Microscopic contrast enhancement methods Transillumination

Operating Instructions

G 41 - 211 / I - e

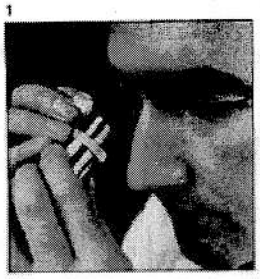
Adjustment for phase contrast

1. Adjust to brightfield illumination with phase contrast condenser – knurled disk in position "J" – and low-power phase contrast objective according to sections 1. through 7. of operating instructions for microscope transillumination.

2. Set knurled disk of phase contrast condenser to the proper position for the objective.

Example: position **2** for NEOFLUAR 25, Ph **2.**

1



3. Replace the eyepiece by a centering telescope and turn in its eyelens – or,

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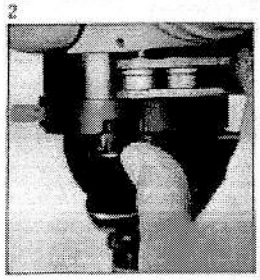
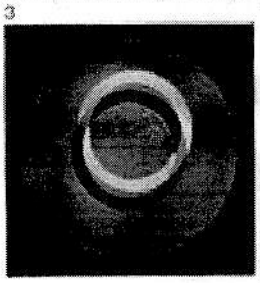


Set the OPTOVAR magnification changer to PH position and focus with the other disk.

3

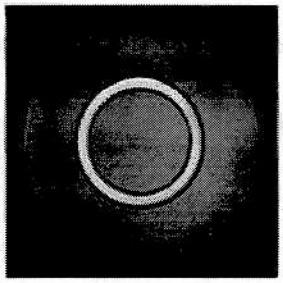


Both a bright and a dark ring must be in focus.

4

4. With the lockable lever and the knob of the phase contrast condenser move the bright ring until it lies exactly within the dark one.

5



5. Replace the centering telescope again by the eyepiece or set the OPTOVAR again to observation (e. g. "1.25"). The phase contrast image can be viewed through the tube.

6. When exchanging the objective, always check the adjustments under 3. and 4. and correct them, if necessary.

Specimen preparation for phase contrast work
For satisfactory results the microscopic specimens must be adapted to the optical conditions prevailing in phase contrast. Because of the high sensitivity of the method the specimens must be most carefully prepared. Use only optically adequate (schlieren and bubble free) specimen slides and coverglasses. Carefully remove any residual detergent solvents from the slides and particularly from the coverglasses. The boundary surfaces of the specimens must be plane. Specimens in suspending drops or even on concave slides cannot be examined. Instead of such preparations we recommend the oil chamber. The required plastic rings are supplied with the mounting media (46 29 29). This chamber has supplied satisfactory results in observing live specimens.

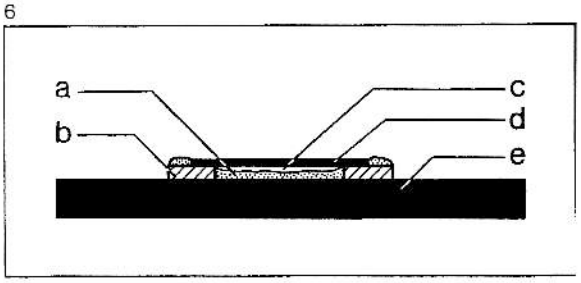
Phase contrast examinations of transparent solid bodies
For phase contrast observation crystals, plastics, fibers or minute inclusions therein should be available as discrete items, e. g. as inclusions in basic matter. The preparations are polished sections or thin sections. Discrete particles are observed in embedding fluid as spread preparations.

For details about preparation techniques and their application in phase contrast microscopy of fine-grained material see:

Correns-Piller, Handbuch der Mikroskopie in der Technik, edited by Dr. H. Freund, Umschau-Verlag Frankfurt a. M., 1953, Vol. IV, Part 1.

Oil chamber
a = paraffin oil
b = plastic ring, 0.5 to 1 mm thick
c = culture medium
d = coverglass
e = specimen slide

6



Differential interference contrast (DIC)

Rp 18 in PM III manual

With this method changes in the optical path length (product of mechanical length and refractive index) are made visible as relief. The contrast also depends on the orientation of the observed structure within the field of view (azimuth effect). The specimen should therefore be rotated around the microscope axis, possibly on a rotary microscope stage.

Pre-conditions for operation
If the DIC system is purchased together with the microscope, the DIC adapter rings (6) are rigidly screwed into objective turret or single objective changer and aligned. The orientation of the slot for the DIC prism slides (5) corresponds to that of the analyzer slot in the intermediate tube (2) or microscope tube head.

If a microscope is subsequently equipped with the DIC system, the adjustment described on page 12 must be made.

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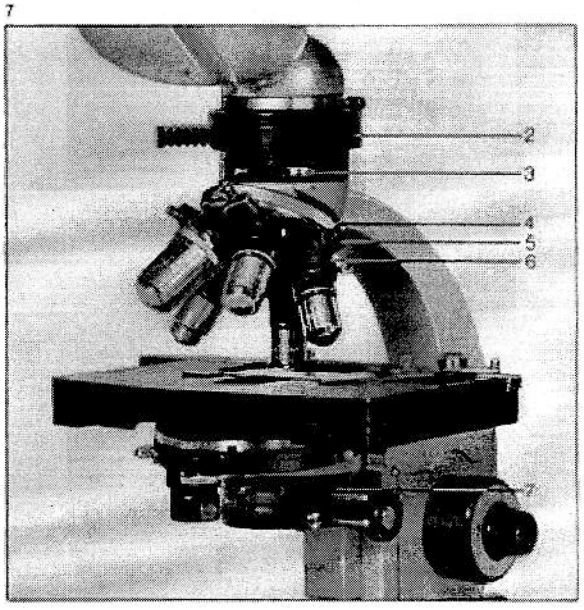
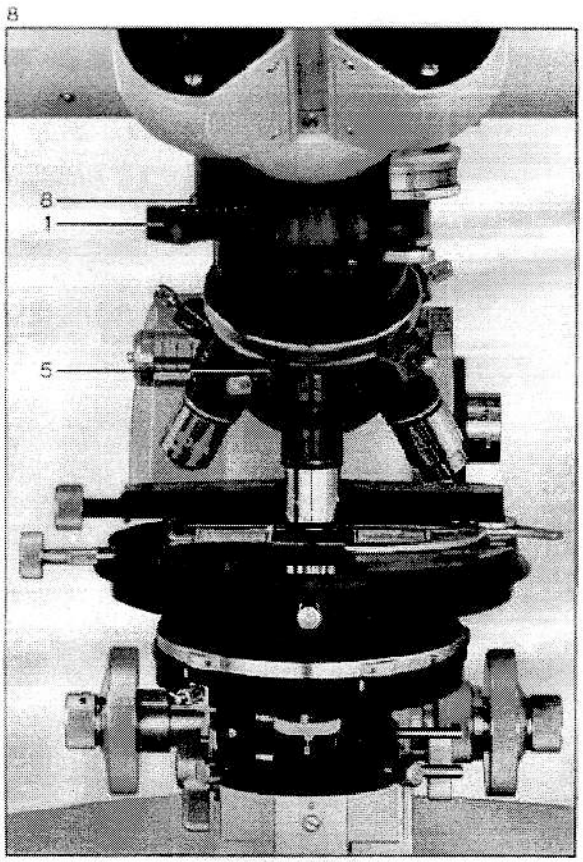


Swing in the polarizer on carrier (47 08 65) (7) and set it to 0 – the readout is on the knob – or the fixed polarizer on carrier (47 08 66). The oscillation direction of the polarizer is East-West.

Analyzer (1)
On STANDARD microscopes insert it in the intermediate tube (47 30 59) (2), on ULTRAPHOT, Photomicroscope and UNIVERSAL in the tube head. Screw in stop screw (8).

Pull out the analyzer as far as it will go.

Turn the turret of the DIC condenser into the proper position for the objective used, either I or II.



Achromatic-aplanatic
DIC phase contrast brightfield condenser
aperture 1.4 (46 52 85)

Condenser positions

Position I DIC prism for Planachromat 6.3/0.16 (incomplete illumination of field of view) Planachromat 16/0.35	Position II DIC prism for LD-Planachromat 40/0.60 corr. Planachromat 40/0.65 Planapochromat 63/1.40 oil Planachromat 100/1.25 oil (all apertures can be fully illuminated)

Position J for brightfield only
Position 2 or 3 for phase contrast with objectives designated Ph 2 or Ph 3.
Position III for special prisms.

Achromatic aplanatic
DIC phase contrast brightfield condenser IV Z/7
aperture 0.63 (46 52 73)
Long focal intercept (distance between front lens and specimen):
7 mm in air, 11 mm in glass, to illuminate thick specimens or specimens in culture vessels.

Condenser positions

Position I DIC prism for Planachromat 6.3/0.16 Planachromat 16/0.35 (all fields of view can be fully illuminated)	Position II DIC prism for LD-Planachromat 40/0.60 corr. Planachromat 40/0.65 Planapochromat 63/1.40 oil Planachromat 100/1.25 oil (apertures up to max. 0.63 can be illuminated)

Position J for brightfield only
Position 2 or 3 for phase contrast with objectives designated Ph 2 or Ph 3.
Position III for special prisms.

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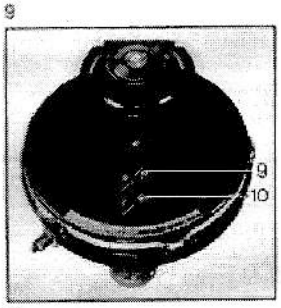

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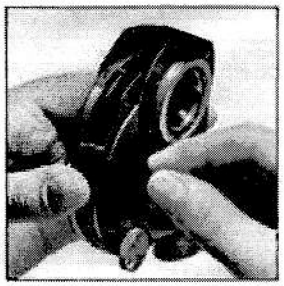

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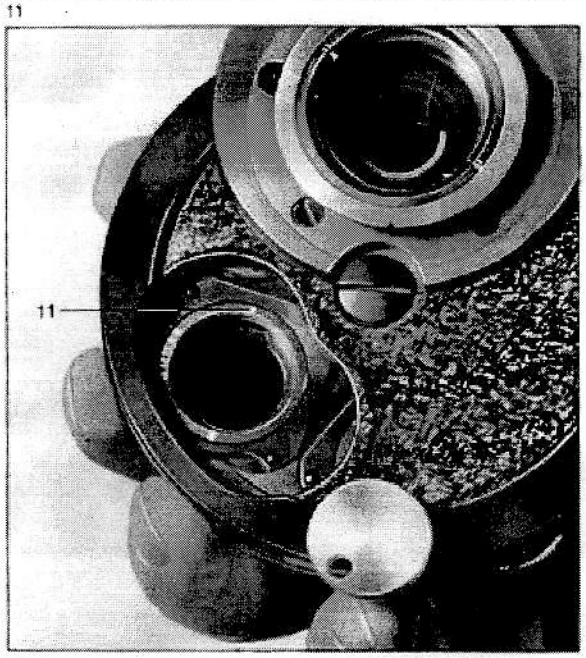

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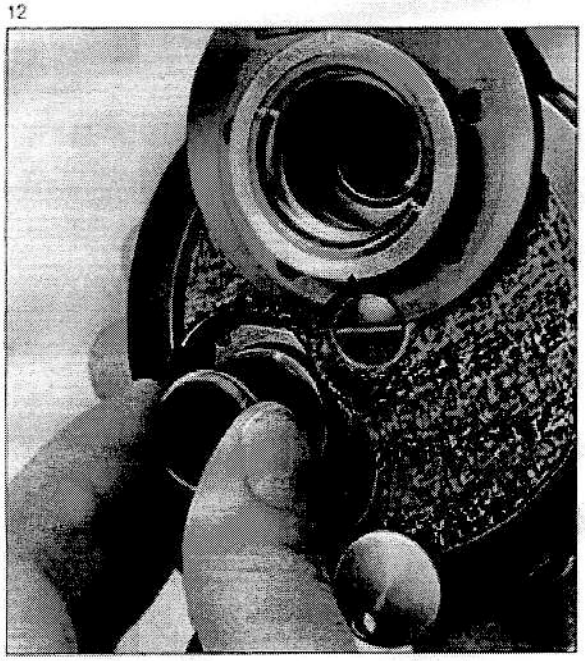

The symbols (9) and (10) on the turret are for adjustment of the prisms in the turret. Take the cover plate off the condenser bottom (Fig. 10) and turn the turret until the prisms are visible (Fig. 11). On the edge of the prism mount there is a white dot (11) and directly opposite a white line, each with a notch underneath; a pin engages this notch. The prism can be removed with a threaded ring (supplied with the condenser) which is screwed into the prism mount (Fig. 12). The notches in the prism mount and the pin in the turret (12) become visible. The symbols (9) and (10) refer to the notches and ensure correct alignment of the prism on STANDARD microscopes and microscopes with tube head (ULTRAPHOT, Photomicroscope, UNIVERSAL): on STANDARD microscopes the pin must engage the notch marked with a dot (•), on microscopes with tube head that marked with a stroke (/). (Fig. 11 shows the correct alignment on STANDARD microscopes).

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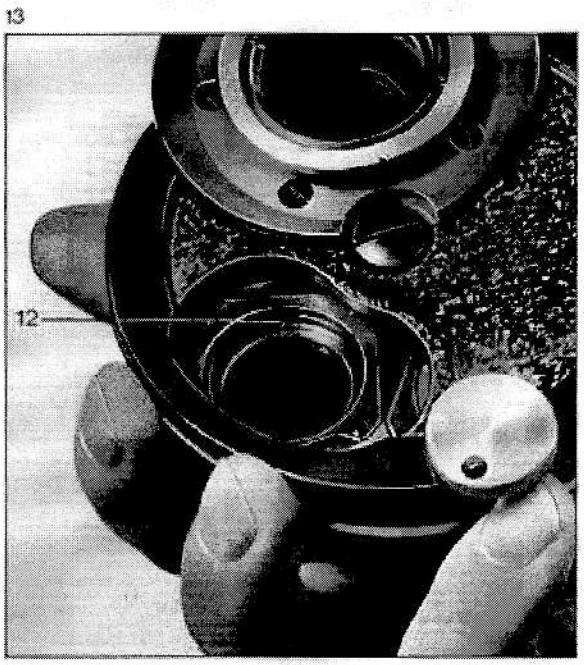

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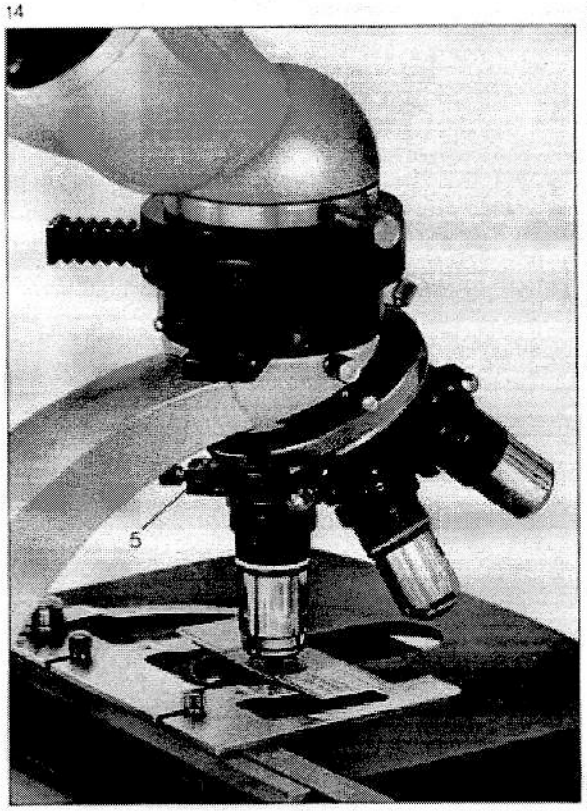

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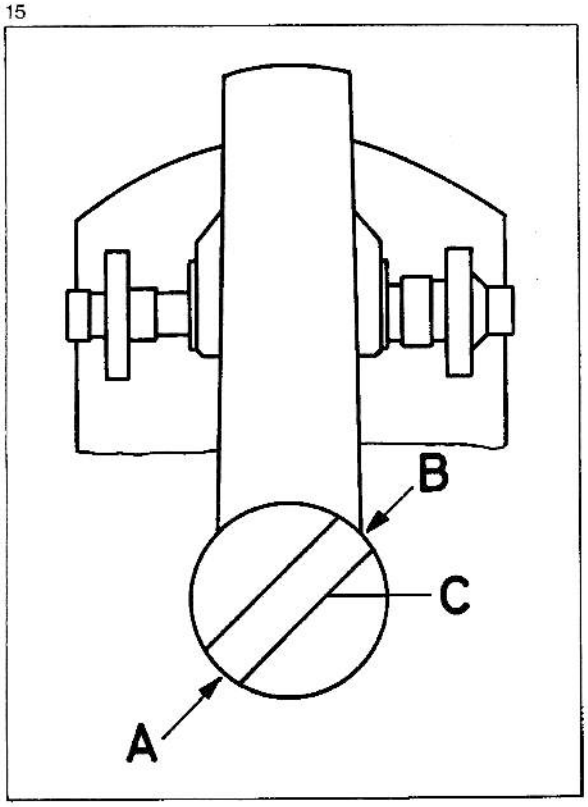


Focus the specimen in brightfield with Planachromat 16/0.35.
Insert the proper DIC slide for the objective (engraving on top) in the adapter ring (6): on STANDARD microscopes (5 Fig. 14) from the side of the tube carrier, on microscopes with tube head (5 Fig. 8) from the side averted from the tube carrier (see diagram). The indicated insertion direction always refers to the objective in the beam path.

A = insertion direction of DIC slide
on Photomicroscope, UNIVERSAL or ULTRAPHOT

B = insertion direction of DIC slide
on STANDARD microscopes

C = direction of slots for DIC prism

DIC slide for Planachromat 6.3/0.16	(47 45 31)
DIC slide for Planachromat 16/0.35	(47 45 51)
DIC slide for LD-Planachromat 40/0.60 corr.	(47 45 64)
DIC slide for Planachromat 40/0.65	(47 45 71)
DIC slide for Planapochromat 63/1.40 oil	(47 45 81)
DIC slide for Planachromat 100/1.25 oil	(47 45 91)

Swing in analyzer (1 Figs. 7 and 8).

Select black-and-white contrast by turning the screw of the DIC slide (4) and adjusting it to specific image details.

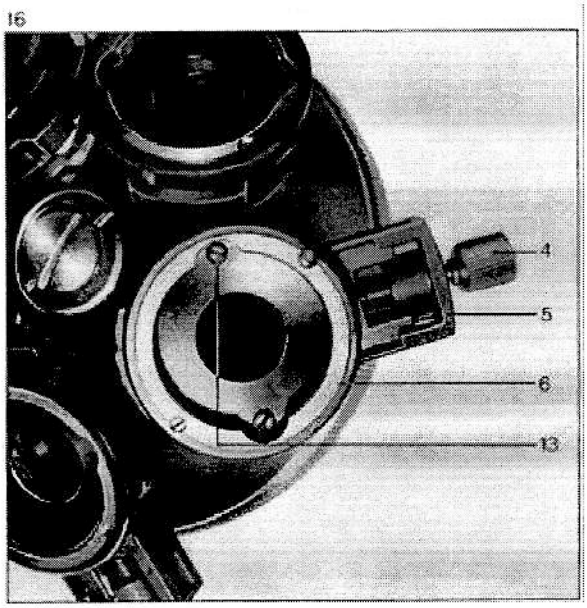
Color contrast is produced by inserting the λ plate (47 37 00) (3 Fig. 7) in intermediate tube or tube head in the opening beneath the analyzer.

"Amplitude contrast" of weakly stained specimens is produced by turning the polarizer (7). Turn the polarizer slightly in each direction until maximum contrast is achieved.

Subsequent fitting of DIC systems

The adjustment described below is required if a microscope is subsequently fitted with a DIC system. Make the adjustment separately for each objective with the proper DIC adapter ring screwed into the objective turret. Orientation of the DIC adapter ring is retained as long as the ring is in one and the same turret opening.

16



1. Aligning the direction of the slot in the adapter ring

Screw DIC adapter ring (6) **rigidly** into objective turret.

Loosen the two opposite inner screws (13) with a screwdriver until the slotted part of the DIC adapter ring can be turned **(not too lightly).** Align this part of the adapter ring so that the slot is directed as shown in the diagram (Northeast-Southwest).

Screw in low-power objective, e.g. Planachromat 16.

Insert the proper DIC slide of the objective (5) — engraving on top — in the DIC adapter ring (direction see diagram), The corresponding condenser prism must be aligned depending on the microscope (STANDARD or tube head type) (see page 11).

Set the turret of the condenser into the position corresponding to the objective (I or II, see page 7).

Remove analyzer or polarizer from the beam path.

In brightfield adjust an empty area in a specimen according to Köhler's rules.

Check the illumination of the objective aperture, either through the empty tube (without eyepiece) or with centering telescope or Bertrand lens.

Open the condenser aperture diaphragm until the pupil (aperture) is completely illuminated (with condenser IV Z/7 this is possible up to a max. aperture of 0.63).

Correct possible inhomogeneous illumination by shifting the light source axially.

Bring polarizer and analyzer into the beam path (their oscillation directions must be crossed).

Slowly turn the DIC ring containing the slide alternately to the right and left and shift the DIC prism with screw (4) until maximum extinction is achieved in the objective aperture or a large part of the aperture center is dark. (It is generally not possible to achieve completely uniform extinction from the center towards the edge of the objective aperture; the higher the objective aperture the brighter the aperture towards the edge).

Retain this position of the DIC adapter ring, unscrew the objective and fix the rotary part of the DIC adapter ring by tightening the set screws (13) with a screwdriver. (Turn the objective turret into a favorable position or remove it from the microscope, if possible).

Screw in objective and insert the eyepiece.

2. Aligning polarizer and analyzer

Check the orientation of polarizer and analyzer before putting the equipment into operation. Remove condenser, objective and eyepiece from the microscope. Switch on the light source, open the luminous field stop, view through the empty tube, swing in polarizer (0 position) and analyzer and check whether the background is dark.

If the darkness can be increased by turning the analyzer, use the analyzer only in corresponding orientation position.

Note

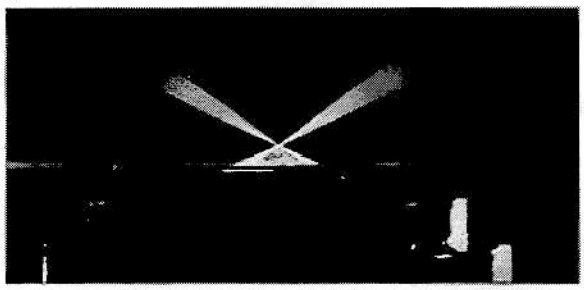
The DIC adapter ring increases the distance between specimen and flange surface of the turret by 11 mm. Objectives not used for DIC should therefore also be equipped with an empty DIC adapter ring to ensure equal height of all objectives and thus parfocalization.

Darkfield

The darkfield condenser illuminates the specimen by a hollow cone of rays whose inner aperture must be larger than that of the objective used for observation. Only the light diffracted by the specimen reaches the objective. The image background remains dark. A high-power light source is required for this method.

Between a clean specimen slide (for immersion condenser 1.1 to 1.3 mm thick) and coverglass the **specimen** must be mounted in a medium with a refractive index higher than that of air.

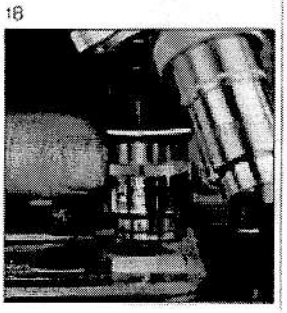
17



Sectional view of the hollow cone of rays of the ultra condenser 1.1/1.4. The specimen lies in the point of intersection.

Adjustment of dry darkfield condenser
Like that of the immersion condenser according to sections 2. through 7., yet without immersion oil on the condenser front lens.

18



Iris diaphragm on immersion objective.

Adjustment of immersion condenser

1. Center the achromatic-aplanatic phase contrast condenser V Z with front lens 1.4 in brightfield and set turret to D or insert ultra condenser 1.1/1.4.
   Apply an adequate amount of immersion oil (bubble-free) to the condenser front lens. Rack down the condenser carrier.

2. Provide the specimen. Rack up the condenser carrier until the oil reaches the specimen.

3. Focus the specimen with a low-power objective 10 to 25.

4. Close the luminous field stop.

5. Adjust the condenser carrier so that the light spot in the image is small, bright and clearly defined.

6. Center this image of the luminous field stop in the field of view with the centering screws.

7. Open the luminous field stop until its image just disappears at the edge of the field of view.

8. For immersion objectives: apply immersion oil to the specimen (bubble-free). Close iris diaphragm of the objective.

9. Focus the image. Improve centering of the luminous field stop according to sections 4. through 7. Open the iris diaphragm of the objective so that the dark background is not brightened.