystal illustrating the complementary information that can be gleaned from the conoscopic images behind each microlens and their spatial raster across the object scene.

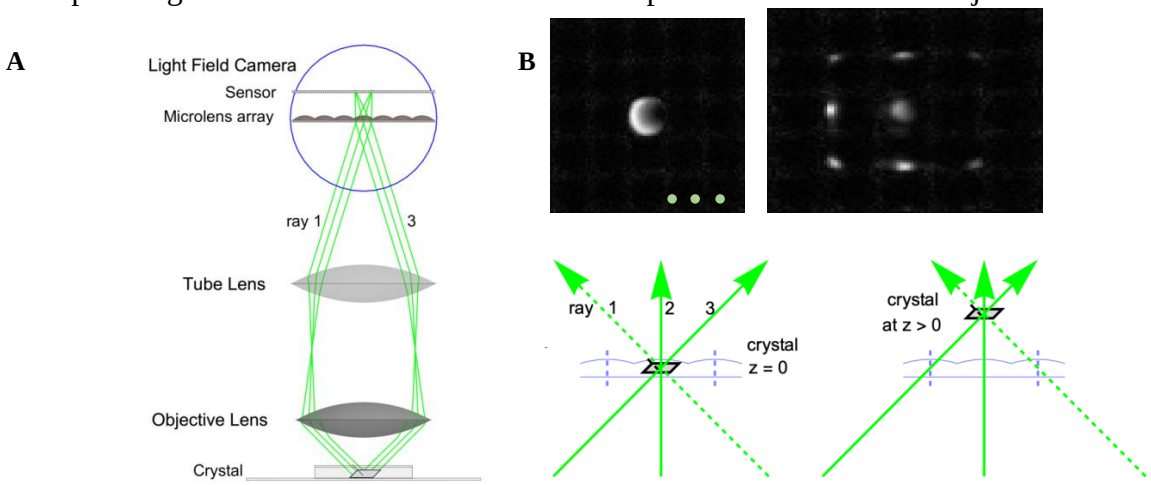

Figure 2. (**A**) Schematic of a light field microscope. The retardance images in (B) were recorded with additional LC-PolScope components in the light path. (**B**) Retardance light field images of a small (dia. ~1μm) calcite crystal, which is placed in the nominal focus plane (z = 0, left image) and out of the plane (right image). On the bottom are two schematics that show the crystal positions in object space in relation to the microlens array, shown in blue, as projected into object space by the microscope objective lens.

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In my presentation, I will discuss this latest approach to elevate polarized light microscopy to the third dimension, capturing both, the full 3D spatial and orientational distributions of complex birefringent samples, such as biological cells, tissues, and artificial materials [8].

References:

1. Oldenbourg, R., Nature, (1996). **381**(6585): p. 811-2. https://doi.org/10.1038/381811a0
2. Oldenbourg, R., and P. Török, Appl Opt, (2000). **39**(34): p. 6325-31. https://doi.org/10.1364/AO.39.006325
3. Kile, D.E., Geochemical News, (2009). **140**, 1-21 Available from: http://www.geochemsoc.org/publications/geochemicalnews/gn140jul09/theuniversalstage.
4. Shribak, M. and R. Oldenbourg, in *Biophotonics Micro- and Nano-Imaging*. (2004). Strasbourg, France: SPIE Proceedings. https://doi.org/10.1117/12.549050
5. Oldenbourg, R., J Microsc, (2008). **231**(3): p. 419-32. https://doi.org/10.1111/j.1365-2818.2008.02053.x
6. Tran, M.T., and R. Oldenbourg, J Microsc, (2018). **271**(3): p. 315-324. https://doi.org/10.1111/jmi.12721
7. Levoy, M., R. Ng, A. Adams, M. Footer, and M. Horowitz, ACM Transactions on Graphics, (2006). **25**(3): p. 924-934. https://doi.org/10.1145/1179352.1141976

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