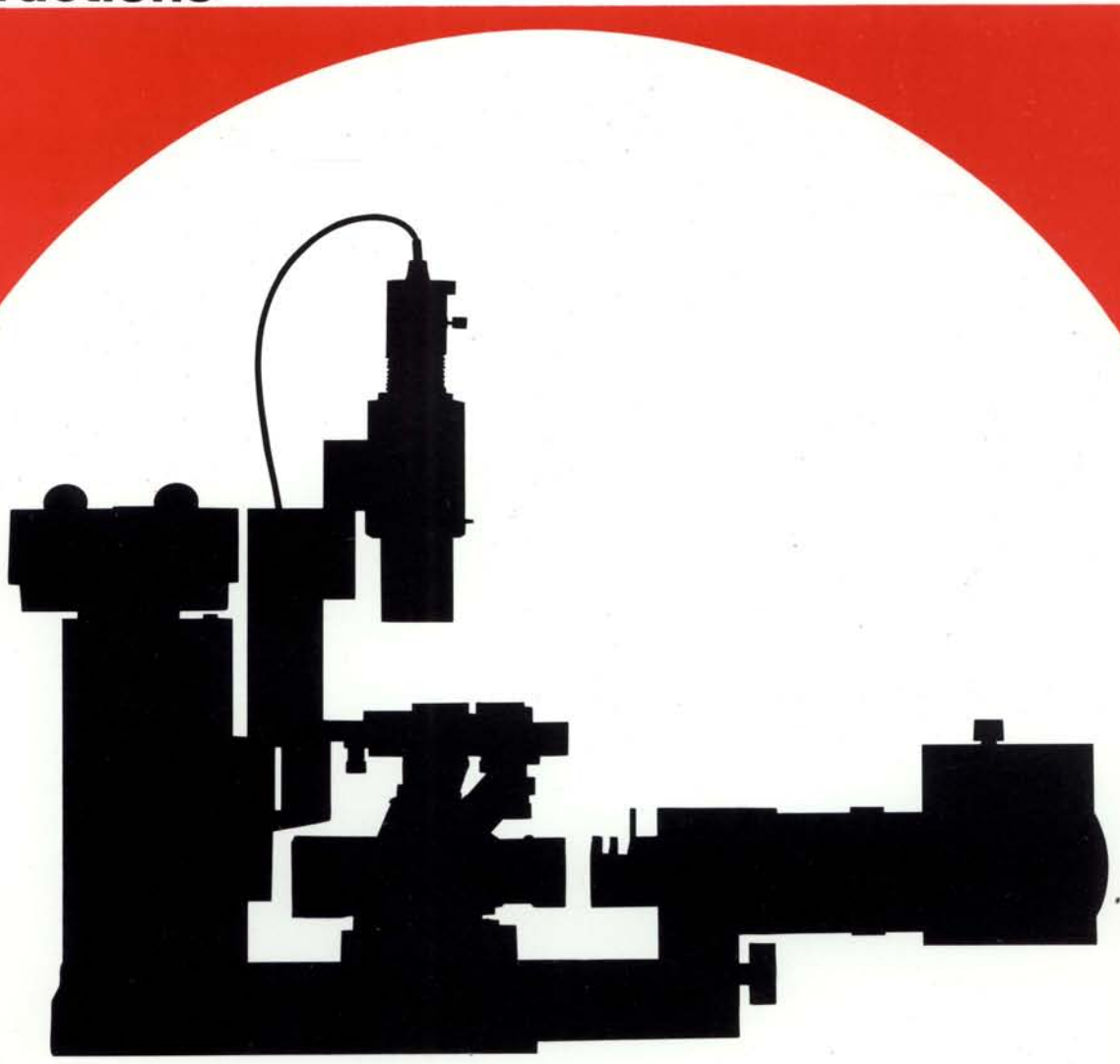
LEITZ Reflections Contrast Device

Instructions



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LEITZ Reflections Contrast Device

Instructions

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1. Introduction

The LEITZ Reflection Contrast Device reveals the most minute phase differences caused by reflections on the interfaces of very thin films. Especially thin cells and their processes as well as fluid layers between cells and coverglass or the bottom of the chamber are suitable objects. The interference fringes observed can basically be interpreted like the contour lines on a topographical map; they join object areas of the same layer thickness.

The interference fringes will become visible only when the disturbing primary reflections of the objectives and the glass surfaces of the specimen are eliminated by the use of the reflection contrast objectives (6.8)*) and the central diaphragms (6.6).

The contact area between the cell and the bottom of the chamber is clearly recognizable as a dark area in the cell in the centre of Fig. 2. Some of the filamentous cell processes lie on the bottom of the chamber (dark), some have no contact (light).

*) (6.8) = Fig. 6, item 8.

Fig. 1 Macrophages in interference contrast

Fig. 2 in reflection contrast

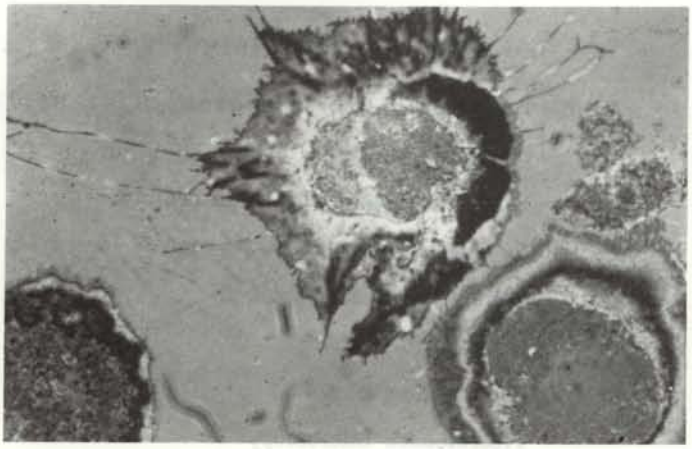
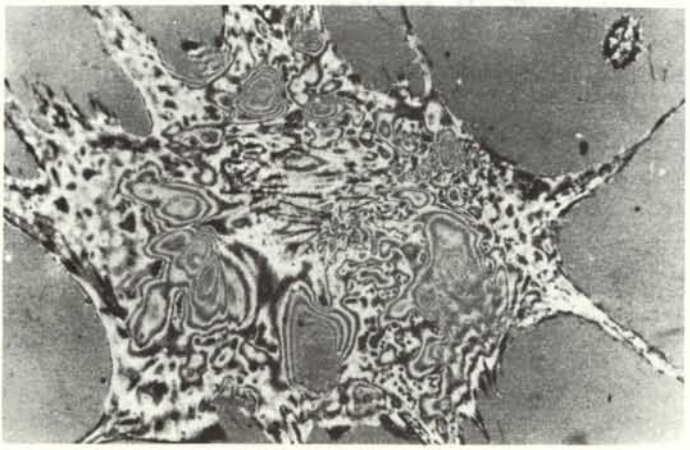


Fig. 3 Human skin fibroblasts in reflection contrast



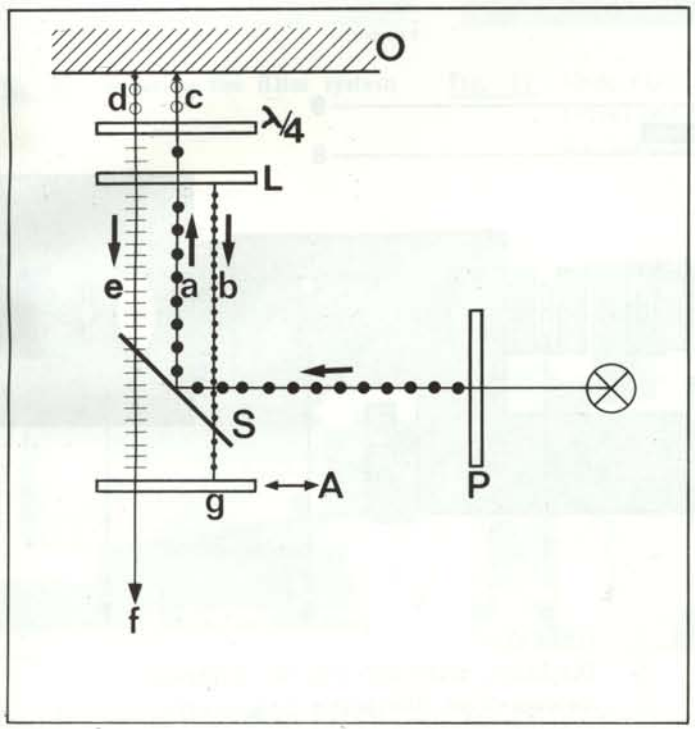


Fig. 4 Increase in contrast according to the immersion contrast method, used in the reflection contrast device

P = Polarizer
A = Analyser
S = Optical flat reflector (beam splitting mirror)
L = Surfaces of the lenses (drawn as a plane-parallel plate in the interest of simplicity)
$\lambda/4$ - plate

Principle :

The straylight portion b reflected by the lens surface L is linearly polarized. It is suppressed by the analyser A with crossed Nicols (g).

The light portion linearly polarized and not reflected by the lenses passes through the front-mounted $\lambda/4$-plate, where it is circularly polarized (c).

The light (d) reflected by the objective 0 is able to pass through the analyser A and produces the contrasty image information (f).

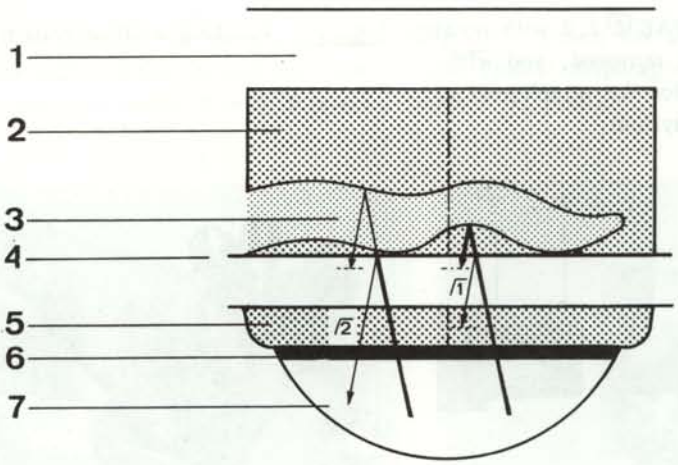


Fig. 5 Production of phase differences in a microchamber

Γ_2 Phase difference between the top and the underside of the cell

Γ_1 Phase difference between the underside of the cell and the bottom of the chamber (coverglass)

1. Top of the chamber
2. Embedding fluid
3. Cell
4. Bottom of the chamber
5. Immersion oil
6. $\lambda/4$-plate
7. Front lens of the objective

Reflections on the interfaces between the objective, the oil, and the underside of the chamber are not shown.

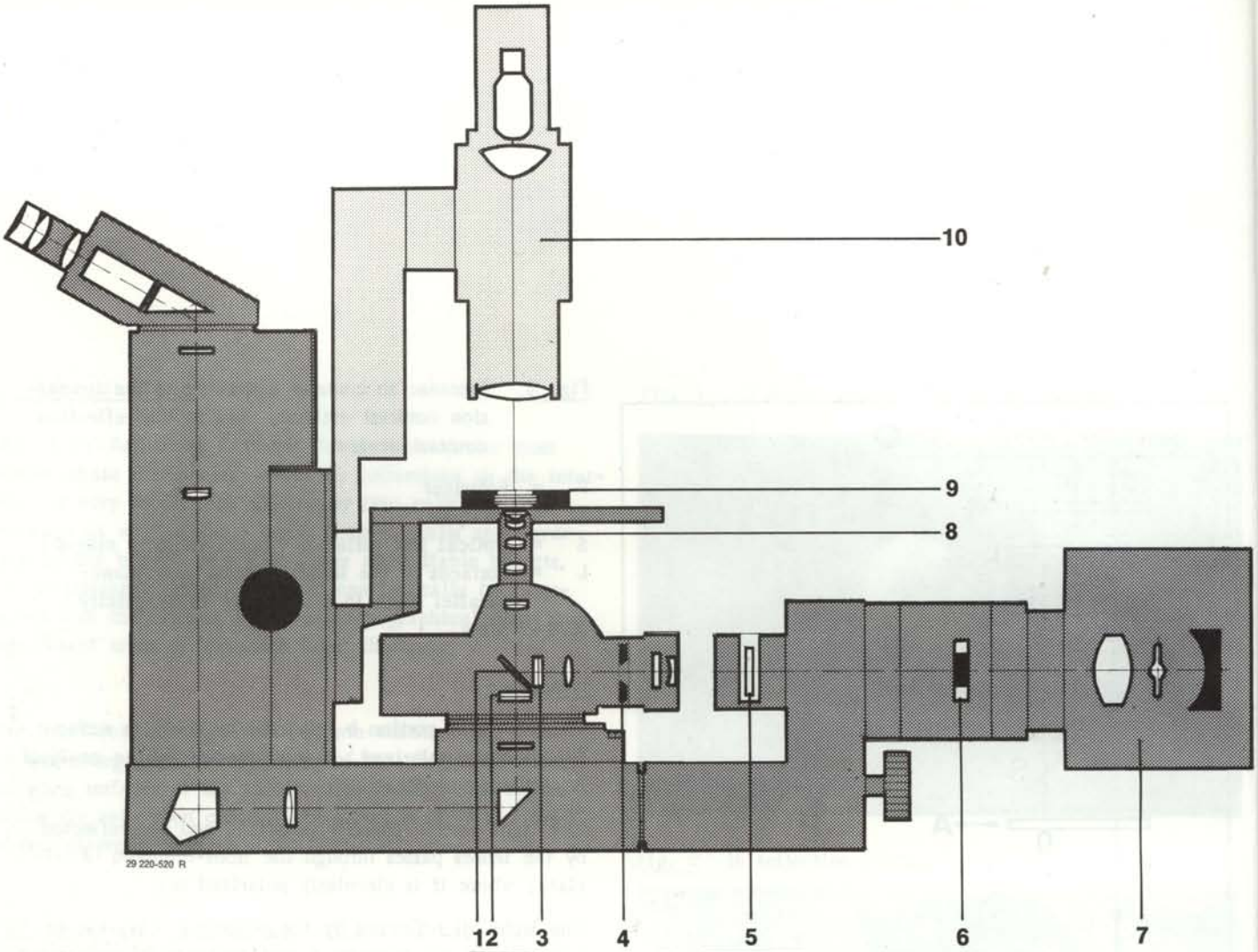


Fig. 6 Diagrammatic representation of the LEITZ Reflection Contrast Device on the DIAVERT ®

1 Optical flat reflector (beam splitting mirror)
2 Analyser
3 Polarizer
4 Field diaphragm
5 Filter slot
6 Diaphragm slide with central diaphragm
7 Incident-light illuminator (Hg lamp)
8 Reflection contrast objectives
9 Object
10 Transmitted-light illuminator

2. Assembling the microscope

Fig. 7 Open the housing cover on the PLOEMOPAK ® 2.2

Fig. 8 PLOEMOPAK ® 2.2 with housing cover (1) removed, and with key (2) for the attachment of a filter system

Fig. 9 Inserting a filter system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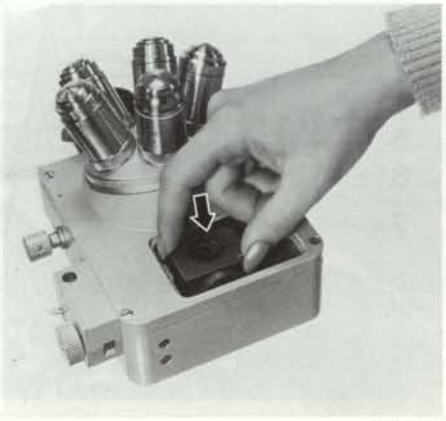
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Fig. 10 Securing the filter system

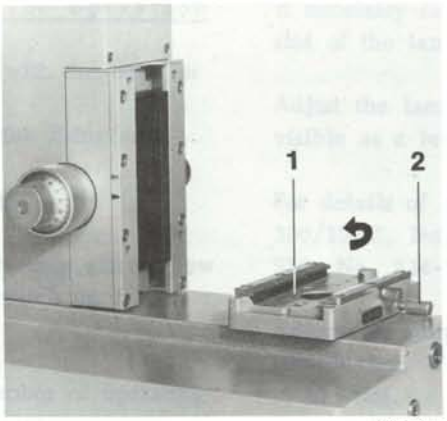


Fig. 11 Undo clamping screw (2), swivel out the fixture for the vertical illuminator (1)

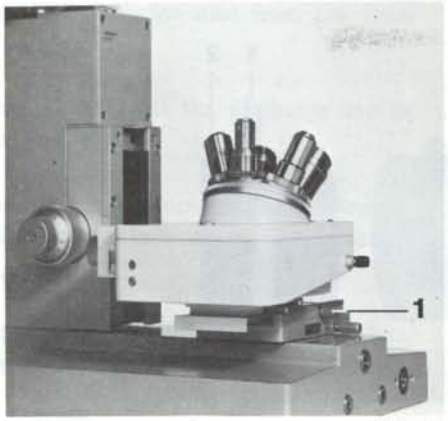


Fig. 12 Insert PLOEMOPAK 2.2 with the reflection contrast objectives screwed in position and secure it with clamping screw (1).

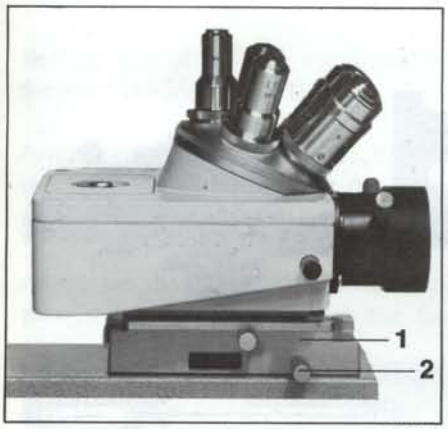


Fig. 13 Swing the fixture for the vertical illuminator (1) in and tighten knurled screw (2)

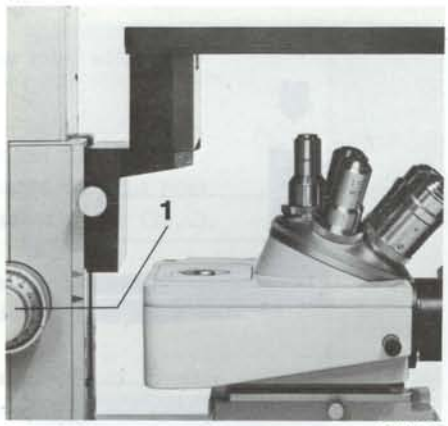


Fig. 14 Lower the stage changer with the coarse adjustment (1) as far as it will go. Insert the object stage.

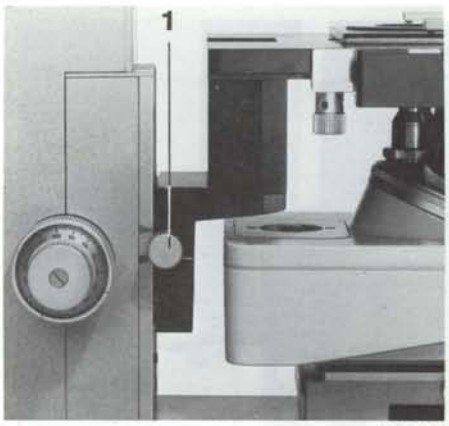
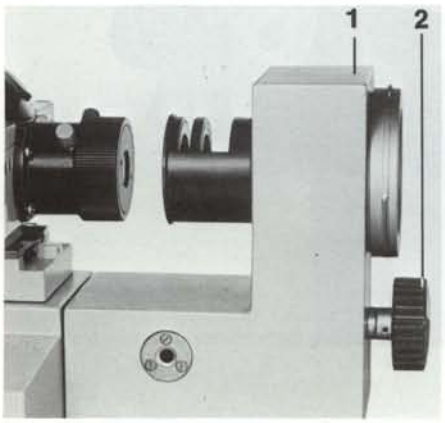


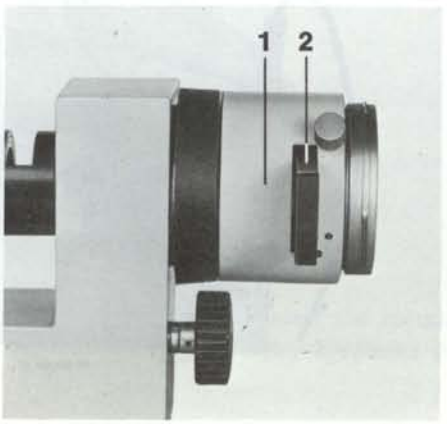
Fig. 15 Lower the object stage until its top or the underside of the specimen is flush with the front lens of the objective. Secure it with knurled screw (1). (Attachment of the object guide see Instructions No. 520-4, P. 7.)

Fig. 16 Attach the lamp carrier (1) to the right of the foot of the microscope and secure it with screw (2).

Fig. 17 Attach the adapter (1) with central diaphragm (2) and lock it in position.

Fig. 18 Insert the lamp according to instructions. Attach the lamp housing and lock it in position. Filter slot (1) for diffusion disc.





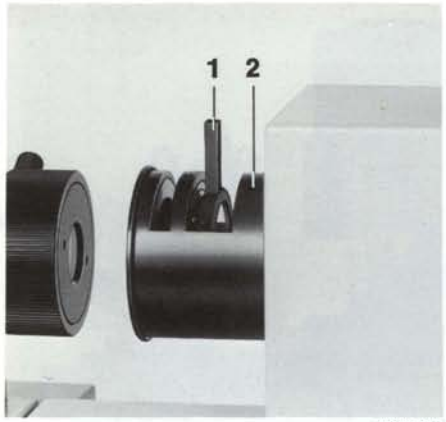


Fig. 19 Inserting a filter (1) in the illuminating tube (2)

Assembling the transmitted-light illuminator

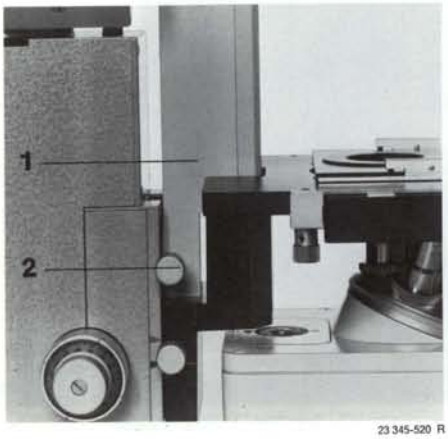


Fig. 20 Insert the illuminator holder (1) and clamp it with clamping screw (2).

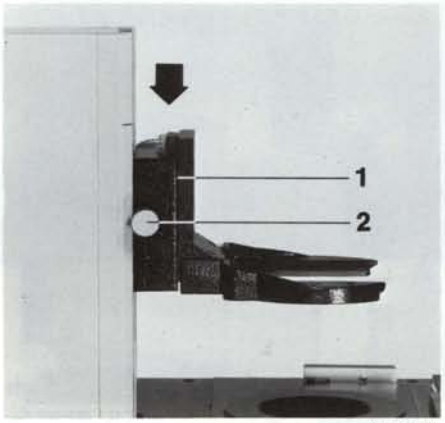


Fig. 21 Insert the condenser fitting (1) and secure it with screw (2)

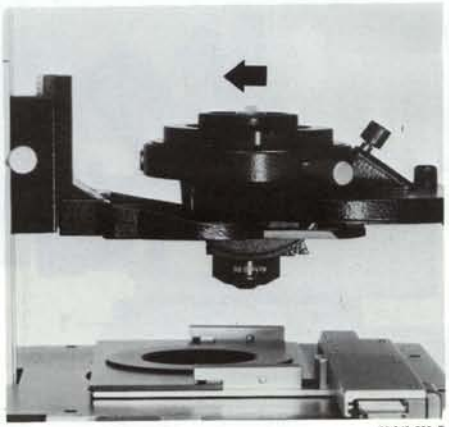


Fig. 22 Inserting the phase contrast condenser

Fig. 23 Inserting the lamp housing fixture, ensure correct position "red and yellow", secure with screw (1)

Fig. 24 Inserting the Lamp Housing 50

3. Preparing the microscope for operation

Compare mains frequency and voltage with the data on the nameplate of the firing unit.
Connect the lamp with the mains via the firing unit.

Switch the lamp on with the firing unit.

Mercury lamps reach their full intensity only after a few minutes' burning. Do not attempt to switch on a hot lamp. Allow it to cool first.

It is useful to keep a record of the number of operating hours (see manufacturer's data sheet).

If the lamp is badly blackened or flickers distinctly, it should be replaced.

Centring the lamp :

Replace one of the two eyepieces in the tube with the focusing telescope (25.1).

Release the knurled screw (25.2) and adjust the top of the focusing telescope until the illuminated circular area appears sharply outlined. Retighten knurled screw (25.2).

Adjust the diaphragm slide (29.3) so that the central diaphragm is not visible through the focusing telescope.

Place the object slide on the object stage without immersion oil. Turn in an oil immersion objective (also without immersion oil) or a dry objective of high magnification.

Turn the POL beam-splitting system into the optical path with knurled knob (28.3). Unblock the light path by pulling the light block (27.2) out. Adjust the object stage until the largest possible luminous area is visible in the focusing telescope.

If necessary remove the diffusion disc from the filter slot of the lamp housing.

Adjust the lamp condenser until the discharge arc is visible as a bright spot.

For details of centring see Instructions Lamp Housing 100/100 Z, No. 514-119 or Instructions Lamp Housing 250, No. 514-72, page 21.

Adjust the lamp condenser until the area is evenly illuminated. If necessary replace the diffusion disc in the slot.

Replace the focusing telescope with the eyepiece, set interpupillary distance. For details see Instructions DIAVERT, page 12.

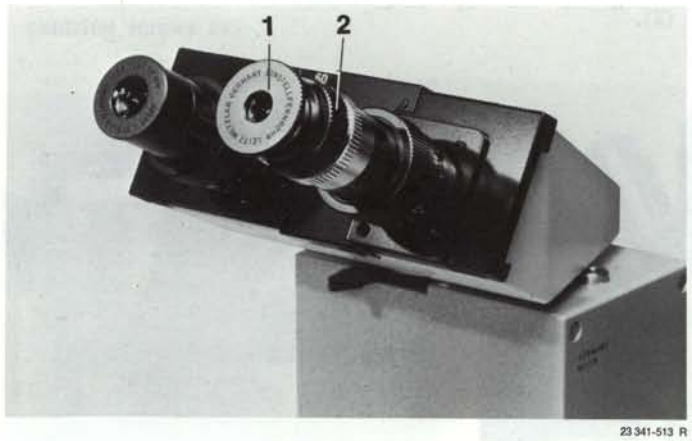


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Fig. 25

Insert the focusing telescope (1) in place of one of the two eyepieces in the tube.

Setting the optimum image contrast

Orientate the objectives (to be carried out only in new instruments or after the reflection contrast objectives have been unscrewed).

Remove the transmitted-light illuminator and object stage from the microscope.

Turn a reflection contrast objective into the beam.

Turn the POL beam-splitting system into the optical path with the control knob (28.3).

Unblock the optical path by pulling out the lever (27.2).

Push the full aperture of the diaphragm slide (29.3) into the illuminating beam.

Open the field diaphragm on the PLOEMOPAK 2.2 .

Again insert the focusing telescope in the eyepiece tube of the tube and focus it on the bright circular area.

Slightly loosen the lateral (smaller) clamping screw (27.1) on the objective with the aid of a watchmaker's screw driver. Only one screw which must be loosened with the Allen key is fitted to NPL FLUOTAR RK objectives. Rotate the objective mount clockwise until a dark, unsharp cross becomes visible in the focusing telescope (Fig. 26). Continue rotating the objective mount clockwise through about 45°, which results in maximum brightness of the circular area visible in the focusing telescope. Tighten (not too firmly) clamping screw (27.1).

Repeat the adjustment for the second reflection contrast objective.

Attach the oil collector (in preparation) to the front mount, which must be carefully cleaned.

Replace the object stage and transmitted-light illuminator.

Apply immersion oil to the front lens of the reflection contrast objective, and if necessary to the underside of the microscope slide.

ATTENTION !

Use only LEITZ immersion oil.
Do not use too much, because surplus oil may enter the spring mount of the objective lens and interfere with the mechanical and optical function of the objective.

Clean the objective immediately after use.

An objective that has become full of oil should be sent to a LEITZ agency as quickly as possible.

Centring the central diaphragms

Replace an eyepiece with the focusing telescope (25.1). If necessary, turn the POL beam-splitting system into the optical path by means of control knob (28.3). Unblock the optical path by pulling out lever (27.2).

Adjust the diaphragm slide (29.3) until the central diaphragm corresponding to the objective used clicks into position.
Focus the specimen; if necessary, temporarily use transmitted-light illumination.

Switch off transmitted-light illuminator.

Observe the central diaphragm through the focusing telescope. Adjust the central diaphragm with the two centring screws (29.4) until the dark patch visible in the focusing telescope is in the centre of the sharply outlined circular bright area.
Remove focusing telescope.
The second central diaphragm need not be adjusted.

Adjusting the field diaphragm

Narrow the field diaphragm with knurled ring (28.2), centre it with the knobs (28.1) and focus it with the knurled knob (28.3).

Open the field diaphragm (28.2) so that the field of view is just fully illuminated.

Fig. 26

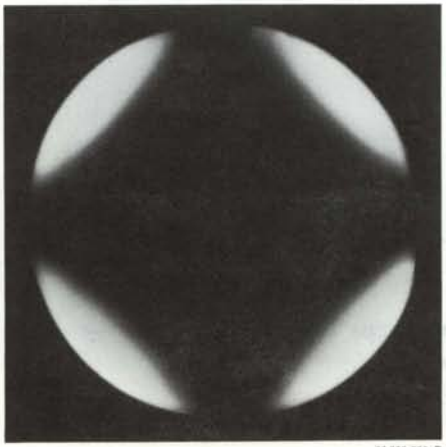

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Fig. 27 Release clamping screw (1) on the objective.
Light block (2).

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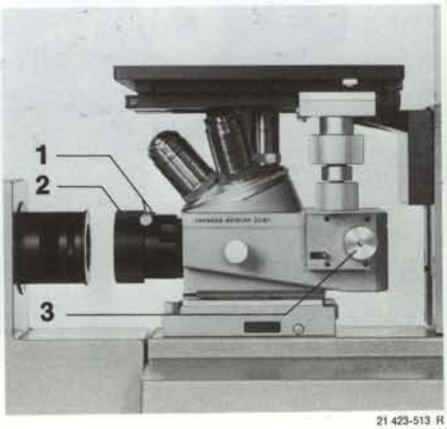
Fig. 28



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Magnification change

When another reflection contrast objective is used, the diaphragm position corresponding to this should be set. As a rule, recentring is not necessary.

A phase ring 2 is built into both reflection contrast objectives. Transmitted-light phase contrast observation is possible through the use of light ring 2 in the lamp fitting. Ensure correct centration of the light ring and vertical adjustment of the lamp fitting.

Adjust, if necessary, the interference contrast device according to special instructions (instead of the POL filter system the insert for normal observation must be turned in). Reflection contrast objectives engraved ∞ cannot be used on the PLOEMOPAK.

Photomicrography

For black-and-white photography the use of a green filter or of an interference filter, especially 546 nm, is recommended. The filters are inserted either in the lamp housing or in the illuminating tube (29.1). It is essential to insert the filter straight.

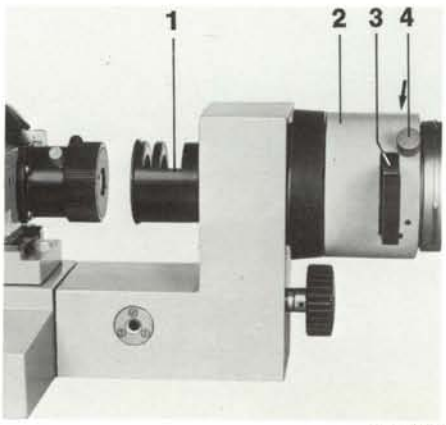
To obtain homogeneous illumination of the object field, the lamp should be centred with particular care (see p. 7). This applies also to the correct adjustment of the lamp condenser. The use of a diffusion disc (18.1) in the lamp housing is essential in photomicrography.

Fluorescence microscopy

A number of special filter systems is available for fluorescence microscopy. They are inserted in the PLOEMOPAK 2 like the POL filter system (9). The central diaphragms are pushed out of the optical path.

Fig. 29

Illumination tube (1), adapter (2), central diaphragm (3), centring screws (4)



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4. Fault tracing

1. Lamp fails to fire

Possible causes :

a) Hot lamps do not fire. Allow a few minutes for cooling.

b) Lamp is used up. This is recognized by a blackening or dimming of the bulb envelope.

c) Fuse or capacitor of the firing unit is defective. Further details see instructions for the power unit supplied.

d) Poor electrical contact in the lamp cable or lamp mount. After disconnecting the mains cable and opening the lamp housing when it is cool, check all the plug-in and screw connections of the electrical supply.

2. No image visible in the microscope

Possible causes :

a) Light block (27.2) on the PLOEMOPAK is closed (pull out the lever).

b) Polarizer and analyser have not been turned into the optical path : turn polarizer and analyser in with the knurled knob.

3. Image quality is unsatisfactory

Possible causes :

a) The object is too thick, so that no visible interferences are produced (if necessary insert interference filter 546 nm in the illuminating beam).

b) Not enough immersion oil has been applied.

c) Wrong central diaphragm (29.3) in the illuminating beam.

d) The refractive indices of objectives, embedding medium, and, where used, coverglass are almost identical, so that extremely low reflection values are the result.

e) The object is viewed through a birefringent medium (culture chamber with plastic bottom). In individual cases rotating the chamber helps, but if possible a glass window of the same thickness as a coverglass (0.17 mm) should be used.

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5. Cleaning

The following points should be observed in the cleaning of optical components :

External surfaces of objectives, eyepieces, condensers

Dust : Remove with a soft, dry sable brush.

Fingermarks : Remove immediately with a damp piece of linen; if necessary use cleaning petrol or methylated spirit

Resistant dirt : Remove with a damp, fine piece of lint or chamois leather. The lens should first be cleaned with a volatile solvent (petrol, chloroform) which is allowed to evaporate.

An additional cleaning method with expanded polystyrene (for instance Styropore) has been found very effective with dirt that is difficult to remove. The granular type of expanded white polystyrene widely use for packaging is particularly well suited. Break a little piece off, push a projecting granule on the fresh fractured surface against the dry lens and rotate it coaxially with it. This removes the most minute residues, for instance of immersion oil or skin grease even from recessed rims of the objective mount, preventing them from spreading across the lens surface and partly counteracting the anti-reflection effect of the coating. Detached granules of polystyrene can be removed by blowing them away or with an absolutely clean sable brush reserved exclusively for this purpose.

A wooden stick wrapped in a piece of cotton wool can also be used for cleaning.

Oil immersion objectives

Clean immediately after use : Remove oil with blotting paper or a piece of lint. Remove residual oil film with a piece of lint soaked in petrol.

6. References

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