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# Scanning aperture polarized light microscope: observation of small calcite crystals using oblique illumination

M.Shribak, R.Oldenbourg*
Marine Biological Laboratory, Woods Hole, MA 02543, USA

## ABSTRACT

The article presents a new design of a polarization microscope with a scanning liquid crystal aperture. The scanning device is based on the earlier reported Pol-Scope technique and includes a liquid crystal universal compensator. It is mounted in the front focal plane of the high numerical aperture condenser lens on the microsccope. By occluding different portions of the aperture, an oblique beam of variable tilt angle and azimuth is created for illuminating the specimen. Birefringence measurements are recorded for different mask configurations and results are evaluated to determine the retardance magnitude, azimuth and direction of optic axis of the specimen. We report measurements using small calcite crystals that confirm our theoretical predictions.

**Keywords**: polarization, microscopy, conoscopy, birefringence, crystal optic

## 1. INTRODUCTION

The polarized light microscope is widely used in biological research [1, 2]. Given adequate sensitivity, it provides the means to observe structures in biological cells and tissues that are otherwise either difficult to see or require staining or labeling with exogenous dyes for adequate contrast. Furthermore, with the polarizing microscope we can measure not only the birefringence magnitude, but also the orientation of the birefringence axes in the specimen, thus providing information on the submicroscopic alignment of molecular bonds and fine structural form. However, the principal axis of alignment, or birefringence axis, is typically determined only in the plane perpendicular to the microscope axis. Only two, very limiting methods exist to observe the birefringence component parallel to the microscope axis. One such method is called conoscopic imaging which typically is applied to single crystals. The other method uses a special Universal Rotation Stage that includes two glass hemispheres encapsulating the specimen. Neither method is compatible with biological specimens which typically require immersion objectives for high resolution imaging (incompatible with Universal Rotation Stage) and include many birefringent structures that are too faint to observe conoscopically. Therefore, we are developing an aperture scanning polarizing microscope that combines conoscopic and orthoscopic type observations in a single measurement process that is applied to all resolvable volume elements simultaneously. We briefly describe the liquid crystal based aperture scanning device which contains no mechanically moving parts. We then demonstrate the application of the scanning device by observing small calcite crystals under oblique illumination. We find good agreement between the measurements of crystal anisotropy and a theoretical estimate that considers the pencil of rays that emanates from the partially occluded aperture and obliquely passes through the crystal.

## 2. APERTURE SCANNING POL-SCOPE

The Aperture Scanning Pol-Scope builds on an earlier Pol-Scope design [3]. The new aperture scanning device is made of a stack of sectored liquid crystal retarders and polarizers (Fig.1). The stack, which is about 7 mm thick, is placed near the front focal plane of the condenser lens. The sectors of the device independently modulate the intensity and polarization state of the illuminating light. The transparency of the aperture is controlled by 8 pie-shaped sectors that can be configured to simulate a rotatable asymmetric mask. The pie-mask is followed in the light path by the universal compensator which has one central sector and 8 sectors around the perimeter. The birefringence retardation of each sector can be configured independently for the purpose of polarization rectification [4] and for measuring polarization properties of the specimen [5].

*Correspondence: E-mail: mshribak@mbl.edu, rudolfo@mbl.edu, Fax: 508-540-6902


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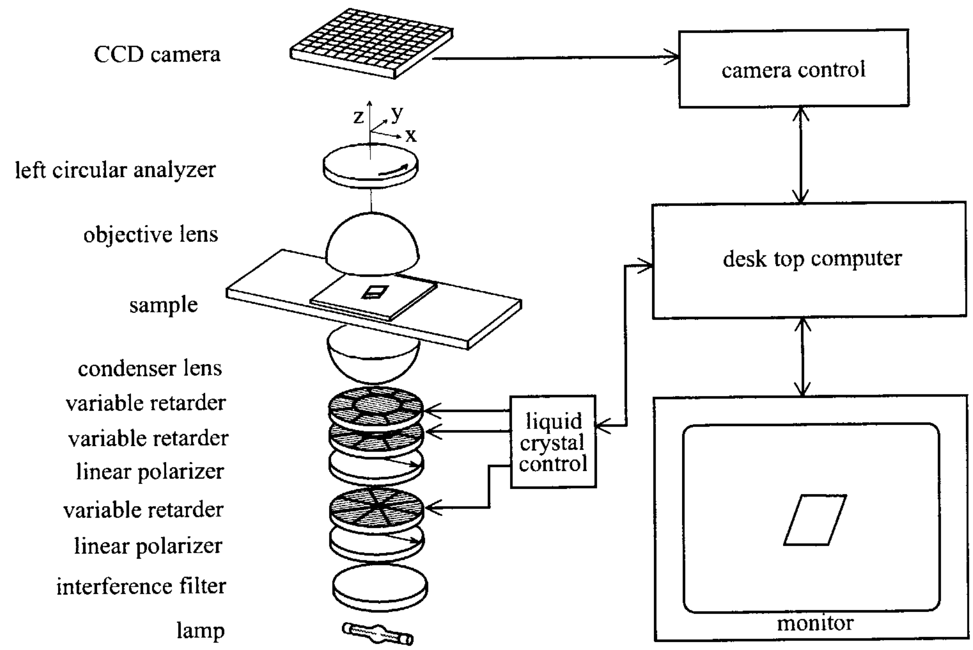


Fig.1. Schematic of the optical and electronic components of the Aperture Scanning Pol-Scope. The aperture scanning device is formed by a stack of sectored variable retarders and polarizers that are located in the front focal plane of the condenser lens. The bottom retarder sandwiched between two polarizers forms a mask of eight pie-sectors whose transparency can be controlled individually. The top two variable retarders form a universal compensator with one central sector and eight sectors around the perimeter. The birefringence retardation of each sector can be configured independently. The working of the universal compensator is described in [5].

## 3. RETARDANCE OF CALCITE CRYSTALS OBSERVED WITH OBLIQUE ILLUMINATION

Using the aperture scanning Pol-Scope we have measured the birefringence retardation of small calcite crystals. The crystals were prepared as described in [6]. Most crystals were several micrometers in diameter and had the well known cleavage form. The crystals were sandwiched between slide and cover glass and were embedded in Permount, a resin that nearly matched the crystal refractive indices. Crystals were generally oriented so that one of the crystal faces was normal to the microscope axis. For the cleavage form of calcite, the crystal optic axis forms an equal angle to the three faces that meet at a blunt crystal corner [7]. Hence, in our experiments the crystal optic axis is oriented at approx. 45¡ to the microscope axis.

We measured the retardance of calcite crystals using several configurations of the pie-mask that is part of the aperture scanning device. Fig. 2 shows the retardance magnitude images of a calcite crystal measured with an open aperture and

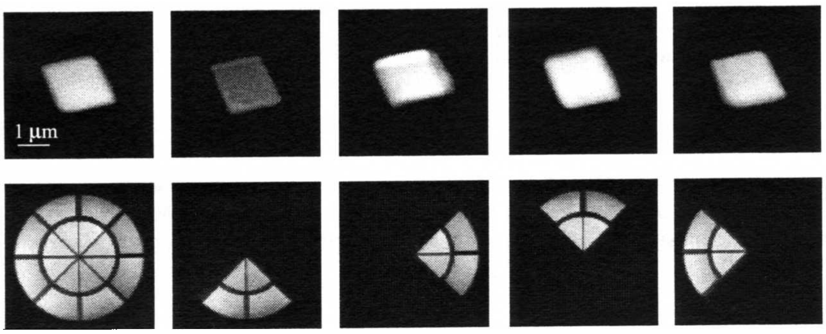


Fig.2. Top row of images show the retardance magnitude images of a calcite crystal that was observed with aperture mask configurations that are shown in the bottom row of images.

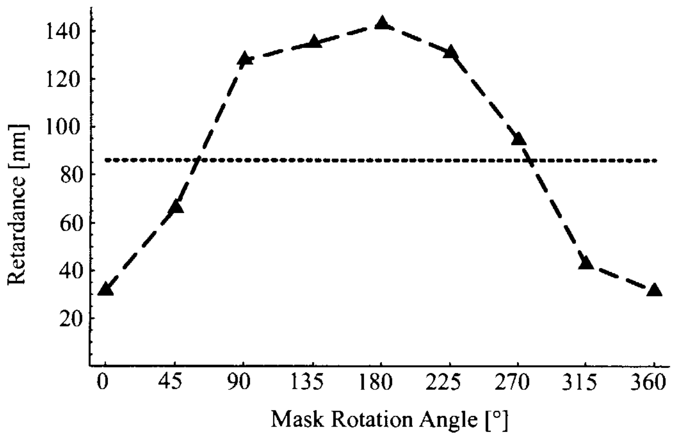


Fig.3. Measured retardance magnitude of a small calcite crystal versus rotation angle of quarter mask. Dashed line connecting data points is drawn to guide the eye. Horizontal dotted line represents retardance magnitude value measured with open aperture.

four different orientations of the quarter mask. Each magnitude image was computed based on raw images that were recorded using 4 settings of the universal compensator as described, for example, in [5]. Fig. 3 shows the plot of the retardance magnitude measured in the central region of the crystal versus the rotation angle of the mask.

The measured crystal retardance strongly depends on the orientation of the quarter mask. This phenomenon can be qualitatively understood by considering the tilt angle of the pencil of rays that pass through a partially occluded aperture mask and converge on the crystal (Fig. 4). In the schematic of Fig. 4A, for example, the mask orientation is such that the tilt angle and the crystal optic axis are nearly parallel; hence the majority of rays should experience little birefringence retardation and the measured crystal retardance is expected to be small. In Fig. 4B, however, the tilt angle is nearly perpendicular to the optic axis; accordingly, most rays should experience a maximum of birefringence retardation and the measured crystal retardance should be a maximum.

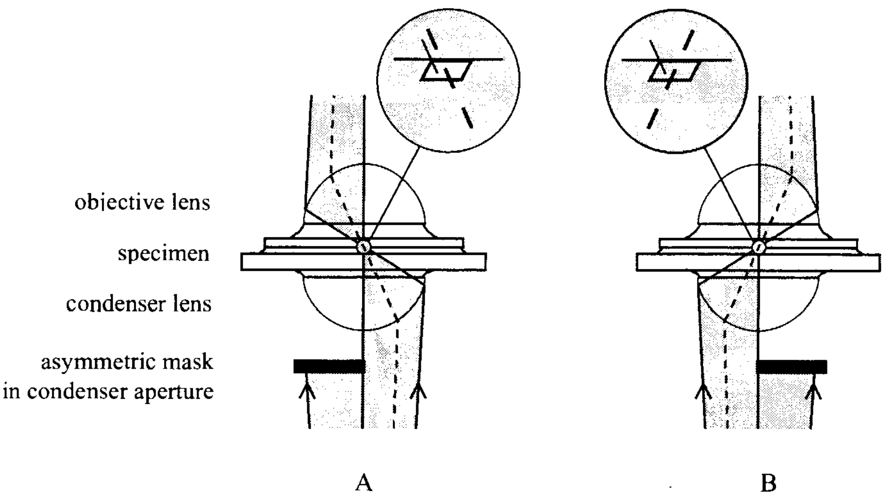


Fig.4. Schematic to illustrate the propagation of an oblique beam created by an asymmetric aperture mask. In A the left portion of the condenser aperture is occluded leading to a center ray that is nearly parallel to the crystal optic axis (see inset). In B the mask covers the opposite part of the aperture and the center ray propagates nearly perpendicular to the optic axis.

## 4. THEORETICAL ESTIMATE OF CRYSTAL RETARDANCE

For a quantitative analysis of the crystal retardance as a function of different aperture mask configurations, we consider the pencil of rays that pass through the center of the crystal. Each ray originates from a point in the condenser aperture and, while passing through the crystal, accumulates a birefringence retardation Δ that depends on the angle σ between the ray and the optic axis of the crystal (see page 699 in [8]). Fig. 5 illustrates the geometric relationship between the light ray, the calcite crystal and condenser aperture with mask. The schematic shows a calcite crystal with optic axis at an angle ω to the microscope axis. One set of crystal faces is perpendicular to the microscope axis and the thickness of the crystal along this axis is *t*.

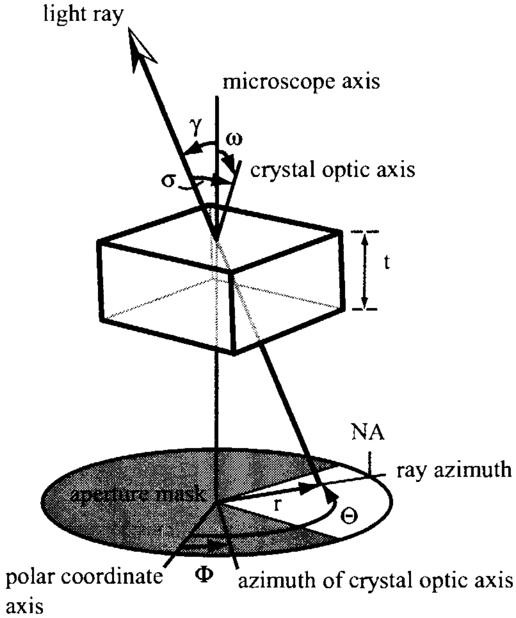


Fig. 5 Schematic of the geometric relationship between the crystal optic axis and the parameters of a light ray passing through the condenser aperture at the point (r,Θ).

The ray is tilted to the microscope axis by an angle γ. The path length of the ray through the crystal is $t/\cos\gamma$. We neglect refraction at the crystal faces because the crystal is embedded in a resin that closely matches the crystal refractive index. Based on these geometric relationships the birefringence retardation Δ can be written as:

$$\Delta = (n_o - n_e)\frac{t}{\cos\gamma}\sin^2\sigma. \tag{1}$$

$n_o, n_e$ are the ordinary and extra-ordinary refractive indices of the crystal.

As illustrated in Fig. 5, the angle σ is a function of the tilt angle ω of the optic axis, the tilt angle γ of the ray, and the azimuth angles Θ and Φ in the aperture plane:

$$\cos\sigma = \sin\gamma\sin\omega\cos(\Phi - \Theta) + \cos\gamma\cos\omega. \tag{2}$$

For the axial ray, for example, expression (1) reduces to:

$$\Delta_0 = (n_o - n_e)\, t\sin^2\omega \tag{3}$$

Expressions (1) (2) and (3) can be combined to obtain the birefringence retardation Δ as a function of parameters $\gamma, \omega, \Theta, \Phi$:

$$\Delta(\gamma, \omega, \Theta, \Phi) = \frac{1 - (\sin\gamma\sin\omega\cos(\Phi - \Theta) + \cos\gamma\cos\omega)^2}{\sin^2\omega\cos\gamma}\Delta_0. \tag{4}$$