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An Improvement of the Instrumental Extinction in the Polarizing Microscope by the Condenser Apodization

A new and simple method of improving the instrumental extinction in a polarization microscope by introducing a segmented hyperbolic diaphragm to the condenser aperture positioned thus that only those parts of the aperture will work, which do not transmit the effect of polarization plane rotation, occuring on lens curvatures.

New applications of the polarization microscope to quantitative examinations in biology and medicine require the devices of considerably better propetries than those appropriate to standard microscopes used in mineralogy and petrography. The detection and measurement of very weak birefringences appearing in biological samples are possible only when systems of high degree of light extinction for the crossed position of polarizer and analyzer are applied. This is necessary to achieve a high contrast with respect to birefringent object the background.

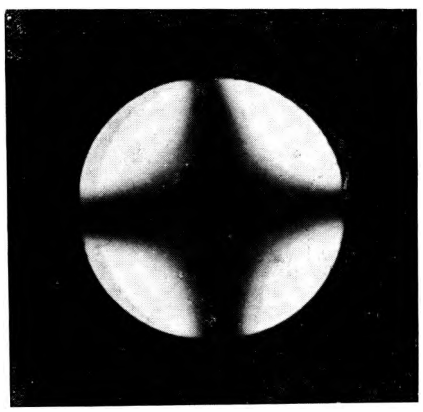

Fig. 1. "Conoscopiccross" in the exit pupil of the objective in the polarization objective for crossed polarizer and analyzer. Objective aperture 0.40

The parameter describing light extinction in the polarizing microscope is the instrumental extinction coefficient E, defined as the ratio light intensity I_0 in the exit pupil of the objective for parallely positioned polarizers to the light intensity I_p for crossed polarizers: $E = I_0/I_p$. Sensitivity of the polarizing system to the birefringence detection increases with the square root of the extinction coefficient [1, 2]. Its maximization is therefore of great importance for polarizing microscopes used in biological investigation.

The extinction coefficient depends on many factors [1]: the quality of used polarizers, stress induced birefringence occuring in the optical elements of the microscope, the cleanliness of the optical elements and anti-reflection layers used for elimination of reflections in the system. Optimal conditions may be achieved by properly selecting the elements of the system [1, 3]. An intrinsic disadvantage here is that especially when objectives of large aperture are used for fine structure observations, an effect of polarization plane rotation occurs on lens curvatures. This phenomenon is manifested by the ap-

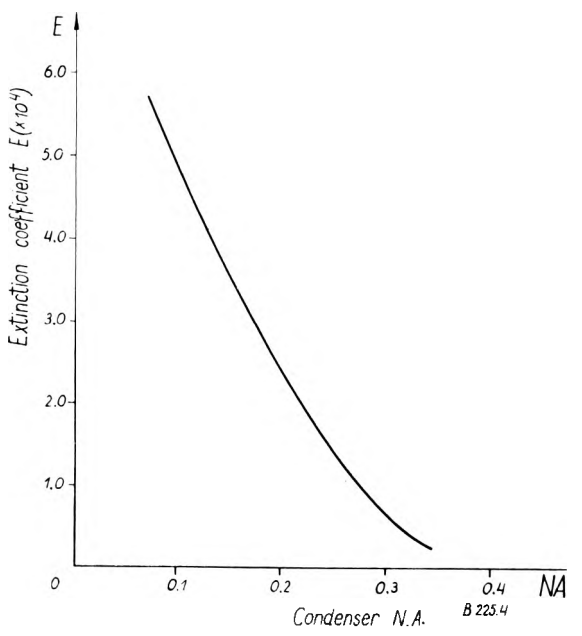

Fig. 2. A decrement of the instrumental extinction E of the system as a function of the condensor aperture NA

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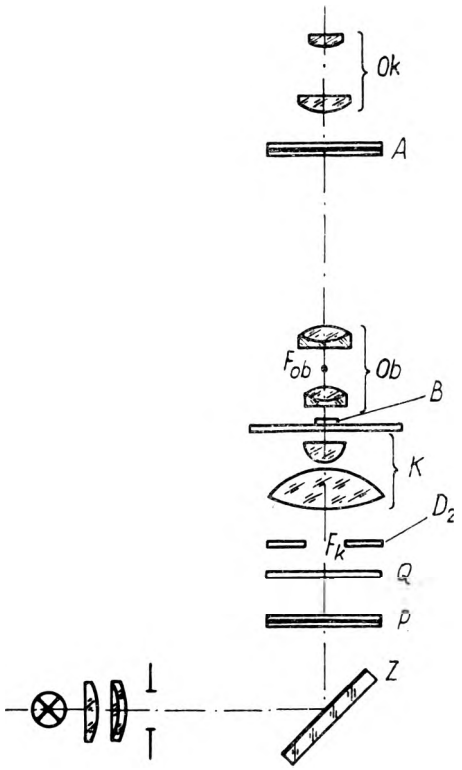


Fig. 3. Scheme of the polarization microscope with the special aperture diaphragm D_2 placed in the focal plane of the condenser

pearance of the conoscope cross in the exit pupil of the objective (Fig. 1), which is, in turn, associated with a rapid decrement of the instrumental extinction coefficient. As may be seen in the Fig. 2 the decrement of the coefficient with the increase of the condenser aperture is considerable, but on the other hand, a covering up of the condenser aperture results in a considerable loss in the resolving power of the microscope and precludes, therefore, an observation of fine details of the sample.

A solution of the problem without diminishing the aperture was given by [4]. Compensation of the said rotation may be accomplished by introducing one under the condenser and the other above the objective two meniscus with a half-wave plate. Some good results have been obtained in this way, however, the system requires a good fitting of both the meniscus to the condenser, objective and illuminating systems, as well as a good matching of the half-wave plate to the single wavelength and a precise adjustment of the whole system.

An improved extinction may be achieved without considerable diminishing the resolution of the system in a simpler way. The method consists in shaping the aperture diaphragm of the condenser in such a way that it covers only those parts of the beam, which are responsible the largest rotation of the polarization plane. The region where there is no rotation of crossed polarizers is visible in the form of

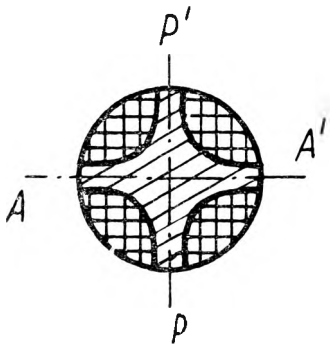


Fig. 5. An image of the exit pupil of the polarization microscope objective with crossed analyzer and polarizer

a dark cross in the exit pupil of the objective, and the diaphragm should consist of four elements of hiperbolic shape located symmetrically with respect to the directions of light vibrations in the polarizer and analyzer, which cover the light regions between the conoscopic cross hands. A scheme of a microscopic polarization system supplied with that kind of diaphragm as well as its operation is presented in Figs. 3–6. The hyperbolic diaphragm is located in the φ/Fe focal plane F_k of the condenser K. It consists of four

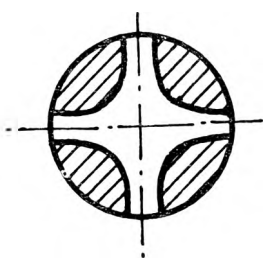


Fig. 4. Aperture diaphragm under the condenser. A view from the upper side

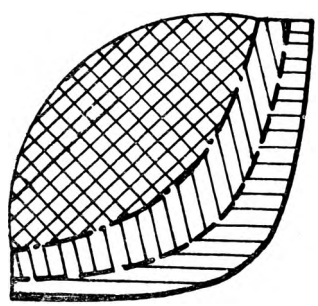


Fig. 6. A diaphragm element consisting of segments

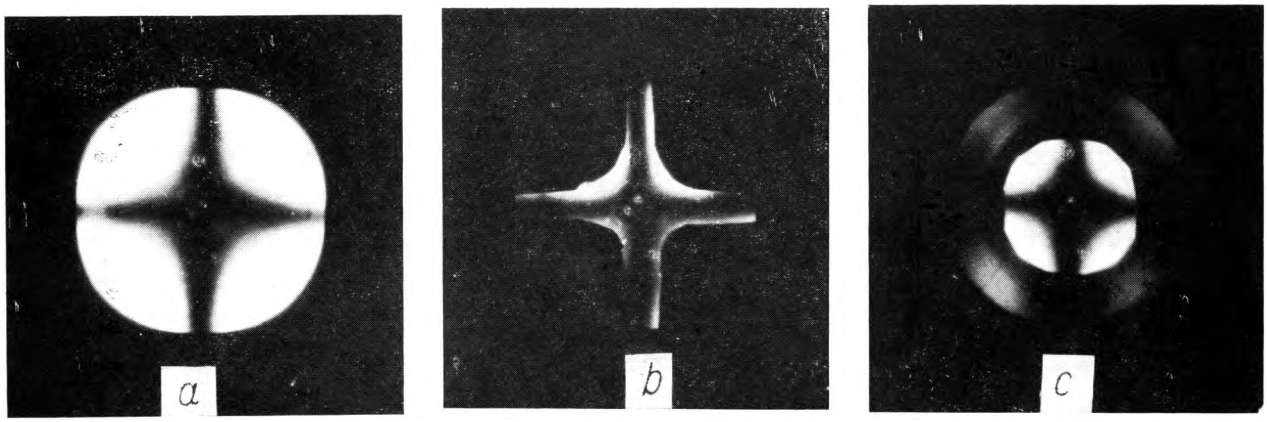
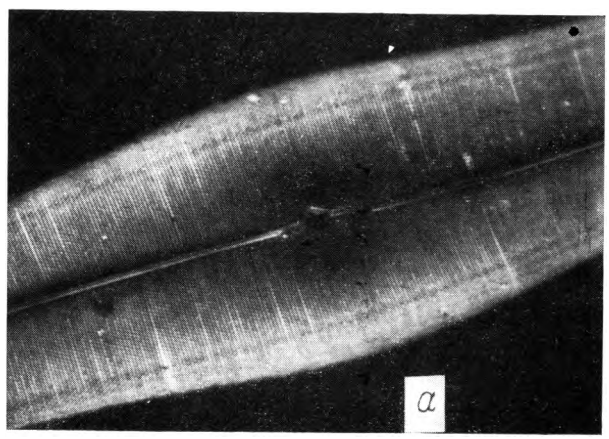


Fig. 7. An image of the exit pupil of the objective 40x/0.68 for crossed polarizers:
a) without diaphragm in the condenser ($A = 0.68$), b) with the diaphragm, c) with a reduced aperture ($A = 0.3$)



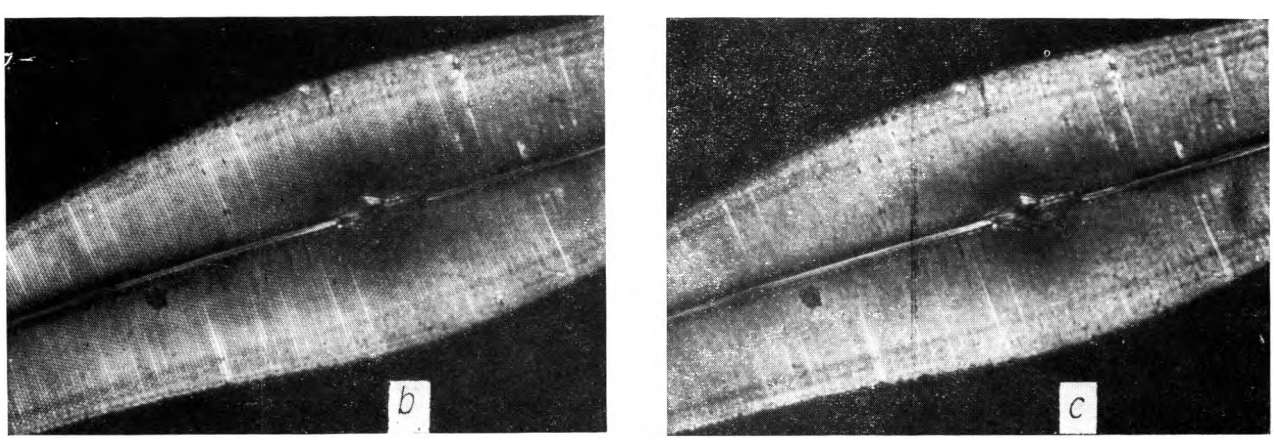


Fig. 8. Pleurosigma angulatum observed through an objective 40x/0.68:
a) with the whole aperture of the condenser ($A = 0.68$), b) with a hyperbolic diaphragm, c) with a reduced aperture of the condenser ($A = 0.3$)

elements, each being shaped as a equiaxial hyperbola (Fig. 4). The diaphragm may be rotated about the optical axis of the microscope and its elements may be shifted perpendicularly to this axis.

The proper position of the diaphragm D_2, with crossed polarizer P and analyzer A and the excluded compensator Q, occurs when the images of its elements are localized in the focal plane of the objective F_{ob} and distributed symmetrically with respect to the dark hands of the cross (Fig. 5). The dark cross determines the directions of the vibrations of both the polarizer PP' and analyzer AA'. The diaphragm elements (the grided regions in Fig. 5) enter the region between the cross hands and block out the fields in which the rotation of the polarization plane appears. The proper positioning of the elements of the diaphragm D_2 in relation to the cross hands may be accomplished by rotating the diaphragm about the optical axis of the microscope and shifting its elements in the direction perpendicular to the microscope axis.

In order to match the diaphragm elements to the dark conoscopic cross better we may apply elements, consisting of several segments, each being shaped approximately like the equiaxial hyperbolas (Fig. 6). Particular segments correspond to hyperbolas of an equally great axis so that it is possible to fit them to a more or less spread conoscopic cross. The respective segment is chosen by shifting it in relation to others so that its boundary is an envelope of the diaphragm elements D_2.

An improvement of the instrumental extinction coefficient in the polarization microscope was easily observed after the employment of the diaphragm. At a whole condenser aperture and with diaphragm elements set in a manner that the intensively shining region is extensively covered the instrumental extinction coefficient was improved by factor 10 and was smaller at a smaller coverage. An equivalent extinction coefficient without the diaphragm was achieved by diminishing the condenser aperture to less than one half of its whole value.

The symmetrical shape of the diaphragm ensures a uniform illumination of the sample. The whole aperture is exploited in two mutually perpendicular directions and by the same means the resolution of the microscope in these two directions is the same as for the completely clear condenser aperture.

An image of the exit pupil of a 40x objective without diaphragm at the aperture $A = 0.68$ is presented in Fig. 7a, while 7b illustrates the situation after the diaphragm has been installed. Figure 7c presents an image of the exit pupil in the same system after the condenser aperture was reduced to the limit, and when the extinction coefficient was the same as that obtained with diaphragm ($A = 0.3$).

The influence of condenser aperture changes on the system resolution is illustrated in Fig. 8. Pleurosigma angulatum was used as a fine structure test sample. It was observed with the help of a 40x microscopic objective with the condenser aperture $A = 0.68$ completely opened (Fig. 8a) and with the hyperbolic diaphragm included (Fig. 8b) as well as with the aperture reduced to 0.3 (Fig. 8c). Photographs 8a, b and c correspond to Fig. 7a, b and c. While the fine structure of the sample is visible in picture 8a and b the fine details in Fig. 8c are no more divided.

It seems that the solution proposed may be successfully used in those systems where a high instrumental extinction coefficient is required simultaneously with the great aperture of the beam illuminating the sample to be examined: the simplicity of the solution being an additional advantage.

References

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