INSTRUCTIONS

LEITZ
phase contrast equipment

after Zernike

Its use with our ORTHOPLAN®, ORTHOLUX®, LABORLUX®, and SM microscopes. General knowledge of microscopy is assumed. For further details we recommend our booklet „The Microscope and Its Application".

Leitz
WETZLAR

513-84/Engl.

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INSTRUCTIONS

This equipment allows examinations in

Brightfield
phase contrast,
and darkfield.

Since each objective is correlated with a certain setting of the annular-stop turret the illumination will always be exactly adjusted. The conditions are easy to control and reproducible at any time. The object is seen either in brightfield, phase contrast or darkfield. Furthermore, the full aperture of the condenser may be utilized in brightfield. It is therefore possible to make full use of the resolving power of the objectives without appreciable effect of the phase-ring during comparative examinations. Darkfield is obtained by introducing a central stop into the illuminating beam.

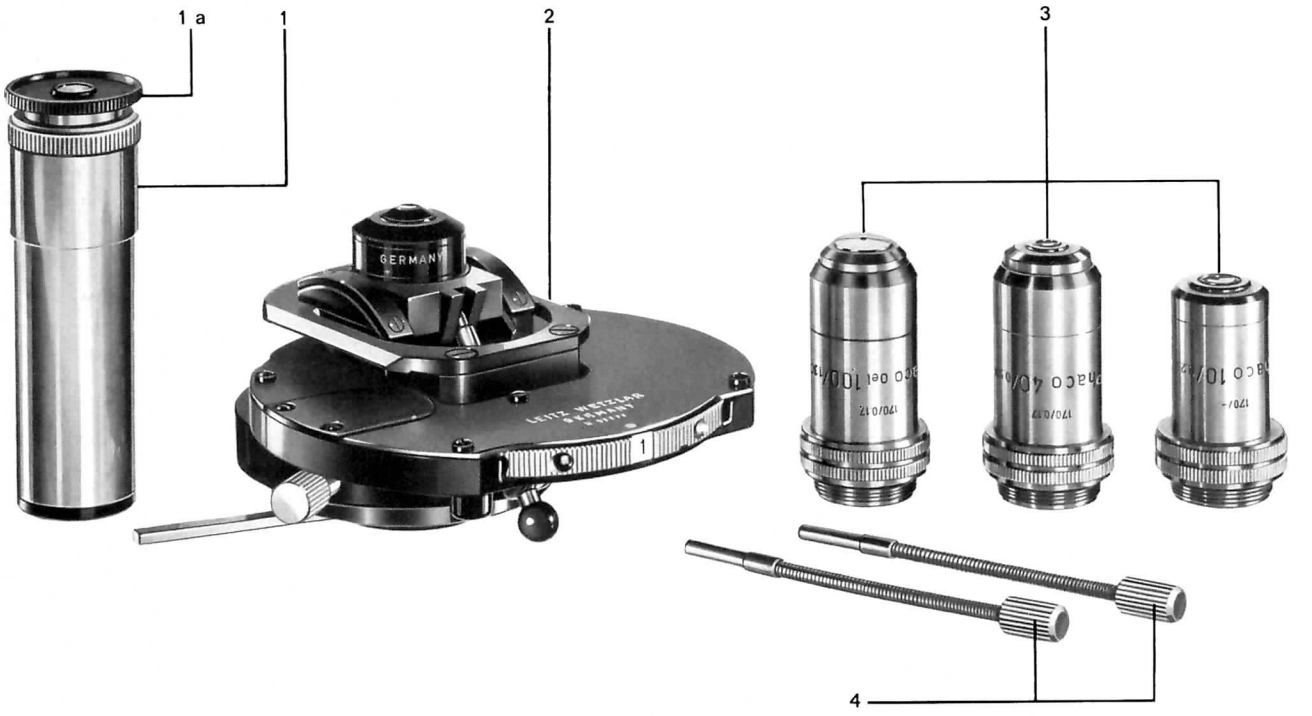




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Fig. 1
Complete phase contrast equipment
1 Auxiliary microscope (focusing magnifier) with
1a Knurled ring for focusing on the phase ring
2 Phase contrast condenser
3 Phase contrast objectives
4 Centring keys for the annular stops of the condenser



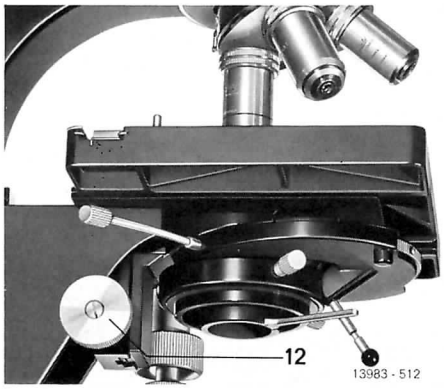


Fig. 3
Phase contrast condenser on the LABORLUX with centring keys in position
12 knob for raising the condenser

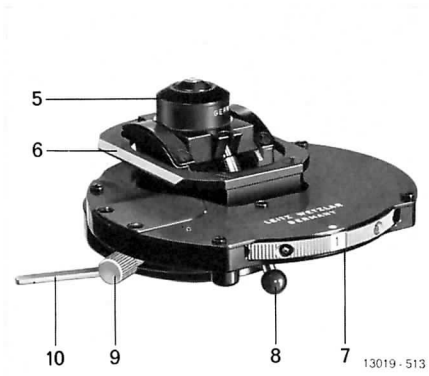


Fig. 4
Phase contrast condenser
5 Swing-out condenser top
6 Dovetail mount
7 Annular-stop turret in position 1
8 Knob for swinging out the condenser top
9 Centring screws (one of which is visible) for the condenser
10 Aperture diaphragm lever



Fig. 5
Rear view of the condenser
11 One of the two sockets for inserting the centring keys 4

Operation

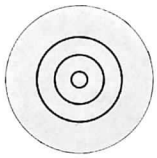
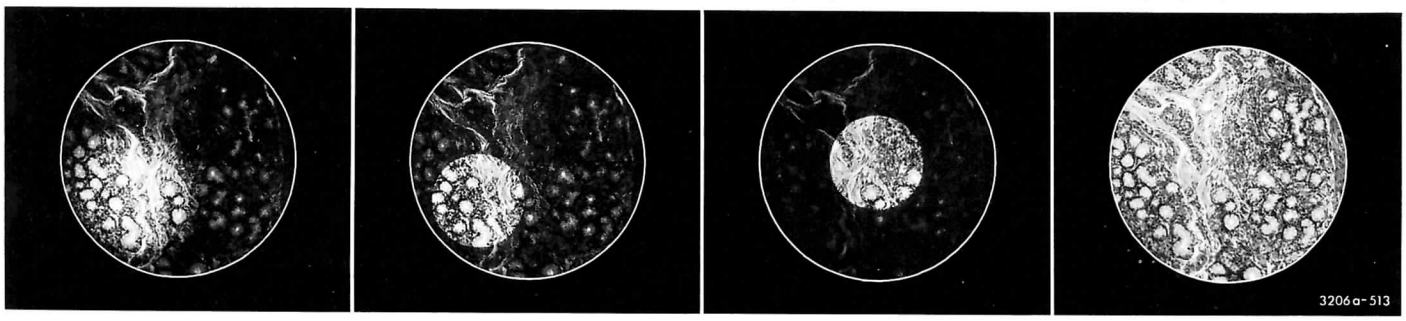


Fig. 7
Groundglass disc for centring the light source, and the condenser of the SM.



Fig. 8
Field diaphragm for installation in an existing LABORLUX. Here the additional lens must be pushed into the condenser from the bottom left (see footnote p. 7).

Fig. 6
Centring the field diaphragm, from left to right
a field diaphragm out of focus b field diaphragm focused c field diaphragm centred d field diaphragm opened



2) The various types of illumination and their relation to the setting of the annular-stop turret

1. "Brightfield (H)" position of the annular-stop turret

Here the condenser functions as a brightfield condenser, and the built-in iris diaphragm as an aperture diaphragm.

2. "Phase contrast" (1, 2, 3) position of the annular-stop turret

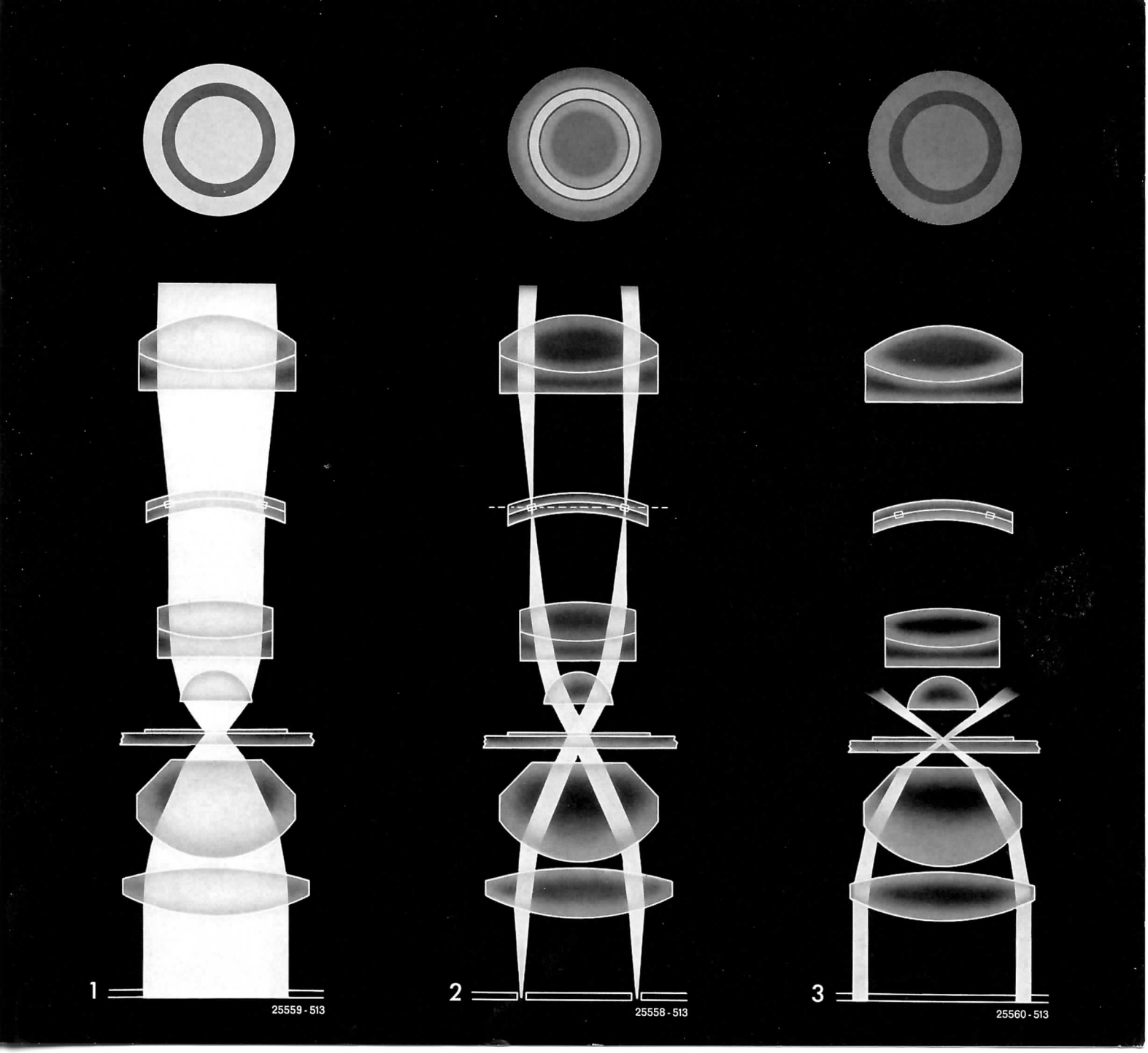
In the positions 1, 2, 3 the light beam emitted by the source is reduced to a ring of light. The diameters of the three annular stops are matched with the diameters of the various phase rings built into the Phaco objectives. The illumination is centred and controlled by the auxiliary magnifier in the rear focal plane of the objectives.

3. "Darkfield" (4) position of the annular-stop turret

In position 4 a darkfield central stop is an integral part of the annular-stop turret; it blocks the entire central illuminating beam. The object is lit exactly as with a darkfield condenser.
For darkfield observation the aperture of the illuminating rays must be larger than that of the objective, so that no illuminating light can enter the objective, which receives light refracted by the object only; as a result, the structure of the object is seen brightly illuminated against a dark background.
In position 4, the effective aperture of condenser 402b is 0.80. Thus, all objectives of apertures smaller than 0.80 can be used for darkroom illumination. In addition to the Phaco objectives which form part of the phase contrast equipment we recommend for darkfield observation:—
Objective achr. 63/0.85 with built-in iris diaphragm
objective achr. 63/0.85 with funnel stop 513069
objective Fl Öl 54/0.95 with funnel stop 513069
Because of their different adjustment lengths these objectives, when used on the same nosepiece as Phaco objectives, must be combined with the adapter ring 519164. This increases the objective magnification by the factor 1.05 (= 5%).

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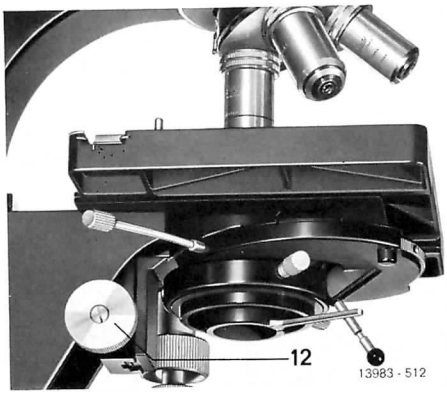


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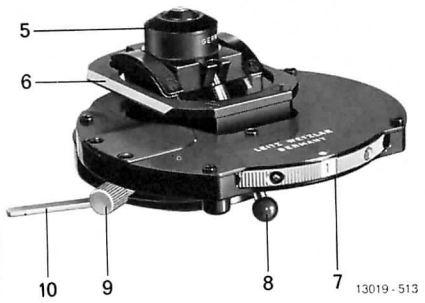


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Rear view of the condenser
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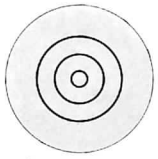
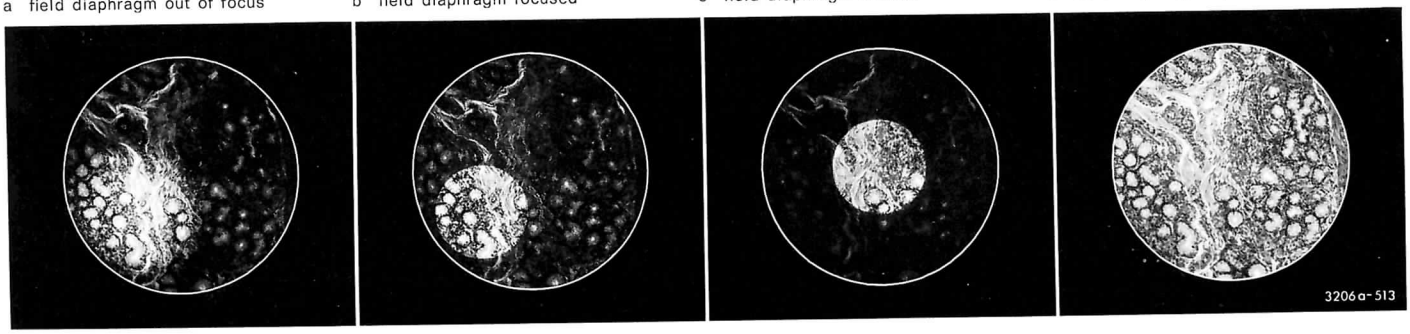


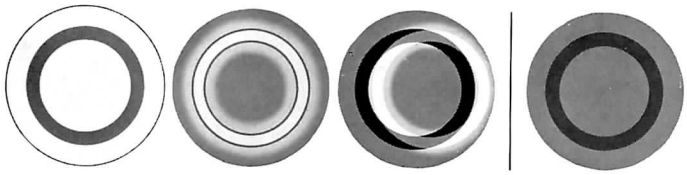
Fig. 7
Groundglass disc for centring the light source, and the condenser of the SM.



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Field diaphragm for installation in an existing LABORLUX. Here the additional lens must be pushed into the condenser from the bottom left (see footnote p. 7).

Fig. 6
Centring the field diaphragm, from left to right
a field diaphragm out of focus b field diaphragm focused c field diaphragm centred d field diaphragm opened





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Fig. 9
Phase ring and light ring as seen in the microscope
From left to right:
9a in brightfield
9b in phase contrast, centred
9c in phase contrast, out of centre
9d in darkfield

3.1 Phase contrast in the ORTHOPLAN, ORTHOLUX or LABORLUX with field diaphragm

Insert the condenser in the dovetail guide of the stand and raise it to its topmost position by means of the rack-and-pinion drive **12**.
Turn in condenser top **5**.
Screw in Phaco objectives.**
Set the annular-stop turret **7** at position 1.
Turn in objective Phaco 10/0.25.
Open aperture diaphragm **10** fully.
Place object on the stage and focus it.
Close field diaphragm fully.
Focus field diaphragm by means of knob **12**.
Centre the field diaphragm with the two knurled screws **9.**
Open field diaphragm so that it is just visible at the margin of the field of view. If necessary, repeat centring.
Insert the auxiliary magnifier 1 in one of the eyepiece tubes, and focus its eyelens on the dark phase ring by means of the knurled ring 1a (Fig. 9a).
The bright light ring of the condenser must now be coincident with the dark phase ring (Fig. 9b). Since the annular stops have already been centred in the factory, recentring is not normally required. Should the rings be out of centre (Fig. 9c), this must be remedied by means of the centring keys 4 which must be inserted in the two rear sockets **11** of the condenser. Make the light ring exactly coincident with the phase ring by turning the centring keys.

* If the Zernike condenser is used on a LABORLUX with a subsequently fitted field diaphragm, an additional lens must be inserted in the bottom opening of the condenser (see Fig. 8). The lens should point upwards during insertion.

** With the ORTHOPLAN microscope a dove-tail slide nosepiece 1.25x has to be used.

Take out centring keys, and check centration with the two other objectives, by setting the annular-stop turret at positions 2 and 3 and turning in objective 40/0.65 and Öl 100/1.30 respectively.
When oil immersion is used, it must be confined to the objective and coverglass. The condenser must not be immersed, i.e. no oil must be introduced between condenser top and the underside of the microscope slide. Re-centre if necessary. Further control of the ring images is not necessary; once adjusted, their centration is maintained. A reduction of image quality will be noticed only with extremely wedge-shaped specimens (wedge error 50 µ per cm object length) with the objective Phaco 10/0.25. In this case the rings must be re-centred. Even this large wedge error remains without effect on image quality with the objectives 40/0.65 and 100/1.30. However, the average wedge error of biological specimens does not exceed 25 µ per cm object length so that the original centration will be sufficient.
Remove the auxiliary magnifier, insert the eyepiece.
The microscope can now be used in the usual manner. After each change of magnification open the field diaphragm so that it just exposes the whole field of view.

3.2 Phase contrast in the SM microscope

Insert the condenser in the changing slide and raise it to its topmost position by means of the rack-and-pinion drive 12.
Screw the phase contrast objectives into the nosepiece.
Turn in objective Phaco 10/0.25.
Set annular-stop turret at position H (brightfield).
As the stand has no field diaphragm, the centring groundglass disc Fig. 7 must be used for centring the condenser:—
Place the centring groundglass disc on the 6v 15W low-voltage lamp.
Close aperture diaphragm 10.
Focus the objective 10/0.25 on the centring groundglass screen.
The concentric rings in the groundglass screen are now visible in the microscope. Centre the condenser with the two knurled screws 9; the centre of the groundglass screen must be precisely in the centre of the field of view.
Remove the centring groundglass screen.
Place the specimen in position, and focus.
Open the aperture diaphragm fully.

Take out an eyepiece and replace by the auxiliary magnifier. Focus auxiliary magnifier on the dark phase ring by means of 1a. Annnular-stop turret at position 1. The bright light ring of the condenser and the dark phase ring must now be coincident (Fig. 9b). Since the light rings have already been centred in the factory, normally recentring is not necessary. Should the rings be out of centre (Fig. 9b), this must be remedied by means of the centring keys 4, which must be inserted in the two rear sockets 11 of the condenser. Make the light ring exactly coincident with the phase ring by turning the centring keys.

Take out centring keys, and check centration with the two other objectives by setting the annular-stop turret at positions 2 and 3 and turning in objective 40/0.65 and Öl 100/1.30 respectively.

When oil immersion is used, it must be confined to the objective and coverglass. The condenser must not be immersed, i.e. no oil must be introduced between condenser top and the underside of the microscope slide. Re-centre if necessary.

Further control of the ring images is not necessary; once adjusted, their centration is maintained.

Concerning extremely wedge-shaped preparations our remarks on the ORTHOPLAN, ORTHOLUX, and LABORLUX microscopes apply.

Replace the auxiliary microscope with an eyepiece.
Check the focusing of the object.

3.3 Use of the phase contrast equipment in darkfield

3.31 ORTHOPLAN, ORTHOLUX and LABORLUX* with field diaphragm

For darkfield the following objectives can be used on these stands:

Phaco 10/0.25
Phaco 40/0.65
Fl Öl 54/0.95 with funnel stop 513 069
Achr. 63/0.85 with funnel stop 513 069
Achr. 63/0.85 with built-in iris diaphragm

+ adapter ring 519 164 for use on the same nosepiece as Phaco-objectives. See also page 4, last para.

* In the LABORLUX accurate centration of the illumination must be ensured.

Turn in objective Phaco 10/0.25
Condenser top turned in, condenser at position H.
Open aperture diaphragm 2/3.
Focus the object.
Close the field diaphragm until it becomes visible in the field of view of the microscope.
Centre the field diaphragm with the two knurled screws 9.
Condenser at position 4 (darkfield).
Open aperture diaphragm fully.
Place the groundglass disc on the dust glass.
If necessary refocus the object, and readjust the illumination.
Slightly lower the condenser by means of the vertical adjustment 12, until a dark spot appears in the field of view (the spot increases progressively as the condenser is lowered further).
Centre the dark spot by means of the centring keys 4.
Raise condenser by means of 12 until the darkfield spreads uniformly over the field of view.
The condenser is now centred also for the higher-power objectives.

3.32 SM Microscope

The objectives listed under 3.31 can be used.
Turn in objective Phaco 10/0.25.
Turn in condenser top: condenser at position H.
Open aperture diaphragm to approximately 2/3.
Focus the object.
As the stand has no field diaphragm the groundglass disc supplied must be used for centring the condenser as described on p. 7, Phase contrast with SM.
Open aperture diaphragm fully.
Condenser at position 4 (darkfield).
If necessary readjust the illumination.
Slightly lower the condenser by means of the vertical adjustment, until a dark spot appears in the field of view (progressively increasing as the condenser is lowered further).
Centre the dark spot by means of centring key 4.
Raise the condenser until the darkfield spreads uniformly over the entire field of view of the microscope.
The condenser is now centred also for the higher-power objectives.



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