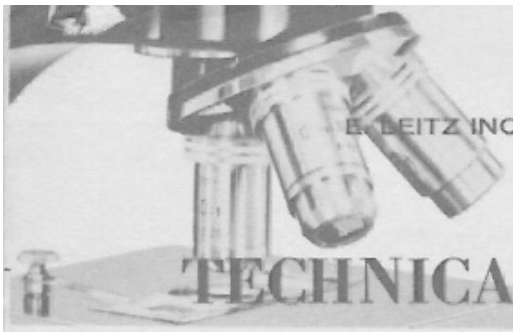

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# TECHNICAL INFORMATION BULLETIN

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## THE MEASUREMENT OF OPTIC PATH DIFFERENCES IN A POLARIZING MICROSCOPE

Polarizing Microscopes are used to study the behavior of materials in polarized light. The specimen is illuminated with linearly polarized light. This light vibrates in the direction of the plane of vibration of the polarizer. The analyzer is mounted above the objective, its plane of vibration has an orientation at right angle to that of the polarizer. The purpose of this examination is to find out whether or not the specimen influences the linear state of polarization of the illuminating light.

If it exerts no such influence, all the light coming from the polarizer will be extinguished by the crossed analyser. However, if it does exert an influence, then the light emerging from the specimen will have a component in the direction of the analyzer, and the specimen will appear bright on the extinguished dark background.

The degree and type of influence caused by the specimen upon the linearly polarized light coming from the polarizer, allows us to draw far reaching conclusions, as to what type of material is present, its orientation, even as to its submicroscopical morphology.

This can be done quantitatively by measuring the change in the state of polarization of the light emerging from the specimen. In general, this light will be elliptically polarized. The ellipticity is determined by two quantities: The geometric thickness "t" of the specimen, and its effective birefringence $(n_2-n_1)$. The product of these two is called the retardation $\Gamma$ .

The method of measurement is almost exclusively one of compensation. So called compensators are inserted below the analyzer. They consist of birefringent plates, the orientation of which can be reproducibly changed. Depending on the orientation, they also introduce path differences, or retardation, or polarize light elliptically. However, they can be oriented so as to create exactly the same influence which the specimen has exerted, only in the reverse. In that orientation, the specimen influence is compensated for exactly and the light leaves the compensator as if it had never passed the specimen; it is again linearly polarized and is completely extinguished by the analyzer.

There are several different types of compensators. Two considerations determine the choice of any one of these. First, the compensator must be able to compensate for a higher retardation than is introduced by the specimen. Otherwise, compensation would not be reached. Second, this overlap should not be too great. If the free measuring range of the compensator just about exceeds the retardation introduced by the specimen, one obtains, of course, a higher measuring accuracy.

The most commonly used compensators are the following:

1) Quartz Wedge

Graduated Quartz Wedges are the simplest means for the measurement of retardations. The accuracy of such wedges is limited when it comes to the measurement of small retardations. However, quartz wedges are excellent means to demonstrate the retardation phenomena.

2) Berek Compensator

This consits of a calcite plate cut vertically to its optic axis. The plate can be tilted, which changes the ellipticity of its index ellipse, and accordingly its compensating retardation. The compensator usually covers a measuring range of four orders, or 2200 m $\mu$ path difference. Compensators with thicker calcite plates for the measurement of a higher number of orders are however also available. The accuracy is approximately 2 m $\mu$ in the lower part of the measuring range. The measurement is carried out by tilting the calcite plate first in one direction until the specimen is compensated, and then in the opposite direction. The angle of tilt of the calcite plate, necessary to compensate for the specimen, is then calculated by

$$i = \frac{a-b}{2}$$

wherein i is the angle of tilt, and a and b the readings on the compensator drum.

The retardation is calculated by

$$\log \Gamma = \log C + \log f(i)$$

Each Berek compensator comes with an instruction booklet which includes logarithm tables and the optical constant C for the specific compensator, both of which are necessary for the calculation.

The compensator can also be calibrated very easily. This is usually done by plotting the retardation against the scale on the compensator drum. The retardation of any compensator setting is then to be read off this calibration curve.

3) Senarmont Method for retardation measurements

This method serves for the measurement of retardations up to one order. A 1/4 wave plate in subparallel position is used. It superimposes a circular vibration over the elliptical vibration emerging from the specimen. The resultant is a linear vibration, but its plane of polarization has been rotated against the analyzer's plane of vibration. This angle can be measured by rotating the analyzer until the specimen appears extinguished. The retardation is a direct function of this angle, and given by:

$$\Gamma = \frac{\lambda \times \alpha}{180}$$

wherein $\alpha$ is the angle of rotation for the analyzer.

If a 1/4 wave plate for 540 m $\mu$ is used and the measurement is carried out in monochromatic light of this wave length, the equation simply reads:

$$\Gamma = \frac{540 \times \alpha}{180}$$
$$= 3 \times \alpha$$

4) Brace-Koehler Compensator

This consists of a thin Mica plate with a retardation of either 1/10, 1/20 or 1/30 wavelength. The Mica plate can be rotated in its mount, thus changing the ellipticity of the elliptical vibration. The maximum measuring range is the retardation of the Mica plate. Brace-Koehler compensators are extremely sensitive, and under favorable conditions a retardation as small as 0.5 Å units can be detected.

All of these compensators are used principally in the same way. The specimen is rotated to extinction and then rotated 45° to a position of maximum brightness. The compensator is then inserted. Now it is adjusted until the retardation of the specimen is compensated, that is, until the specimen appears extinguished again. Since the retardation which is introduced by the compensator at each setting is known, we also know the retardation resulting from the specimen.

References:

Dr. Peter H. Bartels "Polarized Light Analysis", Microchemical Journal 7, 98-119 (1963) Volume 7, No. 1, May 1963.

For technical reasons we supply two types of quartz wedges for Leitz Polarizing Microscopes. The modern microscopes, equipped with a centerable nose piece, require a short quartz wedge, while the microscopes with individual centering clutch can be used either with a short or a long wedge. The prices and code words for the two types of wedges are as follows:

Quartz Wedge 1 to 4th order, in short mount,
for use with centerable nose piece or clutch, KORAZ . . $ 75.00

Quartz Wedge 1 to 4th order, in long mount,
for individual centering clutch, IIVZD . . $ 75.00

HEATING MICROSCOPE FOR HIGH TEMPERATURES

Our catalog No. 73-3a and 3b indicates the possibility of using the Heating Microscope Oven up to 1800° C. We would like to caution users as follows:

If the thermo couple does not lie exactly in the middle of the oven and if the user in some way neglects to make all the necessary adjustments correctly and finally if the inherent error in the reading system is added, then it is possible for the oven to become heated higher than 1800° C and it will burn through. We therefore suggest that the user of the heating microscope does not go beyond 1750° C on the reading instrument, and if the oven is to be heated to a high temperature for an extended period of time, one should not go much beyond 1600°.

EXAMINATION OF SMEARS

The continued high interest in bacteria identification by means of the fluorescent antibody technique suggests a reference to an article by Bernice M.Thomason, William B. Cherry, Betty R. Davis and A. Pomales-Lebrón of the US Department of Health, Education, and Welfare, Public Health Serivce, Communicable Disease Center, Atlanta, Georgia and the Department of Microbiology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

The use of dark field condensers in the illuminating system is recommended, and we quote from the above paper as follows:

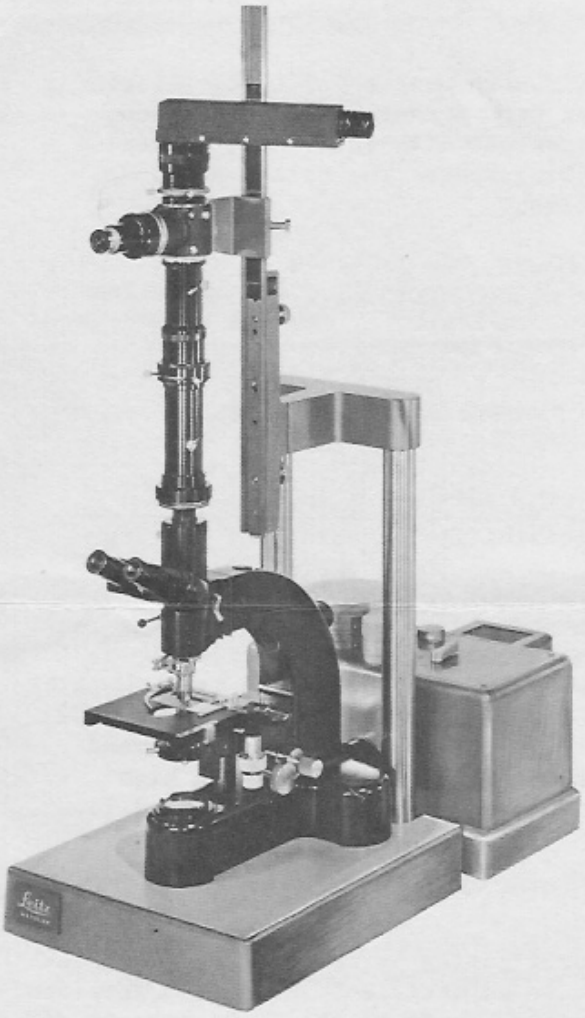
1) Examine smears under a microscope fitted with a cardioid-type darkfield condenser and illuminated by an intense light source such as the Osram HBO-200 mercury vapour lamp. This light source is available in the equipment supplied by several manufacturers. An auxiliary incandescent lamp aids in focussing the smear and supplies useful information concerning the unstained bacteria and debris in the preparation.

2) Oil the condenser to the slide employing fluorescence-free immersion oil. Cargille's Type A, Very Low Fluorescence, Non-drying Immersion Oil for Microscopy may be obtained from Fisher Scientific Co., USA. Its refractive index is 1.515 at 25° C.

3) Bring the area to be examined in focus under the low power objective.

4) Switch to the oil immersion lens.

MICROPHOTOMETRY



The illustration shows a set-up, consisting of a research microscope with photo micrographic accessories and photo multiplier, used extensively for cytochemical tests and previously explained in volume II No. 3 of this bulletin. This measuring method is used more and more to gather quantitative information in microscopic investigations, for instance, for the interpretation of auto radiographs. The microspectrometric measurements of primary or secondary fluorescence can be determined with a sensitive photo multiplier unit. Certain requirements must be met to reduce the signal to noise ratio, which is done by means of a chopper or the artificial cooling of the multiplier.

Microdensitometric measurements of photographic emulsions, such as spectrographs, x-rays, electron diagrams of a non-biological character can also be evaluated by means of this micro photometric method. The evaluation of the degree of reflection within a given wavelength or of the entire spectrum are possible; then there is the field of coal petrography and ore microscopy for the determination of the degree of coking of the coal and the identification of ores. In the last mentioned cases the reflection measurements are generally carried out in polarized light. Furthermore diffuse reflection and its relation to the spectral characteristics of the surface can be measured photometrically on such samples as papers, plastics, lacquer, tapes, etc.

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