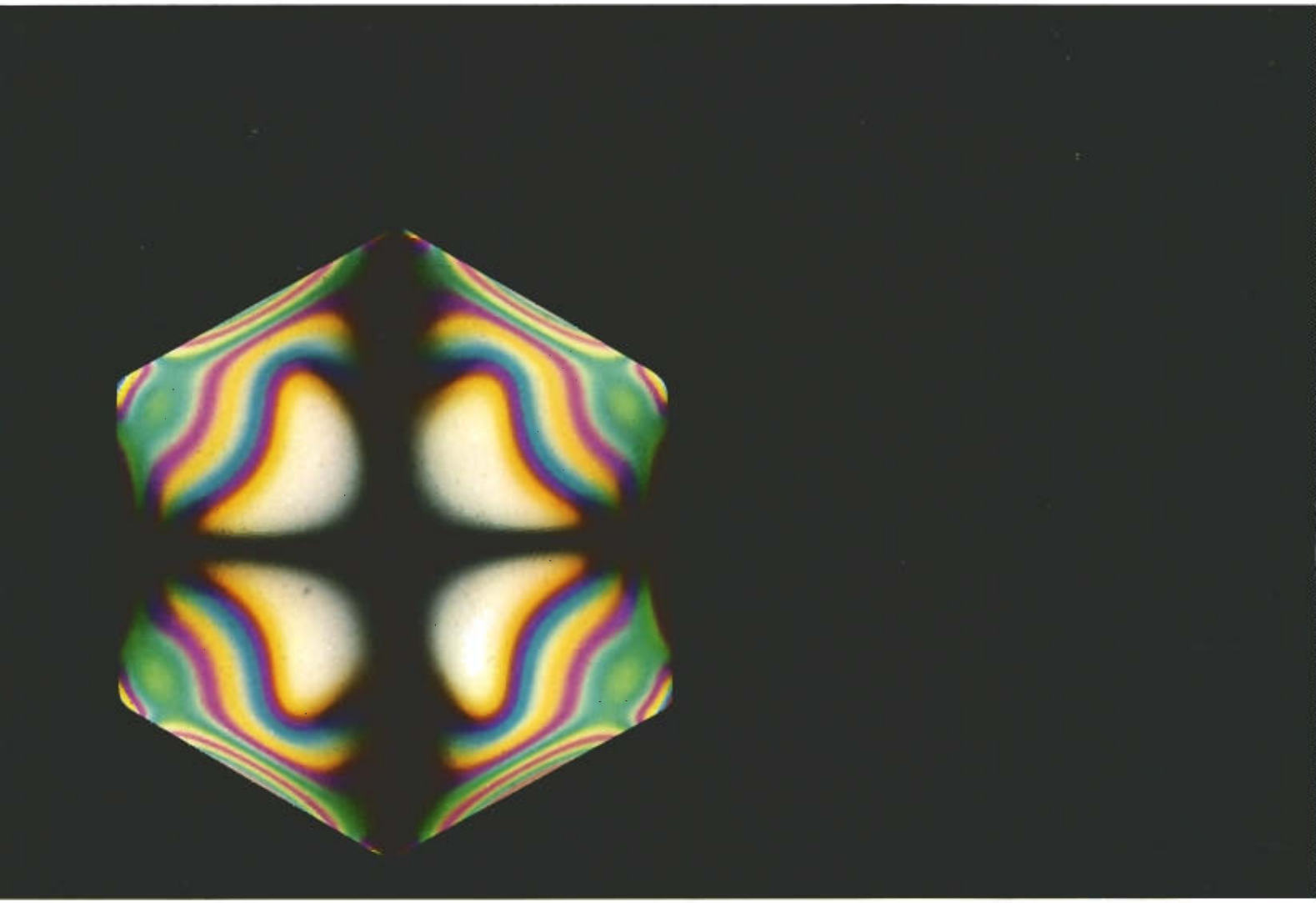
Polarized light microscopy

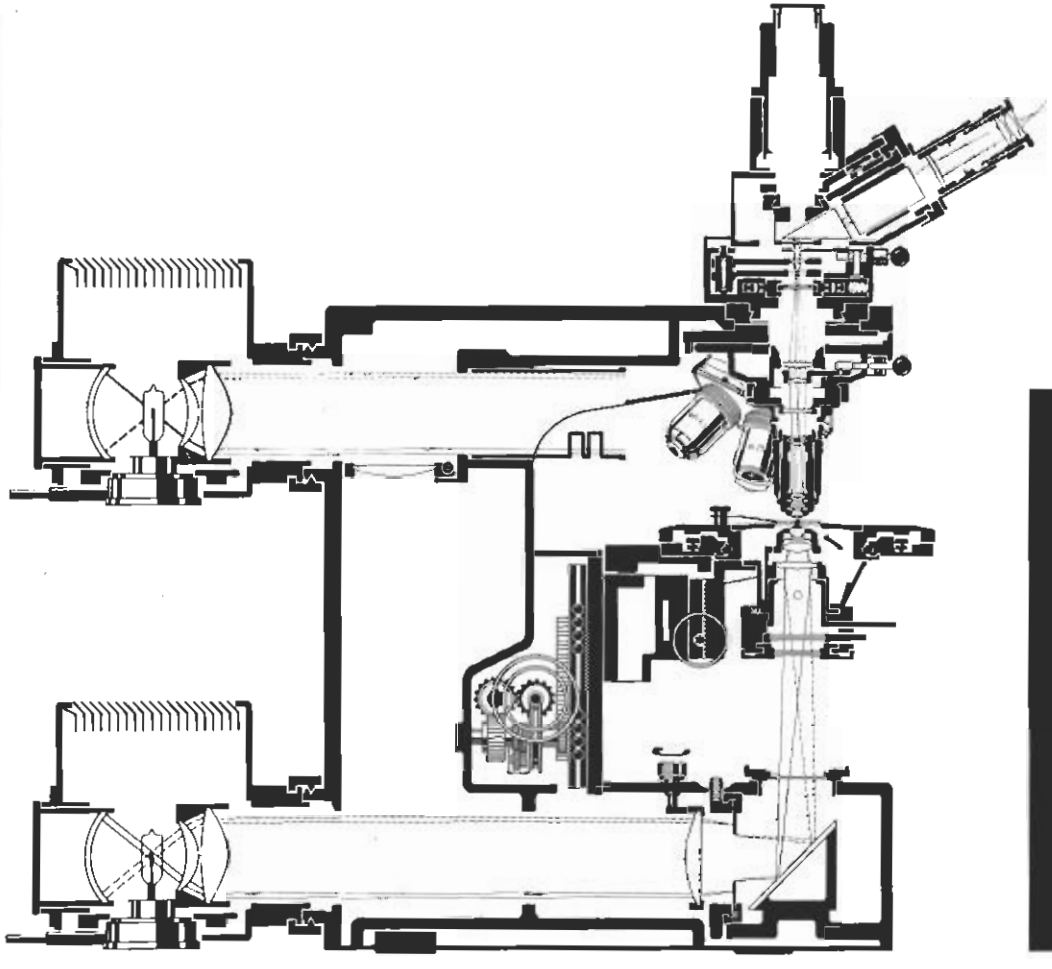
Principles, instruments, applications

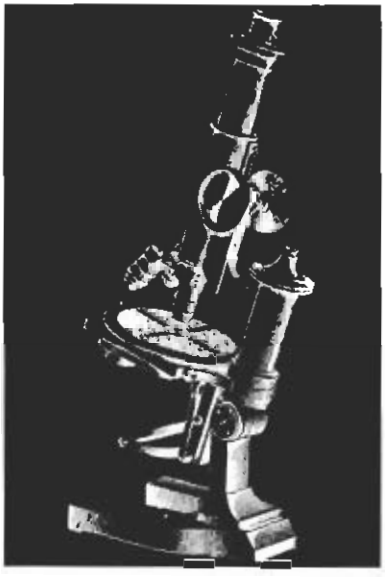
Walter J. Patzelt



Cover-charge

ORTHOPLAN-POL polarizing microscope, beside it the first Leitz polarizing microscope of 1885.





Polarized light microscopy

Principles, instruments, applications

3rd Edition

Ernst Leitz Wetzlar GmbH

Preface

Although the naked human eye cannot detect or analyze polarization of light, the bee, however, uses the part polarization of daylight, caused by the sun's position, for orientation purposes, even on a cloudy day.
This phenomenon was possibly also known to the Vikings before the invention of the compass. Dichroic crystals of optical quality found in graves may have been used as "analyzers", and thus as optical compasses.
Henry Fox Talbot was the first to equip a microscope with polarizers, in 1834. During the next few years, the demand for polarizing microscopes was still rather small. Petrographers were content to fit their existing microscopes with Nicol prisms made by Ernst Leitz in Wetzlar, amongst others. In 1885, the Leitz® company introduced a special polarizing microscope. Transmitted light polarizing microscopes soon became the most important tools for mineralogists and geologists, for they made it possible, even then, to determine several specimen parameters quantitatively with the same instrument.

At Leitz, Max Berek (1886–1949) in particular was intensively engaged in polarized light research. This resulted in numerous crucial publications and important new designs. The polarizing methods used at first only by petrographers were employed increasingly in industry. Pioneering work on the medical and biological application of polarized light microscopy was carried out by W. J. Schmidt at the Justus-Liebig University in Giessen, the closest to Wetzlar.
In the century since then, Leitz has produced more polarizing microscopes than any other manufacturer. They can be found in all corners of the earth, and are still in daily operation after decades of use. The current models, the LABORLUX® 11 POL, LABORLUX 12 POL, ORTHOLUX® 2 POL BK and ORTHOPLAN® POL, reflect this experience.
The first edition of this brochure was published in 1974. It was re-printed many times and has also appeared in German and French. This 3rd edition includes all the most important developments.

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Wetzlar, 1st October 1985
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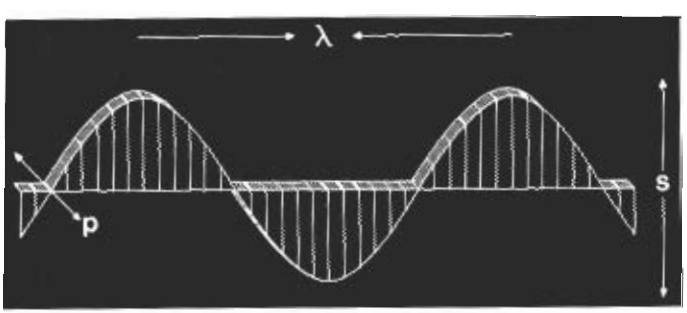
Contents

I. Principles

The wave nature of light

Light is an electro-magnetic vibration. Most phenomena occurring in polarized light and interference microscopy can be explained if light is imagined in terms of transverse vibrations of a rope fixed at one end. If movement is imparted to the rope at the loose end, a wave will progress in the longitudinal direction of the rope.
Determinants for light vibrations (Fig. 1) are:
Wavelength,
frequency,
propagation direction,
vibration direction,
velocity of propagation,
intensity.

Fig. 1:
Wave length λ, vibration direction s, and polarizing direction p of a transverse wave. With electro-magnetic waves s corresponds to the electric vector, t to the magnetic vector.



Wavelength λ

The wavelength is the distance between two identical points on a wave (Fig. 1) such as the distance between two wave troughs (minima) or between two wave peaks (maxima).
The wavelength of electro-magnetic vibrations is generally described with the Greek letter λ (Lambda). The human eye can register only a very narrow range, the "visible range", of the broad spectrum of electro-magnetic waves (Fig. 2).
The unit of measurement in optics is the nanometre (1nm = 10^{-9} m). Occasionally the Ångström (1 Å = 10^{-10} m) or the micrometre (1 μm = 10^{-6} m) is used. Indications of wavelength are always referred to vacuum, because in matter the wavelength becomes shorter (p. 11). The visible portion of the sunlight reaching the earth has on average the spectral composition illustrated in Fig. 3. The human eye sees the brightest region of this spectrum at about 550nm (green).
The eye is unable to select any given wavelength from mixtures of various wavelengths such as sunlight or incandescent light. In fact, it perceives a general impression, such as daylight, which is seen as white. If from this white mixture a part is removed by means of a filter, selective reflection or dispersion, the eye will receive the impression of a color (blue of the sky, a rosy dawn, the color of objects etc).

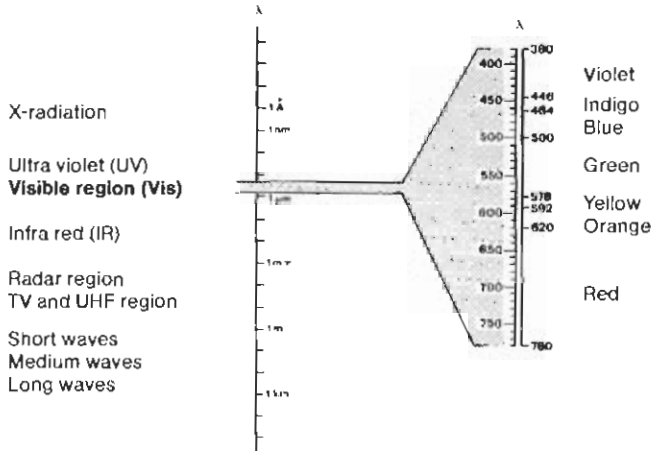
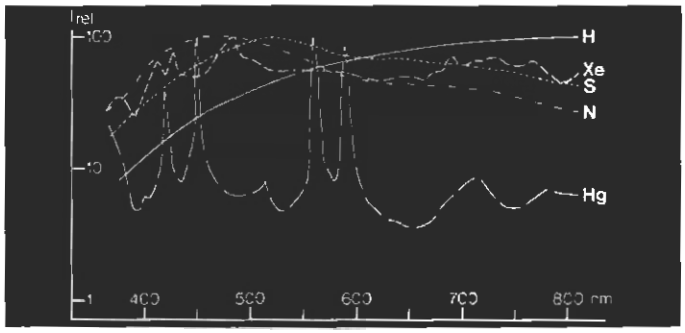


Fig. 2:
The range of electro-magnetic waves

Fig. 3:
Relative spectral composition of various light sources. The brightest area in the visible range is always referred to 100 (logarithmic scale)
H tungsten halogen lamp
Xe ultra-high-pressure xenon lamp
S sunlight (mean)
N north light (mean)
Hg high-pressure mercury lamp



Frequency

The number of vibrations per second is called frequency. Within the visible region its order of magnitude is $10^{15} s^{-1}$ (Hz). The frequency of an electro-magnetic wave is constant. It does not change when the wave penetrates matter but its velocity and wavelength are reduced (Fig. 7).
Whereas in the region of the radio waves (Fig. 2) frequency is used to describe their size (MHz, kHz), in the optical region it is not used in practice. Here, the wavelength λ, referred to in vacuum, is almost always used for the accurate determination of radiation.

Polarization

Linear polarization

If vibrations are generated in a loosely hanging rope, these will usually be propagated in a certain plane. This is called the plane of vibration or vibration direction (see Fig. 1).
Electro-magnetic waves, too, vibrate in certain planes. Light which contains only rays of the same vibration direction is called linearly polarized light. Light which consists of an infinite number of different vibration directions is called natural light. It is immaterial whether this is light of a single wavelength (monochromatic light) or a mixture of various wavelengths (e.g. white light). The direction of the electric vector is called the vibration direction. The polarization direction, on the other hand, corresponds to the magnetic vector, which is perpendicular to the electric one. For the explanation of phenomena of polarized light only the vibration direction is used in our context.

Without aid, the human eye is not able to distinguish between polarized and natural light.

Circular and elliptical polarization

A rope can be excited to exhibit spiral forms of vibration: the vibration direction changes continously.
One can imagine any such three-dimensional vibration to be composed of two linearly polarized vibrations of the same frequency but perpendicular to each other and a certain phase displacement (cf. Figs. 6, 7). In wave optics the phase difference is generally indicated with the Greek capital letter Γ(Gamma). If the phase displacement is precisely $\lambda/4$, $3/4\ \lambda$, $5/4\ \lambda$ ... (Fig. 4), circularly polarized vibration is produced, i.e. its cross section is circular (Fig. 4). With other phase differences an elliptically polarized vibration is produced, i.e. its cross section is elliptical (Fig. 5). Phase differences of 0, $\lambda/2$, $3/2\ \lambda$ produce a linearly polarized vibration.

Polarizers serve for the production of light waves of uniform vibration direction (Fig. 21). Various polarizers are described on p. 24.

Interference

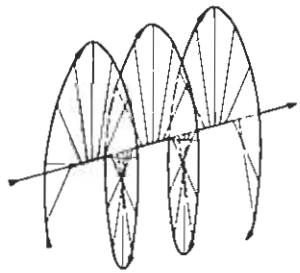
Electro-magnetic waves can combine exactly like mechanical waves provided conditions are suitable, for instance when light is coherent, i.e. two waves meet the following conditions:
a) the same vibration direction (polarization)
b) the same wavelengths
c) common origin in the same point of a light source.

If combining waves vibrate in phase, the amplitudes of the two vibrations (Fig. 6a) add up to a vibration of greater amplitude (intensity).

If, one the other hand, the original vibrations are exactly opposite (phase difference $\lambda/2$, $3/2\ \lambda$ ... wave troughs coincide with wave peaks), both amplitudes will be extinguished (Fig. 6b).

Fig. 4:
Composition of two waves of the same amplitude, vibrating vertically to one another, at a phase difference of $\lambda/4$ from a circularly polarized vibration.

Fig. 5:
Elliptically polarized vibration.



If the phase displacement Γ of the two waves is neither a whole nor half a wavelength, the resultant vibration may be greater or smaller or equal to that of the original wave (Fig. 6c). If only the wavelengths, but not the vibration directions of the two original waves are identical, no interference takes place. The resultant is an elliptically or circularly polarized vibration (Fig. 4, 5).

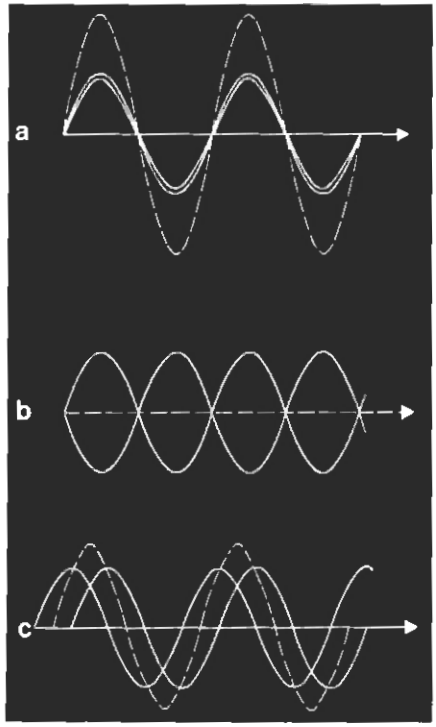


Fig. 6:
Interference of light waves of the same amplitude (intensity)
a Phase difference = 0
b Phase difference = $\lambda/2$
c Phase difference = $\lambda/4$

The resultant wave is always drawn in broken lines.

Propagation of light in vacuum and in matter

Transparent and opaque objects

Substances that transmit visible light are called transparent. Many substances which at first sight appear opaque, such as rocks, become transparent in thin sections and can therefore be investigated with transmitted-light microscopy.
Substances which even in the form of very thin sections are little if at all transparent are called opaque. They include, for instance, coal, metals, and ores. With some opaque objects, transmitted-light investigations can be carried out at a layer thickness of $< \mu$m. Because of considerable difficulties in preparing such sections, incident-light microscopy (p.83) of polished sections is usually preferred to transmitted-light investigation.

Velocity of light and refractive index

The velocity of light in a vacuum is 299550 km/sec for all electromagnetic waves. In transparent objects, however, the velocity of light is less. The factor by which the light is faster in vacuo (air) than in a given medium is called the *refractive index* n. Water, for instance, has a refractive index n = 1.33. The velocity of light in water is therefore 225000 km/sec.
Other examples:

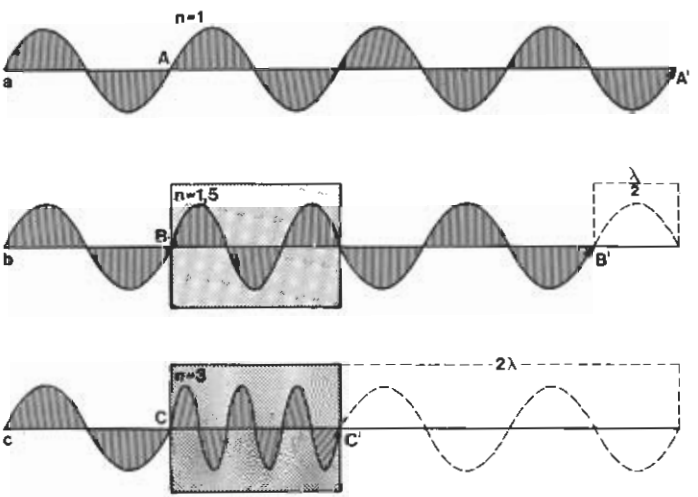
air	1.0003*
ice	1.31*
fluorite	1.43
glasses	1.4...2.1
rock salt	1.54
garnet	1.9
diamond	2.42
rutile (TiO_2)	2.7*

* birefringent

The refractive index is a parameter which is calculated for the identification and determination of solids and liquids (p. 93). The refractive index depends not only on the material, but, within narrow limits, also on the wavelength. This influence of the wave length on the refractive index is called *dispersion*. Temperature, too, affects the refractive index. Temperature and wavelength must therefore be stated with accurate indications of the refractive index, for instance $n_e^{23} = 1.5521$* or $n_D^{20} = 1.5543$**.

* e indicates the line at 546.1 nm for mercury vapour.
** D is the double line of sodium vapour at 589.0 and 589.6 nm. Refractive indices are often stated for one of these wavelengths.

Fig. 7:
Retardation of a wave front (phase displacement) after passage through media of various refractive indices:
a) Vacuum (retardation 0)
b) Medium of refractive index n = 1.5 (retardation $\lambda/2$)
c) Medium of refractive index n = 3 (retardation 2 λ)



Phase differences

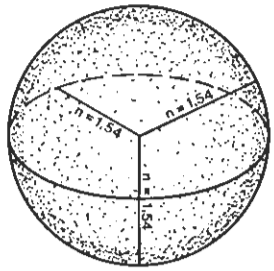
Fig. 7 shows three initially phase-coincident waves a, b, c and their differential retardation as they pass through media of different refractive indices: the three media are reached by the wave trains at the points A, B, C at the same instant (plane wave front).
After three vibrations (phases) the waves reach the points A', B', C'. Wave b has been retarded by half a wavelength ($\lambda/2$) compared with wave a, and c has been retarded by two wavelength units (2λ) (deformed wave front). Such retardations in optics are called *phase differences*. The unit of measurement is referred to in either wavelength units (e.g. $\lambda/2$) or metric units (e.g. 275nm).
With a decrease in the velocity of propagation, the wavelength, too, decreases (Fig. 7). The color impression of the light remains unchanged.
The following relation exists between the velocity of propagation c', frequency ν (constant) and wavelength λ':

$$c' = \lambda' \cdot \nu$$

Isotropy

Substances in which light has the same velocity (refractive index) in all directions are called *isotropic*. They include gases, liquids, glasses, as well as all crystals of the cubic lattice system (e.g. rock salt – NaCl, cf. p. 47). The directionally derived representation of the velocity of light (refractive index) produces a sphere (Fig. 8) for isotropic substances.
Isotropic objects under strain (p. 46) (e.g. rapidly cooled glasses) act anisotropically, their refractive index depends on the direction.

Fig. 8:
Velocity of light as a function of the propagation direction: for isotropic objects a sphere is produced in the spatial representation of the refractive index.



Uniaxial substances

If an object is observed through a piece of calcite of several millimetres (mm) thickness it appears duplicated (Fig. 9). When the piece of calcite is rotated, one image remains stationary, whereas the second, equally bright image rotates around the stationary image in a circular orbit*.
In this phenomenon, known as *birefringence*, each light ray is split up into two part-rays. With vertical incidence one ray passes through the object in a straight line (i.e. as in isotropic objects), whereas the second ray is laterally displaced. The straight ray is called ordinary ray (o or ω), the ray that suffers parallel displacement is called the extraordinary ray (e or ε).

Careful experiments show that the two images cannot be simultaneously sharp when projected: The ordinary and extraordinary rays have different velocities of propagation (refractive indices). The refractive index associated with the ordinary ray is called. n_o, that associated with the extraordinary ray n_e.
The difference between the two refractive indices

$$\Delta n = n_e - n_o$$

is an important constant of a given substance. It is determined with the polarizing microscope. It is generally called birefringence.
A phase difference Γ (cf. p. 11) is produced because of the different velocities of propagation of the two rays.

Fig. 9:
Birefringence in a cleavage fragment of calcite.

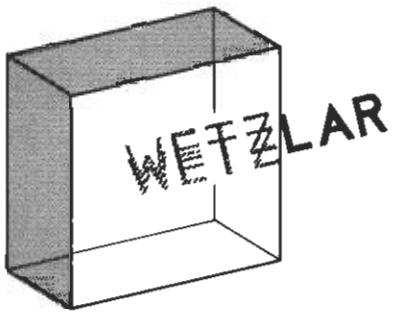
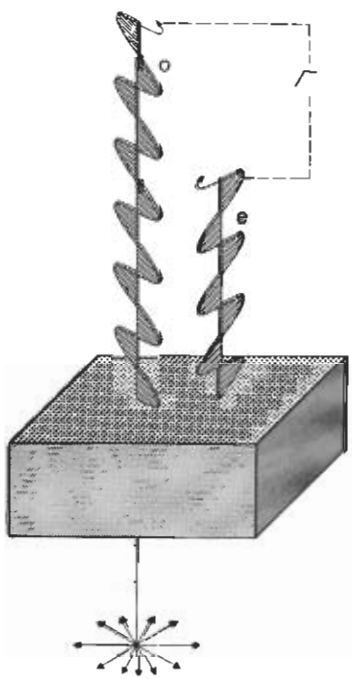


Fig. 10:
Diagrammatic passage of rays at birefringence o = ordinary ray, e = extraordinary ray, Γ = phase difference.



* Observation is possible with polarizing microscopes and objectives of very low power. Specimens for special experiments with polarized light can be obtained from Dr. Steeg and Reuter, D-6000 Frankfurt/M. 56, Berner Strasse 109.

Optical sign

In many birefringent substances (cf. p. 46) the refractive index of the extraordinary ray is higher than that of the ordinary ray. With other substances, on the other hand, the ordinary ray has the higher refractive index.
In the first case, the substance is positively birefringent ($n_e - n_o$ is positive) and in the second case negatively birefringent ($n_e - n_o$ is negative). This specific property of a material called *optical sign* is determined with the BERTRAND lens in conoscopic observation (p. 69). In another group of birefringent substances (biaxial crystals see p. 16) both rays suffer parallel displacement. Two extraordinary rays are produced. Here the higher refractive index is always called n_γ' the lower one n_α'.

Birefringence and polarization

The two rays produced in birefringence differ also in their polarization direction. They vibrate precisely perpendicularly to each other (fig. 10): the vibration directions of the rays are called e and o (extraordinary and ordinary ray) or γ' and α'.
The two polarization directions perpendicular to each other can be demonstrated by observation of the double image produced in a piece of calcite with a polarizer. When the piece of calcite or the polarizer is rotated the "ordinary" and the "extraordinary" image disappear alternately after a rotation through 45°.

Directional derivation of birefringence

The velocity difference Δc changes as a function of the direction of a light ray passing through a birefringent substance. Calcite and quartz, for instance, both have a direction along which neither polarization nor birefringence occur. In this privileged direction, the crystals therefore behave exactly like optically isotropic substances. The privileged direction is called the optic axis.
In both cases, the maximum difference Δn between the two refractive indices occurs in directions perpendicular to the optical axis. This value is called the specific birefringence or maximum birefringence. The intermediate values are designated $\Delta n'$ (Fig. 11).

Examples of uniaxial birefringent minerals

	n_e	n_o	Δn	Optical sign
Quartz	1.553	1.544	0.009	+
Zircon	2.015	1.960	0.055	+
Calcite	1.486	1.658	0.172	–
Corundum	1.760	1.767	0.007	–
Apatite	1.630	1.633	0.003	–

Representation of the refractive indices

If the light velocities or the refractive indices of the ordinary and the extraordinary ray are entered on graph paper as a function of the direction, the following figures are obtained.

a) for the ordinary ray a *circle*, in three-dimensional representation a *sphere* (Fig. 11);
b) for the extraordinary ray an *ellipse*, in three-dimensional representation an *ellipsoid of revolution* (Fig. 11).

In positively birefringent structures the refractive index of the ordinary ray is lower than of the extraordinary ray. When the refractive indices are graphically represented, the circle or the sphere (n_o) is inscribed in the ellipse or the ellipsoid (n_e).
In negatively birefringent structures, on the other hand, the ellipse or the ellipsoid are *inscribed* in the circle or the sphere (Fig. 11).

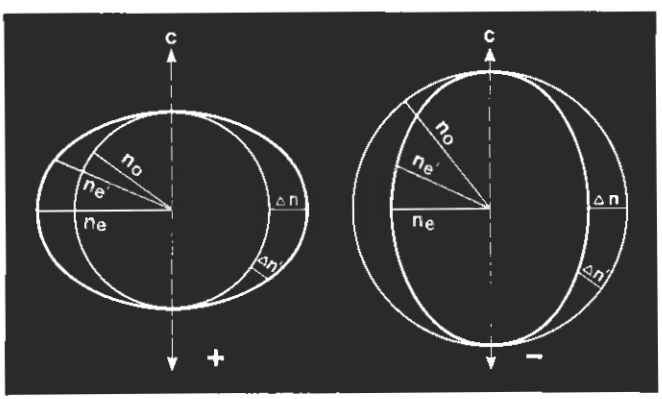


Fig. 11:
2-sheet representation of the refractive indices in a positively uniaxial (left) and a negatively (right) crystal:
c optical axis
n_o Refractive index of the ordinary ray (constant)
n_e maximum refractive index of the extraordinary ray
n_e intermediate value
Δ n maximum birefringence (in directions vertical to the crystal axis)
Δ n' intermediate values

Indicatrix

This representation of the refractive indices by a circle and an inscribed or circumscribed ellipse (or sphere and ellipsoid respectively) is very instructive and easily understood provided the vibration directions of the two rays are not included in it. Since the vibration directions play an important part in crystal optics, a representation showing all the relations between propagation direction, vibration direction, and value of the refractive indices is preferred. This is possible with the ellipsoid called indicatrix. It can be explained as follows in somewhat simplified terms.

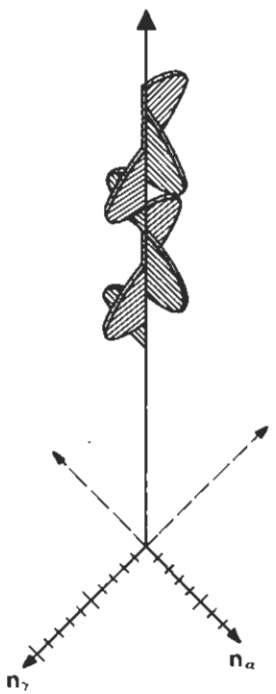
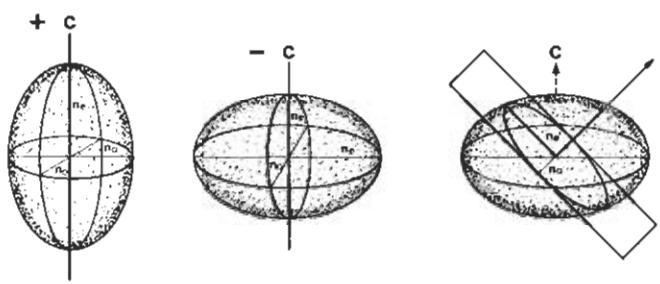


Fig. 12:
Construction of the indicatrix: the magnitude of the refractive indices n_α' and n_γ' is entered in the vibration directions.

The refractive indices corresponding to the ordinary and the extraordinary ray are entered at a random scale in the vibration direction of the two rays (Fig. 12). The end points thus obtained for all possible propagation directions connected with each other produce the indicatrix (Fig. 13), which, in uniaxial structures, is an *ellipsoid of revolution.*
Conversely, refractive indices and vibration directions can be determined from the indicatrix as follows:
A section is cut through the centre of the indicatrix (Fig. 13c) perpendicular to the relevant propagation direction. This section produces an ellipse. The two half axes of the ellipse indicate both the vibration directions and the unknown refractive indices. In the direction of the crystal axis the sectional figure is a circle. There is no privileged vibration direction (apparent isotropy).

Fig. 13:
Indicatrix:
Left: optically positively uniaxial crystal.
Centre: optically negatively uniaxial crystal.
Right: Determination of the vibration directions and refractive indices n_o and n_c' from the indicatrix for a random-cut section of the crystal.



Biaxial substances

Besides birefringent substances which display isotropic behaviour in one direction, there is a second group of birefringent substances with two such privileged directions. These include, for instance, sugar, sodium thiosulphate (fixing salt), felspar and mica. Structures with more than two such axes are not known.

The narrower angle between to two axes is called the axial angle, abbreviated 2V. It may have values between 0° (= uniaxial crystal) and 90°. The value of the axial angle is a function of the structure and chemical composition of the material. The axial angle is determined with the BERTRAND lens in conoscopic observation (p. 69) or with the universal rotating stage (p. 81).

Spatial distribution of the refractive indices and ray propagation are considerably more complicated than with uniaxial structures. As in these the ray is split up into two rays polarized perpendicular to each other, which, however, both suffer parallel displacement: two extraordinary rays are produced. Instead of the refractive indices n_o and n_e of the uniaxial structures, in biaxial structures we distinguish between three characteristic refractive indices.

$n\beta$ = refractive index with light propagation in the direction of the optical axes.

$n\alpha$ = lowest refractive index.

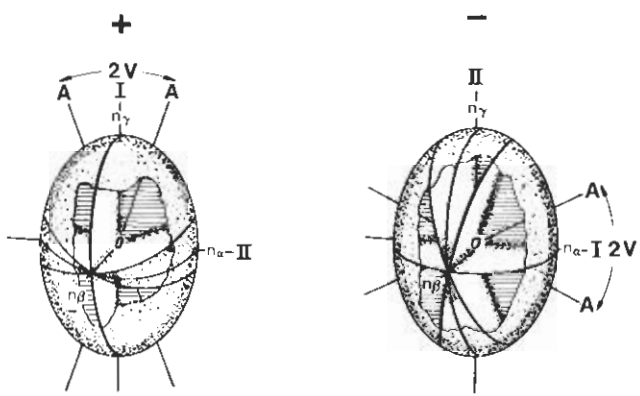
n_γ = highest refractive index.

The intermediate values are called $n\alpha'$ and $n\gamma'$, and their differences $\Delta n'$. The corresponding vibration directions are called α, γ and α', γ' respectively.

For reasons of simplification, the minimum and maximum refractive index is also called $n\alpha$ and n_γ (instead of n_e and n_o) in the uniaxial structures which, after all, are the special case of a biaxial structure of axial angle 2V = 0.

The bisetrices of the obtuse and the acute angle of the crystal axes are called obtuse and acute respectively. The indicatrix (Fig. 14), unlike that of uniaxial structures is not an ellipsoid of revolution, but a tri-axial ellipsoid with the half axes $n\alpha$, $n\beta$, n_γ. Here the optical axes are normal to the two circular sections across the indicatrix. The direction of maximum birefringence ($\Delta n = n_\gamma - n\alpha$) is called the *optical normal* (N). On the indicatrix model it coincides with $n\beta$. The optical charateristics depend on whether the acute bisectrix coincides with n_γ (positive) or $n\alpha$ (negative).

Fig. 14:
Indicatrix:
Left: positively biaxial crystal
Right: Negatively biaxial crystal
2V axial angle
OA optical axes
I acute bisectrix
II obtuse bisectrix



Pleochroism and dichroism

Most birefringent substances exhibit practically the same transmissivity (Fig. 10) for both rays caused by birefringence. In some substances (Fig. 15), on the other hand, transmissivity depends on the vibration direction and on the propagation direction: *pleochroism* (biaxial structures) and *dichroism* (uniaxial structures).

Crystals of the cubic system display neither birefringence nor anisotropy of absorption. Like birefringence, pleochroism and dichroism do not depend on wavelength. Sometimes distinctive color phenomena occur in observation with only one polarizer (p. 52). Quantitative evaluation is carried out with the aid of a microscope photometer.

Pleochroism and dichroism are extremely specific of some minerals such as tourmaline, biotite, cordierite. They are therefore used for identification (table II p. 45). The phenomenon is widespread also in organic structures. It is used, for instance, for the production of filter polarizers. The differential absorption of some substances for laevo- and dextro-rotatory light is called circular dichroism.

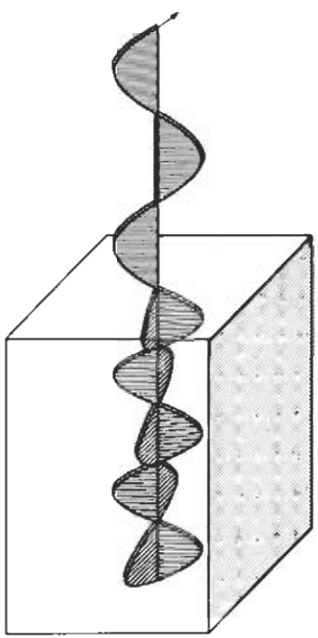


Fig. 15:
Pleochroism, diagrammatic representation: both rays caused by birefringence suffer differential absorption, so that one of the two rays may completely disappear. Such substances can be used as polarizers.

Optical activity

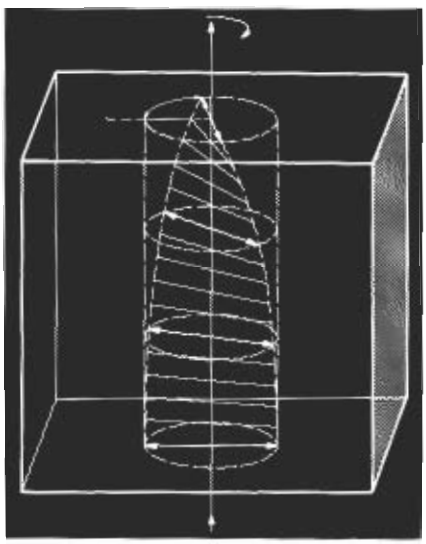
Some solids and liquids rotate the vibration direction of polarized light. They are *optically active*.
The angle through which the vibration direction is rotated increases proportionally with the thickness of the layer the light traverses and, in liquids, also with the concentration. The angle of rotation per mm (solids) and per dm (liquids) is called specific rotation. It depends on the wavelength (optical rotatory dispersion). In quartz, for instance, it has the following values per mm layer thickness for light propagation in the direction of the optical axis:

397nm	51.2°
421nm	42.6°
527nm	27.5°
589nm	21.7°
687nm	15.7°

As the propagation direction diverges from the optical axis optical activity decreases and finally disappears. Depending on the structure of the optically active substance the plane of vibration can be rotated to the right or the left. Example: dextro-and laevo-gyrate quartz, dextrose and laevulose (sugars).
The rotation direction is often referred not to the propagation direction of the light, but to the observation direction (Fig. 16). In microscopic specimens optical activity can only very rarely be observed because of the negligible layer thicknesses. In conoscopic observation (p. 69) of quartz sheets of 1mm thickness cut vertically to the crystal axis the rotation of the vibration direction can be seen in the brightening of the point of intersection of the cross and the so-called "AIRY spirals" (Fig. 71).

Optical activity is used in practice, for instance, in the determination of the concentration of sugar solutions with polarimeters.

Fig. 16:
Optical activity



II. Instruments

Polarizing microscopes

The most important structural elements of polarizing microscopes are illustrated in Figs. 42–45. The technical conception is described on the following pages, Use and Accessories, Chapter III.

Microscopes are classified according to size and possibilities of extension as students' microscopes, laboratory microscopes and research microscopes (p. 38). An extremely important consideration in the purchase of a microscope is the greatest possible versatility of facilities of extension and interchangeability of components according to the modular principle. This is an essential condition to ensure that the instrument can be adapted to the investigation of new problems even after years of use. Table I lists the accessories suitable for combination with the various models.

Microscopes for biology, medicine, and metallography can be adapted with modules for simple investigations in polarized light (orientating polarization (Fig. 18)). For quantitative investigations, however, special polarizing microscopes must always be used.

Polarizing microscopes are characterized mainly by the following components:

Polarizer and analyzer
rotating stage with angle graduation and verniers
strainfree, centring objectives,
tube slot,
polarizing tube,
eyepiece with crosslines

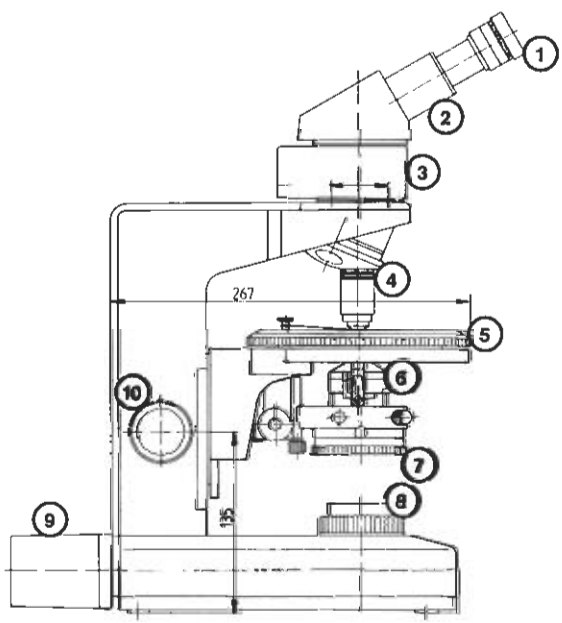


Fig. 17:
Schematic polarizing microscope
1 Eyepiece
2 Tube, pinhole stop, Bertrand lens
3 Intermediate tube with analyzer
4 Centerable objective nosepiece with tube slot and objectives
5 Stage (specimen)
6 Condenser with top and aperture diaphragm
7 Polarizer
8 Field diaphragm
9 Light source
10 Coarse and fine focus controls

Stand

In a narrow sense, the usually aluminium-cast heavy support which serves the permanent connection of light source, stage, and tube is called the stand. The size of its foot decides the degree of stability of the entire microscope, especially when accessories for photomicrography, projection, or photometry are attached to it. Feet made of vibration-damping materials screwed to the underside of the stand reduce the transmission of vibrations of the ground.

The upper part of the stand is called the limb. It supports the tube and objective carrier and, in the ORTHOLUX-POL, the analyzer (Fig. 44).

More generally, the term stand is used for the entire operational microscope. The reason for the angular design of modern stands is not only aesthetic. Structural components and accessories can be attached to and adjusted on plane horizontal and vertical surfaces simply, securely and precisely. In addition, plane surfaces mean a reduction of manufacturing costs, which in the last resort benefits the purchaser.

Fig. 18a:
Polarizing device for orientating investigations, for attachment to existing standard microscopes:
1 Analyzer
2 Lambda/4 plate
3 Polarizer
4 Mount for polarizer and compensator
5 Lambda plate

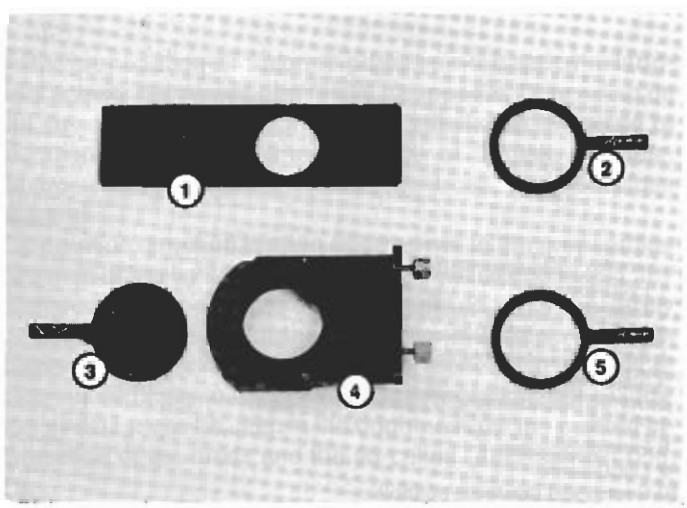
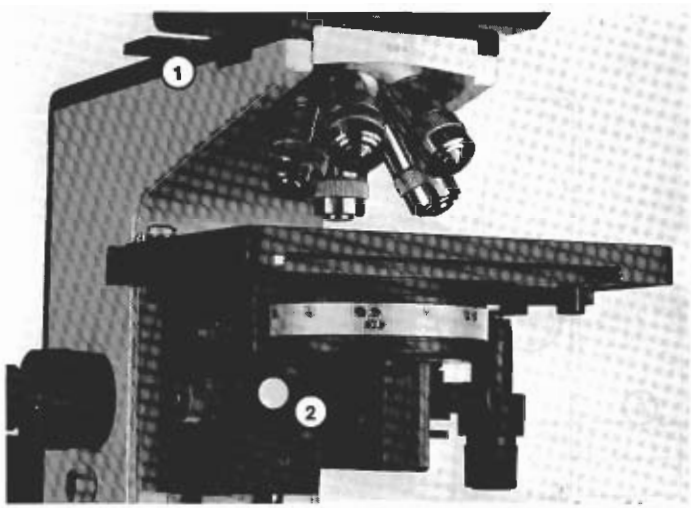


Fig. 18b:
1 Analyzer
2 Mount with polarizer and compensator



Light sources and filters

Modern microscopes are equipped with interchangeable light sources (Fig. 42–44). Before the light source is used for the first time it must be carefully centered for even illumination of the microscopic field. To do this, the luminous spot (incandescent filament or discharge arc) must be precisely centered to the axis of the illuminating beam. Uniform illumination of the field is ensured by adjustment of a lens in the lamp housing. This so-called lamp condenser is usually frosted to eliminate inhomogeneity of the luminous spot (incandescent filament). Lamp housings for high-power light sources also have an adjustable reflector to increase the light utilization.

For simpler light sources, adequate homogeneity is attained through use of diffusers. Centering is thus unnecessary.

Incandescent lamps and conversion filters

Today's microscopes are used almost exclusively with low-voltage halogen lamps. The compact filaments necessary for an operating voltage of 6–12 V give significantly brighter, more homogeneous illumination than lamps with thin filaments connected directly to the mains.
The halogen filling reduces the condensation of tungsten vapour on the quartz due to a closed-circuit process. Brighter illumination, a constant color temperature and longer life is thus guaranteed.
Light sources of 10–20 W are sufficient for exclusively visual use of the microscope. For photomicrography, however, higher powered lamps of up to 100W are recommended, especially for instant photography, when using interference filters or for conoscopy of extremely fine grains.
The spectrum of tungsten filament lamps (Fig. 3H) differs from that of daylight. The proportion of blue is reduced, that of red increased. This is why colors appear different in light from tungsten filament lamps to those seen in daylight illumination. The colors can be corrected, also for photography on daylight color film, by the insertion of a suitable *conversion filter* (CB 1.5 to 16.5) in the illuminating beam. For details the Leitz brochure "The Microscope and its Application" should be consulted. Green filters are recommended for black-and-white photography.

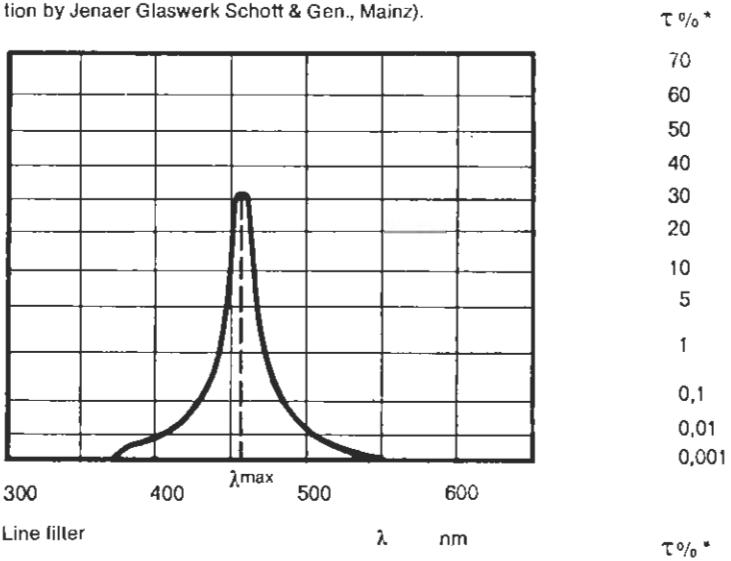
Gas discharge lamps

The spectrum of *high-pressure xenon lamps* resembles that of sunlight (Fig. 3). Because of their high intensity these lamps are used in polarized-light microscopy for projection and in conjunction with interference filters.
High-pressure mercury lamps have a spectrum which shows high intensities in individual lines (bands) (Fig. 3). Their use is appropriate only in conjunction with interference filters. This also applies to halogen arc lamps. For investigations in very closely defined wavelength regions *spectrum lamps* (e.g. sodium vapour lamps) are used. Because observation and orientation of the object is often begun in white light, an additional tungsten filament lamp must be available for alternative use via a *mirror housing*.

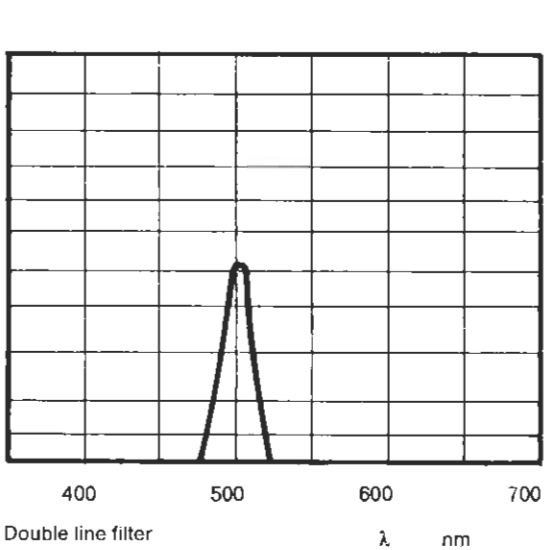
Filters

Interference filters are often adequate for monochromatic illumination. They are placed on the dust glass in the foot of the stand, and, for white-light observation, simply removed from the beam. Interference filters consist of numerous thin films vapour-deposited on a sheet of glass. The coated side must always face the light source. In prolonged use, the filters generally withstand heating of up to about 70° C, and for short periods even 100° C.
Homogeneous interference filters permit the selective transmission of a defined narrow wavelength range (Fig. 19). They can be supplied for the most important spectrum lines and are specially produced for any desired other wavelength on request.

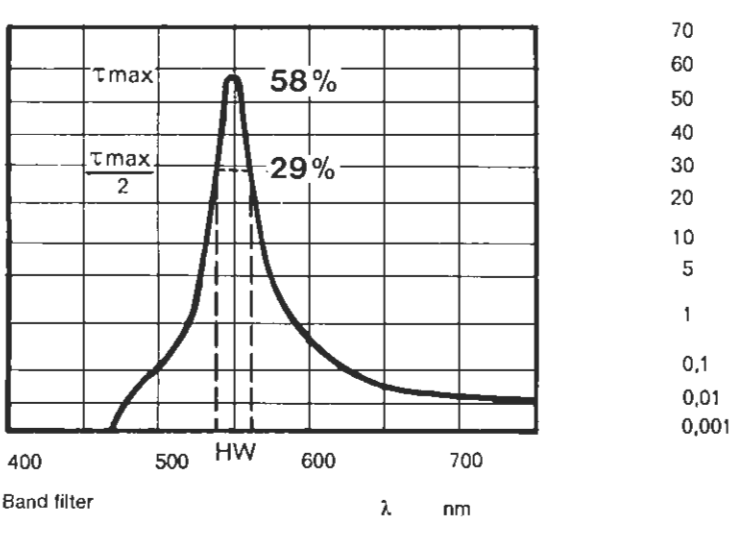
Fig. 19:
Typical transmission curves of interference filters (according to sales information by Jenaer Glaswerk Schott & Gen., Mainz).



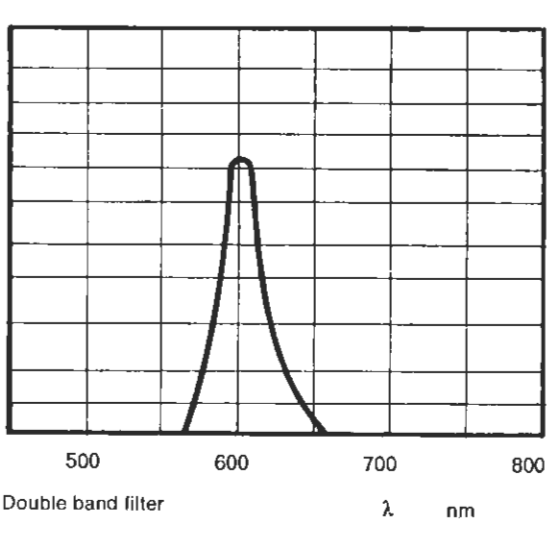
Line filter



Double line filter



Band filter



Double band filter

With *interference graduated filters* (Fig. 20) the visible region can be continuously traversed by adjustment of the filter. Any desired wavelength can therefore be set.

The most important technical data of the filter can be obtained from the transmission curve (Fig. 19):

τ_{max} Maximum transmission

λ_{max} The spectral region of the filter defined by the arithmetrical mean of the wavelengths measured at $\tau_{max}/2$

HW Width of the transmission curve at $\tau_{max}/2$ (half-value width)

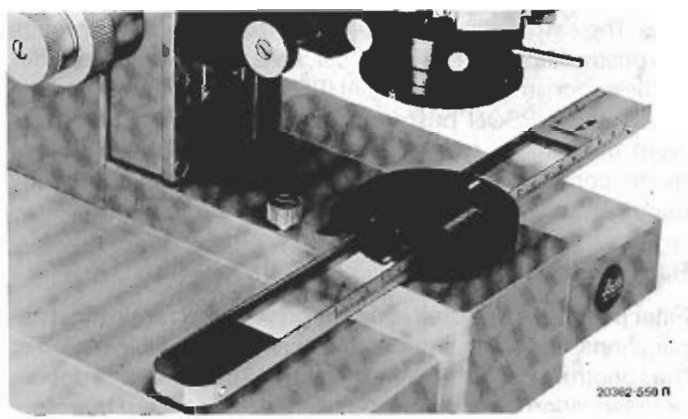
TW Width of the transmission curve at $\tau_{max}/10$

Depending on the width of the transmission curve (band width) of the filters we distinguish between:

Band filters (HW > about 15nm)

Line filters (HW < about 15nm)

Fig. 20:
Interference graduated filter for the continuous adjustment of the wave length in the illuminating beam.



Double-band and double-line filters consist of two identical cemented filters; they suppress the effect of any defective areas of the interference films (Fig. 19).

Monochromators permit a more precise and more narrow-band selection than interference filters. The Leitz grating monochromator, for instance, permits the power traverse of the spectral region from 220–800nm. The spectral band width can be set reproducibly from 3.3 to 26.4nm according to purpose.

Field diaphragm

The field diaphragm is built into the foot of the stand (Fig. 43). Its diameter is adjusted so that an area (field of view) which is only slightly larger than the object field observable through the eyepiece is illuminated. This eliminates contrast-reducing stray light. In small students' microscopes such as the HM-POL the field diaphragm can be omitted. The foot of the stand usually also contains a hinged lens, which must be turned out of the beam when objectives of 1:1 reproduction ratio are used. This ensures uniform illumination of the extremely large field of view (Fig. 45.4).

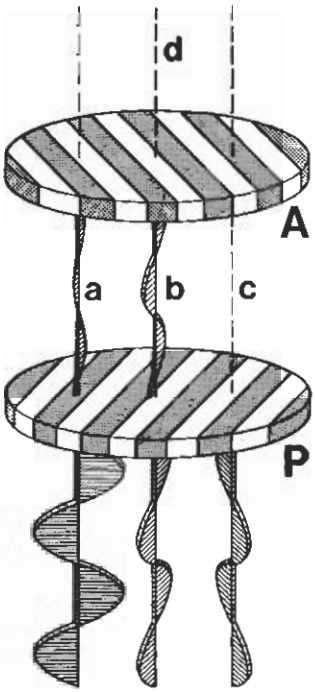
Polarizers

Polarizers are used for the alignment of uniform direction. Rays which already travel precisely in the so-called transmission direction or "vibration direction" of the polarizer, are transmitted with a slight loss of intensity (Fig. 21b). Rays that vibrate perpendicular to the transmission direction are completely suppressed (Fig. 21c).

Rays vibrating "obliquely" to the transmission direction on the other hand (parallelogram of vectors), are divided into a transmitted and an eliminated component (Fig. 21a). Crossed polarizers block the passage of light completely (Fig. 21d). The crossable polarizers of polarizing devices are called *polarizer* (in front of the object; in the polarizing microscope as a rule combined with the condenser) and *analyzer* (behind the object). The collective term for polarizer and analyzer used to be "NICOLS" (after the inventor).

In the manufacture of polarizers today mainly the following three principles are used:

Fig. 21:
Crossed polarizers
P = Polarizer
A = Analyzer



Filter polarizer

Filter polarizers use the differential absorption of the two rays produced by birefringence (dichroism p. 17); they consist of innumerable submicroscopically small dichroic crystals, aligned parallel to each other on a foil by means of a special technical process.

Reflection polarizers

Polarization can take place by reflection from non-metallic surfaces, e.g. a set of obliquely arranged sheets of glass. Both the reflected and the transmitted portions of the light are polarized (Fig. 22). The degree of polarization which can be reached in this way is generally unsatisfactory. Reflection polarizers are therefore combined with filter polarizers. The reflection polarizer serves as a prepolarizer for the protection of the heat-sensitive filter polarizer.

Prism polarizers

Prism polarizers for microscopic purposes are almost exclusively made of calcite. In the NICOL prism (Fig. 23) the ordinary ray, with which the higher refractive index must be associated, is laterally deflected by total reflection on the cemented surface. The extraordinary, linearly polarized ray passes through the prism. Since optically pure calcite is very expensive, differently cut prisms such as the AHRENS prism (Fig. 23) and the GLAN THOMPSON prism are used today. Prism polarizers were the original type of polarizers used in polarized-light microscopy. Dichroic crystals, on the other hand, were already used centuries ago by jewellers (tourmaline tongs).

Range of uses of the various polarizers

Filter polarizers are as effective as prism polarizers in most applications. Only with work at the limits of the visible region of the spectrum (below 430nm and above 750nm) is their degree of polarization is reduced, and prism polarizers are therefore

preferable in these cases. Special filter polarizers adjusted for these regions of the spectrum are, however, available. Filter polarizers are heat sensitive and even after brief heating above 100° C they turn yellow and are destroyed. A heat filter should therefore be inserted in the lamp housing when 50W lamps are used and a second heat filter for the 100W tungsten halogen lamp. More powerful gas discharge lamps can be used only with interference filters or pre-polarizers (condenser 702 fvi) or the polarizing prism (condenser 702pi). The pre-polarizer consists of an inclined vapour-deposited film (Fig. 22), which already reaches a level of polarization of about 98%. Complete polarization is achieved by the filter polarizer inserted behind it, without appreciable exposure of the foil to heat.

Fig. 22:
Polarization through reflection (principle of the pre-polarizer)

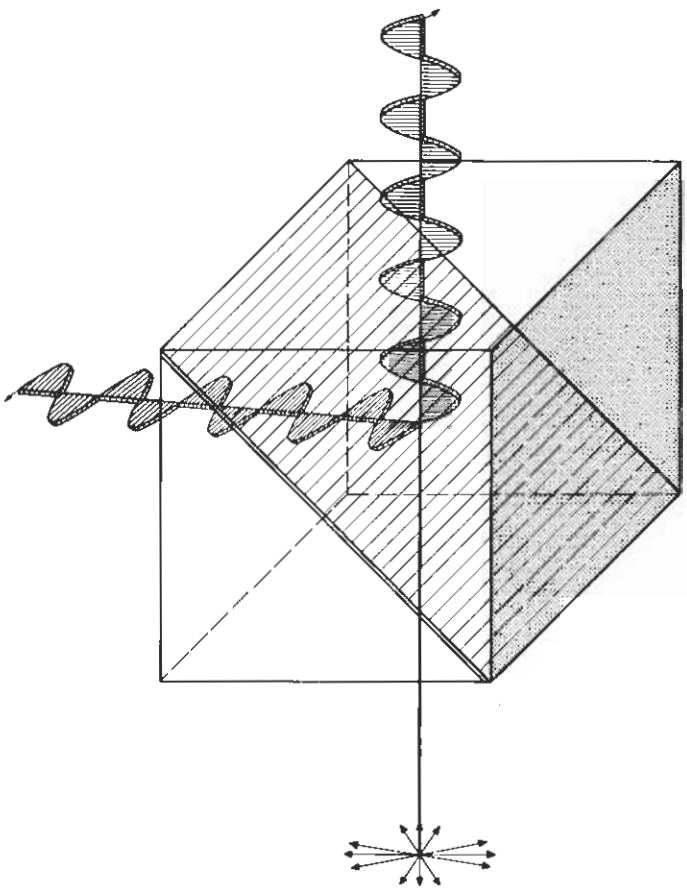
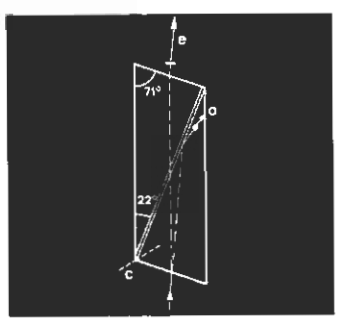
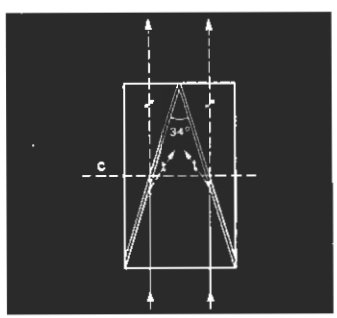


Fig. 23:
Prism polarizers
Beam in the NICOL prism
c Optical axis of calcite

AHRENS prism





Polarizing condensers

It is the purpose of the microscope condenser to illuminate the object at the desired condenser aperture (i.e. with the optimum cone of rays corresponding to the objective used). With correct vertical adjustment of the condenser an image of the field diaphragm is formed in the object plane. Consequently, an image of the light source is formed in (the rear focal plane of) the substage condenser, and again in (the upper focal plane of) the objective.

The optical construction of polarizing condensers corresponds to that of condensers in ordinary microscopes (see also our brochure "The Microscope and its Application"). But the lenses used are in strain-free mounts (strain birefringence see p. 46).

The *aperture diaphragm* serves the adaptation of the field aperture to the objective aperture. In orthoscopy (p. 51) it is opened partly, and in conoscopy (p. 69) fully.

The two *centering screws* serve the accurate centration of the condenser and therefore the illuminating beam to the optical axis of the microscope.

The hinged *condenser top* (also called swing-out lens or front lens of the condenser) provides wider condenser apertures. It is always turned into the beam at objective apertures >0.25. With correct vertical adjustment of the condenser (formation of the image of the field diaphragm in the object plane) the condenser top is immediately below the specimen. After the condenser top has been turned out for the use of low-power objectives, the condenser must be lowered to form a sharp image of the field diaphragm in the object plane. Details can be obtained from the instructions for the use of the microscope.

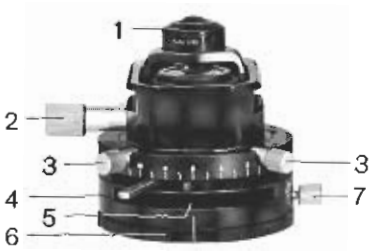
In the Leitz-POL condensers of series PLK and 702 the standard condenser top (n. a. 0.90) can be replaced by other condenser tops:

The condenser top No. 004 P Oil n. a. 1.33 is required for the use

* Numerical aperture (n. a.) is the product A = n x sin α, where α is the angle included by the extreme ray just entering the lens or its projection and the optical axis of the lens; n is the refractive index of the optical medium (air, immersion oil).

Fig. 24:
Leitz polarizing condensers:
Left: condenser 702 fi frontview
Center: condenser PLK sideview
Right: Low-power condenser for the Pl 1/0.04 (P) objective

1. Interchangeable condenser top
2. Knurled screw or lever for swinging out the condenser top
3. Centering screws
4. Lever for the adjustment of the aperture diaphragm
5. Slot for the $\lambda/4$-plate for circular polarization
6. Rotating filter polarizer
7. Clamping screw for arresting the polarizer rotation



with oil immersion objectives (especially in conoscopy). Other special condenser tops are necessary for work with universal rotating stages and heating stages.
The rotating *polarizer* is attached to the underside of the condenser. The following versions of condensers are available for Leitz ORTHOLUX 2 POL BK and ORTHOPLAN POL polarizing microscopes (Fig. 24):
POL-condenser 702**fi** with **f**ilter polarizer
POL-condenser 702**fvi** (with **f**ilter and pre-polarizer)
POL-condenser 702**pi** (with calcite **p**rism polarizer).
All condensers of the series PLK and 702 and the pol-vertical illuminator (p. 84) have index adjustment, so that the zero position of the polarizer can be accurately indicated after the polarizers are crossed.
The slot above the polarizer (Fig. 24.5) accepts a $\lambda/4$-plate for observation in circularly polarized light (p. 68).
Other filters must never be inserted in this slot because of their possible strain birefringence.

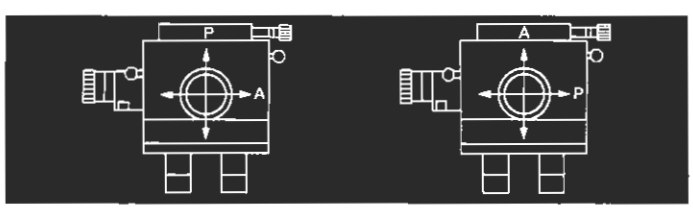
For the illumination of very large fields in conjunction with the PI 1/0.04 (P) objective or with the BERTRAND lens on the ORTHOPLAN POL (p. 82) a low-power condenser is available (Fig. 24).
The modular principle allows the use of other condensers, e.g. for darkground, phase contrast and interference, in the microscopes (see p. 38).

Orientation of the polarizers

Since 1972, when DIN 58879 was laid down, the transmission direction of the polarizers in microscopes have been standardized so that the vibration direction of the polarizer is East-West*, that of the analyzer with crossed polarizers North-South* (Fig. 25). All leading manufacturers of microscopes adhere to this standard. With crossed polarizers, Leitz-POL microscopes show the angle values 0° (polarizer) and 90° (analyzer) engraved in red. For microscope components of earlier manufacture the orientation of the polarizers can also be obtained from Fig. 25.

* The orientation of vibration directions, specimens, etc., is usually described in terms of compass points.

Fig. 25:
Orientation of crossed polarizers (vibration directions)
Left: Conventional — Right: to DIN standards
P = polarizer — A = analyzer



Rotating stages

In almost all investigations in polarized light the objects must be rotated without moving out of the field of view. Rotating stages running on precision ball bearings, graduated in degrees and fitted with verniers on polarizing microscopes allow rotary movements and angle measurements of the highest precision (cf. Table I, p. 38).

The *clamping device* (Fig. 26-5) is used for the temporary clamping of the stage rotation. In research microscopes an engageable *click stop* (Fig. 27-5) permits object rotation in steps of exactly 45°. In students' microscopes, 45° rotations are carried out with the aid of the vernier scale or the engraved 45° markings.

In large polarizing microscopes the *stage plate* (Fig. 27-2) is removed for the adaptation of special accessories such as universal rotating stages (UT see p. 87). Furthermore, the stage can be moved vertically on a sliding track (Fig. 27-6) in addition to the fine adjustment of the stage drive or interchanged with other stages. The micrometer graduation of the fine adjustment permits approximate thickness determination of objects by means of focusing the top and bottom surface of the object. The value obtained must be multiplied by the refractive index of the object.

Fig. 26:
Rotating stage on ball bearings (LABORLUX 12 POL)
1. Threaded and unthreaded bores for the attachment of accessories
2. Stage clip
3. Friction clamp for the stage rotation
4. Vernier for reading the stage rotation to 1/10°

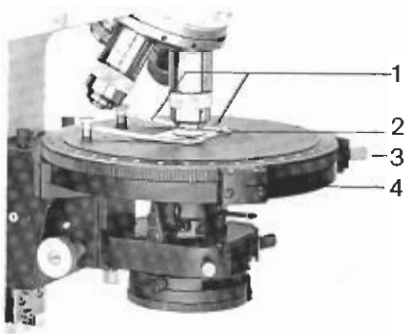


Fig. 27:
Rotating stage No. 837 on ball bearings for research microscopes (ORTHOPLAN POL)
1. Stage clip
2. Stage plate (removable for accessories)
3. Verniers (1/10°)
4. Friction clamp for the stage rotation
5. 45° clickstops
6. Sliding track for stage adjustment and change

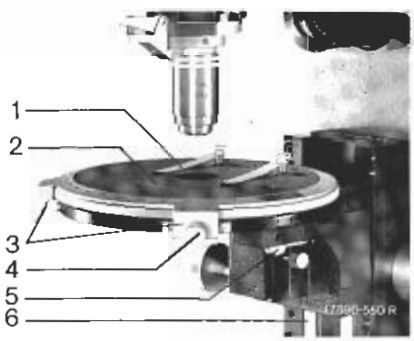
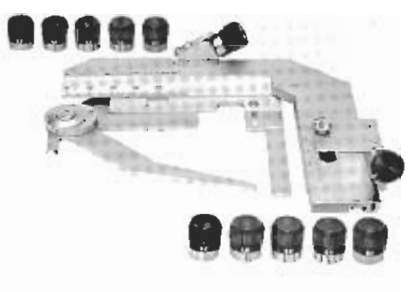


Fig. 28:
Attachable mechanical stage Pol for the accurate adjustment and systematic scanning of specimens. It has interchangeable catch-buttons for point-counting methods.



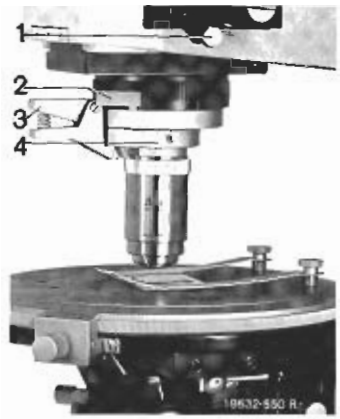


Fig. 29:
Objective centering clutch on a research microscope (ORTHOLUX 2 POL)

1. Clamping screw for the exchange of the object centering clutch against the objective centering revolving nosepiece, pol-vertical illuminator or other accessories.
2. Tube slot (made dustproof by closure with a blank slide)
3. Lever for the attachment of the objective
4. Centering mount

Objective carriers

Objective centering clutch

The objective centering clutch which was always used on polarizing microscopes is today only employed for very specialized methods on research microscopes, particularly for studies with the universal rotating stage (p. 81) and for preparative work. It is also advantageous if immersion objectives are used often (regarding the cleaning). With respect to optical performance and centering accuracy, the clutch is as just as good as the centering nosepiece.

Objective centering nosepiece

The nosepiece is generally preferred because it has the advantage that the magnification is easy to change. Using the special key, the objective mounts in the Leitz centering nosepiece can be centered exactly on the stage's axis of rotation. This ensures that the objectives are always centered and are insensitive to contact.
The so-equipped Leitz polarizing microscopes have the objectives facing the rear, thus simplifying specimen changing and the view of the specimen.

Fig. 30:
Objective centering nosepiece and centering keys on LABORLUX 11 POL (tube slot with compensator above the right-hand key).

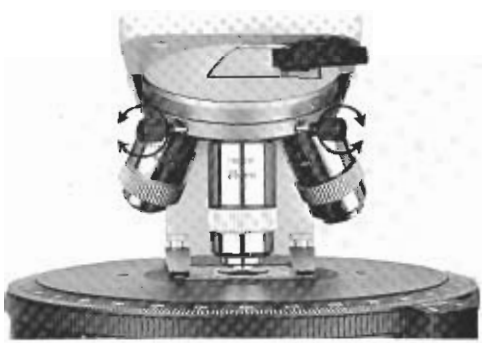
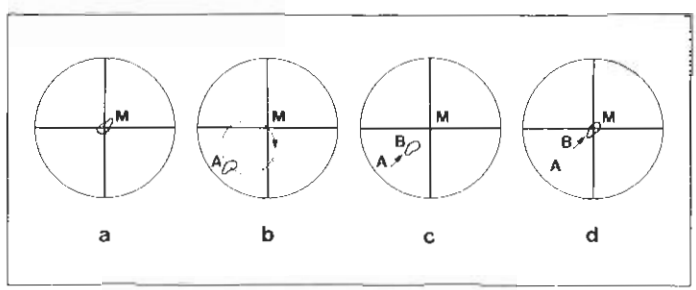


Fig. 31:
Centering a microscope objective:
By rotation of the two centering keys (Fig. 31) the objective is displaced in its holder until an object point B situated within the crosslines M no longer changes position during the rotation of the stage (d).



Tube slots

Tube slots (Figs. 29.2, 30) accommodate compensators* (p. 61). In microscopes of earlier manufacture the tube slots were situated in the tube proper. In modern microscopes they have been moved to the revolving nosepiece holder or the centering clutch. The intermediate optical system has been designed so that the compensators are in the parallel beam to ensure that the image is not displaced when the compensator is pushed in or out. The intermediate optical system (also called tube lens) is required for structural reasons to improve image quality and facilitate operation. A *tube factor,* where engraved, e.g. 1.25, must be allowed for in the calculation of the final magnification.

* The DIN dimensions of the tube slots and the compensators are 20x6mm. In microscopes of earlier manufacture (curved stand design) they are 12x4mm.

Intermediate tubes

The analyzer, the Bertrand lens and the pinhole stop (amongst others) must be accommodated in the tube system of the polarizing microscope. Space problems, caused by the prisms housed in the tube, are particularly common in binocular tubes. The intermediate tube is the solution; it also increases the distance between the objectives and the eyepieces, however. The optical tube length is compensated for by the lens system contained in the intermediate tube.
For the LABORLUX 11 POL and LABORLUX 12 POL microscopes, the intermediate tube for transmitted light is placed between the stand and the observation tube. It is not necessary for incident light as the corresponding illuminator already takes over a similar function.
In the ORTHOLUX 2 POL BK and ORTHOPLAN POL, the intermediate tube is part of the stand or binocular phototube. In this case, it is used in combinatin with the incident light illuminator as well.

Fig. 32:
Intermediate tube for LABORLUX 11 POL and LABORLUX 12 POL.
Intermediate tube 90 with disengageable analyzer (1); also available with Bertrand lens as intermediate tube 90B.

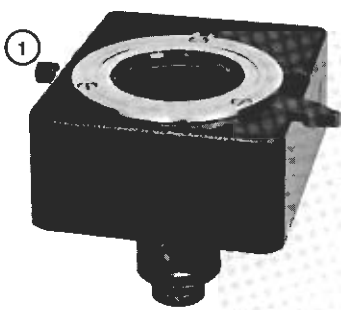


Fig. 33:
Intermediate tube 360B with analyzer (1), rotatable through 360°, Bertrand lens with integrated pinhole stop (2), and disengageable neutral density filter (3).

Analyzers

The polarizer between the object and the observer's eye is called the analyzer. In polarizing microscopes it is generally situated between the objective and the eyepiece. In the ORTHOLUX POL it is in the limb, in the ORTHOPLAN POL (Fig. 44) in the bottom part of the tube. For the LABORLUX 11 POL and 12 POL, the analyzer is located in the intermediate tube (transmitted light) or incident light illuminator, and is disengageable.

In polarizing microscopes the analyzer must always be removable to permit special methods of observation with only one polarizer (dichroism p. 17 and p. 52, BECKE line p. 93).

For simple needs (student's and laboratory microscopes) non-rotatable analyzers are adequate, they must, however, be rotatable for uses such as phase difference measurements according to SÉNARMONT's method (p. 66) and for investigations in incident light (p. 83).

The analyzers of modern polarizing microscopes consist almost exclusively of choice-quality filter polarizers.

A grey filter built into the empty aperture of the analyzer compensates for the intensity difference of the microscope image after removal of the analyzer. This neutral filter can be disengaged, e.g. for fluorescence observation of photomicrography (Fig. 33).

Orientation of the analyzer see Fig. 25.

Polarizing tubes, Bertrand lenses

The principles of construction and function of microscope tubes have already been discussed in the brochure "The Microscope and Its Application". In polarizing microscopes the tubes are orientated to the stand with great precision. To permit interchange between the various tubes, they are aligned by means of a locating pin and a corresponding location groove in the stand and tube respectively.

For precision orientation of the eyepiece crosslines to the vibration directions of the polarizers (or the object in diagonal position) the (right-hand) eyepiece tube has two guide grooves displaced through 45°.

In binocular tubes, interference effects may occur in polarized light owing to the glass prisms (polarization through reflection p. 24). Binocular polarizing tubes therefore contain a thick quartz plate of at least 20 orders' phase difference. The higher-order white this produces thereby (p. 58) eliminates disturbing color interferences.

The BERTRAND lens (Fig. 44.19) serves mainly the observation of interference figures in conoscopy (p. 69). The lens inserted in the beam acts as a weakly magnifying objective, which together with the eyepiece forms an (auxiliary) microscope.

In student's and laboratory microscopes the BERTRAND lens is permanently focused in the factory, so that its use is very simple indeed. In research microscopes it can be focused. Through precise centering and focusing the interference figures seen are particularly clear and suitable for photography. The multicomponent, achromatically corrected BERTRAND lens of the ORTHOPLAN POL further improves conoscopic observation. It can, in addition, be used for special purposes such as observation of fine bores (Fig. 78), for low-power observation of extensive objects, and for the adjustment of phase contrast and interference devices and monochromators. Very small crystals can be isolated for conoscopy in all Leitz polarizing microscopes by means of a pinhole stop (Fig. 33.2). A supplementary lens for conoscopy of very minute

crystals (minimum grain size about 6μm), is incorporated in research microscopes.

The BERTRAND lens is used mainly for the determination of the optical characteristics and the number of axes of minerals. If a students' or laboratory microscope is to be used almost exclusively for orthoscopic observation (p. 51) it is recommended to fit the binocular tube (Fig. 35) or, better still, the binocular photo tube (Fig. 36) instead of the monocular tube (with BERTRAND lens and pinhole stop). Binocular microscopes, provided the eyelenses of the eyepieces have been correctly adjusted, ensure microscopy without eyestrain. The binocular photo tube furthermore allows the adaptation of various photomicrographic attachments, projection screens (Fig. 37), TV cameras and microscope photometers.

For phototubes with back-reflection, a measuring aperture or the film format outlines (photomicrography) are reflected to the viewing port.

Fig. 34:
Monocular tube P 12
1. Adjustable eye lens
2. Knob for engaging the pinhole diaphragm for the conoscopy of small grains
3. Knob for Bertrand lens
4. Opening for focusing the Bertrand lens
5. Tube clamp lever
6. Intermediate tube 90
7. Tube slot (can be covered) with inserted compensator

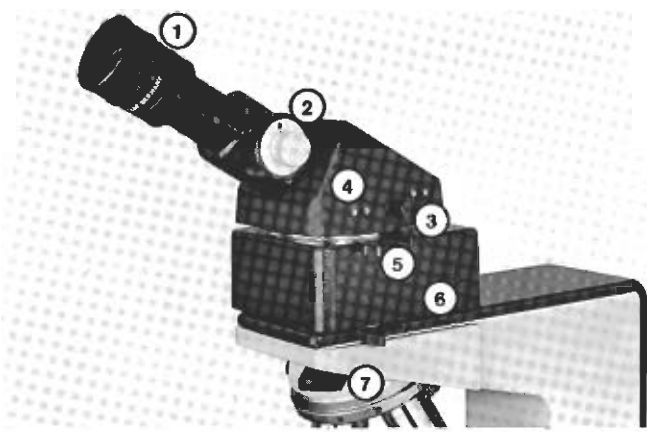
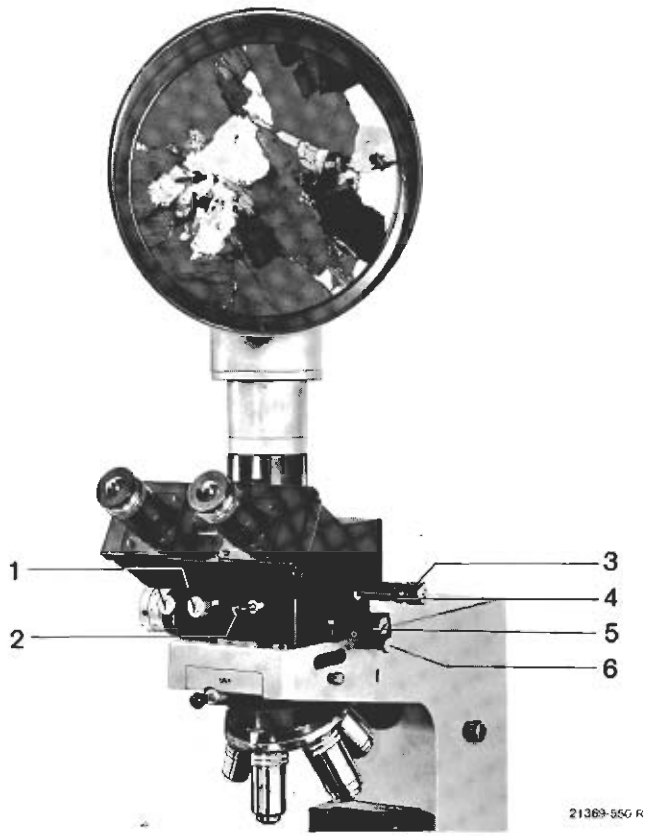


Fig. 35:
Binocular pol tube S 42/30 for LABORLUX 11 POL and LABORLUX 12 POL

Fig. 36:
Binocular phototube 42/30, for LABORLUX 11 POL and LABORLUX 12 POL, with automatic sharpness compensation when the interpupillary distance is changed.

Fig. 37:
Binocular phototube FSA 50 for ORTHOPLAN POL with automatic sharpness compensation, with projection screen in position
1. Centering screws for the BERTRAND lens
2. Lever for the introduction of the pinhole stop for the conoscopy of small particles
3. Focusing screw for the BERTRAND lens
4. Lever for turning in the BERTRAND lens
5. Analyzer slide
6. Tube lock



Objectives

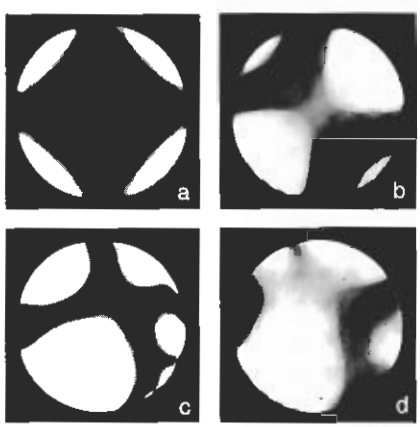
In addition to the fundamental properties of resolving power, contrast, and spherical and chromatic correction, objectives for polarizing devices must, in addition, be free from glass strain. Strains cause birefringence and therefore a brightening of the field of view. This renders precise investigations in polarized light impossible. Strains can arise, for instance, during the cooling of optical glasses from the melt. Frequent other causes are tight lens mounts or damage e. g. because the objective has been dropped.

Strain-free and carefully tested objectives specially produced for polarized-light microscopy are engraved with the letter P (e. g. EF 63/0.85 P). The engraved letter (P) of the objective Pl 1/0.04 (P) means that this type of objective is, within limitations, free from loss of contrast, which here is not caused by strain, but by the size of the object field, which has a diameter of 18mm. Objectives that have been damaged or are not engraved "P" are best checked for strain with exactly crossed polarizers and with observation of an empty area after insertion of the BERTRAND lens. During the test, the objective must be rotated through at least ¼ turn in its mount (thread). Strain-free objectives of medium and high magnifications show a symmetrical, blurred cross* of unchanged shape (Fig. 38a). In low-power objectives the cross is hardly distinguishable.

Asymmetrical loss of contrast which changes its shape as the objective is rotated (Fig. 38c, d) indicates the presence of undesirable strain birefringence. If it does not, the strain is present in one of the condenser lenses or in a tube lens.

Very minute strain occurs in every lens with a temperature change of even only a few degrees. If, for instance, a piece of glass lying on the object stage is warmed up by marginal finger contact, a slight loss of contrast can be observed between crossed polarizers. If the most stringent demands of freedom from strain are made of the optical equipment, a constant-temperature (maximum ±2° C) storage of the entire set of instruments is essential.

Fig. 38:
Upper focal plane of the objective during observation with the BERTRAND lens:
a. with strain-free optical systems and exact crossing of the polarizers (the image resembles the interference figure of an optically uniaxial crystal, cf. Fig. 71)
b. when the polarizers are not exactly crossed (the image resembles the interference figure of an optically biaxial crystal, cf. Fig. 74).
c. heavy strain in an objective between crossed polarizers
d. strained objective as c, but in circularly polarized light.



* If the polarizers are not exactly crossed, the cross is ± opened up.

The lightened sector (Fig. 38a) which can be observed with the BERTRAND lens and objectives of higher magnification are caused by minute rotation of the vibration directions, which is due to not precisely vertical incidence of the light waves on the numerous curved lens surfaces of the condenser and objective system (cf. polarization through reflection p. 24). For almost all investigations in polarized light, this loss of contrast is without disadvantage. The engraved letters indicate the type of correction.

Fig. 39:
Top:
Polarized light objectives for transmitted light
Bottom:
Polarized light objectives for incident light

Objective engraving

The letters and numbers engraved on the objective give the unser information on the correct use of the objective.
The figures **160** and **170** indicate that it is a transmitted light objective designed for an mechanical tube length of 160 or 170mm respectively. Whilst the LABORLUX 11 POL and LABORLUX 12 POL have the DIN tube length of 160mm, the ORTHOLUX 2 POL and ORTHOPLAN POL POL use the classic tube length 170mm. For objectives from power 16:1, however, it is unimportant whether objectives for 160 or 170mm are used. For this reason, Leitz medium and high magnification objectives are only produced for tube length 160mm.
The situation is different for objectives of magnification 10 or under. The correct tube length is important here as otherwise the image position will be different. This has the effect that it is necessary to refocus considerably after every magnification change, without the image being visibly improved. For this reason, some low-powered objectives are available for both 160 and 170mm tube lengths.
For incident light microscopy, objectives for tube length infinity (with engraving ∞) are used.
The use of incident-light objectives on the transmitted-light microscope (and vice versa) produces, especially at low objective magnifications, images of only just acceptable quality. Such odd combinations should, however, not be used, because the advantages of the high quality of the objectives are largely lost. The engraved reproduction ratio and aperture values are no longer correct (exception see below).
The engraved figure **0.17** indicates that the object must be under a coverglass of 0.17mm thickness. The effect of the coverglass thickness is reduced with objectives of magnifications below 16:1 and the object can be observed with or without coverglass. These objectives have (–) engraved instead of the figure 0.17. Incident-light objectives have been computed for use without coverglass: they are engraved **0** For the examination of thin sections without coverglass at high magnifications the incident-light device (p. 83) can exceptionally be used in

combination with transmitted-light illumination. Incident-light objectives must, however, on no account be mounted on the transmitted-light revolving nosepiece or on the objective centring clutch (see above).
The engraved letters indicate the type of correction.
The engraving **PL 5/0.08** indicates the type (normal planachromat), the magnification, and the numerical aperture (cf. p. 27) of the objective.
The numerical aperture (N.A.) is a measure of the ray cone accepted by the objective:

$$N.A. = n \cdot \sin \alpha$$

where n is the refractive index of the medium between the specimen and the objective (air or oil) and α the angle included by the extreme ray entering the objective and the microscope axis. The numerical aperture determines the resolving power of the optical system. As the magnification increases, the aperture is therefore also increased by computation. For maximum magnifications, e.g. with EF 100/1.25 OIL P and PL FLUOTAR® 100/1.32 OIL P objectives, oil immersion is required. With incident-light objectives oil immersion serves to increase not only the resolving power but also the contrast through selective effect on the reflecting power of the object (cf. p. 83). Immersion objectives have a black or white ring engraved round the mount.
For observation with an oil immersion objective 1-2 drops of the Leitz immersion oil (immersion oil by any other manufacturer should not be used) are applied to the specimen. To allow this, the object stage may be slightly lowered or the objective temporarily turned out of the beam. Air bubbles must be strictly avoided. If necessary they may be squeezed out of the oil with a fine wooden stick. After use the immersion oil must be removed from the specimen and the optical system (p. 50).
The engraved letter **P** indicates that the objective is strain-free and therefore fully suitable for microscopy in polarized light (see above). The technical data of all available objectives, condensers, eyepieces, and graticules are listed in our brochure "Image-forming and Illuminating Systems". The choice of the objectives to be acquired depends on the purpose of their use. NPL, NPL FLUOTAR and PL FLUOTAR objectives are used mainly for photomicrography because of their excellent flatness of field. Achromats are less expensive than apochromats and semi-apochromats, and are therefore preferred for student's and laboratory microscopes. The do not have any special data engraved before the magnification value. The **EF** engraving indicate achromates with particularly good flat field performance.
UT objectives are used for work with the universal rotating stage. The objectives, like special heating stage objectives, are eminently suitable for all microscopic methods calling for long working distances. In universal rotating stage objectives the engraved magnification and aperture values change when these objectives are used without glass segment (double engraving).
The dimensions of Leitz objectives are such that after objective changes only minor refocusing of the image is necessary. The so-called *parfocal distance* indicates the distance between the object and the screw-on face of the objective. With the transmitted-light pol objectives it is 45mm (exception: Pl 1/0.04 P: 65.6mm), and with incident-light pol objectives 30mm (exception: NPl 5/0.09 P: 39mm). On students' microscopes all incident-light objectives are matched for 45mm by means of adapter rings. Interference contrast objectives are also matched for 45mm with the WOLLASTON prism adapter.

Eyepieces

Eyepieces serve for the secondary magnification of the object image magnified by the objective and projected into the *intermediate-image plane* (Fig. 50).
The standard eyepieces for all modern Leitz polarizing microscopes are of the PERIPLAN GF 10x M type. The crosslines incorporated (Fig. 31) precisely indicate the vibration direction of the polarizers. The eyepiece can also be engaged in the eyepiece tube at exactly 45° rotation, so that the crosslines indicate the vibration directions of an object in the diagonal position. Microscopy without eyestrain is possible only if the crosslines have been critically focused by adjustment the eyelens with the eye relaxed. Spectacle wearers are recommended to use special high-point eyepieces.
For linear measurements point-counting methods, as well as for the indication of the outlines of formats in photomicrography eyepieces with special graticules are available.

Fig. 40:
Linear measurement in the microscope
Calibrating the graticule in the eyepiece scale on crosswires with the aid of a stage micrometer (right) placed on the object stage. In the example shown 50 graduation lines of the eyepiece micrometer correspond to 1.22mm (distance between 2 graduation lines = 24.4μm, so-called micrometer value).

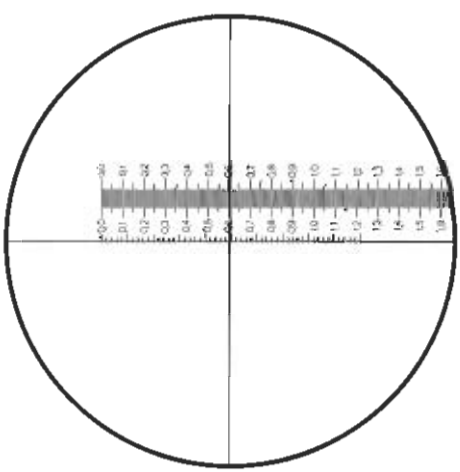


Fig. 41:
PERIPLAN eyepieces in binocular tube.

Table 1: Survey of Leitz polarizing microscopes (as of 1985)

Microscope	LABORLUX 11 POL	LABORLUX 12 POL	ORTHOLUX 2 POL	ORTHOPLAN POL
Use	Teaching and student microscope, transmitted and incident light	Teaching and laboratory microscope, transmitted and incident light	Research microscope transmitted and incident light	Large research microscope transmitted and incident light
Light sources	Transmitted light: built-in 6V 10W halogen lamp Incident light: lamphousing 20 with 6V 20W halogen lamp	Transmitted and incident light with lamphousing 20, 6V 20W halogen lamp	Transmitted and incident light, 6V 15W low-voltage lamp, lamphousing 50 with 12V 50W halogen lamp	Transmitted and incident light lamphousing 100 with 12V 100W halogen lamp; spectral lamps
Field diaphragm	Only incident light	Incident and transmitted light (Koehler illumination)		
Condensers and polarizers (transmitted light)	Non-interchangeable condenser with swing-out top As 0.90 P, foil polarizer	Interchangeable, centerable condenser with swing-out top Achr. 0.90 P, foil polarizer, special condensers for UT stages	Interchangeable, centrable swing-in condenser 702i with iris diaphragm, interchangeable tops, optionally with foil polarizer, pre-polarizer, prism polarizer, overview condenser for PL 1/0.08 objective Special condenser tops for UT stages	
Additional illumination methods	Darkfield	Darkfield, phase contrast, interference contrast T, universal rotating stage methods, circular polarization.		
Incident light illuminators	SR Pol incident light illuminator (semi-reflecting planar glass), centerable field diaphragm, iris aperture diaphragm, can be equipped for interference contrast R TR Pol incident light illuminator (pupil splitting) Incident light fluorescence illuminator		Pol incident light illuminator with compensation prism and planar glass, centrable and focusable field diaphragm, iris aperture diaphragm Incident light interference contrast illuminator, illuminator for metallography, incident light fluorescence illuminator	
Additional incident light illumination methods	Interference contrast R, quantitative incident light interference, microhardness testing, fluorescence		Darkfield, interference contrast R, quantitative incident light interference, microhardness testing, fluorescence	
Stage ball-bearing mounted	Non-interchangeable, diameter 168mm, 0.1° vernier	Interchangeable, diameter 168mm, two 0.1° verniers, friction-damped movement	Interchangeable, diameter 150 mm, 2 verniers, friction-damped movement, 45° click stop	
Focus controls Micrometer scale	Combined coarse/fine Approx. 2 µm	Coaxial coarse/fine Approx. 2 µm	Coaxial coarse/fine 1 µm	
Objective carrier Tube slot	Non-interchangeable quadruple nosepiece with individual centring. Objectives face rear. One tube slot		Interchangeable quintuple nosepiece with individual centring, objectives face rear. Objective clutch. Supplementary lens for conoscopy. Transmitted light: 2 tube slots at 90° to one another. Incident light: one tube slot	
Tubes Bertrand lens	Monocular and binocular observation, and binocular photo tubes with or without back reflection, intermediate tube for monocular and binocular conoscopy, centrable Bertrand lens, pinhole stop for conoscopy of small grains		Binocular phototube with centrable and focusable Bertrand lens and pinhole stop	Binocular phototube with or without back-reflection, with centrable and focusable Bertrand lens and pinhole stop
Analyzers	Disengageable, optionally fixed or rotatable trough 360°, 0.1° vernier and neutral filter (incident light: only rotatable through ±8°; neutral filter as option.)		Rotatable through 360°, 0.1° vernier, disengageable neutral filter	
Eyepieces	PERIPLAN 10x/M high-point eyepieces with or without crosswires. For length measurements, point counting, photomicrography			
Objectives Transmitted light	EF achromats and PL FLUOTAR (tube length 160mm)		PL FLUOTAR, EF achromats (tube length 170/160mm) (tube length ∞)	
Incident light	Achromats, NPL planachromats, NPL FLUOTAR			
Photomicrography	Leitz VARIO ORTHOMAT 2 and Wild MPS systems			
Heating stages	Heating Stage 80 −20 to +80° C	Heating Stages 80, 350, 1350, −20 to +1350° C	Heating Stages 80, 350, 1350 and 1750 −20 to +1750° C	
Photometry Image analysis	ASM 68 K image analysis system		MPV compact microscope photometer ASM 68 K image analysis system	Autoscope, TAS plus

Fig. 42:
LABORLUX 11 POL teaching and laboratory microscope

1. Monocular Pol tube P with pinhole stop and centerable Bertrand lens
2. Intermediate tube 90 with disengageable analyzer
3. Compensator or blank slide
4. Centerable quadruple objective nosepiece
5. Ball-bearing-mounted rotating stage with scale and Vernier; 2 specimen clips
6. Pre-centered polarized light condenser with aperture diaphragm
7. Single-knob combination control for coarse and fine focus
8. Illumination cone with filter holder
9. Foot with built-in transformer and 6V 10W halogen lamp

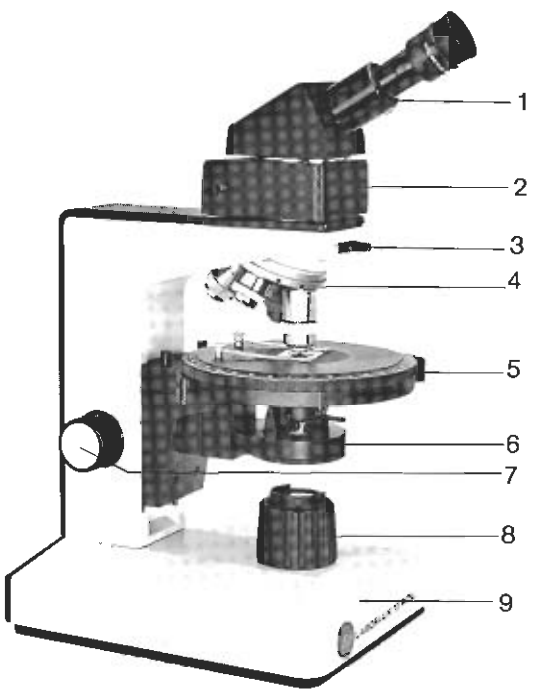


Fig. 43:
LABORLUX 12 POL routine and laboratory microscope

1. Binocular phototube FSA 55 with automatic focus compensation
2. Intermediate tube 360 B with rotatable analyzer, Bertrand lens and pinhole stop
3. Compensator or blank slide
4. Centerable quadruple objective nosepiece
5. Ball-bearing-mounted rotating stage with scale, friction-damped movement and 2 Verniers; attachable Pol mechanical stage
6. Centerable, interchangeable polarized light condenser with aperture diaphragm
7. Coaxial coarse and fine focus controls
8. Field diaphragm with filter holder
9. Foot with built-in transformer
10. Lamphousing 20 with 6V 20W halogen lamp

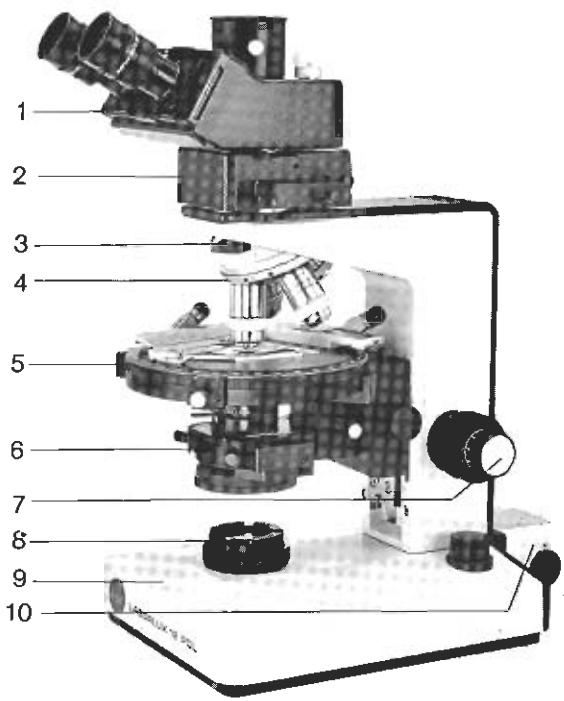


Fig. 44:
ORTHOLUX 2 POL BK and ORTHOPLAN POL (right) research microscopes

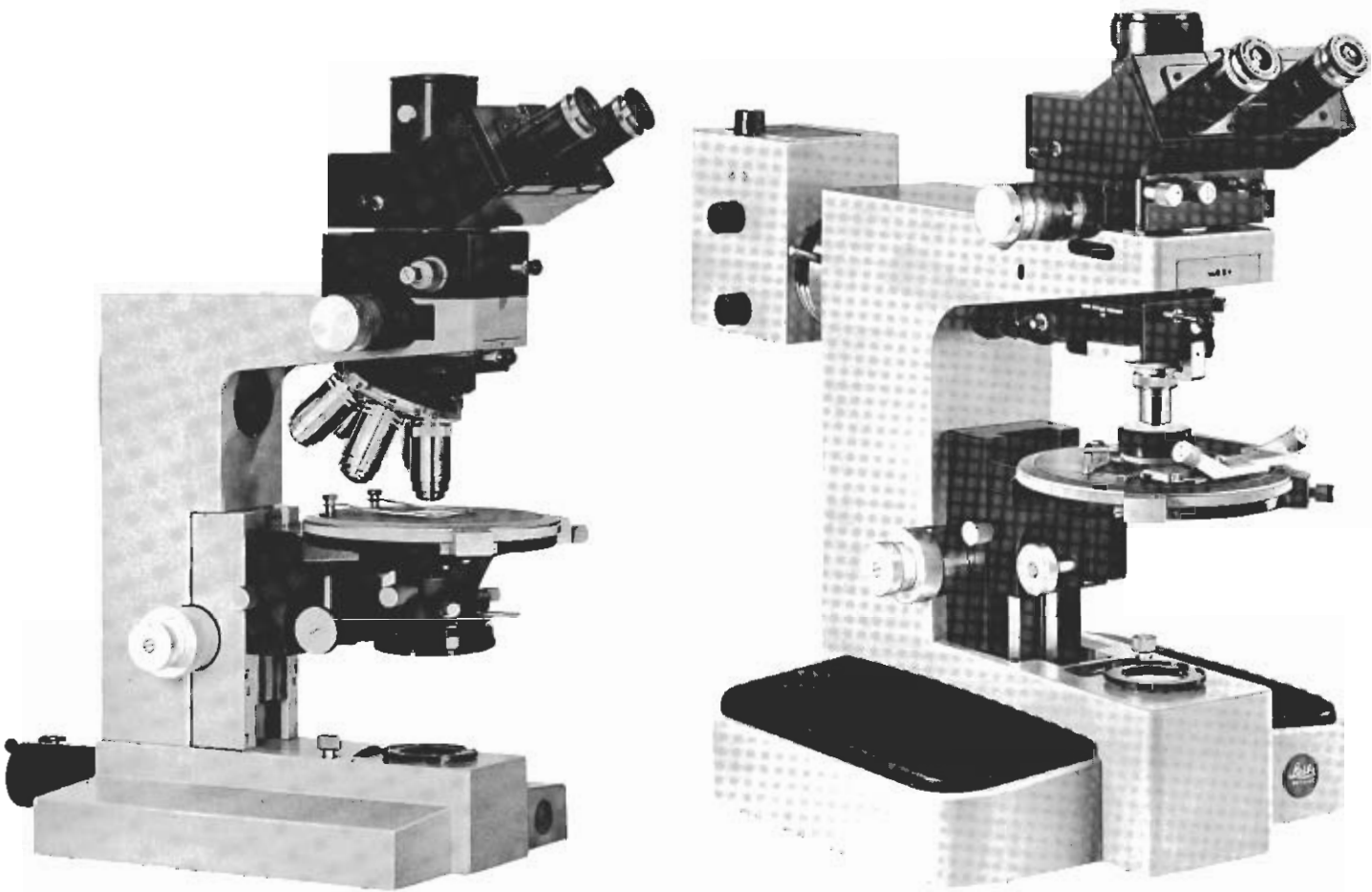


Fig. 45:
Sectional drawing of a research microscope (ORTHOPLAN POL, cf. Fig. 44, sectional drawing incident light Fig. 82).

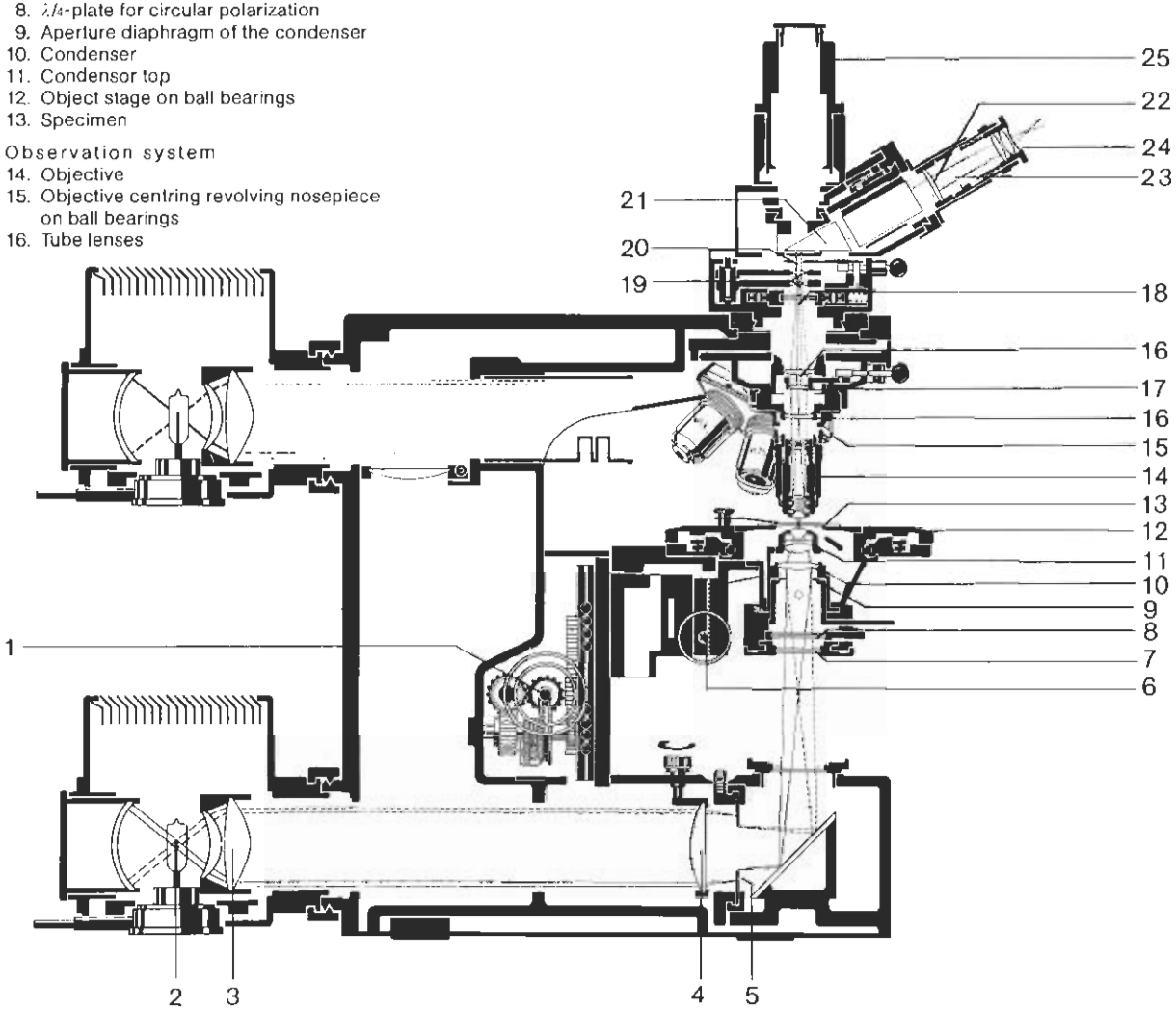
1. Coarse and fine adjustment, actuating the stage

Illuminating system

2. Filament of the halogen lamp in the Lamp Housing 100
3. Lamp condenser
4. Hinged lens
5. Field diaphragm
6. Vertical adjustment of the condenser
7. Polarizer
8. $\lambda/4$-plate for circular polarization
9. Aperture diaphragm of the condenser
10. Condenser
11. Condensor top
12. Object stage on ball bearings
13. Specimen

Observation system

14. Objective
15. Objective centring revolving nosepiece on ball bearings
16. Tube lenses
17. Supplementary lens for the conoscopy of small particles
18. Analyzer
19. BERTRAND lens
20. Pinhole stop for the conoscopy of small particles
21. Deflecting prism for binocular observation
22. Field lens of the eyepiece
23. Intermediate-image plane with crosslines
24. Focusing eyelens of the eyepiece
25. Photo tube for photomicrography, projection, attachment eyepieces, or microphotometry



Polarizing stereomicroscopes

For the low-power observation of object portions larger than 1cm² stereo-microscopes with polarizing attachment are used with the lowest-power microscope objectives (Pl 1/0.04 and Pl 2.5/0.08 P, and observation with BERTRAND lens on the ORTHOPLAN POL P).
For the determination of the vibration directions γ' and α' in the object, λ- and $\lambda/4$-plates can be inserted in the beam. Measurements of phase differences and conoscopy are not provided for. It should be noted that objects of greater phase differences may display different interference colors in the left-hand and right-hand eyepiece because of the directionally-derived birefringence.

TV microscopy

It is no problem to mount a black and white or color TV camera on the microscope. It is especially easy for cameras with C screw lens mount, optionally with magnification changer, either stepped or stepless. Special adapters are in general necessary for 3-tube color cameras with non-standard lens mounts.
The Leitz light regulator, controlled by the video signal, is necessary if the camera does not have a built-in automatic brightness control. It ensures that the camera receives practically the same light intensity, if the color temperature is constant, when the magnification of illumination method is changed.

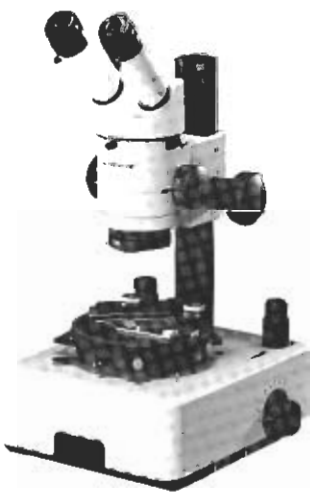
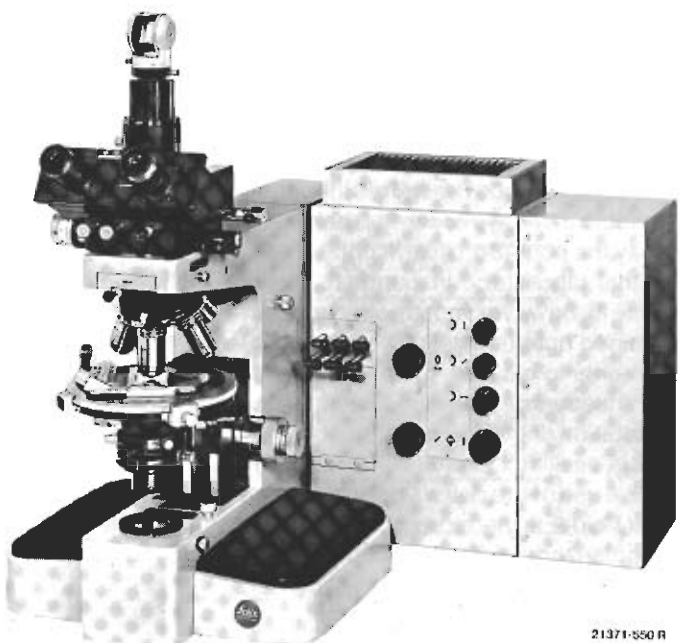


Fig. 46:
Stereo-microscope WILD M3Z with transmitted light base EB and polarizers for orientating investigations in polarized light

Microprojection

For microprojection of detailed structures such as thin sections of rocks the ORTHOPLAN POL with the 450W ultra-high pressure xenon lamp (Fig. 47) must be used. Because of the exposure of the polarizer to intense heat, polarizing condensers with pre-polarizer or prism are necessary (p. 26).
Highly reflecting screens reflect a sufficiently bright projected image of up to 1.2m diameter provided the projection room is completely blacked out. Larger projected images of sufficient intensity could theoretically be realized by the use of more powerful illuminating systems, but most specimens will be damaged by their exposure to intense heat.
The best possible adaptation of the outfit to conditions of space is by means of projection eyepieces with deflecting mirror and of various magnifications. Very low-power eyepieces have been found particularly suitable, since they permit the microscope to be set up behind the viewers; this avoids disturbance through stray light from the louvres of the lamp housing.
If the projection attachment (Fig. 37, diameter of the ground-glass screen 15cm) is used instead of the projection eyepiece, projection is possible also in daylight with the aid of the 450W xenon lamp. The number of viewers will, however, be restricted to about 5 in this case.
If the projection room is dimmed, the projection attachment can also be used in conjunction with the 150W ultra-high-pressure xenon lamp. If the room is completely blacked out, the 50W and 100W tungsten halogen lamps, too, can be used.
Projection with halogen arc or mercury lamps is not advisable because of the spectral composition of their light, which strongly deviates from that of daylight.
In spite of high capital cost, color television is constantly gaining in importance because of the progressive improvement of color reproduction.

Fig. 47:
ORTHOPLAN POL with Lamp Housing 500, Mirror Housing 500, condenser 702 fv, projection eyepiece and projection mirror for micro-projection with polarized light



III. Use of the polarizing microscope and its accessories

The application of polarized-light microscopy was originally restricted almost exclusively to the identification of minerals and rocks. Here the largest possible number of optical as well as morphological parameters (Table II) are compared with identification tables. This versatile quantitative microscopic method is still widely used by geologists and workers in related sciences although other analytical methods such as electron beam-microprobes, x-ray methods, atom absorption etc., are available in many research laboratories.
Today the polarizing microscope is used increasingly in the industrial laboratory. In contrast with the petrographic field (Table II), however, mostly only one or two parameters are determined quantitatively. These are, for instance, strain measurements in glasses, glass-to-metal seals and plastics, (Fig. 66), measurement of the birefringence of fibers (Fig. 64), or orientation of the axis of crystals used in industry (e. g. bearing jewels, oscillator crystals etc). Numerous possibilities of quantitative use of the polarizing microscope also exist in the biological field. Furthermore, many interference methods relying on polarized-light microscopic aids have found increasing practical within the last few years (p.86).

Relationship between structure and birefringence

The phenomenon of birefringence observable in the spectral region from about 380 to 780nm is the result of the microstructure of the substances examined. The order of differences between the structural components, i.e. atoms and molecules, is however, 100-1000 times smaller than the light waves. The components are thus beyond the limit of direct observation not only in the optical microscope but also in most electron microscopes.
In spite of the enormous difference between the dimensions of the components and that of light as observation medium the effect of vibration directions and velocities allows deductions about the latent structural elements.

Gases, liquids, amorphous substances

In gases, liquids, and non-crystalline (amorphous) substances atoms and molecules are in a state of random distribution. A light wave penetrating such substances, it is true, is slowed down, but the velocity is the same for all propagation directions of a given material, i.e. these substances are *isotropic* (Fig. 8).

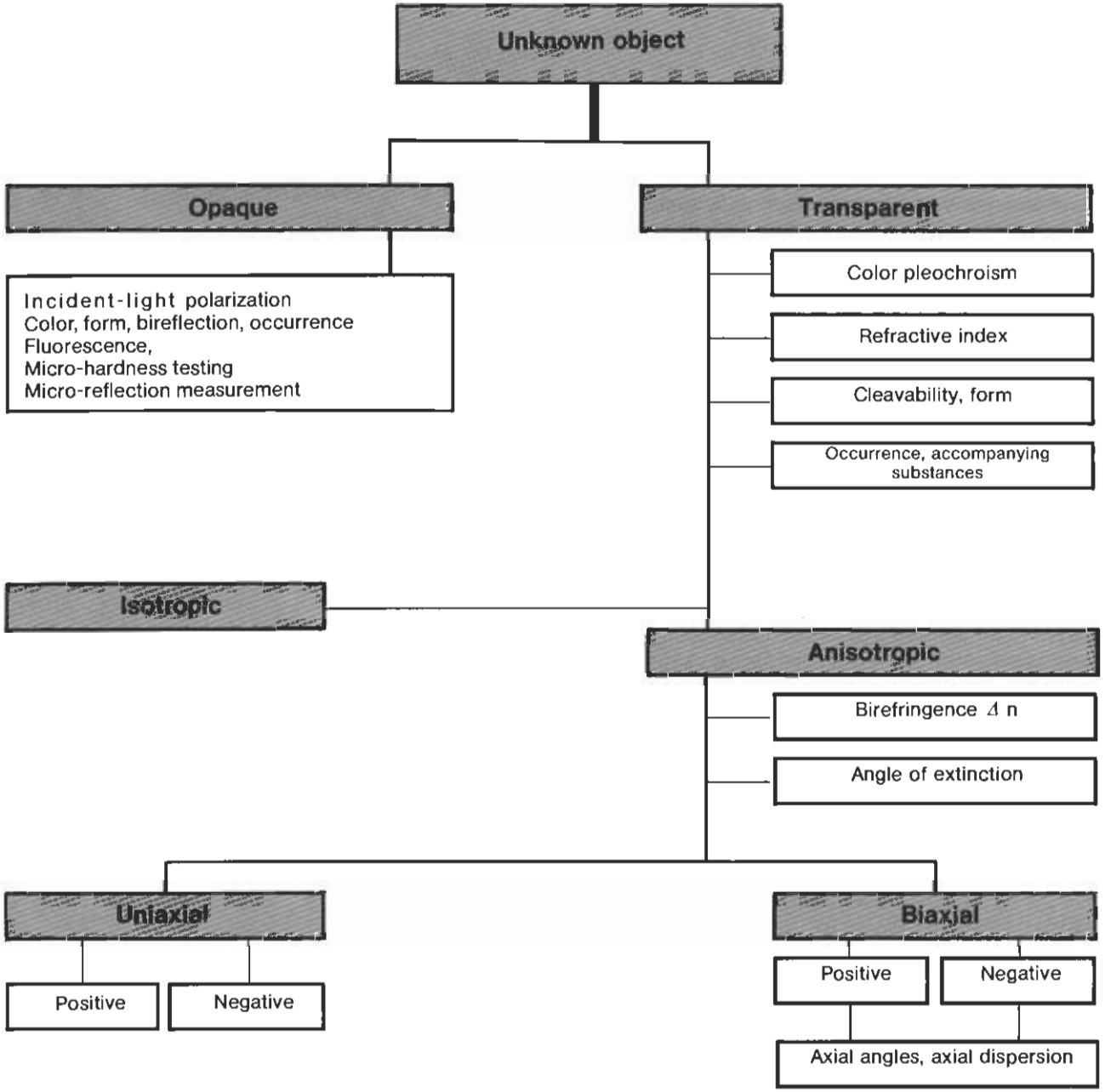


Table II: Procedure of investigation with the polarizing microscope (diagrammatic)

Strain birefringence

If unidirectional strain or stress acts on an optically isotropic substance, the distance between the components is changed in the direction of the strain or stress: the substance behaves optically anisotropically, and birefringence occurs. Glasses subjected to rapid cooling or fixed in a tight-fitting mount (cf. p. 34) or containing metallic inclusions exhibit this effect of strain birefringence very markedly.

Flow birefringence

Particles in a state of flux arrange themselves preferably with their longitudinal axes parallel to the direction of the flow. This also applies to liquids consisting of ± elongated molecules. This arrangement produces flow birefringence, which, however, is very slight.
Synthetic fibres consist of long-chain molecules. The spinning of the fibre from the liquid basic substance is a flow process in which adjustment and therefore birefringence occurs. This birefringence is initially slight. Only when the fibre is stretched does considerably increased adjustment occur. This may produce a specific birefringence of $\Delta n > 0.2$. Since the birefringence of synthetic fibres and foils may supply important information on wear and tear and strength properties, phase difference measurements, (Figs. 64-66) are among the standard methods of the testing of plastic materials.

Biological specimens

Important, but still far too rarely used, is the demonstration of birefringence of biological structures, which consist mainly of long stretched structural elements. W. J. SCHMIDT has conducted basic research in this field.

Liquid crystals

Some liquids also exhibit birefringence owing to preferential arrangement of their molecules within certain temperature ranges. The adjustment can be triggered by the setting up of an electric field or by shearing movements.

Form birefringence

This is caused by fibrous or laminar particles embedded in isotropic media, e. g. glass fibres embedded in a liquid or synthetic resin.

Crystals

Crystals are regular arrangements of atoms and molecules set up according to a lattice system. Depending on the lattice distances and the angles of the individual lattice axes we distinguish between seven classes of crystal.
The optical behaviour of the seven crystal classes is illustrated in Fig. 49.

* A list of the more than 400 scientific publications by W. J. SCHMIDT has been published by the Zoological Department of the Justus-Liebig-University, D-6300 Giessen.

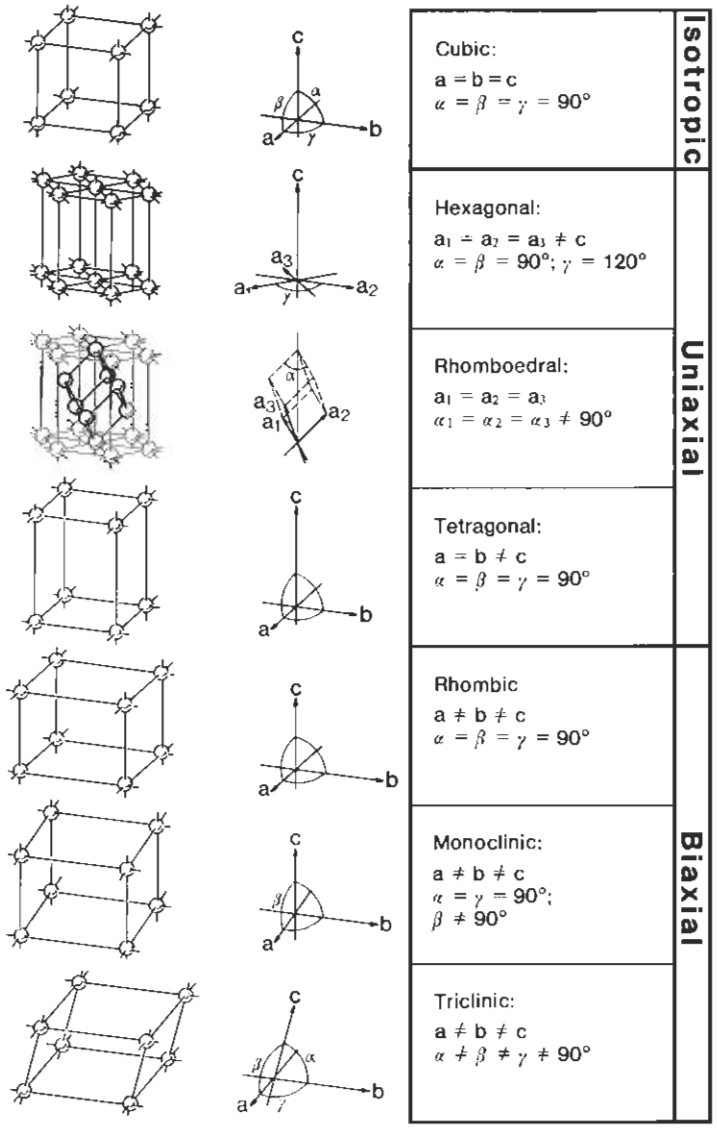


Fig. 48:
Relation between crystal structure and optical behaviour

Specimen preparation

The use of suitable methods of preparation is essential to most microscopic investigations. As a rule, microscopy is possible without preparation only if the object size is at least of the order of mm, and the object bordered by two plane-parallel surfaces. Here it is usually sufficient to place the particles under investigation on a glass plate (microscope slide) of 1mm thickness. For this purpose, versions used in biology and medicine for the preparation of large sections and commercially available (thickness 1.1±01.mm) are preferable to the conventional microscope slides (mineralogical format: 26x45mm; biological format 26x76mm)*.

If necessary, normal glass plates obtainable in any photographic shop for the mounting of transparencies can also be used.

With small objects (grain size <1mm) and with objects with irregular, rough surfaces, embedding in a suitable liquid or transparent synthetic resin is necessary; it is a basic rule here that the refractive indices of the embedding medium and that of the object should be as similar as possible. (cf. p. 96).

* DIN 58884

Powder specimens

Powder specimens are prepared not only from granular or fibrous material, but sometimes also from large objects by the breaking or scraping off of a few very minute splinters. One to two drops of a suitable immersion medium (Table V) are applied to a clean microscope slide. The granules to be examined are now scattered onto the immersion medium, preferably from the tip of a spatula. Now a coverglass is carefully placed on top. With coarser grains (>0.3 mm) mechanical crushing or screening of the objects to be examined is recom-

mended to obtain sufficient transparency and depth of field. Optimum grain size must be determined by trial and error.
It is best to immobilize fibers with the aid of adhesive tape before they are embedded. Stress forces must be avoided by all means, since these influence the birefringence of the fiber.
For specimens to be discarded after microscopy, a liquid of suitable refractive index is adequate as an inclusion medium. When immersion objectives are used the coverglass must be sealed with coverglass cement; if this is not available, nail varnish will be quite satisfactory. It prevents displacement or lifting off of the coverglass. Since at the same time evaporation of the immersion medium is prevented, granular specimens, horizontally stored, can be kept for limited periods. Specimens for filling should be dispersed in hardening synthetic resin, such as Caedax.

Immersion chambers

For the embedding of objects measuring more than 1mm (such as bearing jewels, glass-to-metal seals, plastic parts) it is best to build a small chamber. A large microscope slide or transparency mount serves as a base. A metal or plastic ring is glued on to it with a suitable adhesive (such as Araldite, UHU PLUS, Sigomet, Agumet). Such a ring is best cut off a tube consisting of the available material with a metal saw. If the tube diameter is very small, the chamber must be kept low, since otherwise the objective mount may make contact with the metal ring. For objects of cm size it is best to make a rectangular chamber out of strips of glass.
Before use, tests must be carried out between crossed polarizers to see whether strain birefringence has been produced by the adhesive process in the bottom of the chamber. Any birefringent adhesive residues must be removed from there. Obviously, the adhesive used must not be dissolved by the immersion medium.
Immersion oil ($n_D = 1.515$, $n_C = 1.518$) is often satisfactory as embedding medium. A wide variety of organic liquids (Table V, p. 108) are suitable for objects of higher refractive indices. For the examination of bearing jewels, for instance, methyl iodide has been found particularly useful. For some examinations water, or preferably glycerin, is sufficient. These liquids have the advantage of very easy removal.
Disturbance of the liquid surface caused by vibrations produces an unsteady microscopic image.

Thin sections

Soft biological objects and plastics can be cut on microtomes into "thin sections" of down to 0.5μm thickness and embedded much like a dust specimen. Details can be obtained from our brochure "The Microtome and Its Application".

Thin polished sections

Thin polished sections are produced for the investigation in polarized light of rocks and other objects consisting of microscopically small crystal structures (concrete and other building materials, teeth, bone shells, kidney stones).
For this purpose a piece of the substance to be investigated is cut off with a diamond saw. A surface is ground, polished and cemented on to a microscope slide. Sufficient material is now cut off with the diamond saw so that only a layer of less than 0.2mm thickness remains on the microscope slide. This layer is now reduced to a thickness of about 20μm by means of grinding and polishing, and finally covered with a coverglass.
Melting specimens are produced by the heating of a small amount of substance on a microscope slide. It is best to cover it with the coverglass during the heating process (gas flame) (Fig. 96).

Setting up, adjustment, and maintenance of the microscope

Microscopes are best set up on stable work benches which must not be too high. Benches with drawers to house accessories laid out ready for use are particularly recommended. The special microscope tables of the Leitz production programme have been developed to meet these requirements. Plastic inserts are available for arranging the accessories in the drawers.

It is an unfortunate experience that highly valuable optical, precision-mechanical, and electronic apparatus is often wrongly operated. The possibilities offered by the instruments are therefore not fully utilized. This also applies to polarizing microscopes. Since a frequent cause is lack of experience, the most important steps of adjustment of a polarizing microscope are listed below. Details can be obtained from the instructions for the relevant microscope.

Fig. 49:
Granite, thin section

Orthoscopy

Center the light source
Focus the eyelens of the eyepiece
Center the objectives
Turn the front lens of the condenser into the beam at objective apertures >0.25
Form a sharp image of the field diaphragm by centering and vertically adjusting the condenser, together with one of the specimen (Köhler's illumination)
Cross the polarizers
Adjust the aperture diaphragm

Conoscopy

Use an objective of larger aperture (>0.65).
Fully open the aperture diaphragm
Turn the BERTRAND lens into the beam (in research microscopes also center and focus it)

Care of the optics

The optical parts of the microscope must be kept absolutely clean. The external surfaces of the microscope optical systems are coated with layers of about the same hardness as glass. All these layers, however, are very thin. They must therefore be cleaned with appropriate care.
Objectives must not be dismantled for cleaning. If damage occurs in the interior of an optical system, this should be returned to the factory for repair.

After use, the microscope should be kept under a dust cover. Special measures must be taken in countries with a tropical, humid climate; the local Leitz agency will supply the relevant information.
Repair should always be carried out only by the Leitz agency. Stages, objectives and tubes must on no account be dismantled by the user, since correct assembly is possible only with the aid of special adjustment devices. The table below contains information concerning the cleaning of the optical components:

External surfaces of objectives, eyepieces, condensers	**Dust:** remove with soft, dry sable brush. **Fingermarks:** remove immediately with a damp piece of linen or chamois leather; if necessary use ethanol or petroleum spirit. **Resistant dirt:** Remove with damp piece of fine linen or chamois leather. Clean the lens first with a highly volatile solvent (ethanol, petroleum spirit) and allow all the solvent to evaporate. Additional cleaning with expanded polystyrene has been found very reliable with dirt difficult to remove. The type of white, granular expanded polystyrene well known as packing material for our instruments is particularly suitable. Break a piece off, press it against the dry lens with a projecting grain of the fresh fracture surface and rotate it as coaxially as possible with the lens axis. This removes even from the recessed rims of the lens mount the most minute residues of immersion oil, skin grease, and solvents, which otherwise spread across the surface of the lens and partly counteract the reflection-reducing action of the coating layers. Any adherent grains of expanded polystyrene can be simply blown away or dislodged with an absolutely clean sable brush specially reserved for this purpose. Cleaning is also possible with cotton wool wrapped round a wooden stick.
Oil immersion objectives	**Clean immediately after use:** Dab off oil with a piece of blotting paper or a small piece of linen. Remove the residual oil film with a piece of linen moistened with ethanol. Xylene is not recommended due to its toxicity.
Internal surfaces of eyepieces, condensers	**Dust:** Blow it away softly or clean with a sable brush.

Orthoscopy

Orthoscopy (also direct observation; or, not quite accurately, observation in the parallel beam) is the observation of the magnified image of the object (Fig. 50) in polarized light. Details about the theory of image formation can be found in the brochure "The Microscope and Its Application" or in the special handbooks.

Investigations with a single polarizer

(Analyzer out)

Determination of the refractive indices

A complication occurs with birefringent objects. Except in the direction of the optical axis, two refractive indices must be determined. They are measured also with the embedding method, with the object first moved into the extinction position between crossed polarizers. After the analyzer has been turned out, one refractive index, and after rotation of the object through precisely 90°, the second index can be determined. Since the magnitude of one (uniaxial structures) or two refractive indices (biaxial structures) depend on the position of the observation direction referred to the optical axis (axes), it is recommended that the position of the section be checked conoscopically (p. 69) when the object is birefringent.

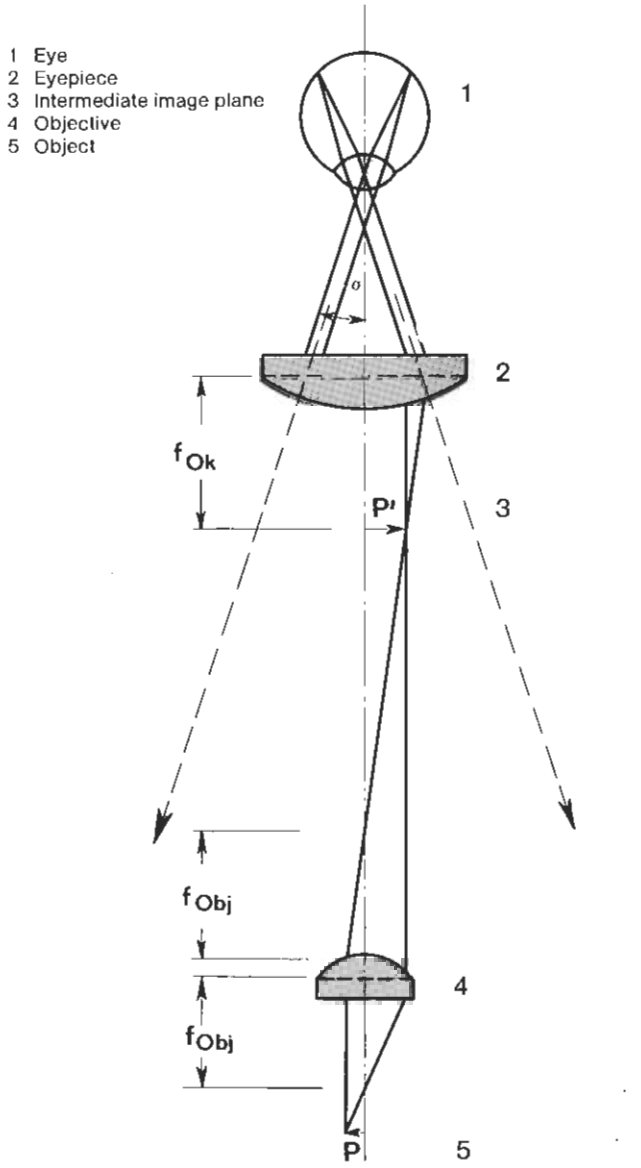


Fig. 50:
Optical path in the microscope (diagrammatic)
The object forms a magnified, real, inverted and wrong-way-round image of the object P, for instance at a scale of 5:1. Through the 8x eyepiece behind the objective this intermediate image appears magnified by a further 8x. The observer therefore sees the image as if he viewed the 5 x 8 = 40x magnified object from a distance of 250mm without instrument. Concerning the magnification the drawing is not true to scale; the polarizers have been omitted.

It is often enough to estimate the refractive indices. This is done by observation of the surface structure (relief), which is the more prominent the more the refractive indices of object and embedding medium differ. (Fig. 93). The BECKE line indicates which of the two refractive indices is the higher one (p. 93).

Pleochroism and dichroism

To recognize dichroism (p. 17) the object stage is slowly rotated. Intensity and color of dichroic substances periodically change during rotation every time the stage has been turned through 180° (Fig. 51).

If color phenomena but no intensity changes occur, the causes may be as follows:

1. The object is isotropic.
2. The object is birefringent, but the color phenomena are the result of embedded dye particles. Example: dyed fibers.
3. The object is dichroic, but this cannot be observed since the observation direction is parallel to the optical axis.

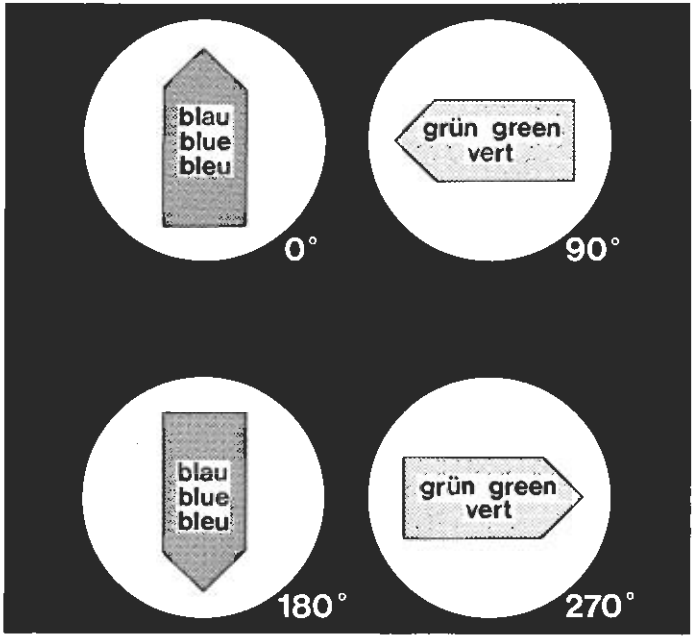


Fig. 51:
Observation of pleochroism and dichroism respectively with a polarizer: Intensity and colour of the object change 4 times during a full object revolution.

Investigations with crossed polarizers

Interference phenomena

The classical phenomenon of image duplication (Figs. 9, 10) can practically never be observed during the microscopic examination of anisotropic structures. Only with objects of a minimum thickness of 1mm and extremely high birefringence such as calcite can, in certain conditions, a duplication of the image of the field diaphragm be seen.

Birefringent (anisotropic) structures can be recognised by another phenomenon. When the object is rotated between crossed polarizers the brightness of the object image changes periodically. During a full rotation the object disappears practically completely after exactly 90° each (Fig. 52). In the intermediate rotation sector the object becomes light. Color phenomena (interference colors) can often be observed. Maximum intensity is situated exactly between two dark positions (45°).

The four dark positions are called normal positions or extinction positions (Fig. 52, left),

The four orientations of maximum light intensity are called diagonal positions (Fig. 52, right) or 45° positions.

Normal positions

Extinction position occurs when the object vibration directions α' and γ' are parallel to the transmission direction of polarizer and analyzer (Fig. 52, 53).

Fig. 53 illustrates the cause of extinction: the light coming from the polarizer P vibrates parallel to one of the two vibration directions of the object. e. g. parallel γ'. The second ray normally produced in birefringence (in the example α') cannot develop.

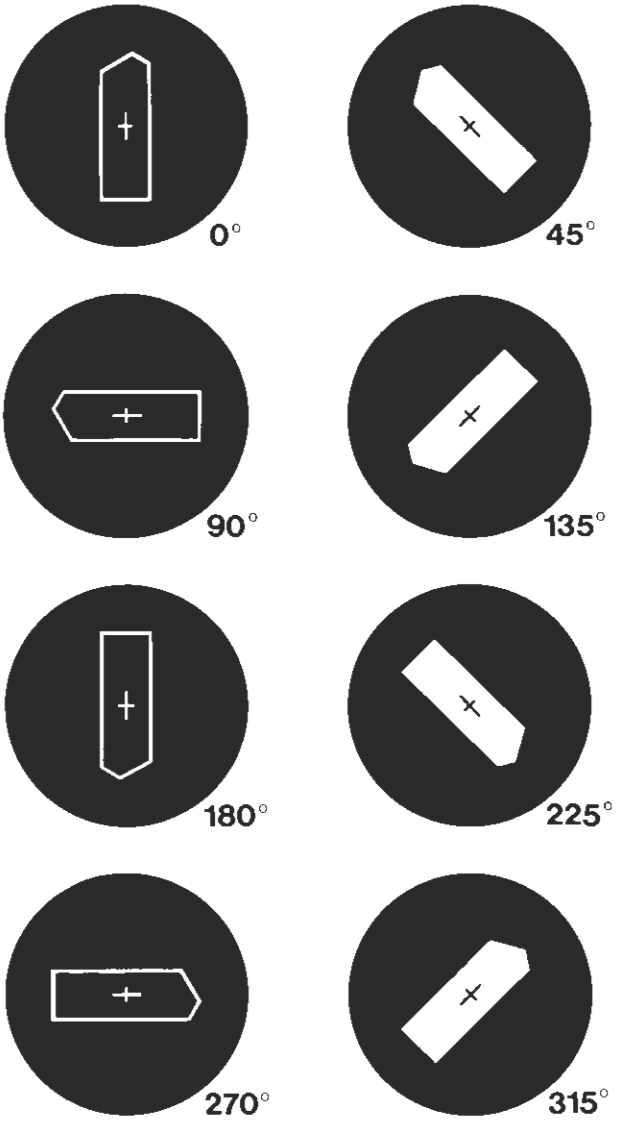


Fig. 52:
Normal positions (left) and diagonal positions (right) of a birefringent object at stage rotation through 360° (straight extinction).

After leaving the object the ray with its unchanged vibration direction γ' (= vibration direction of the polarizer) perpendicularly meets the transmission direction of the analyzer A. Rotation in its transmission direction is not possible (cf. parallelogram of forces); the object remains dark. After a 90° rotation of the object, i. e. in the next extinction position, only the ray with the vibration direction α' is produced in the object. This, too, is blocked by the analyzer.
If the vibration directions and morphological characteristics of the object, e. g. crystal edges, are parallel (Figs. 52, 53) there is *straight extinction* (oblique extinction *see* Fig. 62).

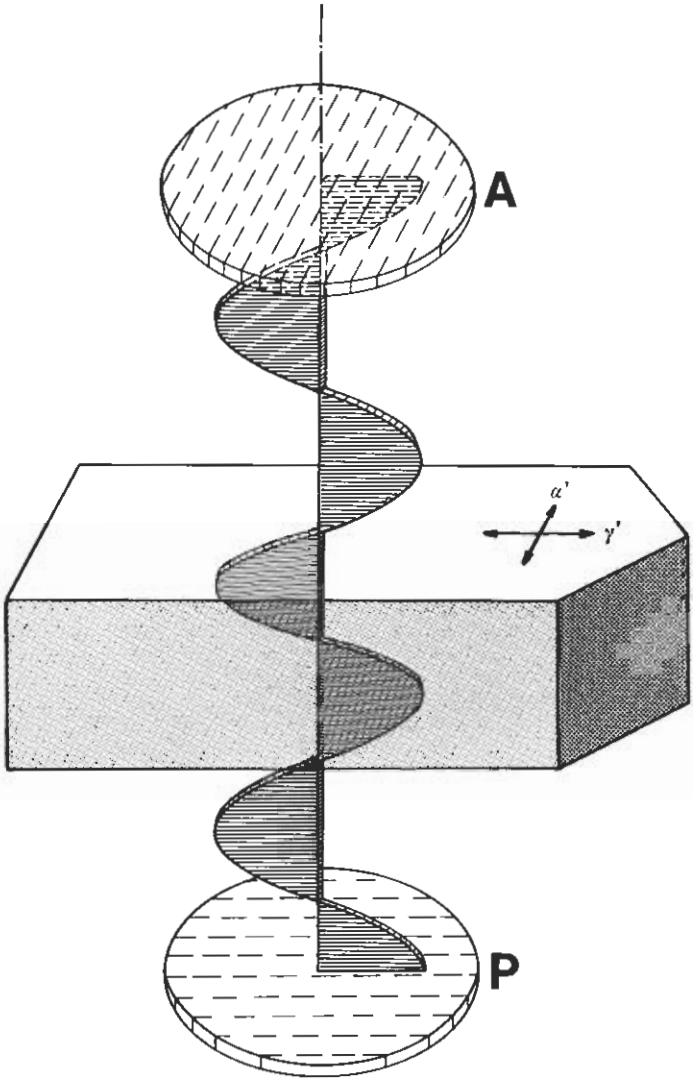


Fig. 53:
Extinction of a birefringent object in normal position.

Diagonal positions and interference colors

The four object positions of maximum intensity are called diagonal positions (Fig. 52, right). They occur after a rotation of exactly 45° from the normal positions. Lightening of the image is explained in Figs. 54-56.

A light ray coming from the polarizer (Fig. 54 P) is split in the object into the two rays of vibration directions α' and γ'. Both rays have the same intensity.

Both rays have the same frequency, and are in phase. The maxima A-A*, B-B*, C-C*, D-D*, E-E* are always reached simultaneously.

Since both rays have different velocities of propagation (different refractive indices $n\alpha'$, $n\gamma'$) a retardation results.

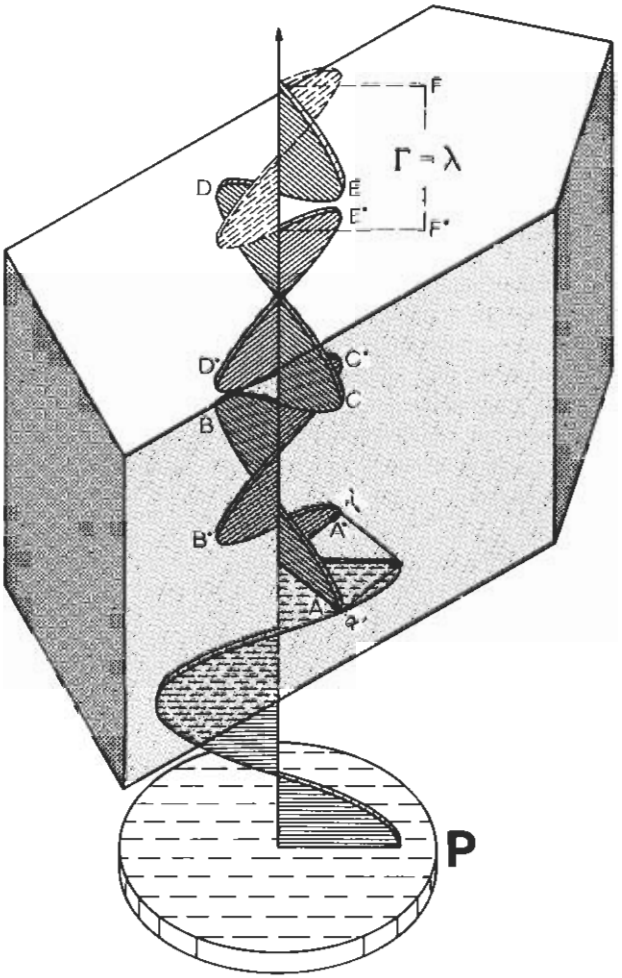
If, for instance, the slower ray γ' just leaves the object in point F* the faster ray α' has already reached point F. The retardation F-F* is called phase difference and designated with the Greek capital Gamma (Γ) (cf. pp. 11, 12). The phase difference Γ is a function both of the thickness d of the object and of the difference in velocity, i. e. of the birefringence $\Delta n'$. It can be calculated according to the following simple formula:

$$\Gamma = d \cdot \Delta n'$$

Example:
A sheet of quartz cut parallel to the optical axis has the specific birefringence (p. 13) $\Delta n = 0.009$. Let its thickness d be 66μm = 66000nm.

Γ = 0.009 x 66000 = 594nm.

Fig. 54:
Diagonal position of a birefringent object:
A phase difference Γ (in the illustration $\Gamma = 1\lambda$) is caused by a differential rate of propagation of both rays.



In practice phase differences are stated almost exclusively in nanometres today (1nm = 1 millionth mm, cf. Fig. 2).
In the explanation and interpretation of polarized-light phenomena, phase differences, on the other hand, are preferably expressed in wavelength units, e. g. $\lambda/4$. Depending on the wavelength the same (metric) phase difference can represent various phase displacements.

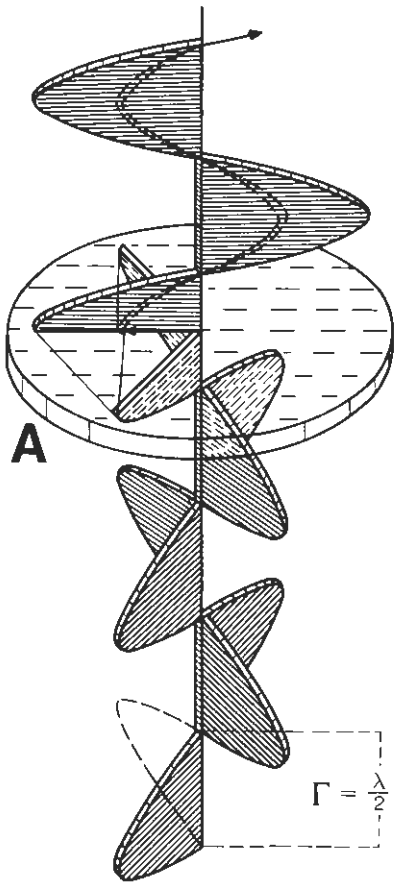
Example: Phase difference Γ = 600nm.

	(wavelength)	Phase displacement	
violet	(λ = 400nm)	600	$3/2\lambda$
orange	(λ = 600nm)	600	(I)λ
infra-red	(λ = 900nm)	600	$2/3\lambda$

After they leave the birefringent object, both waves initially continue to vibrate in the two planes** differing by 90°. The phase difference produced in the object is preserved (Fig. 54). Only by the analyzer (Fig. 55) are both phase-displaced waves deflected into the transmission direction of the analyzer: the deflection can be illustrated by vectorial representation (parallelogram of vectors). This produces the two resultant waves (broken lines).

** In many handbooks phase-displaced waves and those polarized vertically to one another are represented in the form of their resultants as elliptically or circularly polarized vibration (Fig. 4, 5). Interference phenomena in this brochure are explained only with the aid of the two original vibrations. The spatial splitting-up of the two rays is generally extremely small, and absent in special cases. Both waves were therefore represented with a joint axis.

Fig. 55:
Interference between the part-rays caused by birefringence in the analyzer at a phase difference of $\Gamma = \lambda/2$. The amplitudes of the 2 part-rays add up (in the interest of clarity the perspective has been rotated through 90° compared with Fig. 54).



Since both waves now vibrate in the same plane, their merging, i.e. interference, is possible.
Depending on the phase difference the merged wave displays differential intensity:

A. Phase difference: $\lambda/2$, $3/2\lambda$... $\frac{n+1}{2}\lambda$
   Both waves vibrate in phase (Fig. 6) and are additive: the resultant wave has the maximum possible intensity.

B. Phase difference 0, λ, 2λ... $n\lambda$
   Both waves, having reached the analyzer, vibrate in opposite directions, so that their amplitudes cancel each other: the object remains dark even in the diagonal position, provided the light is strictly monochromatic (Figs. 56, 6b).

C In the general case, i.e. when the phase difference is neither a whole nor half a wavelength or a multiple thereof, the intensity will be less than maximum (Fig. 6c).

Intensity as a funtion of phase difference can be particularly clearly observed on a wedge-shaped object. The quartz wedge available with polarizing microscopes is inserted in the tube slot and observed with the BERTRAND lens turned into the beam. It can also be placed on the object stage and viewed at the lowest power.

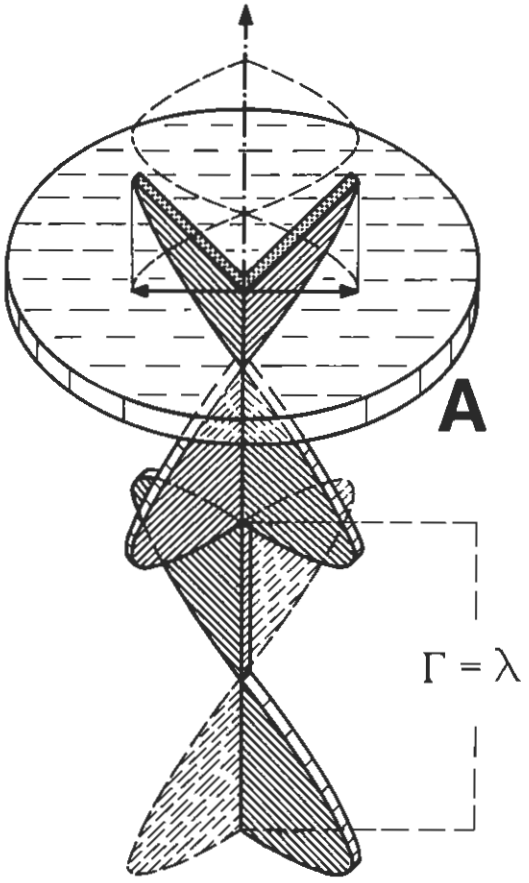
In monochromatic light (use of interference filters p. 21) dark and bright parallel fringes occur in the diagonal position (Fig. 57). The dark fringes correspond to phase differences of

$$0,\ \lambda,\ 2\lambda,\ 3\lambda,\ 4\lambda$$

Bright fringes, on the other hand, occur on all object thicknesses of the following phase differences:

$$\lambda/2,\ 3/2\lambda,\ 5/2\lambda,\ 7/2\lambda,\ \frac{n+1}{2}\lambda$$

Fig. 56:
Interference of the part-rays caused by birefringence in the analyzer at a phase difference of 0, λ, and 2λ:
Both part-rays cancel each other out (cf. Fig. 6).
In the interest of clarity, the vibration directions have been rotated through 90° with reference to Fig. 54.



For blue light (λ = 450nm; Fig. 57a) the dark fringes therefore correspond to phase differences of:

0, 450, 900, 1350, 1800nm,

the light fringes to phase differences of

225, 675, 1125, 1575nm.

For light of longer wavelengths e.g. green and red light (Fig. 57b) the distance between the bright and dark fringes increases.

For red light (λ = 700nm) the dark fringes occur at phase differences of

0, 700, 1400, 2100nm,

and the light fringes at phase differences of

350, 1050, 1750nm.

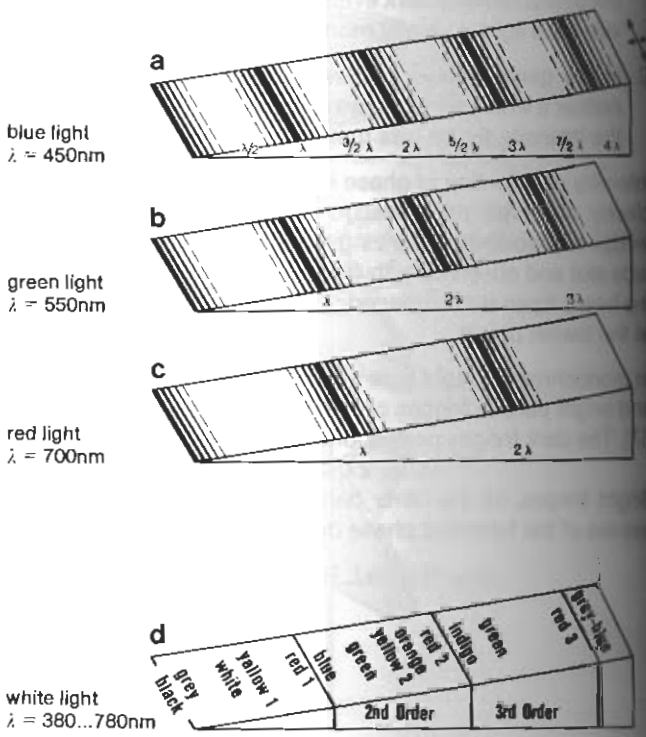
In illumination with white light these effects become superimposed: different colors (= wavelengths) are extinguished in different thicknesses of the quartz wedge, whereas in the same areas different wavelengths are found to have full or reduced intensity. These different mixtures result in an observable sequence of the interference colors (Table III) on the quartz wedge. The same color sequence occurs in almost all birefringent objects. Only with some substances of high dispersion of birefringence (p. 59) can abnormal interference colors be observed.

Interference colours are classified in *orders*. The wavelength 551nm, which represents the region of the solar spectrum which appears visually brightest, has been chosen as measuring unit. Colors of the first order correspond to phase differences from 0 to 551nm, colors of the second order to those from 551-1102nm etc. With a little practice it is possible to estimate the magnitude of a phase difference with the aid of the interference color. With increasing orders the interference colors become paler, gradually approximating a white hue, the *higher-order white*. Variable compensators (p. 65) are used for the precision measurement of phase differences.

Fig. 57:
Birefringent, wedge-shaped object (quartz wedge) between crossed polarizers, in the diagonal position)

a–c in monochromatic light:
Dark fringes occur at phase differences of 0, λ, 2λ, 3λ, 4λ.
Bright rays, on the other hand, will be seen with phase differences of $\lambda/2$, $3/2\lambda$, $5/2\lambda$.
With the increase of the wavelength, i.e. from blue to red, the separation between the fringes also increases.

d in white light:
Since dark and bright fringes of the various wavelengths occur in different portions (thicknesses) of the object, characteristic colour effects, the interference colors, are produced by super-imposition.:



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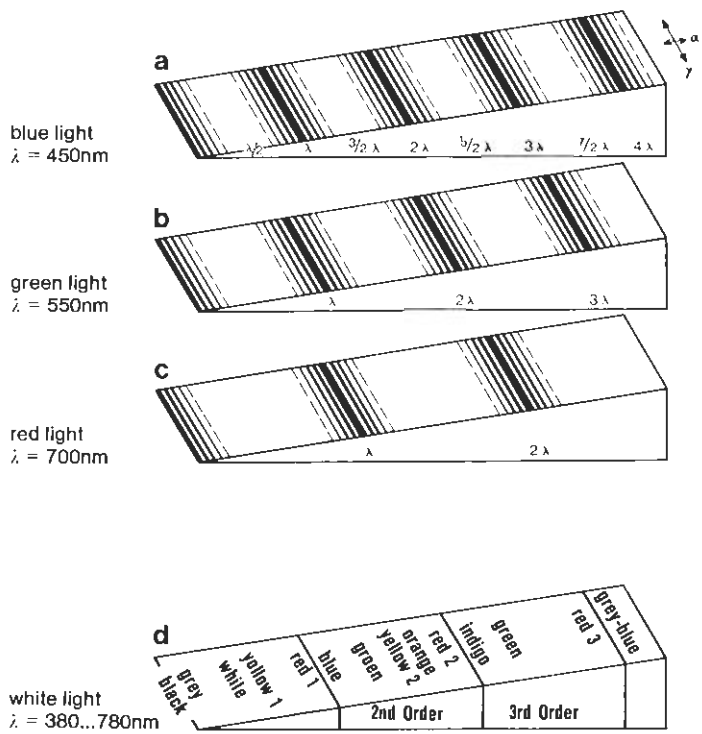
Bright rays, on the other hand, will be seen with phase differences of $\lambda/2$. $3/2$. $5/2\lambda$.
With the increase of the wavelength, i.e. from blue to red, the separation between the fringes also increases.

d in white light:
Since dark and bright fringes of the various wavelengths occur in different portions (thicknesses) of the object, characteristic colour effects, the interference colors. are produced by super-imposition. :



The complementary colors occur between parallel polarizers. Investigation between parallel polarizers, however, is not normal practice.

Aperture diaphragm and interference colors

The interference colors (the phase differences) are a function both of the thickness of the layer traversed, and of the birefringence Δn. Since different layer thicknesses are passed as well as directions of different birefringence in a wide cone of illumination (large condenser and objective aperture), a superimposition of different phase difference may occur in the same area of the object. This results in strikingly pale interference colors. This effect can be eliminated by stopping down the condenser aperture diaphragm.

Dispersion of birefringence

The dependence of the refractive index on the wavelength is called dispersion. Birefringence, too, depends on the wavelength: dispersion of birefringence.
Abnormal interference colors occur in objects of high dispersion of birefringence such as topaz. The measurement of phase differences with the aid of compensators with compensator plates of normal dispersion will then frequently be no longer possible.

Table III
Interference colors from 0 to 4λ (Orders)

	Phase difference	
1st Order	200	Black Lavender grey Grey-blue
	400	Yellowish-white Vivid yellow
2nd Order	600	Red-orange Deep red Indigo Sky blue Greenish blue
	800	Light green Pure yellow
	1000	Orange red
3rd Order	1200	Dark violet red Indigo
	1400	Greenish yellow Sea green
	1600	Greenish yellow Flesh colour Crimson
4th Order	1800	Matt purple Grey-blue Bluish-green
	2000	Light greenish grey Whitish-grey Flesh red

Addition and subtraction of phase differences

The phase differences of superimposed objects can be additive or subtractive depending, how the vibration directions of the objects are mutually orientated.

Case of addition
Example:
A birefringent object in diagonal position displays the interference color yellow 1, the phase difference is therefore about 400nm (table III). After the object has been divided both halves are superimposed with retention of their azimuthal orientation (Figs. 58a, 59a): superimposed areas of the object now show the interference color green (2nd order), corresponding to a phase difference of about 800nm (Table III). Duplication of the object thickness also duplicates the phase difference: addition of the phase differences.

Case of subtraction

If one of the two halves of the object is rotated to the left or right through 90°, the superimposed object areas become extinguished (Figs. 58b, 59b): rays which in the lower objects are initially slower (γ), become the faster rays (α') in the upper object and vice versa, since the vibration directions of the slower and faster rays in both objects are orientated vertically to each other.
The orientation where the vibration directions α' and γ' are aligned is called the addition position; the crossed orientation is called the subtraction position.
If the phase differences of 2 superimposed objects are exactly the same, the subtraction case is also called compensation, and the phase difference is zero.

Fig. 58:
Addition and subtraction as seen in the microscope (cf. Fig. 59). This experiment can be simplest carried out with two lengths of a transparent piece of adhesive tape.

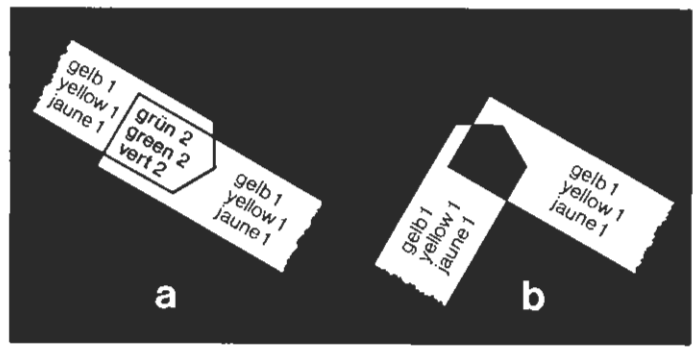


Fig. 59:
Addition (left) and subtraction (right) of phase differences at parallel and crossed super-imposition respectively of birefringent objects, diagrammatic representation.

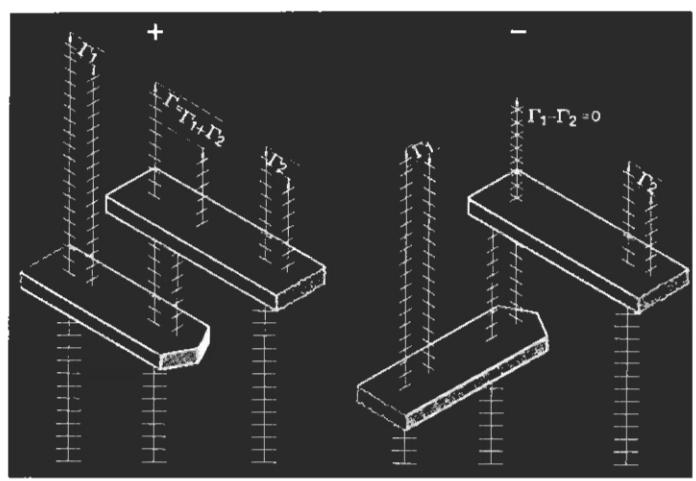
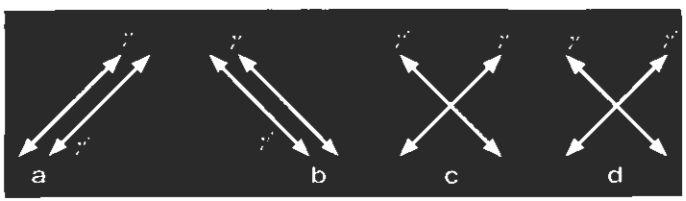


Fig. 60:
Diagrammatic representation of addition (a and b) and subtraction (c and d). For simplicity, only the vibration directions γ and γ' are represented.



Compensators

Determination of the vibration directions

Addition and subtraction cases are used in polarized-light work to determine the vibration directions of the slow (γ') and fast ray (α') of birefringent objects (cf. p. 74). In photo-elasticity, strain ($= \gamma'$) and stress directions ($= \alpha'$) can be determined. Fixed and variable compensators (Figs. 61, 62) are used as reference objects (auxiliary objects) with known orientation of γ and α. The compensators are introduced in the diagonal in the tube slot of the microscope. The vibration direction of the slow ray γ is engraved on the compensators. The following compensators are used on polarizing microscopes:

Fixed compensators

1. $\lambda/4$-plate (Fig. 61a)

   The $\lambda/4$-plate has a phase difference of about 140nm; it thus produces 1st-order gray. In earlier literature, the $\lambda/4$-plate is called a mica plate because the compensator plate is usually made of mica.

2. λ-plate (Fig. 61b)

   The phase difference of the λ-plate is about 550nm and therefore produces the interference color 1st-order red. In older literature the λ-platte is usually called a gypsum plate. On addition and subtraction of small phase differences the interference color changes towards blue or yellow respectively.

Variable compensators

3. λ-plate in sub-parallel position (Fig. 61c)

   In this auxiliary specimen the compensator plate can be slightly rotated from the normal position during observation. Color change towards yellow or blue makes it possible to see even very small phase differences of a few nm, for instance of strained glasses and above all of biological objects. The object vibration directions α' and γ' can be determined as with the ordinary λ-plate (see below).

4. Quartz wedge (Fig. 61d)

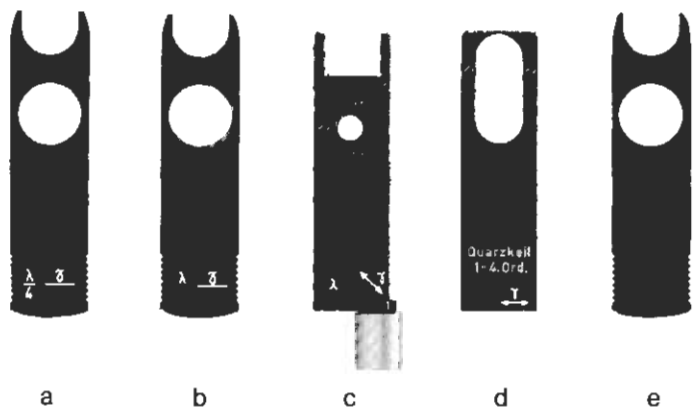
   The quartz wedge produces the interference colors up to the 4th order (Fig. 57). By adjustment of the wedge in the tube slot of the microscope phase differences of 0-4 λ can be set (cf. p. 57).

Practical operation with the polarizing microscope

The object is moved from the extinction position (Fig. 62b) into the diagonal position (Fig. 61c) by accurate stage rotation through 45° to the right or left. The two vibration directions in the object will then run NE-SW as well as NW-SE. The accurate rotation through 45° is made easier with the aid of the degree graduation and verniers or 45° clickstops.
The interference color of the object is noted, for instance 2nd order indigo (phase difference about 600nm, Table IV). The λ-plate is inserted in the tube slot and the interference color of the object again observed: in the example the object shows the interference color 3rd order indigo in the addition case (Fig. 62d).
Result (Table IV): the vibration direction γ' of the object is parallel to the vibration direction λ engraved on the compensator.
In the subtraction case the interference colour gray (Table IV) is produced: the vibration direction γ' of the object is perpendicular to the engraved vibration direction λ of the compensator (Fig. 62e).

In case of doubt, other compensators can be inserted in the tube slot to confirm the result. In the above-mentioned example, use of the $\lambda/4$ plate produces the interference color 1st order red-orange (Table IV, Fig. 62f) in the subtraction case, and the interference color 2nd order green (Table IV, Fig. 62g) in the addition case.

Fig. 61:
Compensators for orientating investigations
a $\lambda/4$-plate (mica plate)
b λ-plate (gypsum plate, also called 1st order red)
c λ-plate in subparallel position
d Quartz wedge 0-4
e Dust slide (must always be inserted in the tube slot when no compensators are used).



Further examples:

Color of object	[nm]	Addition		Subtraction	
		λ	$\lambda/4$	λ	$\lambda/4$
Yellowish-white	270	bright-green	vivid yellow	yellowish-white	gray-blue
Lavender-gray	100	sky blue	white	red-orange	black-gray
Higher-order white	>6000	Determination possible only with quartz wedge, or tilting compensators			

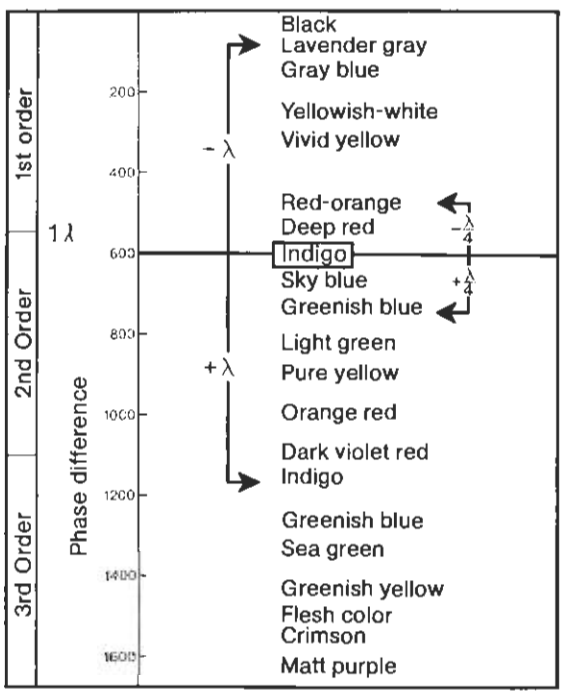


Table IV:
Color changes of a birefringent object (indigo 2) with addition and subtraction of compensators (λ and $\lambda/4$).

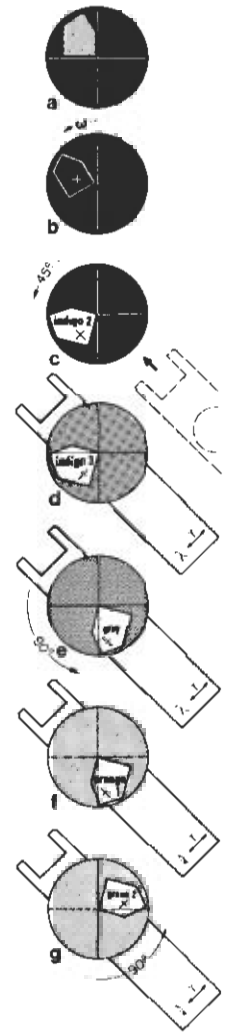


Fig. 62:
Determination of the vibration directions and the angle of extinction.

Angle of extinction

In many birefringent objects morphological charateristics, such as crystal edges, cleavage cracks, twinning planes, etc. are parallel or perpendicular to the vibration directions. In the extinction (normal) position these morphological characteristics are therefore parallel or perpendicular to the transmission directions of the polarizer. This is called *straight extinction* (Fig. 52). An example of this is a birefringent man-made fiber which in the extinction position is orientated precisely North-South or East-West.

In many crystals the vibration directions are not parallel to the morphology, so that in the extinction position these form an acute angle with the transmission direction of the polarizers. This angle is called the extinction angle or *obliquity of extinction.* For precision measurement the angle graduation and verniers on the microscope, the crosslines of the eyepiece, and compensators are used as follows:

The eyepiece is clamped so that the crosslines are orientated North-South and East-West, indicating precisely the transmission directions of the polarizers. The morphological characteristic (e.g. the longitudinal edge of a crystal, Fig. 62a) to be measured is lined up exactly parallel to the North-South line of the crosslines and the angle α_1 obtained from the vernier scales.

The object is rotated exactly into the extinction position (Fig. 62b) and the angle α_2 is noted.

The difference between the two angles $\delta = \alpha_2 - \alpha_1$ (or of the complementary angles $90° - \delta$) produces the angle of extinction. In most cases this angle should be referred to the vibration directions α' or γ'. These vibration directions must therefore be determined according to Fig. 62d-g with the aid of a compensator. To avoid errors it is recommended to make a rough sketch of the object investigated and to rotate is synchronously with the object stage.

Use: identification of minerals and chemical compounds.

Phase difference measurements

The phase difference Γ_o of an object is determined by the thickness d of the object and the birefringence $\Delta n'$.

$$\Gamma_o = d \cdot \Delta n' \text{ [nm]}$$

When the object thickness d is known the phase difference Γ_o can be estimated with sufficient experience and the birefringence $\Delta n'$ approximately determined:

$$\Delta n' = \frac{\Gamma_o}{d}$$

Since the estimation of the color is very inaccurate and no longer possible at high phase differences (higher-order white), variable calibrated compensators are employed. Depending of the phase difference (Fig. 65) of the objects to be measured, one of the following compensators is used:

Tilting compensators (Fig. 63a)

Tilting compensators contain a birefringent crystal plate which can be tilted. The tilting axis is parallel to the tube slot; the tilting angle i can be read off a coarse and a fine scale.

The birefringent crystal plate, the so-called compensator plate, is cut perpendicular to the crystal axis. In the 0 position of the compensator the crystal axis is parallel to the microscope axis: the compensator does not produce a phase difference.

When the compensator plate is tilted both the birefringence $\Delta n'$ (cf. Fig. 11) and thickness of the radiated layer increase, so that any phase difference can be adjusted continuously from 0 to the stated maximum measuring range (Fig. 65).

For measurement the object is moved precisely into the diagonal position (Fig. 64a), the compensator inserted in the tube slot and adjusted until the object area to be measured is in the maximum extinction position (Fig. 64b, c). Compensation is possible only if compensator and object are in the subtraction position.

To increase the accuracy of measurement the compensator plate is tilted in both directions and the sum of the two tilting angles i' and i'' = 2i used for the calculation of the phase difference. The measuring accuracy can be further increased by.

a) the setting of the compensation positions in monochromatic light (interference filter),
b) taking of the mean of several measurements.

Depending on the required measuring range (Fig. 65) the following compensators are available:

1. Tilting compensator **B** (Berek) with magnesium fluoride (MgF_2) for maximum phase differences of 2800nm. The special advantage of the tilting compensator B is that the phase difference can be directly obtained from a table.
Main use: mineralogical and petrographic objects, thin fibers and plactic foils.

2. Tilting compensator **K,** measuring range up to about 10 orders, main use: man-made fibers and plastics.

3. Tilting compensator **K,** measuring range up to about 30 orders. Main use: thick man-made fibers, plastics, and crystals.
Both versions of the tilting compensator **K** are equipped with calcite compensator plates. For evaluation, a tabulated value depending on the sum of the compensating angles 2i and a given calibration constant are added. The phase difference can be directly obtained from a second table.

4. The EHRINGHAUS tilting compensator **E** contains 2 quartz or calcite plates of exactly the same thickness, cemented together in subtraction position and cut parallel to the crystal axis. The measuring range is 20 λ. As for the tilting compensator B, the phase difference can be obtained directly from the tables.

Fig. 63:
Compensators for phase difference measurements
a) BRACE-KÖHLER compensator
b) SÉNARMONT compensator (λ/4-plate in sub parallel position)
c) Tilting compensator

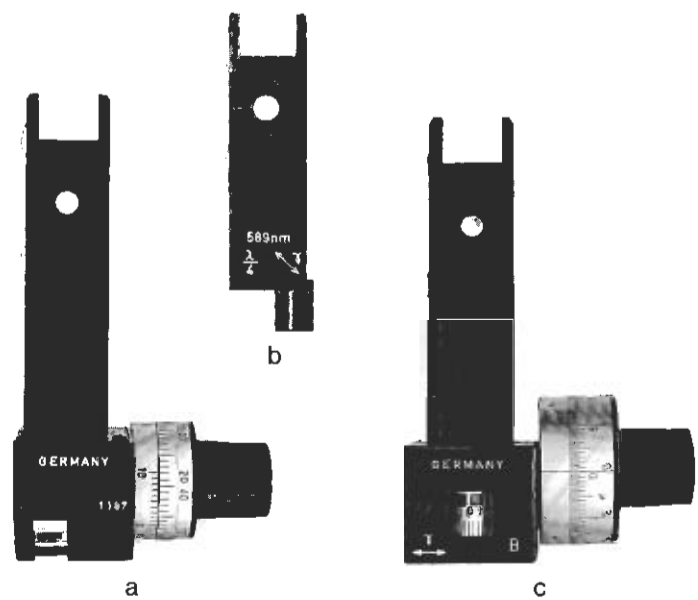


Fig. 64:
Phase difference measurement of a man-made fiber with tilting compensators
a) Fiber in the diagonal position (compensator removed or in the 0 position).
b) Compensation set for the center of the fiber (tilting compensator B).
c) Compensation on the same fiber, but with tilting compensator K.
Through differential orientation of the axis of tilt to the vibration direction γ of the 2 compensators, parallel (b) or hyperboloid (c) interference fringes are produced.

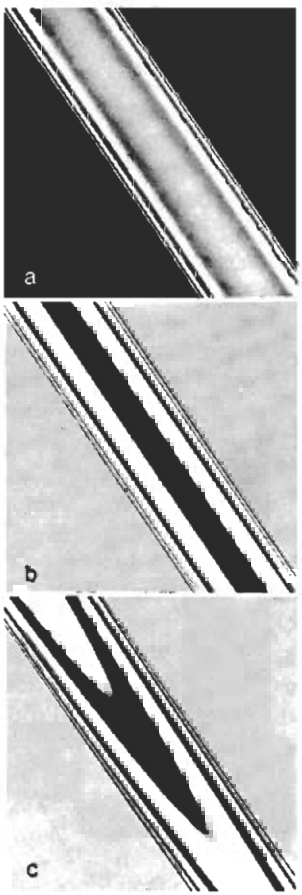
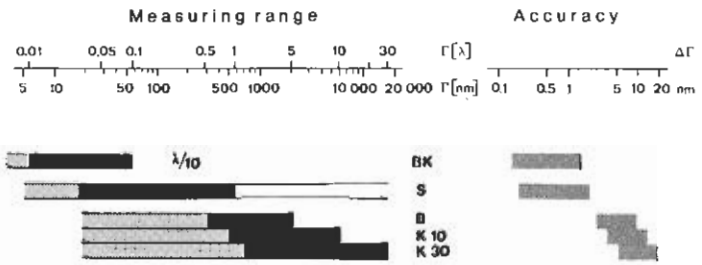


Fig. 65:
Measuring ranges (left) and approximate accuracy (right) of the Leitz compensators. The measuring accuracy of the tilting compensator E is between those of the compensators for 10λ and 30λ.
BK BRACE-KÖHLER compensator with λ/30, λ/20, λ10 plates
S SÉNARMONT compensator (546 and 589nm)
B Tilting compensator B
K10 Tilting compensator K for up to 10λ
K30 Tilting compensator K for up to 30λ



Rotary compensators

1. Elliptical compensator according to SÉNARMONT (Fig. 63b) ($\lambda/4$-plate in sub-parallel position).
The SÉNARMONT compensator is equipped with a compensator plate, which can be rotated around the microscope axis, of $\lambda/4$ phase difference. The $\lambda/4$-plate is adjusted exactly in the extinction (normal) position by means of a centering key. Readjustment is generally required only when the microscope is changed. The object, on the other hand, is oriented in exactly the diagonal position (γ': SW-NE). Compensation is obtained by rotation of the analyzer (Fig. 66).

The phase difference is calculated according to the following formula:

$$\Gamma = \frac{\text{analyzer rotating angle } \beta \cdot \text{wavelength } \lambda}{180} \text{ (nm)}$$

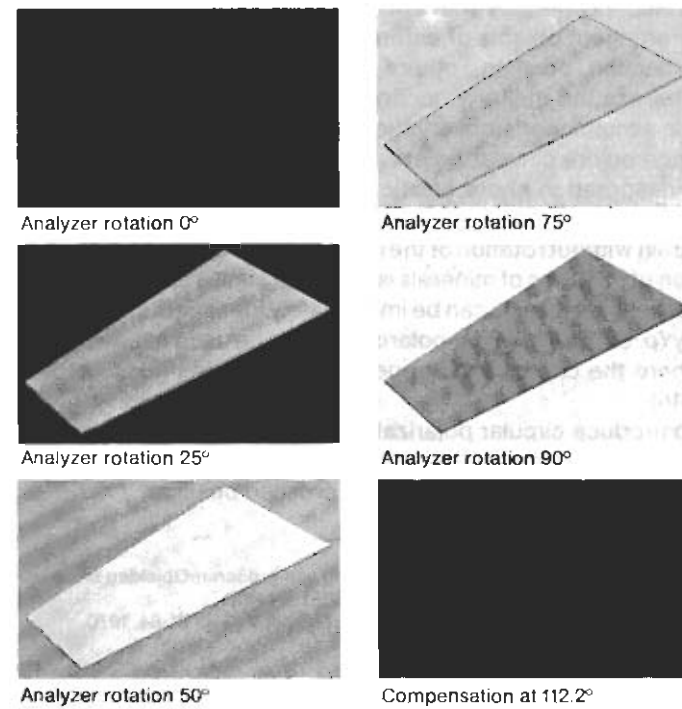
Normally this compensator is used for the measurement of phase differences of up to 1 order (1λ). Higher phase differences, however, can also be measured. To begin with, the measurement will not produce the entire phase difference, but only the value exceeding a whole wavelength or a multiple thereof. Whole wavelengths must be determined by means of the interference color or with a tilting compensator. Accuracy is higher than with the tilting compensator alone.
The measurement must be carried out in monochromatic light (λ = 546nm; use interference filter).
Most important uses: biological objects, strain birefringence, non-stretched fibers.

2. BRACE-KÖHLER elliptical compensator (Fig. 63c).
This rotating compensator consists of a compensator plate which can be rotated round the microscope axis through about 45° and has a small phase difference. Its normal position corresponds to the 0 position of the compensator.
For measurement the object is brought into a random diagonal position. The compensator is inserted in the tube slot and the compensator plate rotated by means of the setting drum until compensation occurs. The measurement can be carried out with white or with monochromatic light.
The compensator is available for the maximum measuring range $\lambda/_{10}$. The phase difference is calculated as follows from the rotating angle S and the given calibrating constant Γ_c:

$$\Gamma_o = \Gamma_c \cdot \sin 2S$$

Most important uses: biological objects, glasses of low strain birefringence.

Fig. 66:
Compensation process on a plane-parallel object (plastic foil) with SÉNARMONT elliptical compensator. Compensation has been achieved at a 112.2° angle of rotation.



Circular polarization

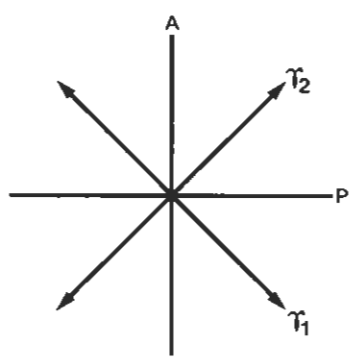
Birefringent objects display the well-known extinction effect (normal position p. 53) between crossed polarizers after 90° rotation. Especially with low-power observation, a number of birefringent objects of different orientation are usually in the extinction position, others are in the diagonal position, whereas the majority occupy intermediate orientations. For the simultaneous observation of all objects in their interference colors circular polarization is used. This is particularly widespread in photo-elastic investigations, since strain-free (isotropic) zones can be instantly distinguished from zones of strain without rotation of the object (Fig. 38d). During observation of mixtures of minerals isotropic granules and crystals cut vertically in an axis can be immediately identified. In conoscopy (p. 69), too, circular polarization can be used to advantage. There the crystal axes appear as dark points (Figs. 71f and 74h).

To produce circular polarization, the $\lambda/4$-plate (Fig. 61b) is inserted in the tube slot, and an additional $\lambda/4$plate (Fig. 67) in the slot of the pol-condenser in the crossed position.

Reference:
MEDENBACH, K.: Über die Untersuchung von größeren Objekten im linear- und zirkularpolarisierten Licht am ORTHOPLAN POL.
Leitz-Mitteilungen für Wissenschaft und Technik **V** (3-4), 81-84, 1970.

Fig. 67:
$\lambda/4$-plate for circular polarization for insertion in the slot of the 702 condensers (Fig. 24-5).
The $\lambda/4$-plate can also be inserted in conjunction with the polarizing device (Fig. 17) for the determination of vibration directions.

Fig. 68:
Vibration directions of circular polarization
P Polarizer A Analyzer
γ_1 Lower $\lambda/4$-plate (condenser)
γ_2 Upper $\lambda/4$-plate (tube slot)



Conoscopy

Conoscopy differs from orthoscopy (p. 51) in that an interference figure (Fig. 69) is observed instead of a magnified image of the object. Number and angles of axes and optical characteristics can be determined (Figs. 71-74) from the shape of the interference figure and its modification by compensators.
In orthoscopy it is a matter of chance whether a crystal axis or the direction of maximum birefringence coincides with the observation direction. Axial angle and optical characteristics cannot be directly determined orthoscopically.
It is therefore obvious that the specimen should be tilted for these measurements for observation of its anisotropic behaviour in as many different directions as possible. This method is carried out with the universal rotating stage (Figs. 76), but for routine investigations the instrumentation is rather elaborate.

Uniaxial crystals

A simpler method of judging the polarized-light behaviour simultaneously in different directions is conoscopy, also known as indirect observation, observation in the divergent, convergent beam and observation of interference figures.
The conoscopy of crystal fragments several mm thick is possible without the microscope as follows:
A piece of birefringent crystal is clamped between two crossed filter polarizers and held in front of the eye at a distance from 1 to 2cm (Fig. 69). Depending on the type of crystal and the direction of its axes typical interference figures are observed. These differ completely from the external shape and colour of the crystal.
The observation is interpreted as follows: the eye receives light rays from the most varied directions (Fig. 69). These light rays, too, pass through the crystal and the two crossed polarizers at the most varied angles.

All parallel rays o, which are vertically incident on the crystal, are combined at point 0 (= focal point of the lens of the eye) on the retina, irrespective of whether they have passed through the margin or the centre of the crystal.
All rays coming from the directions 1, 2, ..., are combined in points 1, 2, ... on the retina.
During the observation of a uniaxial crystal whose axis points in direction 0 (so called axial section, i.e. section perpendicular to the crystal axis) the interference figure shown in Fig. 69 F is produced. It consists of a dark, blurred cross and concentric fringes of the same color sequence as with the quartz wedge (Table III).

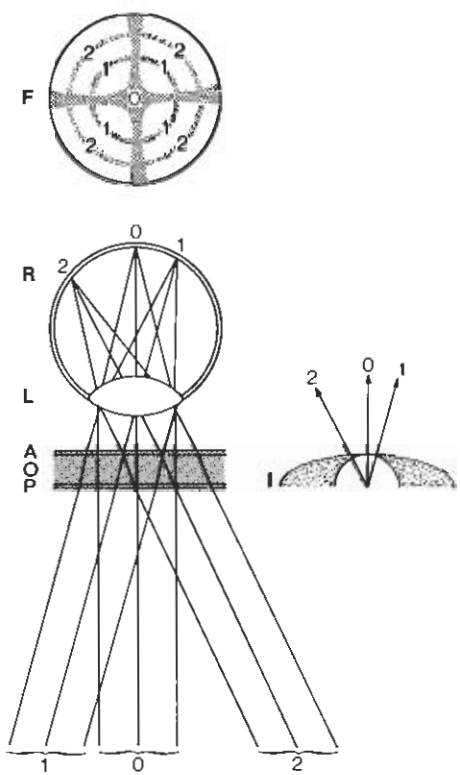
Formation of the concentric fringes

No rays from the direction 0 (= direction of the crystal axis) suffer birefringence, because they proceed in the direction of the crystal axis. Extinction is therefore observed at point 0: intersection of the cross.

The rays 1, 2... incident obliquely to the crystal axis suffer birefringence. With increasing angle of incidence both the birefringence and the path lengths traversed increase. This produces continuously increasing phase differences which cause the concentric sequence of the interference colors beginning in the centre of the cross.

The higher the maximum birefringence or the thickness of the object, the more closely spaced are the colors (Fig. 71). In monochromatic light, there is a sequence of alternatively bright (phase difference $\lambda/2, 3/2\lambda, 5/2\lambda \ldots$) and dark (phase differences 0, λ, 2λ, 3λ) fringes, the so-called *isochromes* are produced.

Fig. 69:
Production of an interference figure during observation of a large crystal plate (between crossed polarizers) from a short distance
0, 1, 2 different directions of the passage of rays through the crystal and co-ordinated points of the interference figure (o = crystal axis)
F visible interference figure
R retina
L lens of the eye
A analyzer
C crystal plate
P polarizer
I direction-derived distribution of the refractive indices in the uniaxial crystal plate (cf. Fig. 11)



Formation of the interference cross

In conoscopy the object is irradiated with a cone of illumination of as wide an angle as possible. The interpretation of the indicatrix (Fig. 13) shows that with changing angles of incidence and azimuths of the light rays, the azimuths of the object vibration directions, too, must change (Fig. 72a):
With uniaxial crystals the extraordinary rays vibrate in planes which include the crystal axis. These radial planes are also called principle sections.
The ordinary rays, however, vibrate perpendicularly to the extraordinary rays, that means "tangentially" to the interference fringes (Fig.72a, b).
Areas in the interference figure of vibration directions parallel to the polarizers, i.e. North-South and East-West, therefore undergo extinction, producing the interference figure of a cross.
This figure is also called *isogyre* (= sites of identical vibration directions).
The interference cross disappears in circularly polarized light.
The direction of the optical axis is represented by a dark point surrounded by concentric interference fringes (Fig. 71f).

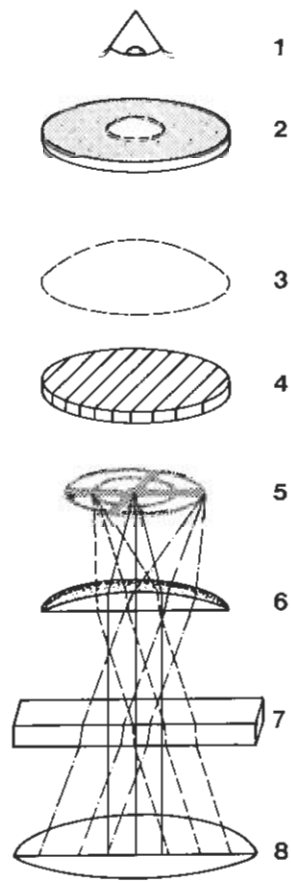


Fig. 70:
Observation of an interference figure in the upper focal plane of an objective of large aperture.
1) Eye
2) Eyepiece
3) BERTRAND lens
4) Analyzer
5) Focal plane (pupil) of the objective with interference figure of the uniaxial crystal
6) Objective of large aperture
7) Uniaxial crystal, crystal axis parallel to the microscope axis
8. Condenser of large aperture (the polarizer has been omitted). The interference figure can be observed even when BERTRAND lens and eyepiece are removed; it will, however, be relatively small then.

Conoscopy with the polarizing microscope

When birefringent objects are set up in any polarizing microscope, interference figures are produced according to the same previously described principle. The rays passing through the object in various directions at larger apertures produce the interference figure in the upper focal plane of the objective. This plane is as a rule situated within the objective mount, i.e. below the connecting screw thread. The interference figure can be observed there with the unaided eye after removal of the eyepiece from the tube (Fig. 70).

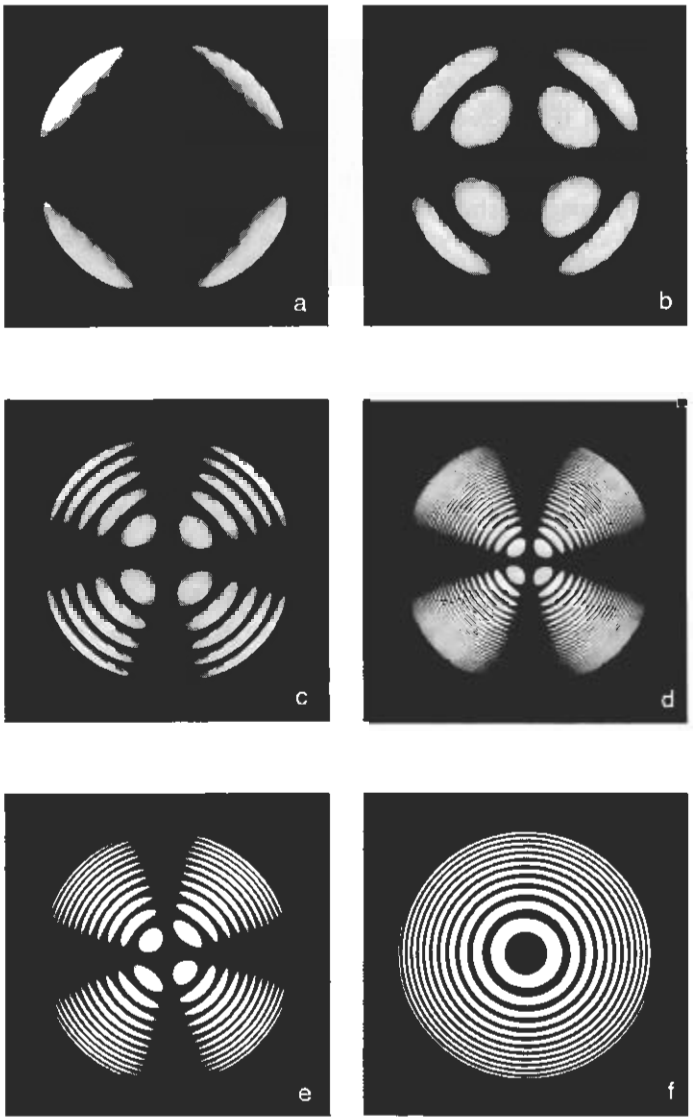


Fig. 71:
Interference figures of uniaxial structures of various orientation, thicknesses, and birefringence in monochromatic light
a-h Sections perpendicular to the crystal axis:
a Crystal of small thickness and/or birefringence. A similar interference figure would be observed also with the the objects b-d, if objectives of smaller aperture were used.
b-d Crystals of increasing thickness and/or birefringence
e Determination of the optical sign (in the example uniaxial negative) with a $\lambda/4$-plate:
The concentric interterence fringes are displaced inwards in the second and fourth quadrant, and outwards in the first and third quadrant
f Observation of a crystal cut vertically to the crystal axis in circularly polarized light (cf. Fig. 68).
g Optically active crystal (quartz, cut perpendicular to the crystal axis).
h AIRY spiral of a quartz twin, thickness about 4mm (set of specimens for the polarizing attachment for 35mm projectors)
i, j Crystal axis inclined about 45° to the plane of the section
k Crystal axis inclined about 70° to the plane of the section
l Crystal axis parallel to the plane of the section.

The conditions permitting the observation of an interference figure are:

a. Object of suitable orientation and size

In uniaxial structures the section planes perpendicular to the crystal axis (so-called axial sections) are particularly suitable. Orthoscopically they can be recognized in that the intensity of the grain does not change as the object stage is rotated. In larger condenser and objective apertures these grains, unlike isotropic areas of the specimen, become somewhat paler.
If the grains are too small, a disturbance of the interference figure may occur owing to neighboring grains unless these have been masked by optical means (see below).

b. Large condenser aperture

This is established when the condenser is in its topmost position below the object stage, the front lens of the condenser is turned into the beam, and the aperture diaphragm is fully open.

c. Large objective aperture

This is established by the use of high objective magnifications. The Fl 63/0.85 P is the standard objective. Its angle of observation is about 64°. For teaching purposes, the P 40/0.65 objective (angle of field 45°) is adequate with selected specimens. For a conoscopic angle of field of 120° the P Oil 100/1.30 and NPl Oil 100/1.30 P objectives are used. Here, the normal condenser top (N. A. 0.85) must be replaced by the condenser top No. 004 P Oil 1.33 for conoscopy, since the condenser and objective aperture should be approximately the same. The rendering of the small, bright interference figures recognizable with the unaided eye is improved if a pinhole stop (dioptre) is pushed on to the eyepiece tube.
This method is usually adequate when binocular tubes are used for occasional conoscopy on students' and laboratory microscopes. Instead of the pinhole stop, the focusing tele-

scope, as required, for instance for phase contrast, can be inserted in the eyepiece tube.
A magnified interference figure will be seen if the upper focal plane of the objective is observed in an "auxiliary microscope". For this purpose a simple lens is swung into the tube. This auxiliary lens (Fig. 70.3), known as a BERTRAND lens (or AMICI lens or AMICI-BERTRAND-lens), takes over the function of a low-power objective. The eyepiece (Fig. 70.2) remains in the tube and produces a secondary magnification of the interference figure projected into the intermediate-image plane by the BERTRAND lens.
For frequent conoscopic work, tubes with a swing-out BERTRAND lens can be attached to all Leitz polarizing microscopes. They also contain a hinged additional stop, to mask disturbing penomena caused by areas of the specimen close to very small crystals.
The ORTHOLUX 2 POL BK and ORTHOPLAN POL research microscopes have a *supplementary lens* (Fig. 45-17) for the isolation of particularly small grains (>6μm). In research microscopes the BERTRAND lens can be focused and centered, in students' and laboratory microscopes it is adjusted in the factory so that when objectives provided for conoscopy are used the interference figure appears at its optimum adjustment.

Determination of the optical sign of uniaxial crystals

The radial and axial orientation of the vibration directions of a crystal observed at large apertures is illustrated in Fig. 72a. The extraordinary rays always vibrate radially and all ordinary rays tangentially to the optical axis.
The optical sign (p. 13) is defined as follows:
Positive $n_e > n_o$
Negative $n_e < n_o$

With positive birefringence it is therefore the radially vibrating ray which has the higher refractive index, whereas with negative birefringence it is the tangentially vibrating ray (Fig. 72a). By the insertion of a compensator (γ': SW-NE) the vibration directions of n_e and n_o can be determined as in orthoscopy. With uniaxial positive crystals addition occurs in the first and third quadrant, but with uniaxial negative crystals in the second and fourth quadrant (Fig. 72b, c).
The following features appear when the various compensators are used:

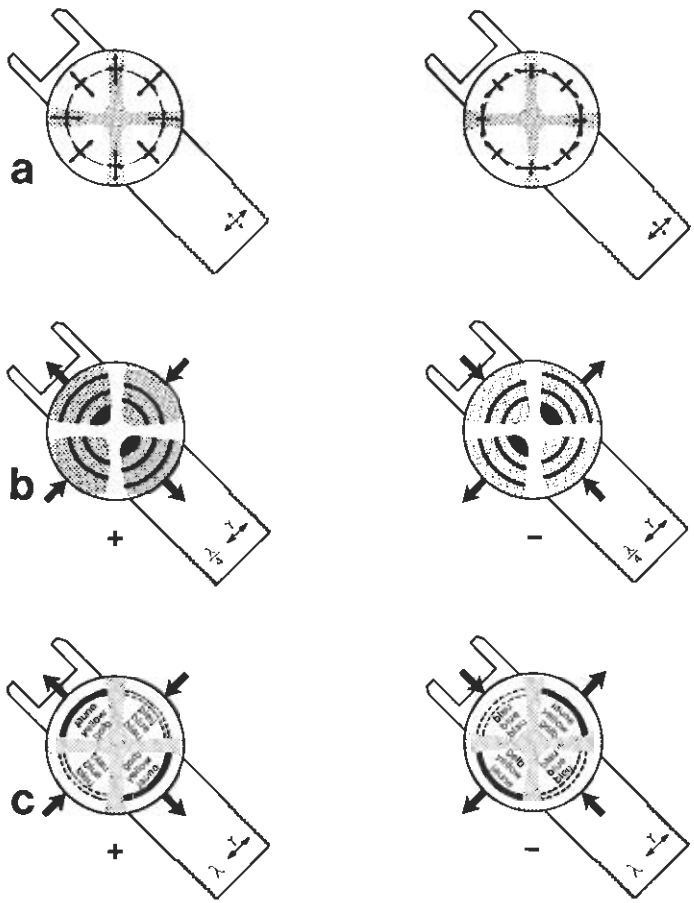
λ-plate

The vertices of the quadrants of addition appear blue, those of the quadrants of subtraction yellow (Fig. 72c).

$\lambda/4$-plate

The vertices of the quadrants of subtraction appear black (Fig. 72b).

Fig. 72:
Determination of the optical sign of uniaxial structures.
Left: Positively uniaxial crystal, cut perpendicular to the crystal axis.
Right: Negatively uniaxial crystal, cut perpendicular to the crystal axis.
a) Representation of the vibration directions in the object and in the compensator
b) Change of the interference figure when a $\lambda/4$-plate is used
c) Change in the interference figure when a λ-plate is used.



Variable compensators (quartz wedge or tilting compensator)

For addition the interference fringes move inwards towards the center of the cross, for subtraction outwards (Fig. 72b, c).

Sections cut obliquely to the crystal axis

Depending on the inclination of the crystal axis and the available apertures the center of the interference figure may lie inside or outside the observable area (Fig. 71i, j, k). Determination of the optical sign is possible even when the center of the cross can no longer be observed.

Sections cut parallel to the crystal axis

The interference figures resemble optical normal sections of biaxial crystals. The interference figures can be observed particularly well in monochromatic light (Fig. 71.l). Determination of the optical sign may not be possible. The distinction between interference figures of biaxial structures is very difficult.

Conoscopy is used not only for the identification of minerals but also in industrial practice, for instance to orient industrial crystals (sapphires for bearing jewels, oscillator crystals).

Biaxial crystals

Biaxial crystals have two axes, i. e. directions, of isotropic behaviour. The angle between these two axes is called the axial angle 2V (cf. p. 16). It is specific for most optically biaxial crystals and can therefore be used for identification.

Sections cut perpendicular to an optical axis

Biaxial structures with a section perpendicular to one of the two axes, like axial sections of uniaxial crystals, exhibit no intensity changes in the parallel beam (orthoscopy) during rotation of the object stage. Distinction between uniaxial and biaxial crystals is possible only in the conoscopic beam.

Instead of the symmetrical cross of uniaxial crystals, only a single bar is observed with axial sections of biaxial crystals (Fig. 73, 74). Whereas in uniaxial crystals circular interference fringes or color fringes (isochromes) occur, here, figure-8-like fringes of the same phase difference are found (Fig. 74). When the object stage is rotated the bar rotates in the *opposite* direction. That portion of the interference figure which always remains dark during rotation corresponds to the crystal axis. The curvature of the bar which occurs during rotation depends on the axial angle (Fig. 73). With very acute axial angles and high observation apertures the second axis may also appear in the image (Fig. 74).

Fig. 73:
Biaxial crystals: section perpendicular to one axis.
Relation between axial angle and curvature of the isogyre (diagrammatic representation, individually the curvature also depends to some extent on the objective aperture used).

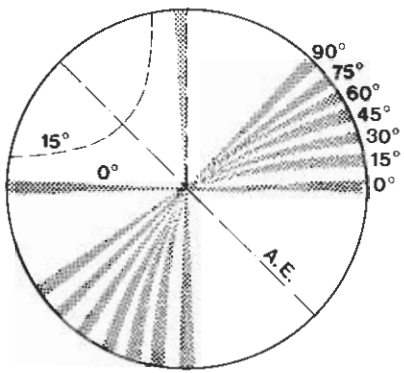


Fig. 74:
Interference figures of optically biaxial crystals:
a-j Sections cut perpendicular to the acute bisectrix

a) Axial angle about 10°
Thin specimen
(or specimen of low birefringence)

b) Axial angle about 10°
Thick specimen
(or specimen of high birefringence)

c) Axial range about 30°
Axial plane sligthly inclined to the microscope axis

d) Specimen as c.
Determination of the optical sign (negative) through introduction of a $\lambda/4$-plate into the tube slot

e) Axial angle about 50°
Thin section or section of low birefringence

f) Axial angle about 50°
Specimen (slightly thicker than e)

g) Specimen as f, but in normal position

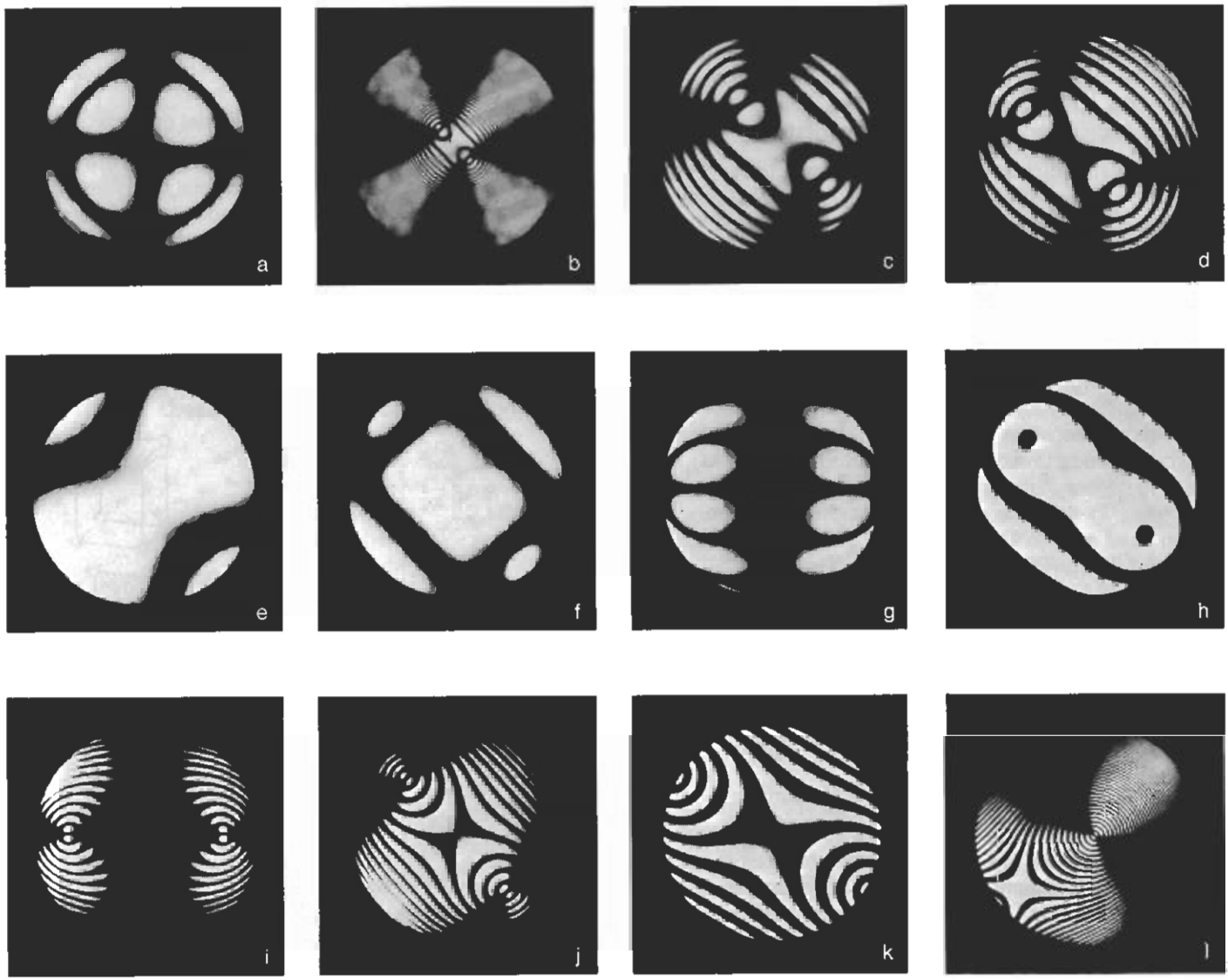
h) Specimen as f and g in circularly polarized light.
The optical axes are indicated by the two dark points

i) Axial angle about 50°
Thick specimen (normal position)

j) Specimen as i, diagonal position

k) Axial angle wider than the available microscope aperture (2V about 70°)

l) Section cut perpendicular to the optical axis, axial angle about 40°, very thick specimen (sugar)


a
b
c
d
e
f
g
h
i
j
k
l

Sections cut perpendicular to a bisectrix

Sections perpendicular to the acute or obtuse bisectrix show, exactly like uniaxial crystals of general sections position in the orthoscopic beam, alternate brightness (diagonal position) and extinction (normal position) during rotation.

Sections perpendicular to the acute bisectrix (cf. p. 16) show the cross-shaped interference figure illustrated in Figs. 74a-k in the conoscopic beam. When the object is rotated, the two bars open up into two hyperbolic arms. Areas whose intensity remains unchanged during rotation denote the point of emergence of the crystal axes. These areas are visible only when the microscope aperture is larger than the acute axial angle. The bars and hyperbolic arms are surrounded by figure-8 interference fringes whose position rotates in the *same* direction as the stage rotation.
The distance between the points of emergence of the two axes can be used to estimate the axial angle 2V (Fig. 74). More accurate determinations are possible with an eyepiece micrometer (Fig. 40); it is calibrated with the aid of objects of known axial angles. The most accurate measurements are possible with universal rotating stages (Fig. 76).

In sections vertical to the obtuse bisectrix the points of emergence of the axes are generally not recognizable since the aperture of the microscope is not sufficient. Only if the obtuse axial angle is slightly larger than 90° will interference figures appear similar to sections vertical to the acute bisectrix (use oil immersion objective).

Sections cut perpendicular to the optical normal.

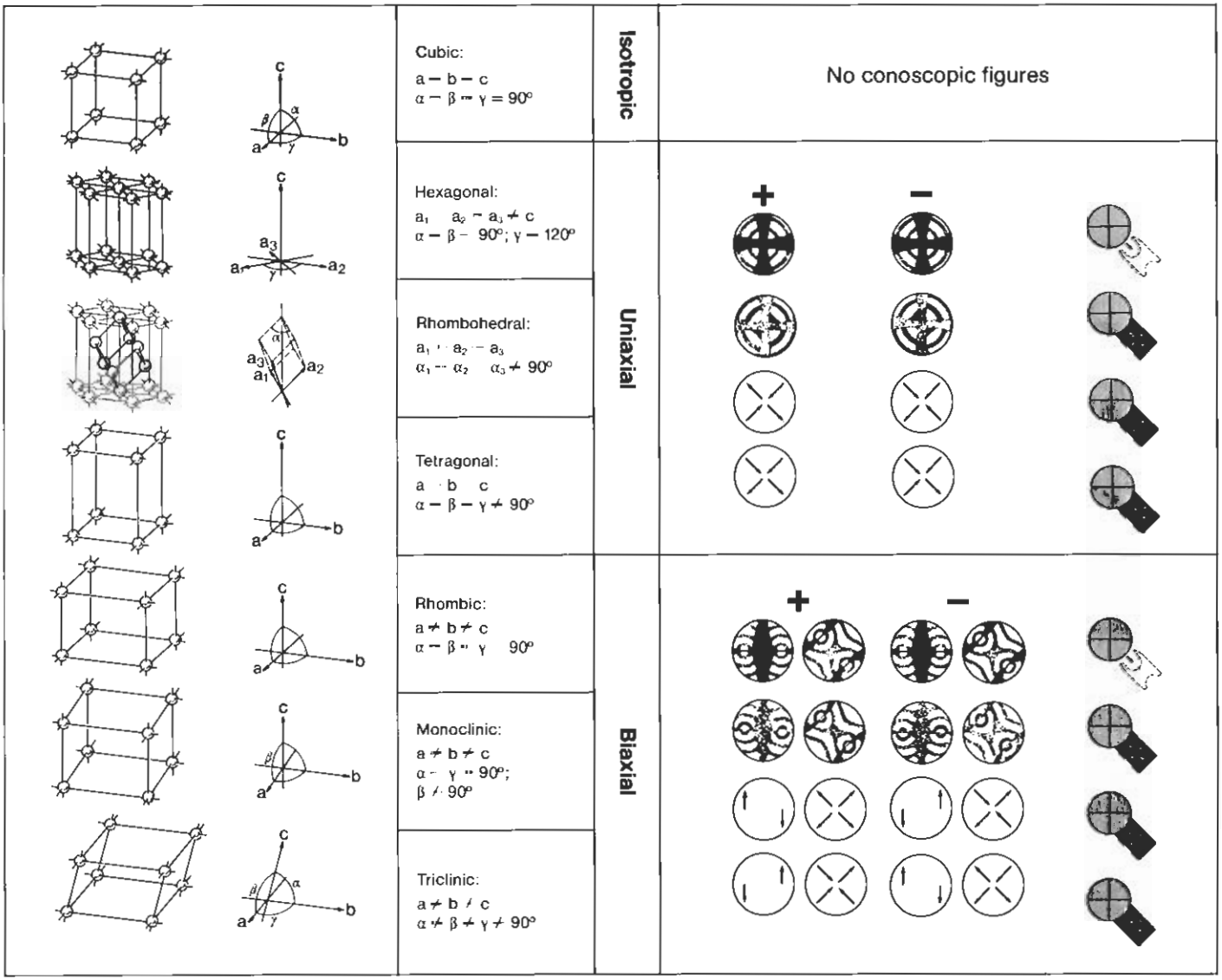
In sections parallel to the axial plane (= section perpendicular to the optical normal) the highest interference colors occur in the orthoscopic beam (p. 16). In the conoscopic beam a blurred dark cross is observed in the normal position, opening up into hyperbolic arms with slight rotation of the object. Interference fringes can be seen only in particularly suitable specimens and at large microscope apertures. The interference figure thus closely resembles section positions of uniaxial crystals cut parallel to the crystal axis.

Determination of the optical sign of biaxial crystals

For the determination of the optical sign, sections are suitable in which the angle bisector of the two crystal axes runs parallel to the microscope axis (section perpendicular to the acute bisectrix). In the conoscopic beam a dark cross is seen, which opens up when the object stage is rotated into two hyperbolic arms, the so-called isogyres. The cross and the hyperbolic arms are surrounded by colored interference fringes.

The optical sign can be determined according to Fig. 78 or the rules mentioned below from the displacement direction of these fringes when compensators are used.

Fig. 75:
Determination of the optical sign of uniaxial and biaxial crystals


Cubic:
a = b = c
α = β = γ = 90°
Isotropic
No conoscopic figures
Hexagonal:
a1 = a2 = a3 ≠ c
α = β = 90°; γ = 120°
Rhombohedral:
a1 = a2 = a3
α1 = α2 = α3 ≠ 90°
Uniaxial
Tetragonal:
a = b ≠ c
α = β = γ = 90°
Rhombic:
a ≠ b ≠ c
α = β = γ = 90°
Monoclinic:
a ≠ b ≠ c
α = γ = 90°;
β ≠ 90°
Triclinic:
a ≠ b ≠ c
α ≠ β ≠ γ ≠ 90°
Biaxial
+
−

The plane of symmetry of the isogyres (= axial plane) must be perpendicular to the γ-direction of the compensator

a) Biaxial positive structures

The interference fringes move from the convex to the concave side of the isogyres.

b) Biaxial negative structures

The interference fringes move from the concave to the convex side.

Usually the optical sign can be determined even when only one of the crystal axes points in the viewing direction of the observer. In the parallel beam the brightness of specimens orientated in this way does not change. In the conoscopic beam, only one of the two isogyres will be visible here (Fig. 73).

Universal rotating stages

In orthoscopic and conoscopic observation the determination of maximum birefringence and of axial angles is possible only in certain viewing directions, which depend on the orientation of the object. Universal rotating stages (UT) permit orthoscopic and conoscopic quantitative measurements in various directions through tilting of the specimens through a maximum angle of about 45°. In structural studies, universal rotating stages are also used for the statistical survey of the crystal axes of certain minerals such as quartz.
The specimen is arranged between two glass segments with the aid of immersion oil, and observed through special objectives (UT objectives). Depending on the refractive indices of the minerals investigated glass segments of various refractive indices are available. For conoscopic work special segments and objectives are required. The condenser is equipped with UT condenser tops.

Fig. 76:
Universal rotating stage on the LABORLUX 12

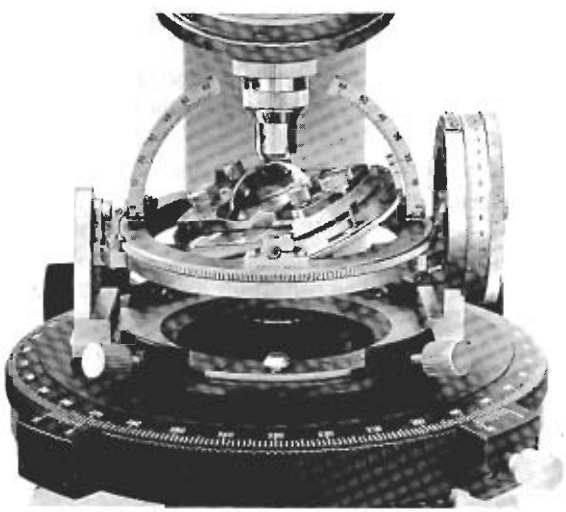
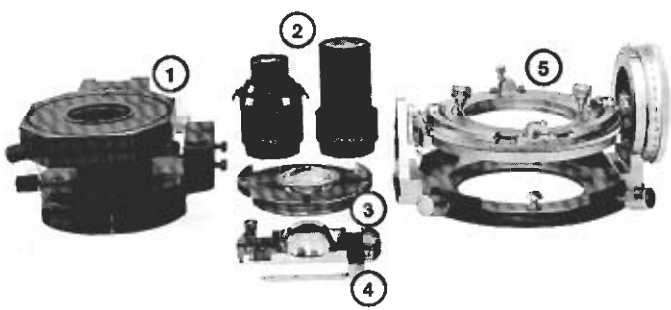


Fig. 77:
1. Condenser holder with polarizer and drive (for LABORLUX 12 POL)
2. Special UT and UTK condensers
3. Segment base
4. Upper segment with parallel guide sledge
5. Universal rotating stage



Special uses of the BERTRAND lens

The focusing BERTRAND lens can be used not only for conoscopic observation but also for other methods (cf. Table I):

Adjustment of accessories

such as phase contrast, interference contrast, monochromators and microscope photometer.

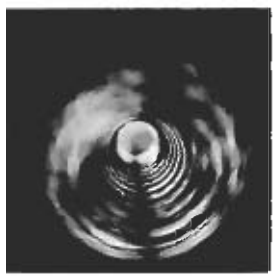
Observations of the internal walls of fine bores and threads

Observation is carried out as in conoscopy with an objective of larger aperture, such as EF 63/0.85 P or NPL FLUOTAR 40/0.70 P in conjunction with the BERTRAND lens. The image can be improved by the use of circularly polarized light (p.68) or through illumination with a darkfield condenser and additional incident-light illumination.

Low-power observation of large fields

A survey can be carried out of large object fields of up to about 34mm diameter on the ORTHOPLAN after removal of the revolving nosepiece and insertion of the BERTRAND lens in the beam. The reproduction ratio is slightly changed by the vertical adjustment of the stage. Focusing is carried out with the focusing BERTRAND lens, which acts as a weakly-reducing objective.

Fig. 78:
Inner wall of a badly worn bearing of a pocket watch, photographed with the aid of a BERTRAND lens.
(ORTHOPLAN POL, objective FI 63/0.85 P, LEITZ VARIO ORTHOMAT 2)



Microscopy in polarized incident light

A detailed description of microscopy in polarized incident light is outside the scope of this brochure. Microscopic examination is generally carried out in polarized light on non-transparent, i.e. opaque objects (p. 9) with often optically anisotropic behaviour (reflecting power as a function of the polarization direction) such as ore minerals, coal, ceramic products, cement clinker.

Precision grinding and polishing of the surfaces to be examined is essential to microscopic observation. The polished section is joined to the microscope slide by means of a piece of plasticine and a hand press so that the polished surface is precisely perpendicular to the microscope axis. Some object details can be seen only after additional contrast-increasing measures have been taken. To increase the contrast immersion objectives and interference attachments (see below) are used. Incident-light illuminators for normal polarization for the various incident-light interference methods can be attached to all Leitz polarizing stands (Table I). Here the objective also functions as condenser. In students' microscopes the illuminating ray is deflected by a semi-silvered glass plate above the objective.

In research microscopes a switchover is possible from an optical flat to a trapezoidal deflecting prism (compensating prism according to BEREK, Fig. 82-9). This prism is used whenever exacting demands are made of the polarization. For the highest possible resolution, i.e. large condenser and objective apertures, however, the optical flat is preferred.

Objectives for polarized incident-light are computed for infinity tube length. They are therefore engraved ∞.

The ORTHOLUX 2 POL BK and ORTHOPLAN POL research microscopes also accept special illuminators for incident-light darkfield and fluorescence excitation.

Fig. 79:
Incident light device SR on LABORLUX 12 POL
1. Disengageable pinhole stop
2. Centering opening for BERTRAND lens
3. Disengageable BERTRAND lens
4. Insertable analyzer
5. Polarizer
6. Aperture diaphragm
7. Field diaphragm
8. Dust protection slide

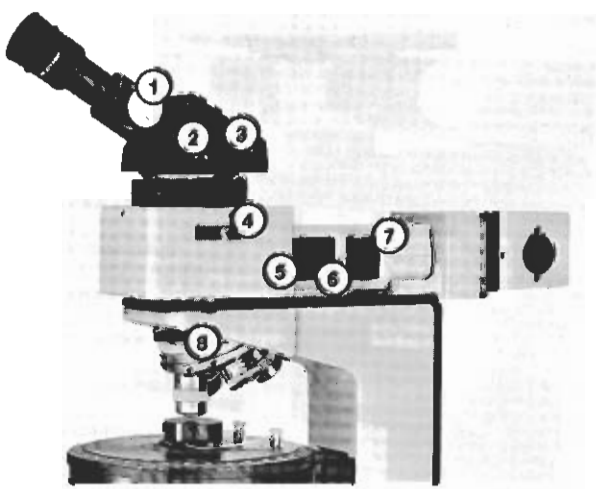


Fig. 80:
Pol-vertical illuminator on the ORTHOLUX 2
POL BK stand with binocular phototube FSA 55

1. Rotating analyzer, reading to 0.1°
2. Arresting screw for rotating analyzer
3. Fixing screw for the polarizer insert
4. Clamping screw for polarizer rotation
5. Lever for aperture diaphragm
6. Centering screw for field diaphragm
7. Lever for the setting of the field diaphragm
8. Lever for objective change
9. Objective centering
10. Clamping screw for the vertical adjustment of the stage
11. Optical flat/BEREK compensating prism selector lever

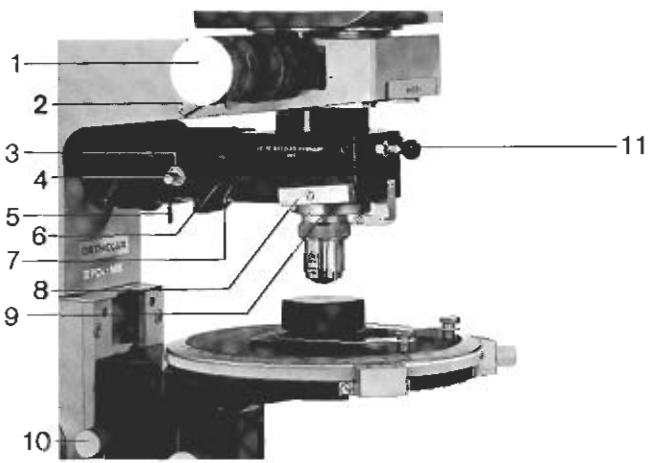
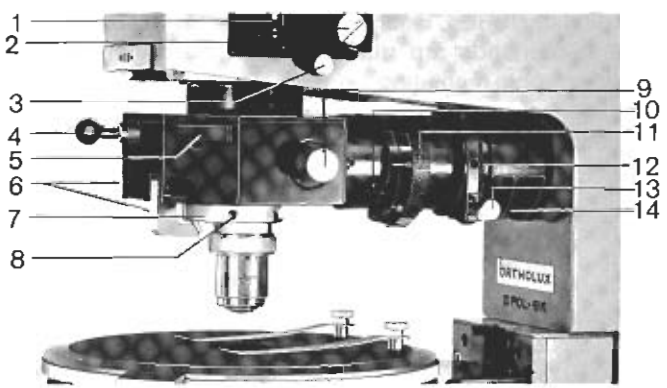


Fig. 81:
Pol-vertical illuminator

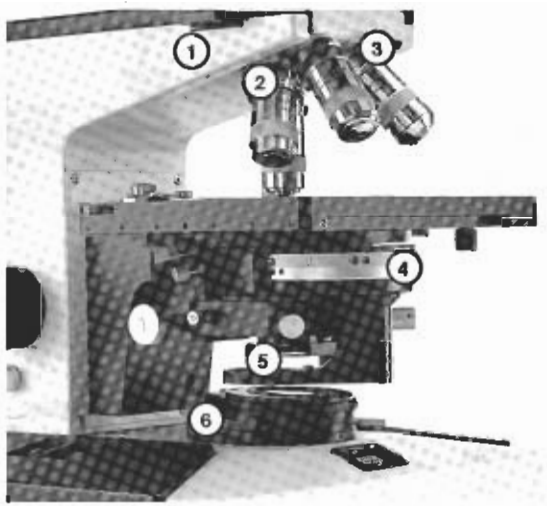
1. Disengageable neutral-density filter in the space for the analyzer as glare protection when the analyzer has been removed from the optical path (only in the version with rotating analyzer)
2. Interchangeable analyzer slide
3. Clamping screw for the Pol-vertical illuminator
4. Optical flat/BEREK compensating prism changing lever
5. Tube slot
6. Lever for objective change
7. Objective centering mount
8. Objective centering screws (left-hand screw obscured)
9. Field diaphragm focusing control
10. Centering of field diaphragm
11. Polarizer (rotatable through 90°) with index adjustment
12. Half stop (to be turned in only in connection with the compensating prism)
13. Vertical adjustment of the aperture diaphragm
14. Closing tube (filter holder)



Interference contrast T

Transmitted-light interference contrast T is also based on the principle of polarization and birefringence.

Fig. 83:
Transmitted-light interference-contrast device T on a biological microscope (DIAPLAN)
1. Filter slot with analyzer
2. Special objective with Wollaston prism
3. Objective with intermediate ring for bright field or phase contrast
4. Revolver-disc with Wollaston prisms and phase contrast light-rings
5 Lambda plate
6 Polarizer with $\lambda/4$-plate



Principle

In Fig. 85 the basic principle is diagrammatically represented for a plane wave, i. e. for parallel light. For a better understanding, however, the function of the *Wollaston prism* must first be explained.
A Wollaston prism consists of two birefringent prisms cemented together, whose crystal axes from a right angle. One axis lies in the drawing plane, the other is perpendicular to it (Fig. 84). In addition, both axes are parallel to the entrance and exit faces of the double prism.
If a light ray is vertically incident on the Wollaston prism, it is split into two rays polarized vertically to each other. If the point of divergence is in the center of the separating face, i. e. where both prisms have the same thickness, the rays emerging from the Wollaston prism will be in phase. If the points of divergence are not in the center of the separating face, a phase difference occurs between the two part-rays polarized vertically to each other. If the double prism is introduced between two polarizers, a series of straight-line interference fringes can be observed in monochromatic light. In white light, the interference fringes appear colored between two crossed polarizers.
After these introductory explanations the basic principle of the interference contrast method can be better understood (Fig. 85). Let O be the specimen through which a plane wave passes. This is deformed during its passage through the object. Let the new wave front be Σ. The Wollaston prism behind the objective Ob splits the wave Σ into the two part wave trains Σ_1 and Σ_2, which after passing through the analyzer interfere with each other so that intensity or color differences become visible in the intermediate-image plane.

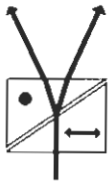


Fig. 84:
Principle of the Wollaston prism
The Wollaston prisms shown in Fig. 86 are greatly simplified. The angle included by the long side of the prism and the base is in reality only a few minutes of arc.

In principle, this arrangement requires a very small condenser aperture, which, naturally, considerably reduces the resolving power. In practice, therefore, the variant illustrated in Fig. 86 is used, in which the condenser aperture is not reduced and which nevertheless ensures the ability of the part-beams to interfere. The natural light coming from the light source is linearly polarized by the polarizer 9. From there it reaches the Wollaston prism 7; this is orientated in the diagonal position, in which the linearly polarized ray is split into two divergent part-rays of the same intensity and polarized perpendicular to each other. Since the point of divergence is situated in the focal plane 8 of the condenser 6, the angular splitting inside the Wollaston prism becomes a lateral separation in the object space. As a result, the part-rays pass through the object 5 in two different points where their phases, too, are differentially affected.

The magnitude of the split has been chosen so that it is below the resolving power of the microscope (Fig. 87). No visible double image is therefore produced in the microscope.

The objective 4 combines the two part-rays in its rear focal plane 3. This is where the second Wollaston prism 2 is situated, which recombines the two part-ray bundles (without this Wollaston prism the part-rays would again diverge behind the

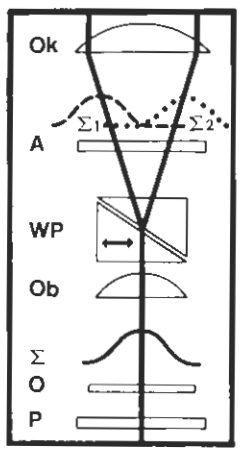


Fig. 85:
Principle of interference contrast
P Polarizer
O Object
Wave train
Ob Objective
WP Wollaston prism
A Analyzer
OK Eyepiece

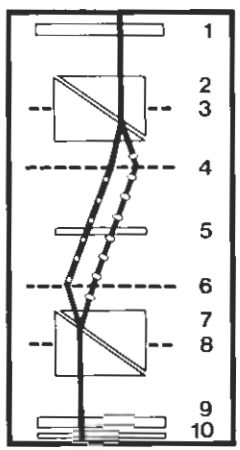


Fig. 86:
Diagram of the Leitz interference contrast device T
For explanation see text.

point of recombination). They now pass through the analyzer 1 and, made to vibrate in a common direction, can now interfere. The interference color or intensity in every point of the field of view now depends on the phase difference between the two part-ray bundles and therefore on the thickness and refractive index of the two object points.

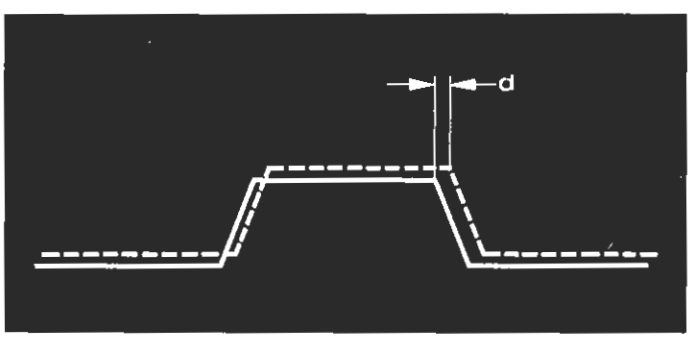
Function of λ- and $\lambda/4$-plate

The $\lambda/4$-plate fitted below the Wollaston prism, in conjunction with the polarizer P, acts as a phase-displacing compensator. Together with the λ-plate which can in addition be inserted in the beam, brightness and color differences in the surrounding field and object can be varied (cf. incident-light interference contrast device R).

Uses

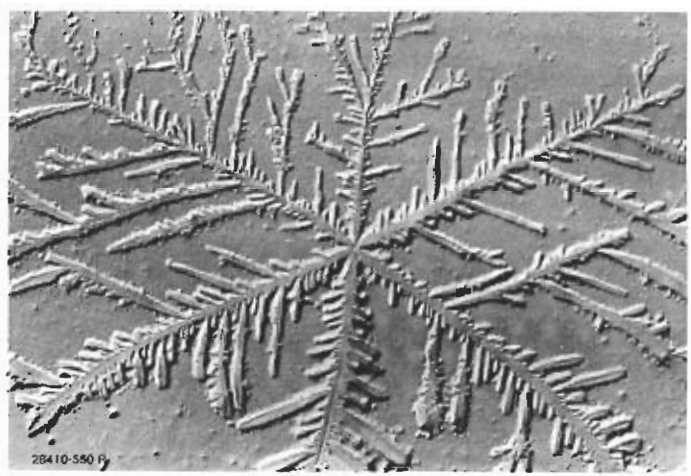
The interference contrast method serves the qualitative representation of phase structures.
In the microscope, the objects and especially their fine structural details appear in a relief-like contrast. But stained specimens, too, can be observed to advantage in interference contrast in this way.
The main use lies in the field of biological microscopy where especially those object structures can be demonstrated which cannot be recognized at this level of clarity with the conventional methods of microscopy (for instance filamentous elements, flagellae, cilia, etc.).

Fig. 87:
Latent image splitting with the interference contrast method. The split d is smaller than the resolving power of the optical system. The split leads to a phase displacement.



Surfaces and inclusions can, however, be rendered three-dimensionally also in transparent non-biological objects (e.g. glass or plastics).
The method therefore supplements phase contrast microscopy and the quantitative interferometric methods. The device can be fitted to all research microscopes (Table I).

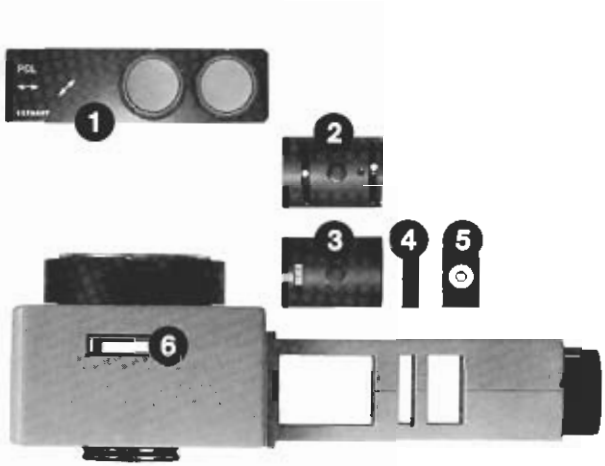
Fig. 88:
NaCl solution crystallized out on a microscope slide (skeletal growth)
Interference contrast T, objective NPI P 25/0.50



Interference contrast R

Incident-light interference contast is based on the same principle as transmitted-light interference contrast T, but the Wollaston prism and objective are traversed twice. Both therefore fulfill the additional function of the illuminating device.

Fig. 89:
Incident-light interference contrast device R
1. Analyzer
2. Slide with variable diaphragms
3 Slide with fixed diaphragms
4 Lambda plate
5 Polarizer
6 Slot for the analyzer

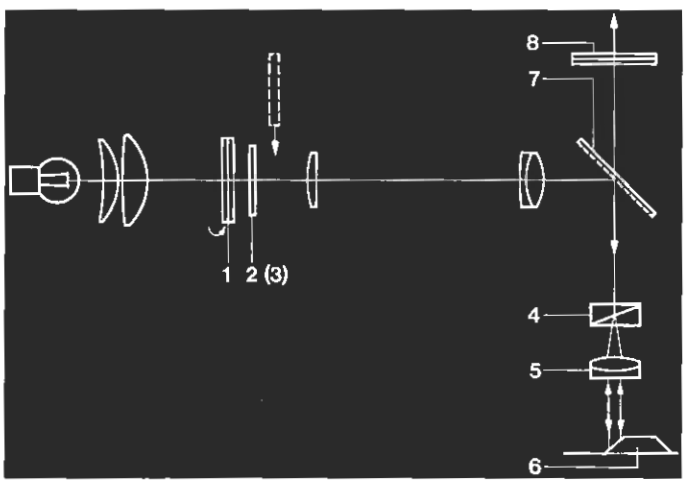


Principle

The light, linearly polarized by the polarizer (Fig. 90.1) (funtion of the $\lambda/4$- and λ-plate see below) and reflected by the optical flat (Fig. 90.7), is split by the Wollaston prism (Fig. 90.4) into pairs or rays vertically polarized to one another. The image-splitting effect is smaller than the resolving power of the microscope objective (latent image duplication, Fig. 87).

After reflection from the surface of the object both part-rays are recombined in the Wollaston prism. Interference occurs in the analyzer (Fig. 90-8).
The origin of the three-dimensional pictorial impression is explained with an object portion of trapezoidal cross section (Fig. 97; considerably simplified):

Fig. 90:
Principle of interference contrast R
1. Rotating polarizer
2. $\lambda/4$-plate
3. Additional λ-plate
4. Wollaston prism
5. Objective
6. Specimen
7. Optically flat reflector
8. Analyzer



1. Horizontal surfaces of specimens (Fig. 91 b, d, f) are reached by the pairs of rays always at the same instant: no phase difference is produced ($\Gamma_o = 0$). Plane, horizontal portions of the specimen therefore appear initially dark in the microscope (Fig. 91 B, D, F).

2. Inclined surface (Fig. 91 c, e):
Both part-rays travel along paths of different lengths. Phase differences Γ_o occur which depend on:
   a. the inclination of the surface,
   b. the separation between the two rays; this in the last resort depends on the objective used. The higher the magnification, the smaller must be the splitting of the two rays to avoid a visible double image. With low magnification a more pronounced relief will be observed than at higher magnifications.
   c. the refractive index of the immersion medium (air or oil).

In practice, phase differences generally do not exceed half a wave length. In the quoted example let $\Gamma_o = 90$nm (cf. Table III). In the microscope the inclined flanks of the trapezoidal structure appear gray (Fig. 91 C, E).

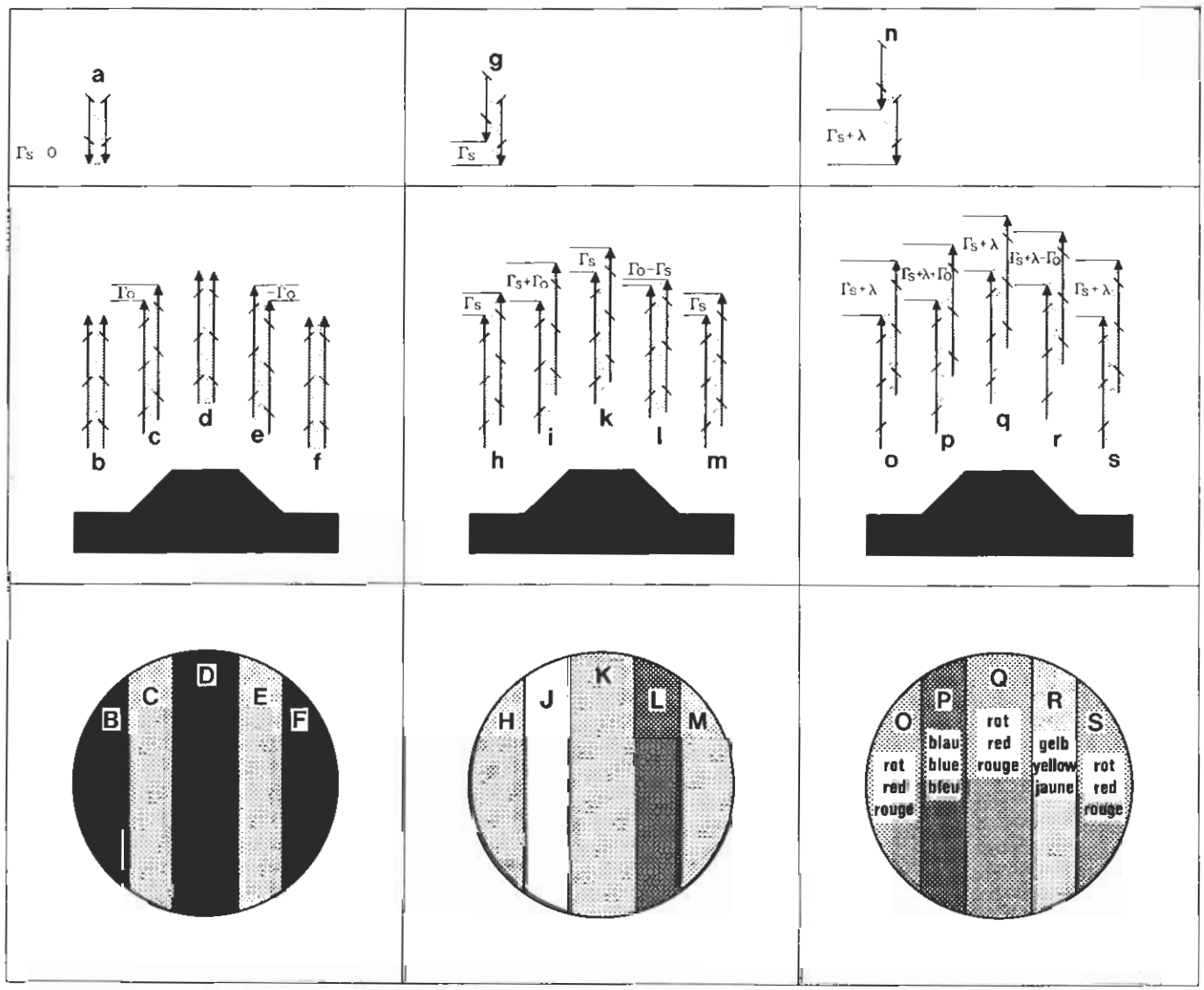
Fig. 91: (center column):

3. On horizontal surfaces (Fig. 91 h, k, m) the given phase difference $\Gamma_s = 80$nm remains unchanged. The horizontal surfaces therefore appear gray (Fig. 91 H, K, M).

4. On the left flank of the structure, for instance
$$\Gamma_s + \Gamma_o = 80 + 90 = 170\text{nm}$$
is produced through addition of the phase differences.
The flank therefore appears white (Fig. 91 J).

5. The phase differences are subtracted on the right flank (Fig. 97-I):
$$\Gamma_o - \Gamma_s = 90\text{–}80 = 10\text{nm}$$
The left flank (Fig. 91 L) therefore appears almost black.

Fig. 91:
Interference contrast R: production of a relief image, highly diagrammatic.
Top row: Phase difference Γ_s established in the microscope
Center row: Section through the specimen with phase differences
Bottom row: Representation of the object in the microscope



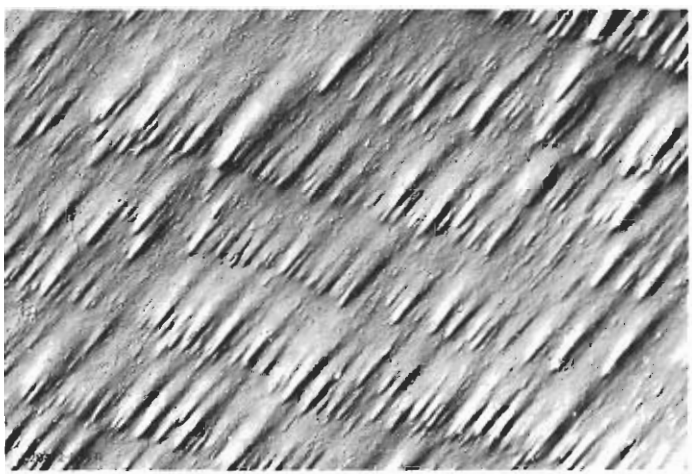
A considerably more pronounced three-dimensional representation is possible if the $\lambda/4$-plate (Fig. 90.2) is placed in the beam behind the polarizer. Any desired phase difference Γ_S within the first order can be set up continuously, as with the SÉNARMONT compensator, by slight rotation of the polarizer against the plates. This given phase difference of, for instance, Γ_S = 80nm (dark gray Table III) is superimposed on the object phase difference Γ_O.

The general impression in the microscope is that the trapezoidal projection is obliquely illuminated from the top left (North-West).
Function of λ-plate (1st-Order red) Fig. 91, right. Color contrast is obtained by the addition of the λ-plate in the interference polarizer (Fig. 90-3).
In the quoted example horizontal areas appear red, the flank inclined to the left blue owing to addition of the phase differences, the right flank, however, yellow owing to subtraction of the specimen phase difference from the given phase differences.

Uses

Quality control of surfaces of polished sections, investigation of crystal faces, evaluation of micro-hardness indentations, rendering visible of grain boundaries in ceramic products, production control of electronic components. The device can be attached to all Leitz pol-microscopes and metallographic microscopes as well as to the majority of the biological stands.

Fig. 92:
Fracture surface of glass
Interference contrast R with objective NPI 20/0.40 P



Determination of refractive indices

Principle: an unstained object immersed in a liquid can no longer be seen if both object and liquid have the same refractive index.

Even a tiny splinter of the material to be investigated is sufficient for the immersion method. It is introduced between the microscope slide and the cover-glass in various liquids and observed in the microscope. The object appears the more three-dimensional the greater the difference between the refractive indices of object and immersion liquid. With increasing agreement between the two refractive indices relief effect and grain boundary disappear. With some practice the refractive index can be estimated already by the degree of relief.

The decision whether the liquid or the object has the higher refractive index is possible with the aid of the BECKE line. If the object is first critically focused and then the object stage slightly lowered or raised, a light fringe (the BECKE line) can be observed along the grain boundaries. This bright line moves always into the medium of higher refractive index as the stage is lowered and can be recognized only after radical reduction of the condenser aperture (narrowing of the aperture diaphragm of the condenser). When the refractive indices are identical, the BECKE line will disappear. Especially sensitive methods of observation are limiting darkground and phase contrast. (JUDA and MEDENBACH 1967).

It is best to cement two strips of coverglass between microscope slide and coverglass so that a bridge is produced. In the hollow underneath various liquids can be conveniently introduced. Before the immersion liquid is changed the previously used liquid is removed with a piece of blotting paper and the hollow space washed with a suitable solvent.

Reference:
JUDA, K. and K. MEDENBACH: Eine neue Praeparationstechnik von Koernergemischen zu kontinuierlichen Untersuchungen nach der Einbettungsmethode. Leitz Mitteilungen fuer Wiss. u. Techn. **IV** (1-2), 36-39, 1967.

Fig. 93:
BECKE line (glass splinter immersed in water, aperture diaphragm closed)

To avoid repeated change of the immersion medium, the following methods can be used.

Lambda variation method

A liquid of the highest possible dispersion is used (Fig. 94) for immersion. The relatively steep dispersion curve intersects the dispersion curve, which as a rule is flat, of the object at a certain wavelength λ.
When the wavelength is changed by means of an interference graduated filter (Fig. 19) or a monochromator the object will disappear at this wavelength. The refractive index of the object n can then be obtained from the previously refractometrically determined dispersion curve of the liquid.

Temperature variation method:

In some immersion liquids the refractive index can also be stongly varied by a change in the temperature. Observation is carried out in monochromatic light with a microscope heating stage (see p. 38).
The advantage of the immersion methods is the need for a minimum quantity of sample, which subsequently is available even for other methods (non-destructive testing). For the investigations a simple laboratory microscope with phase contrast or darkfield facilities and interference graduated filter as well as a refractometer are adequate. In the last resort the accuracy depends on the accuracy of the refractometer used. Accuracies of ± 0.001 are sufficient for routine operation. The refractive indices of the liquids used must be continually checked, since changes may occur owing to aging.

Fig. 94:
Principle of the λ-variation method:
In observation with the wavelength λ the refractive indices n_x of the object and n_m (inclusion medium) are identical, so that the grain boundaries are no longer visible.

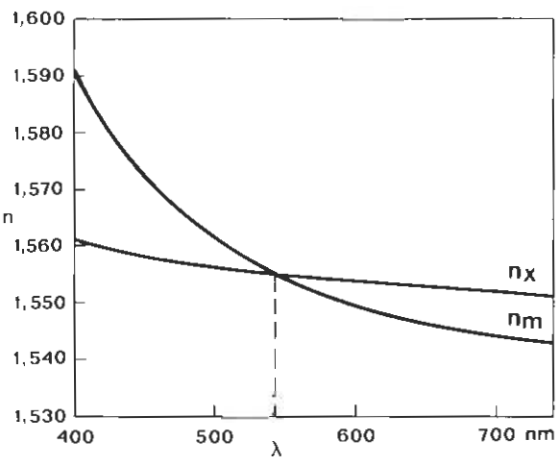


Table V: Refractive indices (n_d) of frequently used immersion fluids
Since the refractive indices may in time change owing to contamination, evaporation of components, or chemical decomposition, the refractive index should always be checked with a refractometer before use. Most fluids listed are obtainable commercially:
Embedding fluids (n up to 2.1) are marketed by Buehler Met AG, CH-4023 Basle, POB Switzerland.

Methyl alcohol	1.3288
Distilled water	1.3332
Sea water	1.343
5% cane sugar solution	1.341
10% cane sugar solution	1.347
30% cane sugar solution	1.376
Ether	1.3522
Acetone	1.3592
Ethyl alcohol	1.3631
Propan-2-ol	1.3759
Satured solution of potassium acetate	1.370
Propan-1-ol	1.3840
Glycerin-distilled water 1:1	1.397
$CaCl_2$ solution 90%	1.401
Paraldehyde	1.404
Crystal oil	1.4369
Chloroform	1.443
Cyclohexanone	1.450
Glycerin	1.456
Cineol	1.458
Carbon tetrachloride	1.461
Lavender oil	1.463
Cyclohexanol	1.465
Bergamott oil	1.465
Decalin	1.4687
Olive oil	1.4702
Turpentine	1.471
Walnut oil	1.477
Methyl benzoate	1.481
Terpineol	1.481
Euparal	1.481
Castor oil	1.482
Liquid paraffin	1.482
Methacrylic acid butyl ester	1.483
Diatex	1.490
Methacrylic acid methyl ester	1.4913
Xylene	1.4929
Phthalic acid di-n-butyl ester	1.493
Toluene	1.494
Eukitt	1.494
Benzene	1.4978
m-xylene	1.500
Laevulose syrup	1.5
Entallan	1.501
Pentachloroethane	1.5017
Ethyl benzoate	1.5034
Cedarwood oil	1.504
Sandalwood oil	1.508
Immersion oil	1.515
Ethyl iodide	1.516
Ethylsalicylate	1.5216
Monochlorobenzene	1.525
Acetophenone	1.536
Oil of wintergreen	1.5363
1,2-Dibromoethane	1.5368
Methyl salicylate	1.537
Ethyl bromide	1.538
Fennel oil	1.538
Safrol	1.540
Eugenol	1.541
Canada balsam	1.541
Clove oil	1.543
Tetralin	1.544
o-Nitrotoluene	1.5453
Nitrobenzene	1.5526
Aniseed oil	1.556
Anethole	1.560
Monobromobenzene	1.560
Dimethylaniline	1.561
Benzyl benzonate	1.5692
Toluidene	1.5704
o-Toluidene	1.575
Anilin	1.5838
Cussine oil	1.586
Bromoform	1.597
Oil of bitter almonds	1.6
Oil of cinnamon	1.602
Monoiodobenzene	1.618
Tetrabromoethane	1.6218
Cinnamic aldehyde	1.624
Quinoline	1.6242
Carbon disulphide	1.626
Alphachloronaphthalene	1.6318
Phenyl mustard oil	1.6491
Alphabromonaphthalene	1.6584
Alphaiodonaphthalene	1.6994
Barium mercury-2-iodide	1.742
Methyl iodide	1.7424

Other accessories for polarizing microscopes

In addition to the accessories specially designed for polarized-light microscopy, many other microscopic attachments can be used on polarizing microscopes (cf. Table I):

Darkfield (transmitted and incident light)

Phase contrast (transmitted and incident light)

Fluorescence (transmitted and incident light)

Contrasting and cathodoluminescence device

Heating and cooling stages −20° C ... +1750° C

Facilities for photomicrography
(35mm and large format, with and
without automatic exposure control)

Micromanipulator (e.g. for the removal of
most minute particles)

Incident-light interference attachments for the
quantitative measurement of object roughness
in the interference fringe field

TV display

Microhardness tester

MPV microscope photometer for absorption,
reflection, and fluorescence measurements

Quantitative image analysis method

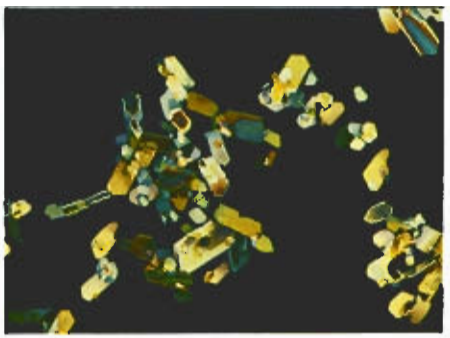
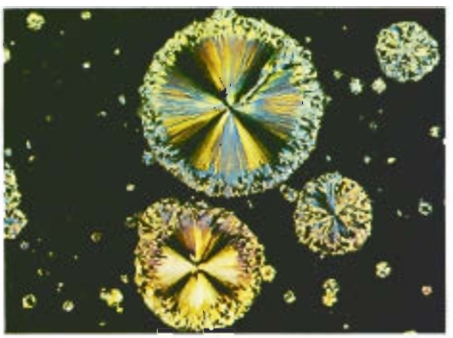


Fig. 95:
Honey
ORTHOPLAN POL
Objective NPl 6.3/0.20 P

Fig. 96:
Hippuric acid
Spheroliths (melt specimen)
ORTHOLUX POL MK
Objective NPl 16/0.40 P



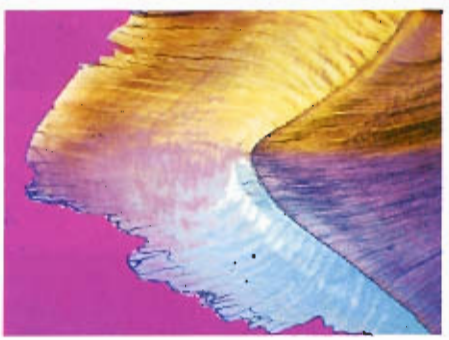
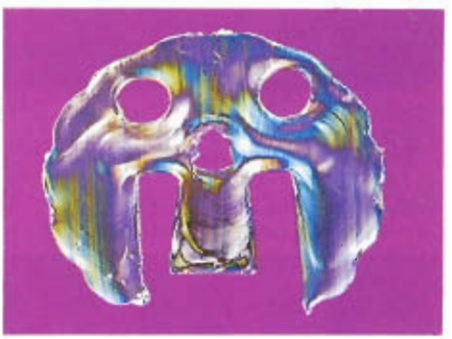


Fig. 97:
Cross section through a tooth of a porpoise (with λ-plate)
ORTHOPLAN POL
Objective Pl 2.5/0.08 P

Fig. 98:
Cross section through a plastic component (with λ-plate)
80mm PHOTAR f/4.5 lens
9x12cm large-format camera



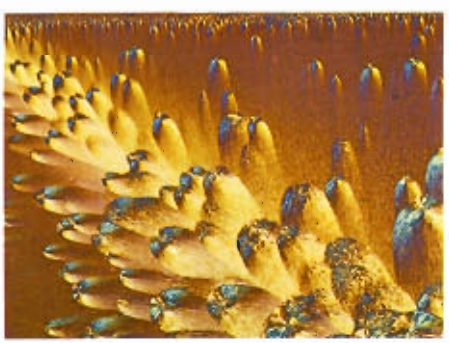


Fig. 99:
Plastic substance (fault)
Microtome section
ORTHOLUX POL BK
Objective NPl 6.3/0.20 P

Fig. 100:
Plastic substance (fault)
Microtome section
ORTHOLUX POL BK
Objective NPl 6.3/0.20 P

The improved versions of the NPL objectives used for this photographs are in the current range as NPL FLUOTAR and PL FLUOTAR series.

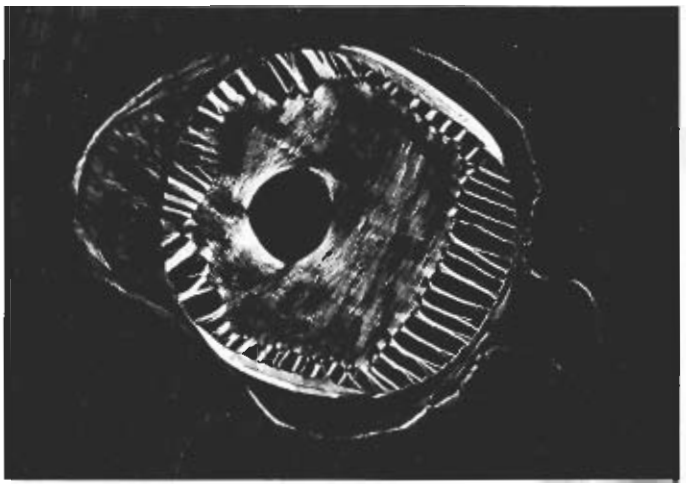


Fig. 101:
Muscle of a leech, transverse section
ORTHOPLAN POL with objective PI 2.5/0.08 P

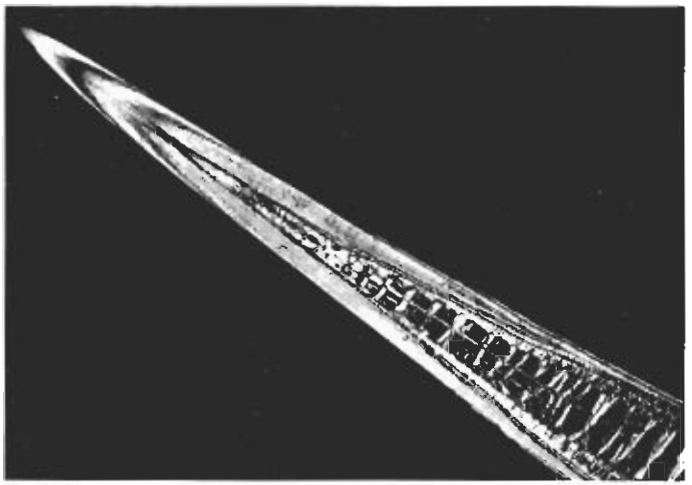


Fig. 102:
Quill of a sea urchin
ORTHOLUX POL BK with objective PL 2.5/0.08 P

Fig. 103:
Tentacles of a squid, longitudinal section
ORTHOLUX POL with objective PI 2.5/0.08 P

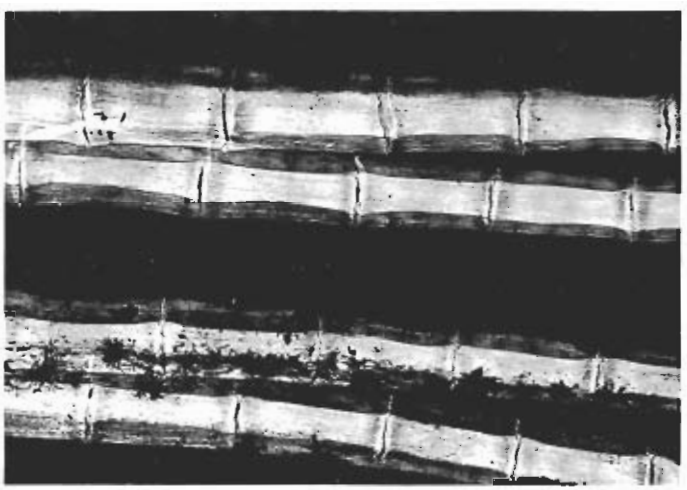


Fig. 104:
Starch granules
ORTHOLUX POL BK with objective NPI 40/0.65 P

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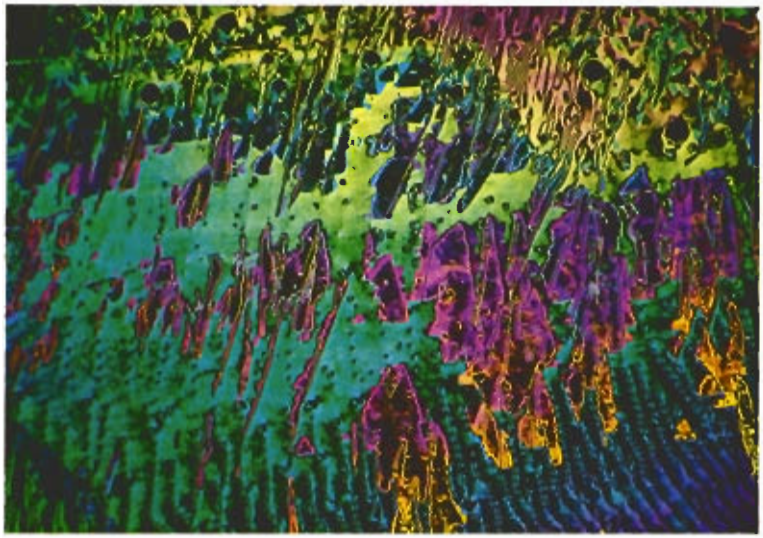
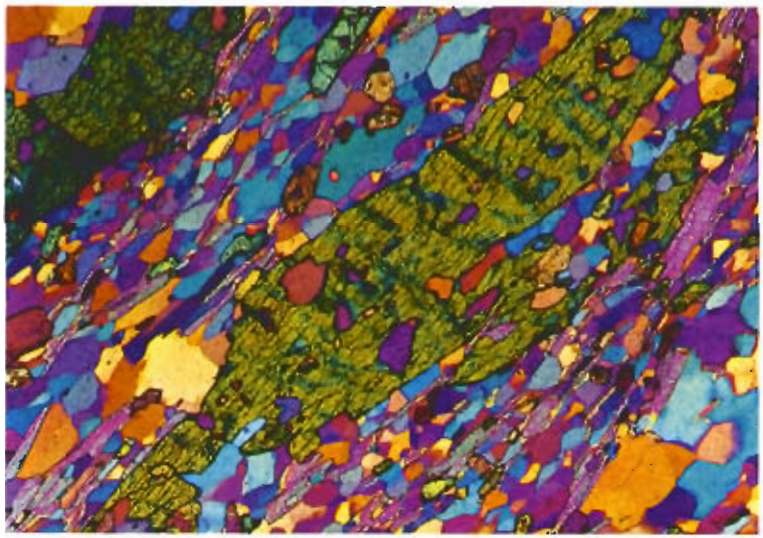


Liquid crystal (MBBA)
ORTHOPLAN POL, objective NPl 6.3/0.20 P
9x12cm large-format camera



Thin section of rock
ORTHOPLAN POL, objective Pl P 2.5/0.08
9x12cm large-format camera

Front cover:
Strain birefringence of an artificially strained hexagonal sheet of glass. ARISTOPHOT macrophotographic apparatus with 9x12cm large-format camera with fully automatic exposure control, 120mm PHOTAR f/5.6 lens

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