A new view on polarization microscopy

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Improvements to the traditional polarization microscope have enhanced the direct analysis of the molecular architecture in living cells with new electro-optical devices, polarization algorithms and digital image processing.

THE Pol-Scope was recently developed at the Marine Biological Laboratory in Woods Hole, Massachusetts, for the analysis of molecular architecture directly in living cells[1]. Its capabilities were achieved by augmenting the traditional polarized light microscope with novel electro-optical devices, algorithms to compute specimen birefringences and today's digital image capture and processing capabilities. Figures 1 and 2 exhibit the exquisite resolution, high contrast and analytical strength of images taken with this new microscope.

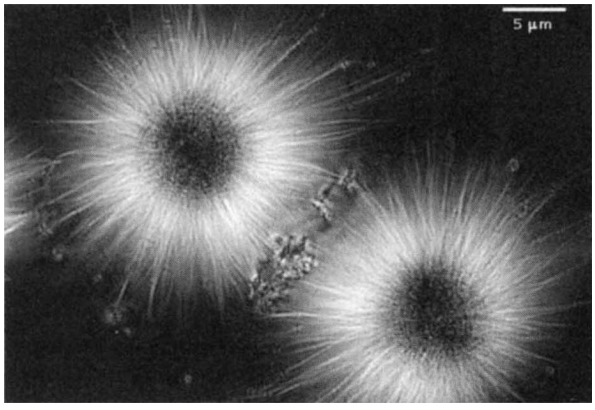

FIG. 1 Mitotic spindle isolated from fertilized sea urchin egg and imaged with the Pol-Scope (*Strongylocentrotus purpuratus*, preparation by John Murray, University of Pennsylvania, Philadelphia, USA). Retardance magnitude image, white: 5 nm retardance; black: 0 nm. Microtubule bundles radiate out from two black centrosomes; chromosomes visible between the two star formations (asters).

The polarized light microscope has the capability to image and measure submicroscopic molecular order. The partial alignment of molecular bonds or of submicroscopic particles leads to birefringence, which alters the state of passing polarized light. Common examples include birefringent crystals, stretched plastic films and individual thin filaments and membranes, which can exhibit minute amounts of birefringence or dichroism. (Birefringence refers to the difference in refractive index for two orthogonally polarized light beams. Dichroism is the difference in absorption of those two light beams.) A careful analysis of the change in polarization of transmitted or reflected light reveals, and can be used to quantify, molecular order.

Based on its analytical power, the traditional polarized light microscope has found numerous applications in fields such as biology, mineralogy, metallography, chemistry and forensic studies[2–5]. In biology, the polarized light microscope has the potential to measure submicroscopic molecular order dynamically and nondestructively in samples that, in general, can be kept in native environmental conditions. Furthermore, specific structures, such as filaments and membranes, are highlighted owing to their intrinsic optical properties, without the need to stain or label them. With the traditional polarized light microscope, however, single images display only those anisotropic structures that have a limited range of orientations with respect to the polarization axes of the microscope. In addition, rapid measurements are restricted to a single image point or single area that exhibits uniform birefringence or other forms of optical anisotropy[6], while measurements comparing several image points take an inordinately long time[7].

To overcome the limitations of the traditional polarized light microscope, we incorporated a precision universal compensator made of two variable retarder plates[1]. The optical setup (Fig. 3), in addition to a video camera and a specially designed, computerized image analysis system, provide fast measurements of specimen anisotropy (retardance magnitude and orientation; the word retardance was introduced by W. A. Shurcliff[8] and refers to the relative change in phase between two orthogonally polarized beams traversing a birefringent specimen. Expressed in distance, the retardance magnitude is the birefringence multiplied with the pathlength through the specimen. The slow axis orientation or retardance azimuth refers to the orientation of the principal axis with the largest refractive index). The retardance is measured at all points of the image and irrespective of orientation of the birefringence axes. Raw image data are recorded in less than half a second at a resolution of 640 by 480 image points and converted to retardance magnitude and orientation values in less than one second using a desktop computer. Retardance values as low as 0.02 nm and as high as several wavelengths can be measured, at a spatial resolution of 0.2 µm or larger, depending on the lenses used. Measurements are displayed as images representing submicroscopic molecular order at an unprecedented level of clarity and detail.

The optical design (Fig. 3) builds on the traditional polarized light microscope with two essential modifications: the specimen is illuminated with nearly circularly polarized light and the compensator is replaced by two electro-optical modulators (Fig. 4). The modulators are variable, linear retarders with their slow axes permanently adjusted at an angle of 45° to each other. While the axes are fixed, the magnitude of retardance can be changed by varying the voltages applied to the modulators. We have called this combination of two vari-

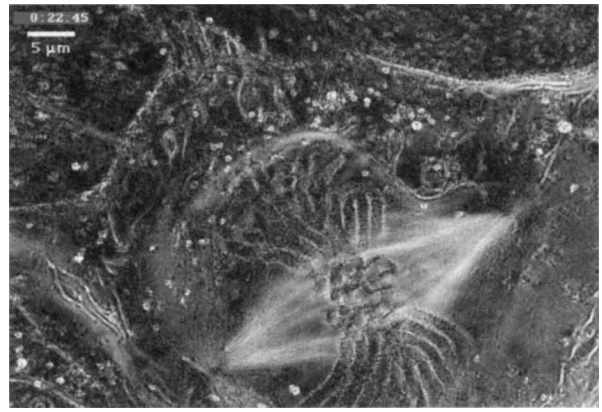

FIG. 2 Newt lung epithelial cell in mitosis (retardance magnitude image). Bright spindle fibres made up of microtubules locate the large chromosomes between the spindle poles. Chromosomes outlined by birefringence near their edges. (Collaboration with P. Tran and E. D. Salmon, University of North Carolina, Chapel Hill, North Carolina, USA.)

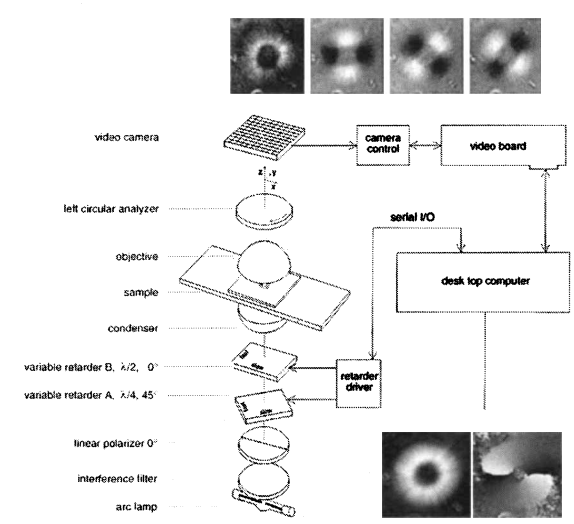

FIG 3. Schematic of the new Pol-Scope. The optical design (left) builds on the traditional polarized light microscope with the conventional compensator replaced by two variable retarders A and B forming the universal compensator. Images of the specimen (top row, aster isolated from surf clam egg) are captured at four predetermined retarder settings. The first image is in circularly polarized light (retarder A set to quarter wave and retarder B to half wave retardance). Subsequent images are in elliptically polarized light of different axis orientations. The monitor inset shows the computed retardance magnitude on the left image and on the right the azimuth image with brightness indicating slow axis orientation, from horizontal (black) to vertical (grey) to horizontal again (white).

able retarders a universal compensator for two reasons. First, it replaces the traditional compensator in the optical path of the polarizing microscope. Second, it can compensate any specimen birefringence, regardless of its orientation and magnitude, by changing the magnitude of retardances, that is the voltages applied to the modulators, so that a minimum of light equal to extinction passes through the analyser of the microscope.

To illustrate the workings of the universal compensator, four different images of the same birefringent specimen are shown in the top inserts of Fig. 3. The specimen is an aster (similar to one half of Fig. 1) formed in lysate prepared from eggs of the surf clam[9]. The aster is made from bundles of stiff filaments (microtubules) radiating from the centrosome in all directions. The bundles each possess a retardance of a fraction of a nanometre to several nanometres with the slow axis orientation parallel to the bundle axis. We emphasize that during the recording of all four images, no part of the microscope setup, including the specimen, was mechanically turned or moved. Instead, voltage were applied to the modulators were changed. Therefore, all four images recorded with the CCD camera are in perfect register, with corresponding picture elements representing intensities of the same object element.

Using the raw image data, the retardance magnitude and orientation images were computed. The algorithms were developed in our laboratory. They are based on a theoretical analysis of the polarization properties of the optical setup including the universal compensator, polarizers and the birefringence of the specimen. Recently, generally applicable algorithms have been developed by us for small and large specimen birefringences, measuring retardances of up to several wavelengths.

Many variations to the design shown in Fig. 3 are possible and already some have been implemented. For reflective samples (see Fig. 5), using an epi-illumination system, we have placed the universal compensator in the imaging path, after the half mirror, separating the illumination and imaging path in the epi-illumination microscope. Moving the universal compensator from the illumination (as shown in Fig. 3) to the imaging side does not alter the algorithms significantly for computing retardance magnitude and orientation. In the future, we hope to show that the most general design of the Pol-Scope uses a linear polarizer and pair of linear retarders in both the illumination and the imaging path, sandwiching the sample between two universal compensators. We believe that this arrangement can measure all possible polarization optical parameters, including linear and circular birefringence and dichroism in the specimen, at the highest resolution of the light microscope.

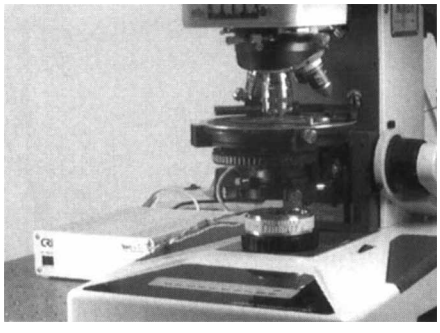

FIG. 4 The new universal compensator adapted to an upright microscope (Microphot SA, Nikon, Melville, New Jersey). Two liquid crystal (LC) variable retarders in rotatable mounts are located in the illumnation path on top of the field lens near the base of the microscope stand. The electronic driver unit for the LC devices is sitting on the table to the left of the microscope stand. (LC devices and electronic dnver unit from Cambridge Research & Instrumentation, Cambridge, Massachusetts, USA.)

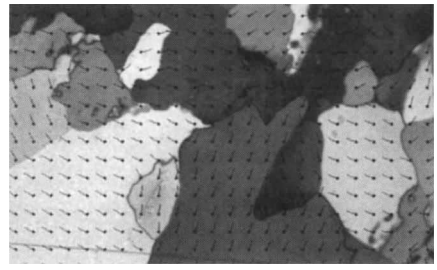

FIG. 5 Anodized aluminium surface observed in reflected light. Retardance magnitude shown as grey values, short lines indicate slow axis orientation. The retardance (up to 40 nm) is due to a thin layer (~1 mm thick) of birefringent alumina (Al_2O_3). The birefringence visualizes the grain structure at the surface of the metallic aluminium–lithium alloy. (Sample by Gary Laughlin, McCrone Research Institute, Chicago, Illinois, USA.)

With the approaching introduction of a commercial version of the new Pol-Scope (a commercial version of the instrument, called the LC-PolScope, is being developed by Cambridge Research and Instrumentation, Cambridge, Massachusetts, USA), we expect applications to develop in many different areas of pure and applied research. Because of its fast speed, high sensitivity, versatility and ease of use for measuring optical anisotropies, the new instrument significantly advances the analytical power of the polarizing microscope in all its traditional application areas. □

Rudolf Oldenbourg, Marine Biological Laboratory, Woods Hole, Massachusetts, USA. *The author thanks Shinya Inoué of the Marine Biological Laboratory for his invaluable inspiration and support from the start of this development project. The instrument development is supported by the National Institutes of Health. For more information fill in reader enquiry number 100.*

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