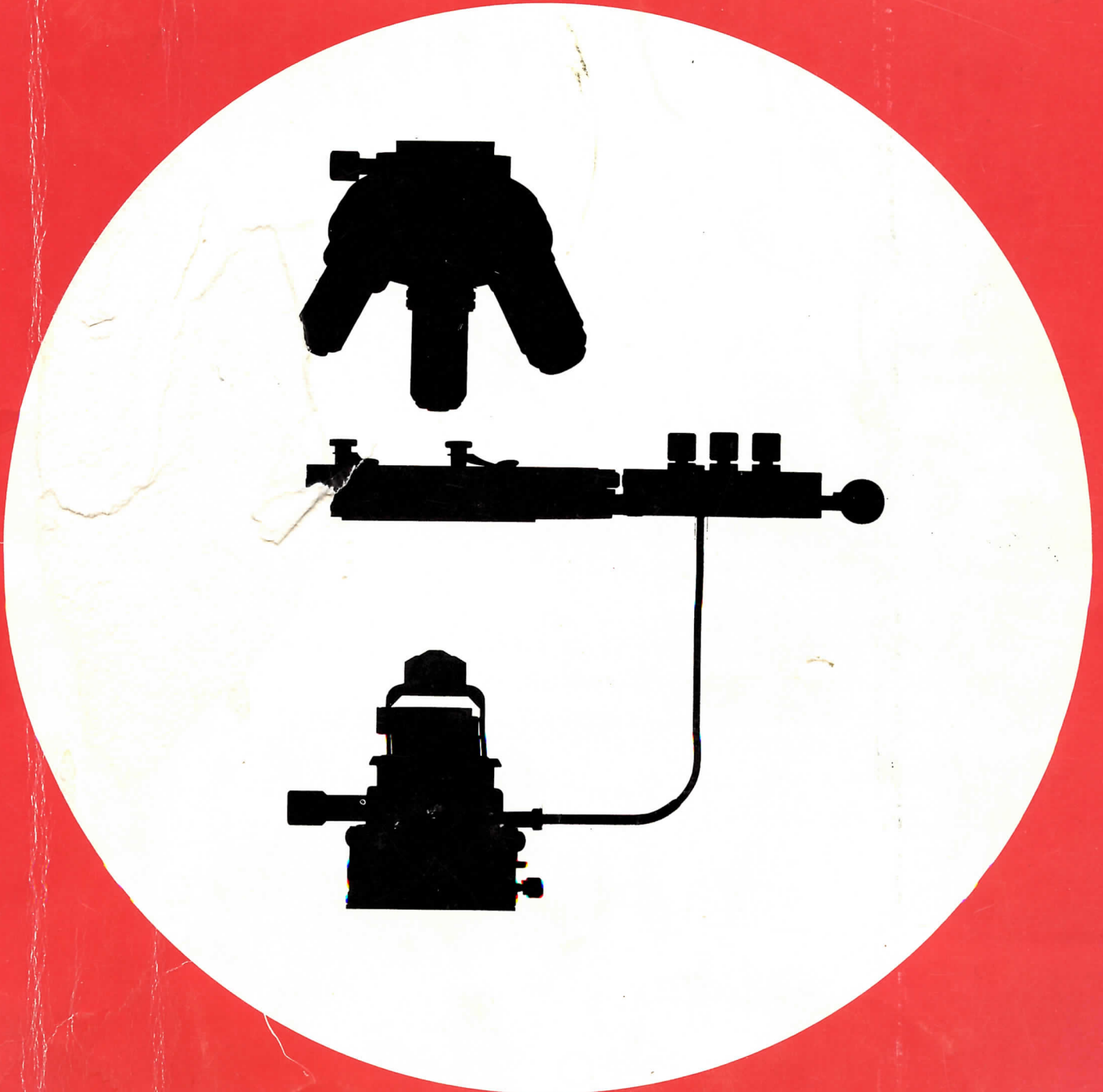# Pol interference device
according to Jamin/Lebedeff

Instructions

Pol-Interference Device
according to Jamin/Lebedeff

Leitz WETZLAR
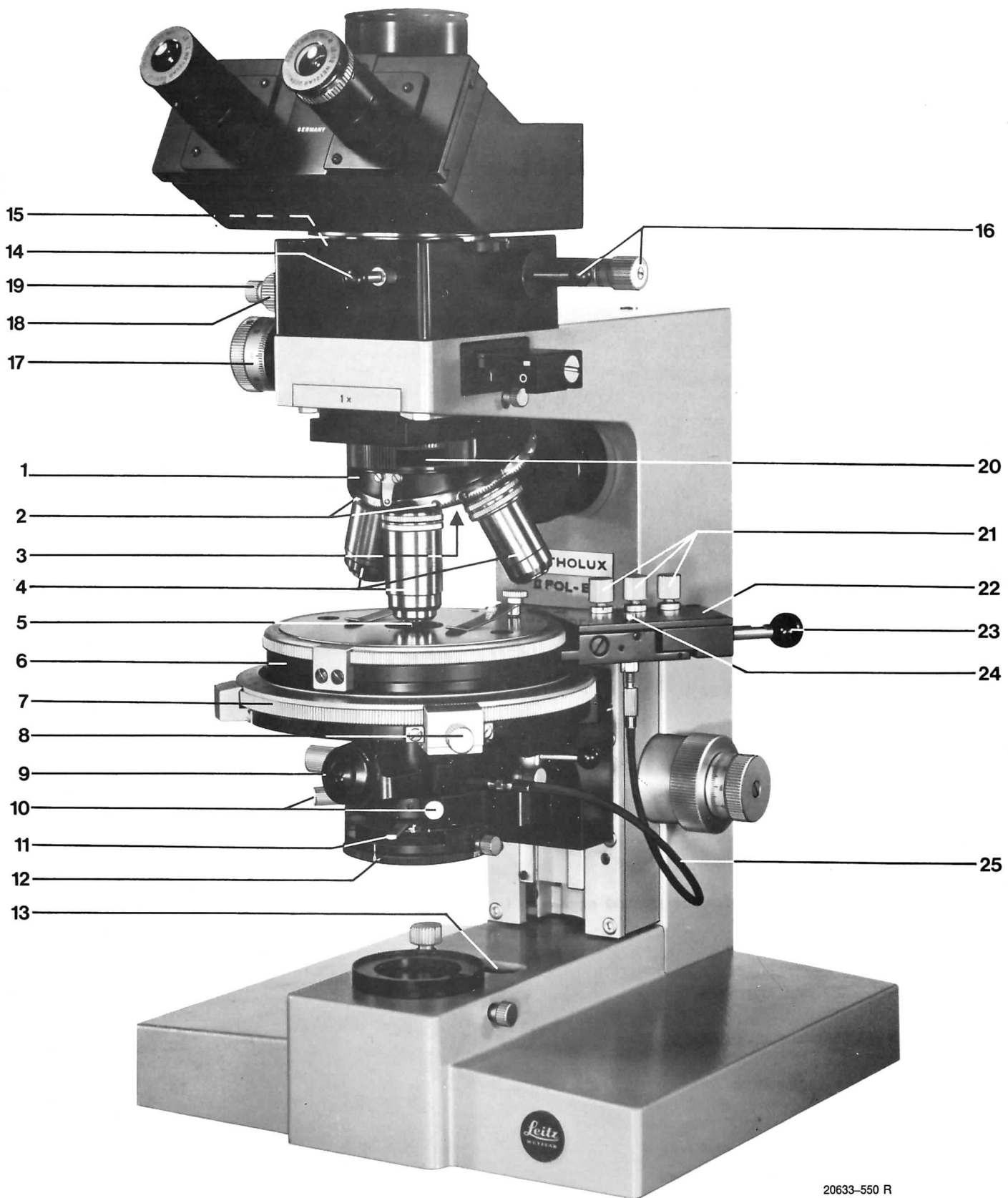
Instructions

CONTENTS

Fig. 1 shows the
Pol-interference device according to Jamin/Lebedeff on
the ORTHOLUX 2-Pol-BK.

The versions for other LEITZ microscopes differ
from the one illustrated in minor details.

20633–550 R

1. Introduction

The LEITZ double-beam interference device according to Jamin/Lebedeff combined with a LEITZ polarizing microscope (ORTHOPLAN-Pol, ORTHOLUX-2-Pol MK or BK) is used for the demonstration and measurement of phase differences in isotropic and anisotropic individual samples. The device is also available for earlier polarizing microscopes (DIALUX-Pol, PANPHOT-Pol, and ORTHOLUX-Pol).

Sample thicknesses,
refractive indices, and
concentrations and dry weights
can be determined with great accuracy from the measured phase differences.

This interference method based on polarization thus opens up a wide range of possibilities of investigation, mainly in

> biology, medicine (cytology)
> mineralogy, geology, and soil science
> dust investigation,
> textile technology,
> crime investigation,

and numerous other scientific and applied fields.

Fig. 1 Pol-interference device according to Jamin/Lebedeff

1. Revolving nosepiece
2. Objective centring screws
3. 2 vacant threads in the revolving nosepiece (not visible in the illustration)
4. Special objectives (with recombination plates)
5. Beam splitter slide with 3 beam splitters and $\lambda/2$-plates in the rotating auxiliary stage
6. Rotating auxiliary stage
7. Rotating microscope stage
8. Friction clamp
9. Condenser top
10. Condenser centring screws
11. Lever for adjusting the aperture diaphragm
12. Polarizer
13. Field diaphragm
14. Slide for pinhole diaphragm
15. Slide for changeover between the binocular and monocular tubes (behind 18 and 19)
16. Lever for actuating the Bertrand lens, with knurled knob for focusing
17. Drum for rotating the analyser
18. Knurled knob for the vertical centring of the Bertrand lens
19. Knurled knob for the horizontal centring of the Bertrand lens
20. Tube slot for the compensator
21. Adjusting screws for tilting the beam splitters
22. Auxiliary stage attachment
23. Switch rod
24. Securing button
25. Bowden cable

2. Principle of the interference method according to Jamin/Lebedeff

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Fig. 2   Diagramatic representation of states of polarization and phase diplacements in a sample

1 Analyser
2 Beam recombination plate
3 Sample
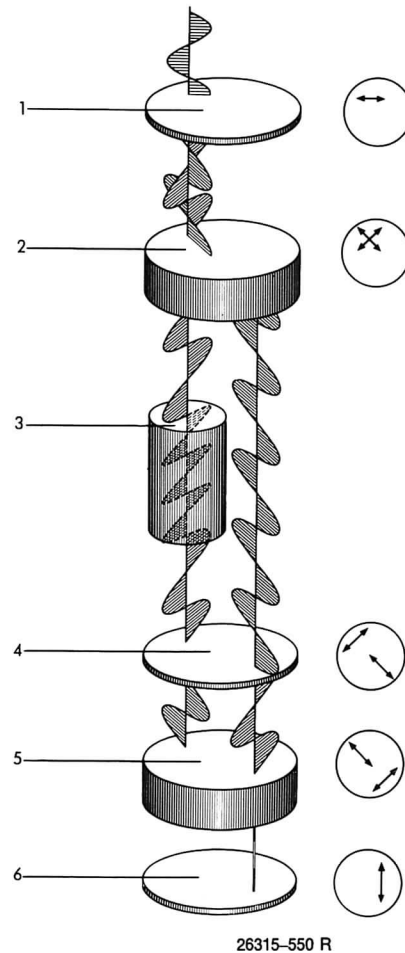4 λ/2 plate
5 Beam splitter
6 Polarizer

The method is based on the principle of polarized-light microscopy. To understand it, basic knowledge of crystal optics and of the use of the polarizing microscope is therefore required. The text books listed in the reference section will offer an introduction to polarized light microscopy.

The light emitted by the low-voltage lamp of the microscope is linearly polarized in the polarizer (2/6). After passage through the special condenser the polarized light beam reaches the crystal beam splitter, (2/5), which splits it into two rays vibrating perpendicularly to each other, with the "ordinary ray" passing without deflection and serving as "reference beam", and the "extra-ordinary ray" being laterally displaced and serving as sample beam.

The vibration directions of both rays are now rotated through 90° through a λ/2-plate (2/4). In the recombination plate the ordinary ray thus becomes the extra-ordinary ray, and the extra-ordinary ray the ordinary ray. The optical paths of both rays are therefore equally long. Since the λ/2-plate is adjusted for a certain wave length (546 nm), precision measurements should be carried out at 546 nm.

After passage through the recombination plate, which must be precisely orientated to the beam splitter, both beams are reunited, they interpenetrate each other as it were; but their planes of vibration are perpendicular to each other.

The sample (2/3) is inserted between the λ/2-plate and the recombination plate. Whereas the sample ray passes through a neighbouring, empty portion (embedding medium or air) of the same thickness.

If no object is in the beam, the path lengths of both beams are identical, both rays vibrate "in phase".

But if the sample - and the reference beams traverse media of different refractive indices (i.e. the speed of light) the wave in the more highly refractive medium is slightly retarded compared with that in the medium of lower refractive index, it shows a phase difference.

The phase difference will be the greater the thicker the sample and/or the greater the difference between the refractive indices. The following formula produces the value of the phase difference Γ :

$$\Gamma = d\,(\,n_o - n_1\,)$$

where
    d = sample thickness (= thickness of the reference medium)
    n_o = refractive index of the sample
    n_1= refractive index of the reference medium

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Naturally, the formula is equally valid when the reference medium is more refractive than the sample; here it should be written $\Gamma = d(n_1 - n_0)$. Air ($n_1 = 1.0003$) can of course also be used as reference medium.

In the analyser (2/1) the components vibrating in its absorption direction are extinguished, whereas the components of the two rays vibrating in the transmission direction are transmitted.

Since the two transmitted components now vibrate in a single plane they can become superimposed, producing a resultant vibration (interference).

If the phase difference between sample and reference beam is zero or a multiple of λ (λ, 2λ, 3λ etc.), the two vibrations extinguish each other, and the sample appears dark. If the phase difference does not exactly correspond to this condition the sample will appear more or less bright. Maximum brightening occurs when the sample produces a phase difference constituting an odd multiple of $\lambda/2$ ($1/2\lambda$, $3/2\lambda$, $5/2\lambda$, etc.).

Phase differences are measured in <u>nm</u> (nanometres)

$$1 \text{ nm} = 10^{-3} \text{ um} = 10^{-6} \text{ mm} = 10^{-7} \text{ cm}.$$

Depending on the wave length used a sample of certain phase difference can appear in all intermediate stages between maximum darkness and maximum brightness as shown by the following example:
Let a sample produce a phase difference $\Gamma = 900$ nm; it is to be observed successively in monochromatic light from about 360 to 780 nm (= visible region).

At $\lambda = 360$ nm the sample appears at maximum brightness, because 900 nm are exactly $5/2\lambda$. When the wave length is increased (which can be done with a monochromator or with an interference wedge filter) the brightness of the sample is visibly reduced.

At $\lambda = 450$ nm (blue) the sample will be in the dark position because the phase difference of 900 nm exactly corresponds to two wave lengths (2λ). If the wave length increases further, the brightness of the sample increases again.
Maximum brightness occurs at $\lambda = 600$ nm, because the phase difference is exactly $3/2\lambda$. When the wave length is further increased, brightness decreases again.

If the sample were observed in the whole spectral region from 360 - 780 nm simultaneously, the intensities of the various wave lengths would be changed very differently. A well-known example is white-light illumination. Owing to the brightness reduction in the region of certain wave lengths the sample no longer appears colourless, but in a certain interference colour which is quite specific to a given phase difference.

Table 1: Interference colours (1st - 4th orders);

Order	Phase difference (nm)	Colour
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	BRIGHT GREEN / PURE YELLOW
	1000	ORANGE RED
3RD ORDER	1200	DARK PURPLE RED / INDIGO / GREENISH BLUE / SEA GREEN
	1400	GREENISH YELLOW / FLESH COLOURED / CARMINE
	1600	MATT PURPLE / GREY BLUE / BLUEISH GREEN
4TH ORDER	1800	BRIGHT GREENISH GREY / WHITISH GREY
	2000 nm	FLESH RED

26961-550

Interference colours are classified in orders. The unit chosen for this classification is the wave length 551 nm which approximately corresponds to the brightest region of the solar spectrum. Colours of the 1st order correspond to a phase difference of 0 - 551 nm, those of the second order to that of 551-1102 nm, etc. With a little practice the degree of phase difference can already be estimated by means of the interference colour. With phase differences of more than about 4 orders, the interference colours become increasingly pale, approaching a white tone, the higher-order-white.

Compensators are used for precision measurements. Their function consists in compensating the phase difference, so that the sample appears dark.

The sequence of the interference colours can be studied very well if the beam-splitting plate is tilted by rotation of the knurled screw. The colour fringes observed during adjustment with the Bertrand lens (page 9), also appear in this sequence.

Plane-parallel samples show a uniform colour hue. Samples of varying thickness (or refractive index) display colour differences in white light, brightness differences in monochromatic light. If the phase difference varies within the range of several orders, dark and bright fringes occur, which join areas of the same phase difference and therefore the same thickness or refractive index (and areas of the same concentration in solution).

As a rule, plant and animal cells have phase differences of less than a whole wave length.

The interference device is available in 4 slightly different versions for the following LEITZ polarizing microscopes :

a) for the DIALUX-Pol   tube slot
b) for the ORTHOLUX-Pol   12 x 4 mm
c) for the ORTHOPLAN-Pol   tube slot
d) for the ORTHOLUX 2-Pol MK/BK 20 x 6 mm

Each version can be used only on the stand for which it is designed.

The following parts of the polarizing microscope must be removed before assembly :

1) Condenser

Lower the condenser by means of its control and remove it from the dovetail guide.

2) Revolving nosepiece or objective centring clutch

This is removed as follows :

from the DIALUX, ORTHOLUX 2-Pol MK/BK and ORTHO-PLAN-Pol after release of the knurled screws on the side of the stand.

From the ORTHOLUX :

a) after release of the spring-loaded lever on the side of the stand the tube is lifted out of its dovetail guide; it is advisable first to remove the eyepieces from the tube.

b) After release of the knurled screws on the side of the stand the revolving nosepiece is lowered and removed.

3) Ring plate in the object stage

The plate is easily lifted out of the rotating stage (Fig. 3).

It is advisable to store all the parts removed carefully to protect them against dust and avoid damage.

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Fig. 3 Removing the ring plate from the object stage

3.   Assembly

The interference attachment consists of the following parts, which replace the corresponding standard equipment of the polarizing microscope (Fig. 4) :
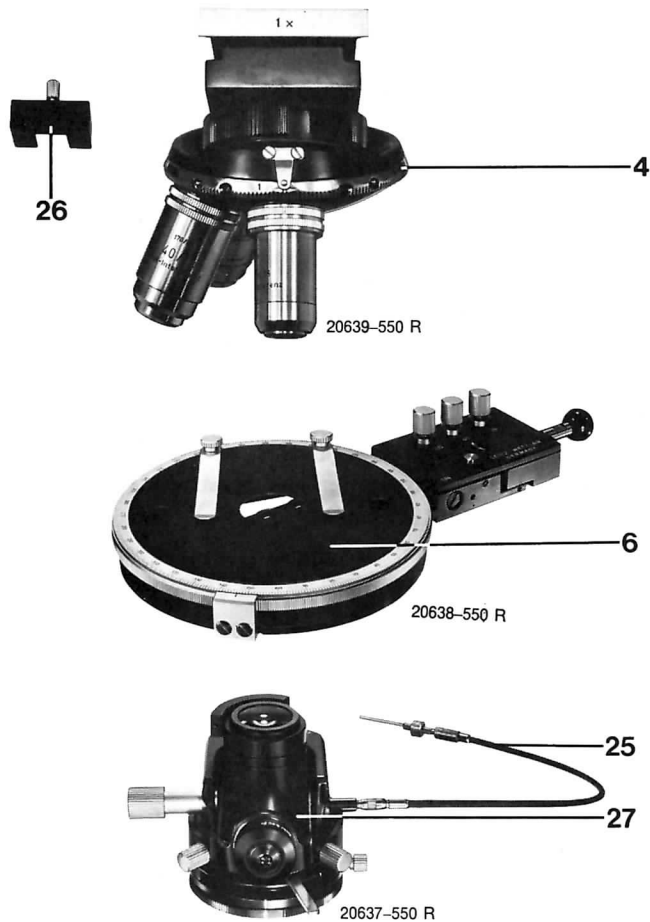
Fig. 4 Components of the interference device

4 Revolving nosepiece

    illustrated version : for the ORTHOLUX 2-Pol BK (with 3 orientated special objectives incorporating a beam splitter each)

6 Rotating auxiliary stage

    (with attachment and built-in, tilting beam splitters and $\lambda/2$ plates)

27 Special condenser

25 Bowden cable

    (for blocking the condenser top when the beam splitter slide is in position).

Additional accessories supplied : **1 Allen key** (2.5 mm)

26 Adapter (for the ORTHOLUX-Pol only)

    (to be pushed onto the dovetail guide of the tube).

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Fig. 5   Attaching the auxiliary stage

The interference attachment should now be assembled in the sequence described below :

1. Auxiliary stage

Before the auxiliary stage is screwed on it is best to set the microscope stage at 90⁰ (or 270⁰) by means of the verniers. In addition the two red dots on the outer rim of the auxiliary stage must be made to coincide. The auxiliary stage can now be screwed on to the microscope stage (Fig. 5) with the Allen key supplied.

2. Bowden cable

The Bowden cable prevents the simultaneous insertion of the condenser top and the sensitive beam splitting plates, which can damage the latter.

The end of the Bowden cable, which is fitted with two knurled screws, is screwed into the attachment of the auxiliary stage from below; the other end is screwed into the condenser (Fig. 4). The position of the screw at which the condenser top cannot be raised if the plate with the beam splitters is already turned into the beam must be found by trial and error, and the larger knurled screw tightened to secure it.

3. Condenser

The condenser is pushed on to the fork of the condenser adjustment and slightly raised with it.

4.   Revolving nosepiece

DIALUX, ORTHOPLAN-POL, ORTHOLUX 2-Pol MK/BK.

The revolving nosepiece is inserted into the dovetail guide and secured with the knurled screw on the side of the stand.

ORTHOLUX-Pol

a) First the adapter supplied is inserted and clamped in the dovetail holder of the tube (4/26).

b) The spring-loaded lever on the side of the stand is released, and the tube pushed into the dovetail guide of the stand (the eyepieces, if they have been removed, can now be replaced).

c) The revolving nosepiece is inserted in the dovetail guide from below, and the knurled screw on the side tightened to secure it.

NOTE :

The three special objectives are attached to the nosepiece in a certain position and must on no account be disturbed by rotation, because this destroys the orientation of the recombination plate.

5) Special accessories such as the object guide, Wright eyepiece, photographic attachments etc. can now be fitted.

4. Adjustment and operation

1) **Adjustment**

1.1 Check and if necessary adjust the lamp position (see instructions for the microscope).

1.2 Check the crossing of the polarizers by

a) moving the beam splitter slide (1/5) to position "40" and swinging it out of the beam (1/23)

b) removing the screw cover from the vacant aperture of the revolving nosepiece

c) turning the vacant aperture of the revolving nosepiece into the beam (an ordinary pol-objective can also be inserted; it can later be used as a spotting objective)

d) turning the Bertrand lens (1/16) into the beam.

On the ORTHOLUX 2-Pol MK stand (with binocular tube only) a focusing telescope must replace the Bertrand lens in the tube

e) turning the analyser (1/17) into the beam.

If the analyser is rotatable turn it into the 90° position (on earlier stands, such as the DIALUX-Pol and ORTHOLUX-Pol, the analyser must be turned into the 0° position)

f) turning the polarizer (1/12) until the maximum extinction position (dark cross) has been reached, fixing the position of the polarizer (tighten the knurled screw on the side of the polarizer).

1.3 Turn out the Bertrand lens (1/16) or replace the focusing telescope with the eyepiece.

1.4 Close the vacant aperture again by inserting the screw cover or a spotting objective.

1.5 Rotate the microscope stage so that the attachment of the auxiliary stage (1/22) points to the rear right at about 45°.

1.6 Turn the beam splitter slide (1/5) into the beam and engage beam splitter plate "16" by actuating the switch rod (1/23).

1.7 Turn in the 16/0.35 objective and focus it on the sample (a stage micrometer or dust particles on an object slide are suitable as test objects; samples with rich detail, such as tissue sections, are not suitable).

1.8 Centre the objective with the two centring keys to the rotation of the auxiliary stage; the microscope stage is temporarily clamped during this operation by means of the friction clamping (1/8).

1.9 Adjust the condenser according to the conditions of Koehler's illumination:
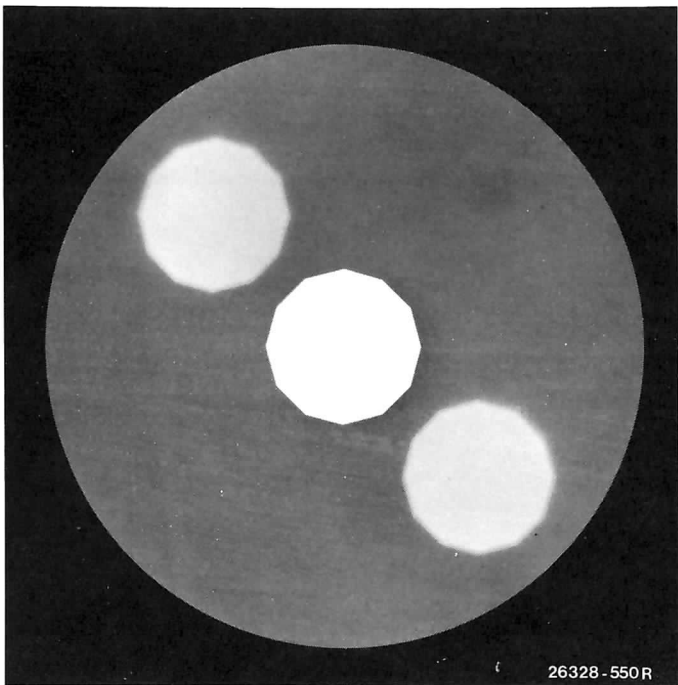
a) close the field diaphragm (1/13) fully

Fig. 6 Multiple image of the field diaphragm

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b) leave the aperture diaphragm (1/11) slightly open

c) focus the field diaphragm by vertical adjustment of the condenser. Instead of the usual single image of the field diaphragm seen in an ordinary microscope, several such images, of varying brightnesses (Fig. 6), will now appear.

d) Centre the condenser on the brightest of the diaphragm images by means of the two centring screws (1/10).

1.10) Open field and aperture diaphragms about halfways each (1/11 and 1/13).

1.11) Turn in the Bertrand lens (1/16) or the focusing telescope. An interference fringe system is seen mostly at once. Failing this, check the focusing of the Bertrand lens; try to find the fringes by slowly rotating the focusing screw of the Bertrand lens (1/16) (the ORTHOLUX 2-Pol MK has no focusing facilities; the Bertrand lens setting is fixed).

If this method does not make the fringes visible, tilting the beam splitter with the knurled screws on the attachment (1/21) of the auxiliary stage will as a rule have the desired effect.

1.12 After the fringe system has been found the fringes must be moved, if possible into the centre of the field of view (rotate the knurled screws on the attachment of the auxiliary stage (1/21)).

1.13 Now widen the fringes by rotating the microscope stage (1/7) until the colour of the field of view appears nearly uniform.

1.14 Clamp the microscope stage in this position with the friction clamp (1/8).

1.15 Turn out the Bertrand lens (1/16) (or replace the illumination setting magnifier with an eyepiece).

1.16 Close the aperture diaphragm (1/11). The field of view now appears in a uniform interference colour, whereas the objects display other interference colours depending on the phase differences they produce. As a result of the principle of birefringence on which the interference attachment according to Jamin/Lebedeff is based every object produces a ghost image, which is pale and poor in contrast and displaced from the image proper towards the bottom right (Fig. 7).

1.17 When the desired colour of the background has been found (measurements are carried out on a dark background, i.e. phase difference $\Gamma = 0$), another check of the condenser centration, and, if necessary, correction is recommended.

2. Change of Magnification

2.1 Turn the desired objective into the beam path by rotating the revolving nosepiece (if necessary apply immersion oil to the sample).

2.2 Introduce the associated beam-splitting plate into the beam path by means of the switch rod (1/23).

2.3 Focus the sample.

2.4 Centre the objective to the rotation of the auxiliary stage (1/2) with the centring screws.

2.5 Check, and, if necessary, correct the adjustment of the microscope stage with the Bertrand lens (1/16) as described in the previous chapter (a slight correction after each objective change cannot be completely avoided owing to the use of spring-loaded objectives).

2.6 Check the centration and focusing of the condenser.

3. Observation of the sample as in the ordinary biological microscope (brightfield)

3.1 Turn the beam splitter slide out of the beam path.

NOTE: The beam splitter slide must not be removed from the beam path except in position "40".

3.2 a) Lower the condenser.

b) Turn the condenser top into the beam (the special condenser can also be replaced with an ordinary one).

c) Raise the condenser again.

3.3 Set the rotating analyser at 135° in stands with 12 x 4 mm tube slot, 225° in stands with 20 x 6 mm tube slot (this does not apply to the ORTHOLUX 2-Pol, in which the analyser is fixed).

3.4 Focus and recentre the condenser.

An ordinary objective can be screwed into the vacant aperture of the revolving nosepiece and the position "40" observed with the beam splitter slide turned out. Analyser and polarizer must be removed from the beam path. The condenser top must be turned in for objectives of more than 10x magnification.

4. Use as polarizing microscope

For investigation in polarized light a pol-objective is necessary, which is screwed into the vacant aperture (1/3) of the revolving nosepiece. Polarizer and analyser are in the beam path. The condenser top must be turned in for objectives of more than 10x magnification. The beam splitter slide (1/5) is turned out (position "40").

1) The ghost image

When a sample is observed, a darker, pale ghost image is seen at the bottom right of the primary image (Fig. 7). This ghost image is by no means due to a defect of the optical system or wrong adjustment, but the result of the beam splitting principle of the interference method :

The λ/2-plate must rotate the vibration direction of sample and reference beam through 90° each. However, this condition is precisely met only if the wave length of the light used and the λ/2-plate are matched. Polarized light which does not meet this condition does not have its vibration rotated through 90° but is converted into two vibrations (elliptically polarized light). The additional component thus produced results in the ghost image. For further details about the origin of the ghost image the publications listed in the reference should be consulted.

In theory no ghost image is produced when matched light of extremely high monochromasia and perfect polarization is used. Since these conditions are never completely achieved, the ghost image cannot be entirely suppressed in practice. With large objects the primary and ghost images become superimposed and prevent quantitative investigations. For such large objects the large LEITZ transmitted-light interference microscope (system MACH-ZEHNDER) is more suitable, because of its 62 mm working distance between sample - and reference beam.

The maximum object width to permit undisturbed investigations is based on the distance between reference and sample beam, which is

0.25 mm	with the	16/0.35
0.10 mm	with the	40/0.65 and
0.04 mm	with the Oel	100/1.20 objective.

Elongated samples such as fibres can be examined if their longitudinal axis in the field of view runs from bottom left to top right. You can obtain this orientation by rotating the auxiliary stage.

Marginal parts of extensive samples, such as tissue sections or foils, can be investigated by alignment of the edge in the same direction. Here, the left upper third of the field of view must be empty (Fig. 8).
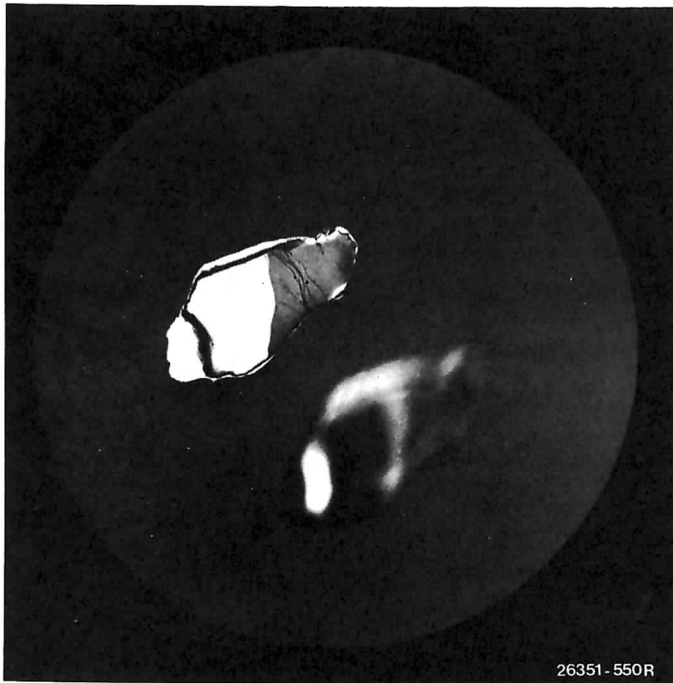

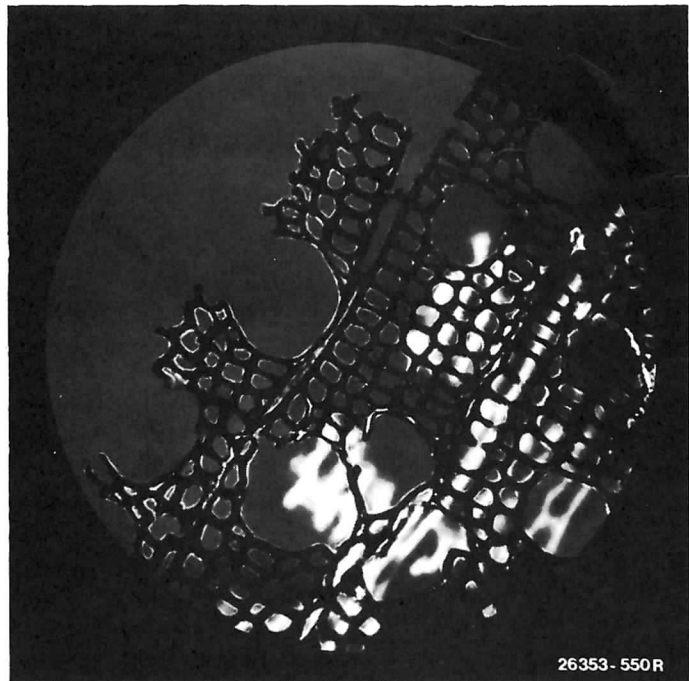Fig. 7   Image and ghost image of an isolated object
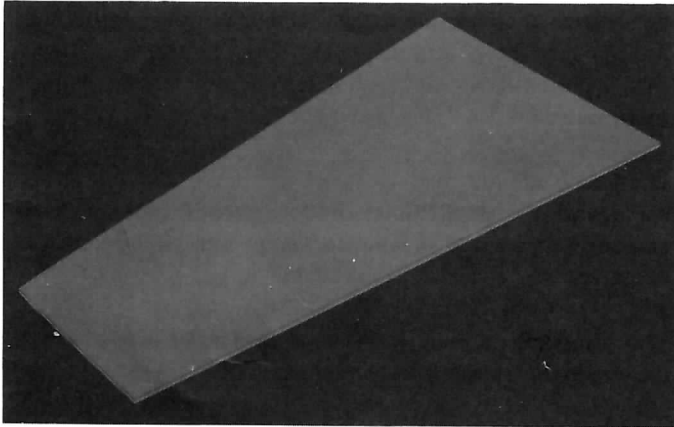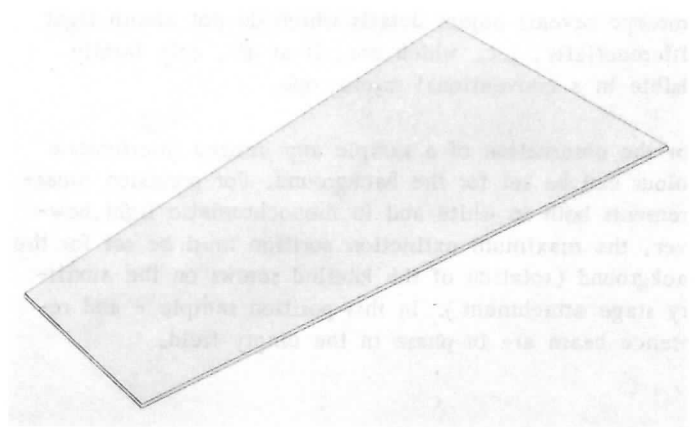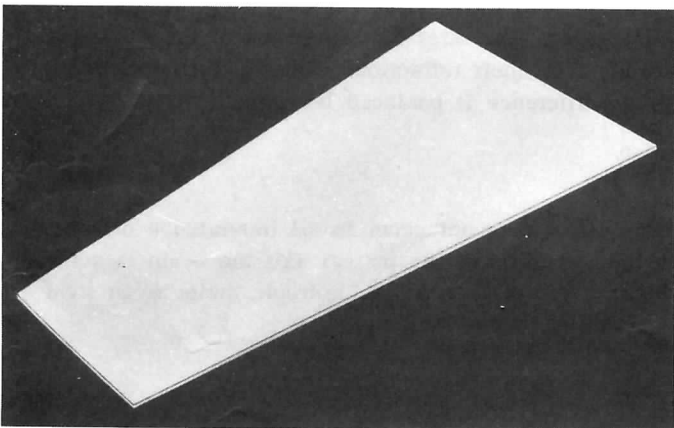(mica scale)

Fig. 8   Image and ghost image of an extensive object
(cross-section of wood) with the sample orientation shown in the illustration. Superimposition-free observation is possible only in the marginal region.
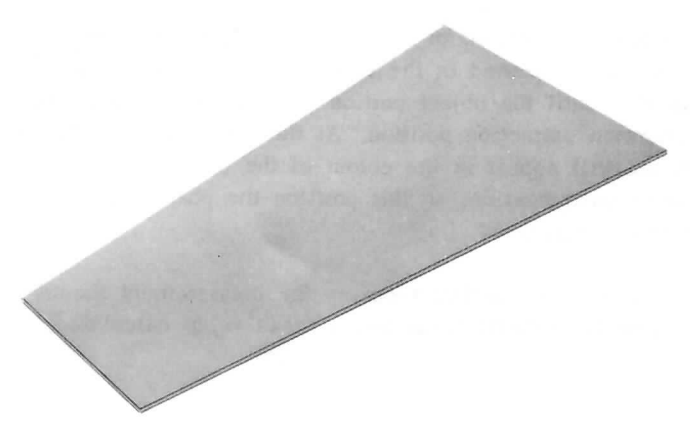
Rotation of analyser in 0°

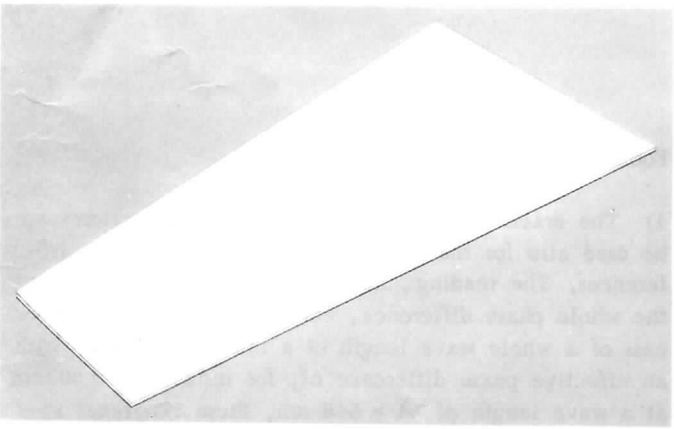Rotation of analyser in 75°

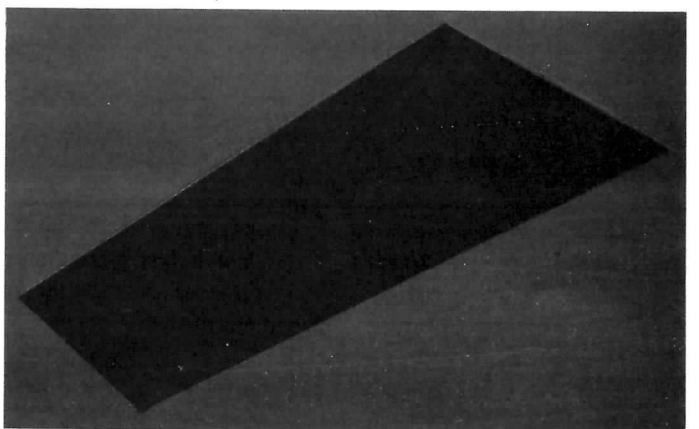Rotation of analyser in 25°

Rotation of analyser in 90°

Rotation of analyser in 50°

112.2° analyser rotation = compensation **position**

26371-550R

Fig. 9   Process of compensation (Sénarmont)

2. Observation and measurement in interference contrast

Like the phase contrast microscope the interference microscope reveals object details which do not absorb light differenctially, i.e. which are, if at all, only faintly visible in a conventional microscope.

For the observation of a sample any desired interference colour can be set for the background. For precision measurements both in white and in monochromatic light, however, the maximum extinction porition must be set for the background (rotation of the knurled screws on the auxiliary stage attachment). In this position sample - and reference beam are in phase in the empty field.

Measurement of phase difference with compensators

The phase difference produced by the sample can be eliminated by means of compensators. The compensator is set at 0, inserted in the tube slot (Fig. 1-20), and adjusted until the object portion to be measured is in the maximum extinction position. At the same time the background will appear in the colour of the object portion before compensation. In this position the phase contrast is compensated.

To improve the setting accuracy the measurement should be repeated several times and a mean value calculated.

From the angle value thus obtained the phase difference can now be determined according to the instruction enclosed with every compensator. Monochromatic light can be used to increase accuracy. The maximum extinction position can be determined with even greater accuracy with a half-shadow device (Wright eyepiece) or photometrically.

Depending on the magnitude of the phase difference to be measured and on the desired degree of accuracy compensators of various measuring ranges and slightly different operation are available (see Table 2.).

The principle of compensation is explained below with the example of the tilting compensator.

This type of compensator incorporates a tilting, birefringent crystal plate. A light ray passing through it is split into two wave trains vibrating perpendicularly to each other. The rates of propagation of the two wave trains, i.e. their refractions, differ slightly, so that a phase difference is porduced between them.

This effect does not occur in all transmission directions. In the direction of the optical axis the beam is not split because the plate acts like isotropic material on light entering it in this direction.

Table 2   Compensators

Type of compensator	Maximum measuring range	Accuracy (with use of a half-shadow plate of photometer
a) Brace-Koehler	$1/30-1/10\,\lambda$ [1]	up to $\pm \dfrac{\lambda}{2000}$
b) Sénarmont [2]	$1\,\lambda$ [1]	$\pm \dfrac{\lambda}{500}$ to $\dfrac{\lambda}{2000}$
c) Tilting compensator [3] (4 orders)	$4\,\lambda$	$\pm \dfrac{\lambda}{500}$

Footnotes :

1) The Brace-Koehler and Sénarmont compensators can be used also for the measurement of greater phase differences. The reading, however, will then not represent the whole phase difference, but only the amount in excess of a whole wave length or a multiple thereof. With an effective phase difference of, for instance, $\Gamma = 600$ nm at a wave length of $\lambda = 546$ nm, these two types of compensator will indicate only 54 nm, (= 600 - 546 nm). The whole wave length, in our example 546 nm, would have to be estimated from the interference colour or determined with the tilting (Berek) compensator. To avoid measuring errors of the magnitude of a whole wave length or its multiples it is therefore urgently advisable to estimate the magnitude of the phase difference visually from the interference colour of the sample before the measurement, or to use, when in doubt, a tilting (Berek) compensator for comparison.

But the more the transmission direction diverges from the optical axis of the crystal plate, the stronger the effect of birefringence; the phase difference between the two waves increases.

The orientation of the vibration directions, however, remains unchanged (the vibration direction of the more strongly refracted wave is marked γ on the compensator). In the tilting compensator the plate is orientated so that these vibration directions are identical with those of the wave trains coming from the beam recombination plate. Let us assume that the phases of these wave trains are already displaced; with the compensator in the 0 position, the two rays pass through the crystal at the same speed, and the phase difference remains unchanged.

If the compensator is tilted from the 0 position, the two wave trains pass through the crystal at different speeds. At a certain angle of tilt one wave train will lose its lead over the other, and both wave trains will leave the crystal in phase. Owing to the effect of the analyser the phase coincidence of both rays can be recognized from the extinction position.

Other types of compensator are based on a slightly different principle; however, they all share the principle of birefringence and polarization.

The following LEITZ compensators can be used (Fig. 10)

17184–550 R

a) Brace-Koehler compensator for ORTHOPLAN-Pol and ORTHOLUX 2-Pol

17849–550 R
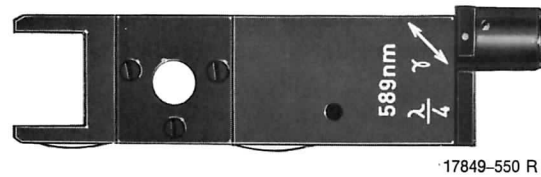
b) Sénarmont compensator (λ/4-plate in sub-parallel position) (for rotating analyser only)

2) The Sénarmont compensator can be used only in connection with a rotating analyser.

Existing ORTHOPLAN-Pol MK stands can be fitted with a rotating analyser at any time unless a Wright eyepiece is preferred. This eyepiece can be mounted on straight tubes only.

3) Versions for up to 30 orders are available.

17183 - 550 R

c) Tilting compensator for ORTHOPLAN-Pol and ORTHOLUX 2-Pol MK/BK

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Measuring procedure

1) Rotate the object to be measured with the auxiliary stage so that both the primary and the ghost image of the object to be measured are not superimposed on the primary and ghost images of neighbouring objects.

2) Bring the surrounding (empty) field to maximum extinction in white light (by rotating the knurled screws on the attachment of the auxiliary stage (= colour of the 0-band). The instrument must be precisely adjusted for this purpose (see page 8).

3) Estimate the phase difference of the sample portion to be measured from the interference colour, and choose a compensator of the appropriate measuring range (with biological objects the phase differences are as a rule smaller than 1λ).

4) Insert the compensator in one of the two compensator slots on the revolving nosepiece. If during the operation of the compensator an intensification of the interference colours (addition position) occurs instead of compensation, insert the compensator in the other slot. (If the compensators according to Sénarmont are used the important directions below must be followed without fail.)

5) Bring the object area to be measured into the extinction (Fig. 9) by means of the compensator and read off the relevant angle. Repeat the measurement several times and calculate the mean value.

6) To improve accuracy repeat the measurements with monochromatic light.

7) Determine the phase difference from the angle obtained with the aid of the compensator tables.

Anomalous interference colours

Substances whose refractive index changes strongly with the wave length (dispersion) usually display divergent interference colours: the black compensation fringe mostly appears broadened.

Such effects can be reinforced or reduced by the dispersion of the embedding medium and the compensator plate. With phase differences not exceeding one order these phenomena are not normally very obvious. Samples of high dispersion should therefore be examined in an embedding medium of similar refractive index, so that only small phase differences are produced. Samples with wedge-shaped edges should be examined near the edge.

Important directions for the use of compensators according to Sénarmont

To avoid faulty measurements, the Sénarmont compensator must be inserted in the correct tube slot according to the table below. The choice of the tube slot depends upon whether the refractive index of the object or that of the embedding medium is higher.

If it is not yet known which is the higher refractive index, an auxiliary compensator ($1/4 \lambda$ -plate, λ -plate, quartz wedge or Berek or tilting compensator) allows the determination of the magnitudes and therefore the choice of the correct tube slot.

The practical use is as follows:

Introduce the auxiliary compensator into the microscope so that its vibration direction γ is oriented SW-NE ⬈. Now the following conditions apply :

a) Microscopes with 12 x 4 mm tube slot

If the phase difference of the sample becomes greater (smaller) the Sénarmont compensator must be introduced into the tube so that the engraved direction γ is north-south (east-west)(cf. Table).

b) Microscopes with 20 x 6 mm tube slot

If the phase difference of the sample becomes greater (smaller) the Sénarmont compensator must be introduced into the tube slot so that its vibration direction is east-west (north-south) (cf. Table).

Table 3

Orientation of the auxiliary compensator, if not	↗ ↗	
Magnitude relationship of the refractive indices: Sample (n_0) to surrounding medium (n_1)	$n_0 > n_1$	$n_0 < n_1$
Change of phase difference with introduction of auxiliary compensator	Addition (increase)	Subtraction (decrease)
Required orientation of the Sénarmont compensator a) microscopes with tube slot 20 x 6 mm (e. g. ORTHOPLAN-Pol) b) microscopes with 12 x 4 mm tube slot DIALUX-Pol, ORTHOLUX-Pol, PANPHOT-Pol *)	←—→ ↗ ↕ ↗	↕ ↗ ←—→ ↗

After the removal of the auxiliary compensator the compensator according to Sénarmont is introduced into the appropriate tube slot. The compensator plate must now be fineadjusted, and the sample measured and evaluated according to the directions accompanying the compensator.

*) The rotating compensator with 1/4 λ -plate (Code No. 553 003/553 034) can be used only in the tube slot leading from the rear left to the front right. Here the compensator plate is oriented by rotation in the 0° and 90° positions respectively.

In position 0° ↗ runs E-W (←—→).

in position 90° N-S (↕).

6. Practical uses

Details about the practical uses of the Jamin/Lebedeff interference attachment can be obtained from publications listed in the enclosed reference section. We are mentioning below once again the most important methods and formulae for quantitative evaluation.

The use of the formulae in the left-hand or right-hand column depends on whether the sample or the reference media have the higher refractive index.

The determination of the higher refractive index can be carried out according to table 3.

$$n_o > n_1 > n_2 \qquad\qquad n_o < n_1 < n_2$$

$$\Gamma_2 > \Gamma_1 \qquad\qquad\qquad \Gamma_2 > \Gamma_1$$

Basic formula

$$\Gamma = d\,(n_o - n_1)\ [nm] \quad (1) \qquad \Gamma = d\,(n_1 - n_o)\ [nm] \quad (1a)$$

6.1 Determination of the refractive index and thickness of isotropic samples

a) Determination of the thickness when the refractive index is known

$$d = \frac{\Gamma_1}{n_o - n_1}\ [nm] \quad (2) \qquad d = \frac{\Gamma_1}{n_1 - n_o}\ [nm] \quad (2a)$$

b) Determination of the refractive index when the thickness is known

$$n_o = n_1 + \frac{\Gamma_1}{d} \quad (3) \qquad n_o = n_1 - \frac{\Gamma_1}{d} \quad (3a)$$

c) Determination of refractive index and thickness

$$d = \frac{\Gamma_1 - \Gamma_2}{n_2 - n_1}\ [nm] \quad (4) \qquad d = \frac{\Gamma_2 - \Gamma_1}{n_2 - n_1}\ [nm] \quad (4a)$$

$$n_o = \frac{n_2 \Gamma_1 - n_1 \Gamma_2}{\Gamma_1 - \Gamma_2} \quad (5)$$

For $n_1 < n_o < n_2$ the following formula applies:

$$d = \frac{\Gamma_1 + \Gamma_2}{n_2 - n_1}\ [nm] \quad (4b) \qquad n_o = \frac{n_1 \Gamma_2 + n_2 \Gamma_1}{\Gamma_1 + \Gamma_2} \quad (5a)$$

In this special case the compensator must be displaced after measurement of Γ_1 by $90°$ into the second tube slot.

Where:

d = thickness of sample $[nm]$

n_o = refractive index of sample

n_1 = refractive index of the first reference medium

n_2 = refractive index of the second reference medium

Γ_1, Γ_2 = phase difference of the sample with the first and second reference medium respectively $[nm]$.

2. The examination of birefringent substances

In birefringent objects (crystals, plastics) the sample ray is divided into two rays polarized perpendicularly to each other. If the sample is rotated so that one of the two vibration directions coincides with that of the sample ray no second ray will be produced. In this position it is possible to measure the associated phase difference in the usual manner. By subsequent rotation of the sample through $90°$ the phase difference of the other ray can be determined.

Procedure

a) Screw ordinary pol-objective into the vacant thread of the revolving nosepiece.

b) turn the beam splitter out of the beam; with objectives of magnification higher than 10x turn the condenser top into the beam

c) turn the sample into the extinction position by rotating the auxiliary stage

d) turn the beam splitter and the corresponding interference objective into the beam (turn out the condenser top)

e) rotate the sample (auxiliary stage) through $45°$ to the left or right. Now one of the two vibration directions will be parallel to that of the sample beam; the corresponding refractive index can be determined in the usual manner by measurement of the phase difference

f) rotate the auxiliary stage through $90°$ to the left or right. The corresponding refractive index can now be determined.

3. Surface investigation

The depth of individual scratches in a plane surface can be determined according to formulae (2) or (4).

Plane-parallel, transparent plates of suitable dimensions can be investigated directly under the microscope. With formula (5) the refractive index can also be determined.

Opaque or unwieldy samples can be examined after a replica of the surface is obtained (Kohaut 1954).

4. Determination of the refractive index of liquids

a) Determination by embedding a birefringent substance

The method was described in detail by BOGUTH and KERN (1963) and is based on the following principle : some ultra-thin wafers (10-50 μm) of a substance of known birefringence are embedded in the liquid to be examined. The phase differences Γ_1 and Γ_2 of a wafer in the two main vibration directions (45° position, see section 2) are now determined. The refractive index of the liquid can then be calculated according to formula (5). Wafers of gypsum are particularly well suited. The two refractive indices are 1.5231 and 1.5319 at 20-25°C and 546 nm wave length.

b) Determination with the aid of a scratch on an object slide

Make a fine scratch on an object slide with a glass cutter or scratch diamond. The depth of a certain point in the scratch as well as the refractive index of the glass can be determined by means of two phase difference measurements (e.g. with water $n_1 = 1.333$, and with air $n_2 = 1.0003$) and evaluation according to formulae (4) and (5).

The liquid to be measured is now transferred to the dry object slide by means of a coverglass and the phase difference measured. The refractive index of the liquid can be calculated according to formula (3) from the previously determined scratch depth and the refractive index of the glass.

Where :

n_o = refractive index of the sample

n_m = refractive index of the embedding medium

n_w = refractive index of water (= 1.334)

$\alpha \left[100 \dfrac{cm^3}{g} \right]$ = specific increment of refraction (0.0016 .... 0.0018)

$\chi \left[\dfrac{cm^3}{g} \right]$ = $100\, \alpha$ (0.16 .... 0.18)

$C \left[\dfrac{g}{cm^3} \right]$ = concentration of the solutes

$T \left[g \right]$ = dry mass of the sample

$d \left[cm \right]$ = sample thickness

$Q \left[cm^2 \right]$ = base area of the sample

$\Gamma \left[cm \right]$ = phase difference between sample and reference medium

$1\ nm = 10^{-3}\ \mu m = 10^{-6}\ mm = 10^{-7}\ cm$

The thickness can de determined according to formula (4).

5. Cytological investigations

$$n_o = n_w + \alpha\, C \tag{6}$$

$$C = \frac{\Gamma}{\alpha\, d} + \frac{(n_m - n_w)}{\alpha} \quad \left[\frac{g}{100\ cm^3} \right] \tag{7}$$

$$T = \frac{Q\, \Gamma}{\chi} + (n_m - n_w) \frac{Q\, d}{\chi} \quad [g] \tag{8}$$

If water ($n_m - n_w \doteq 0$) is used as an embedding medium, formulae (7) and (8) are simplified as follows :

$$C = \frac{\Gamma}{\alpha\, d} \quad \left[\frac{g}{100\ cm^3} \right] \tag{7a}$$

$$T = \frac{Q\, \Gamma}{\chi} \quad [g] \tag{8a}$$

7. Accessories

A. Monochromatic interference filters

1) Interference wedge filter

The interference wedge filter with its diaphragm housing
is inserted in the foot of the microscope in place of the
dust glass. By adjustment of the filter (VERIL B 200 or
VERIL S 200) the wave length of the illumination can be
varied continuously between 400 and 750 nm.

2) Homogeneous filter

Filters of a certain wave length (e.g. 546 nm) can be
supplied on special request. Various types of filter are
available to suit individual half-width requirements. It is
best to place homogeneous filters on the dust glass in the
microscope foot.

B) Light sources

When interference filters are used in conjunction with the
low-voltage lamp built into the foot of the microscope,
the brightness of the microscope image is naturally reduced.
To obtain a brighter image, especially with high magni-
fications and for photomicrography, the use of more power-
ful light sources is recommended.

ATTENTION!

Whereas the light of a low-voltage lamp will not damage
the polarizers even after hours of exposure, unfiltered
light of powerful sources can during prolonged exposure
generate strong heat and destroy them. The use of power-
ful light sources without filter should be limited to short
periods of observation and to photography. If possible, the
low-voltage lamp should always be used for the adjustment
of the instrument and for the location of object details.
The illumination is changed by means of the mirror housing.

1) Lamp Housing 100 and 100 Z

The Lamp Housing 100 accepts the 12v 100 W tungsten
halogen lamp. The Lamp Housing 100 Z also accommod-
ates gas discharge lamps.

On the ORTHOPLAN-Pol the lamp housing is mounted
directly on the stand, and on the other Pol-stands by
means of appropriate adapters.

The microscope can also be placed on an existing
ARISTOPHOT stand. The mirror housing and the lamp
housing are attached to the two columns by means of a
special holder.
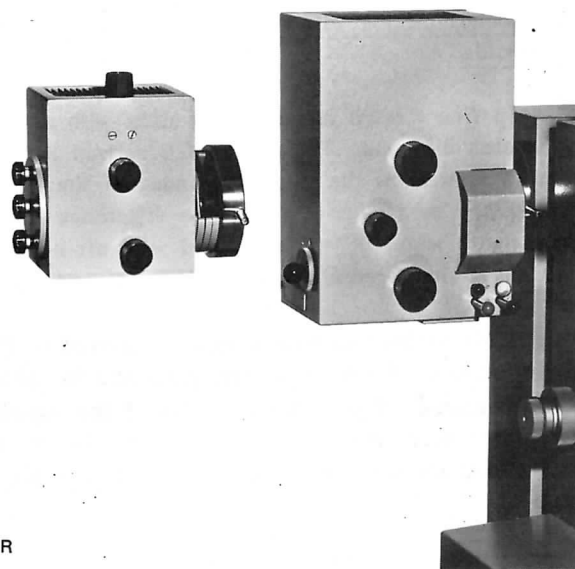
20634–550 R

Fig. 11

Powerful light sources can be used in the Lamp Housing
100 and 100 Z or in the Lamp Housing 250 with the mirror
housing. For the combination ORTHOLUX and LH 100 a
special adapter is available.

2) Lamp Housing 250

The Lamp Housing 250 is suitable for all air-cooled ultra-high pressure lamps up to 250 W (e. g. Xe 150 W, Hg 200 W, CSI 250 W) as well as for a wide range of special lamps (such as deuterium lamps, spectral lamps). It is mounted together with the mirror housing in combination with the appropriate supports and carriers or on the ARISTOPHOT (Fig. 12).
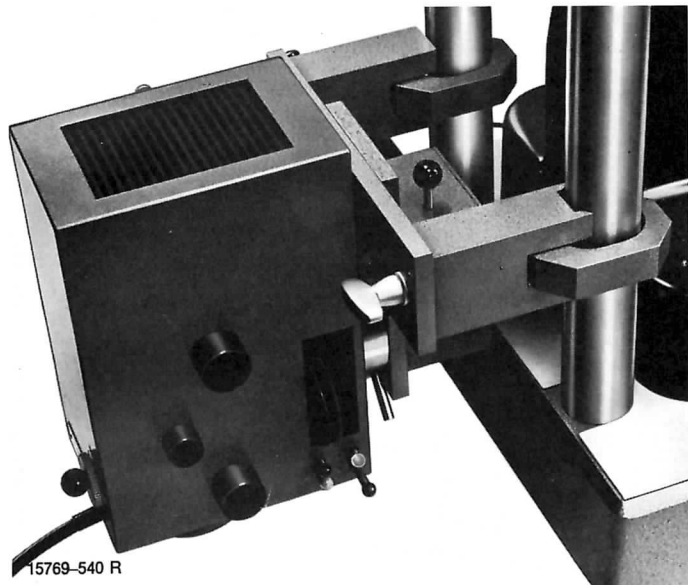

15769–540 R

Fig. 12

Lamp housing holder on the ARISTOPHOT for 100/100 Z and 250 versions (shown here : ORTHOLUX-Pol)

C. Mechanical stage

The Mechanical Stage No. 42 is screwed to the auxiliary microscope stage. The co-ordinates of the samples can be determined by means of the engraved vernier scales.

D. Heating and Cooling Stage 80

The Heating and Cooling Stage 80 can be used with certain restrictions concerning the use of the microscope objectives, for investigations at temperatures other than room temperatures.

Since the crystal beam splitters and lenses have different thermal expansion coefficients, sudden temperature fluctuations should be avoided. The following minimum and maximum temperatures of the heating stage should not be exceeded for the various interference objectives:

16/0.35	-20°	70°C;
40/0.65	-10°	60°C;
Oel 100/1.20	0°	40°C.

A special version of the heating and cooling stage for the interference device according to Jamin/Lebedeff is not available.

Existing standard and pol versions can be used, although they cannot be mounted on the auxiliary stage. To avoid heating the auxiliary stage and the crystal beam splitters it is recommended to insert a thin sheet of cardboard with a light aperture between heating stage and auxiliary stage.

Because of the increased distance between the object and the condenser, however, a sharp image of the field diaphragm cannot be obtained, but this does not appreciably impair image quality and measuring accuracy.

The Heating Stages 350 and 1350 are too deep to be used in combination with the interference device.

E. Photographic attachments

All devices suitable for use with LEITZ microscopes can be used for black-and-white and colour photography. For details please consult the relevant lists.

8. Maintenance of the instrument

As a precision instrument the interference device must be treated with great care. Particularly the following points should be observed :

Protect the instrument from dust and direct sunlight.
Keep the instrument away from sources of heat.
Remove traces of immersion oil immediately after work (use xylene only).
Have any damage repaired by an expert only; if necessary, send the instrument to your nearest LEITZ agency.

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