

Fig. 1: Axioskop 20 microscope for transmitted-light brightfield

- 1 Binocular tube 30°/20
- 2 PL eyepieces
- 3 Nosepiece 6×
- 4 Specimen holder with spring clip
- 5 Mechanical stage 75×30 mm
- 6 Condenser 0.9 Z
- 7 Condenser carrier
- 8 Luminous-field diaphragm
- 9 Knob for vertical condenser adjustment
- 10 Coaxial coarse/fine focusing control

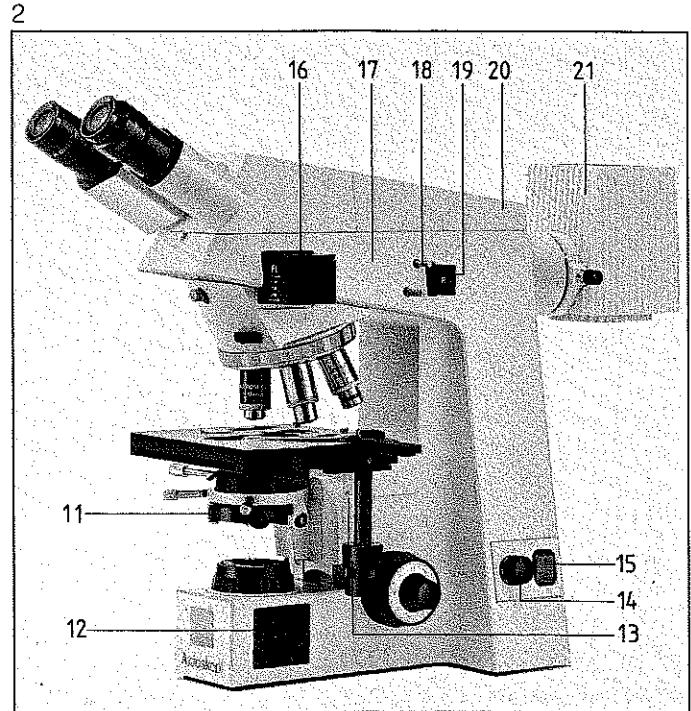


Fig. 2: Axioskop 20 microscope for transmitted light and incident-light fluorescence

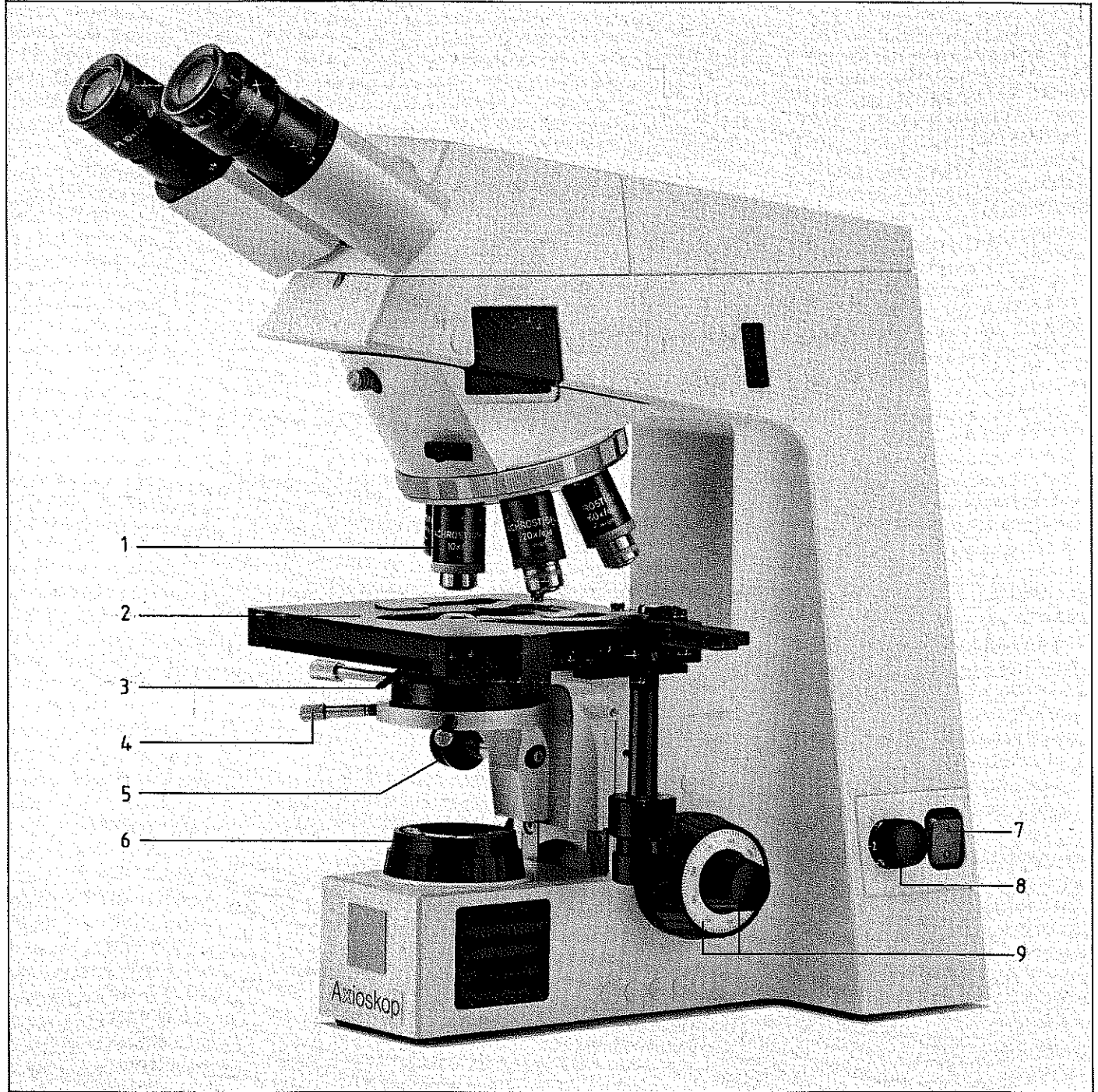
- 11 Carrier with annular phase-contrast diaphragm Ph 2
- 12 6V 20W in-base illuminator
- 13 Coaxial stage drive for movement in x and y
- 14 Potentiometer for in-base illuminator
- 15 ON/OFF switch with power signal lamp
- 16 Reflector slider FITC
- 17 Incident-light system FI
- 18 Luminous-field diaphragm for reflected light
- 19 Slider with the positions light shutter, filter BG 38 and free light path
- 20 Tube panel
- 21 Illuminator with HBO 50 mercury lamp

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Special notes

- The 6- to 10-digit numbers, e.g. 453522, are ordering numbers of instruments or components.
- Caution!
The instruments shall not be used in environments with explosion hazards.
- The instruments shall be changed and/or repaired only by the manufacturer or his authorized representative.
- Specifications subject to change.

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Special note: Framed numbers like **1.1** refer to the description of the instrument starting on page 6.

- Check whether the data on the nameplate on the instrument back comply with the local power data. Plug in microscope power cable, switch on microscope with (7) and set to 2–3 V with (8).
- Load a high-contrast specimen on specimen stage (2) with the cover glass face up!
- Turn in 10× objective (yellow ring) (1) on nosepiece, check 0-positions on the eyepiece scale. Move condenser (front lens not swung out) to topmost position with (5).
- Close the aperture diaphragm about half with (3).

You should now see light spots (the exit pupils) behind the eyepieces. If the microscope is equipped with a binocular phototube with sliding prism, the pushrod must be pushed in. Through the tube you will see a bright circle (the eyepiece stop) with each eye. Turning the two eyepiece tubes to your PD will merge the two circles in one.

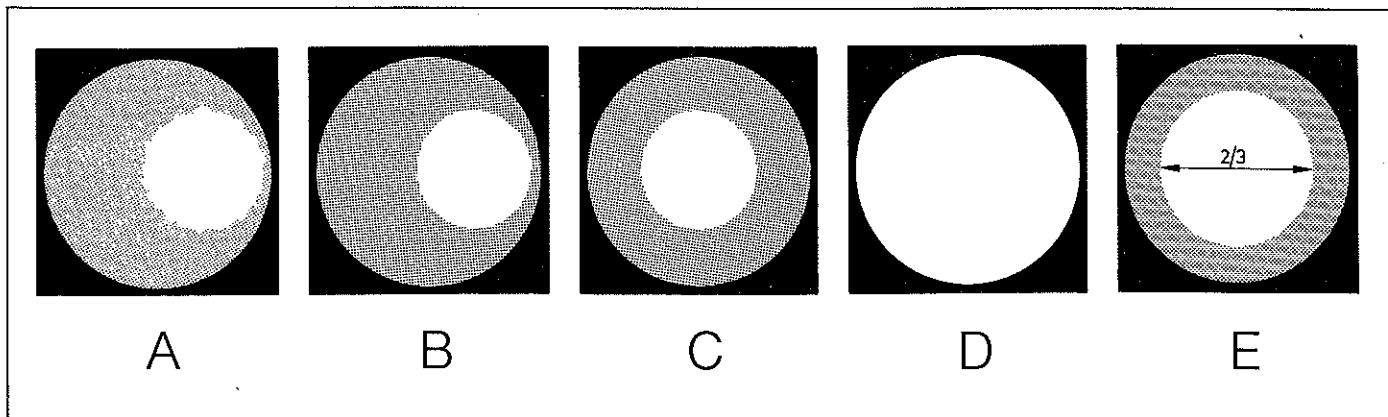
Further steps of Köhler illumination adjustment:

- Focus the specimen with coarse/fine focusing control (9). (If your eyes have different powers and for work without eyeglasses → **5.3**).
- Close luminous-field diaphragm (6) moderately; it will become unsharp in the image (A).
- Focus the diaphragm image by lowering the condenser slightly with (5) (B).
- Center the diaphragm image in the field of view with screws (4) (C), and
- open luminous-field diaphragm (6) until it just disappears from the field of view (D).

The contrast is adjusted for each specimen with the condenser diaphragm (3). If you are not certain how far to stop down: approx. $\frac{2}{3}$ of the rear element of the objective (visible at the tube bottom if the eyepiece is removed from the tube) should be illuminated if a specimen is of moderate contrast (E). Field of view and objective aperture change with each objective exchange so that the last-mentioned steps must be repeated.

When a low-power objective images more than the condenser can illuminate, the condenser front lens must be swung out. For a full description of the procedure see page 8.

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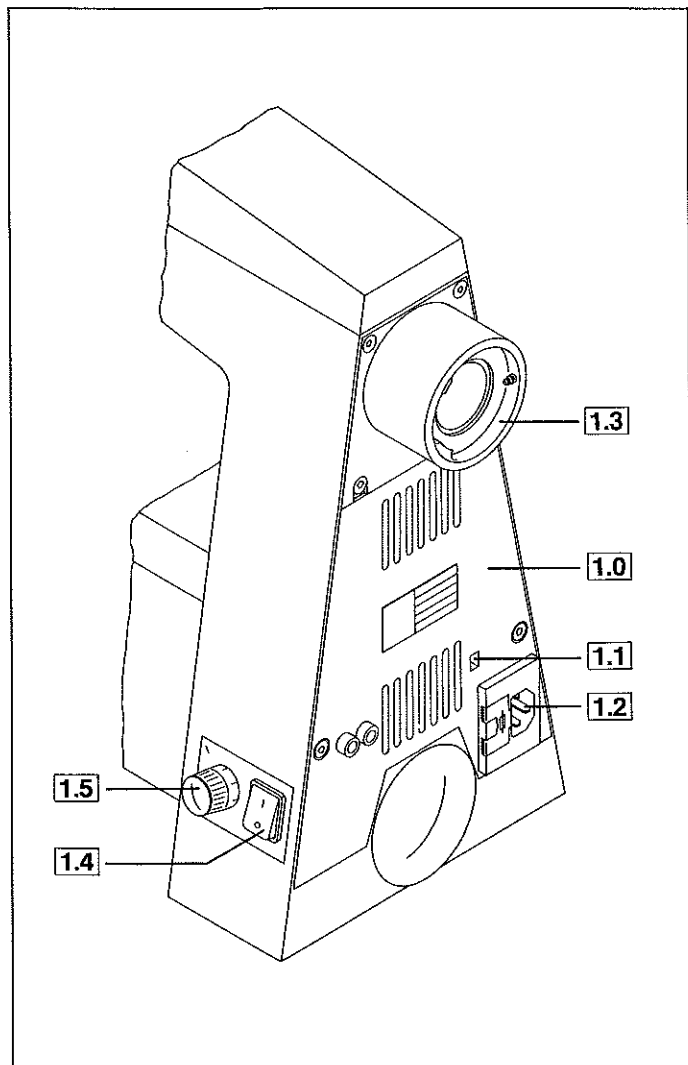


Special notes

Place the Axioskop 20 on the supplied mat (Fig. 1), which assures correct ventilation of the in-base illuminator. Do not use any other soft mat which would cover the ventiducts at the bottom of the stand base.

For information about further accessories see G 42-110. Almost all relevant screws, e.g. to clamp the tube, or for the fluorescence illuminator, are socket head cap screws for which the wrench SW 3 with the red handle is supplied.

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**1.0 Lamp power supply**

The 6V 20W lamp power supply is integrated in the stand.

1.1 Window displaying the internally adjusted input voltage:
230 V for 220...240 V or
115 V for 100...127 V line voltage.

Change by vertical shift with a screwdriver.

Voltage tolerance + 10% / - 15%; frequency 50...60 Hz.

Stabilized output voltage, variable from 1...6 V.

Power consumption max. 40 VA.

The instrument is radio-screened, short-circuit-proof, and complies with VDE, IEC, CSA, and UL regulations.

1.2 Power plug with 2 integral fuses for 230 V or 115 V (as adjusted on voltage dial). The fuses are accessible for exchange when the dust cover is swung aside.

Spare fuses for:

230 V: 0.25 A SB (380127-0140)

115 V: 0.5 A SB (380142-2860)

1.3 Adapter for fluorescence illuminator (see p. 11)

1.4 Power switch with integral power signal lamp

1.5 Potentiometer which supplies 6 V when turned fully clockwise. The adjusted voltage is displayed by the index.

2.0 Stand base

2.1 Coaxial coarse/fine focusing control acting on the stage carrier with condenser carrier which runs in two guide tracks and moves the specimen stage up. Turning the control anti-clockwise lowers the stage. Total focusing range (including fine focusing control): 25 mm.

Coarse focusing control: one turn corresponds to 2 mm travel

Fine focusing control: gear ratio reduced 1:10

The index line on the coarse focusing control can be used to roughly measure the specimen thickness: 1 interval corresponds to approx. 2 μm .

2.2 In-base illuminator; it includes a factory-adjusted lamp socket with 6V 20W halogen lamp (38.0079-9690). A diffusing screen and a collector on top of the lamp provide for correct field and pupil illumination. For lamp exchange see p. 17.

Technical data of halogen lamp

Voltage	6 V
Power	20 W
Color temperature at 6 V	2800 K*
Luminous flux	280 lm
Mean life	1000 hrs
Luminous area	2.0×2.0 mm

* The conversion filter CB 3 (46 78 52) provides for the right color temperature in the beam path for photography with artificial-light color reversal film which is sensitized for 3200 K.

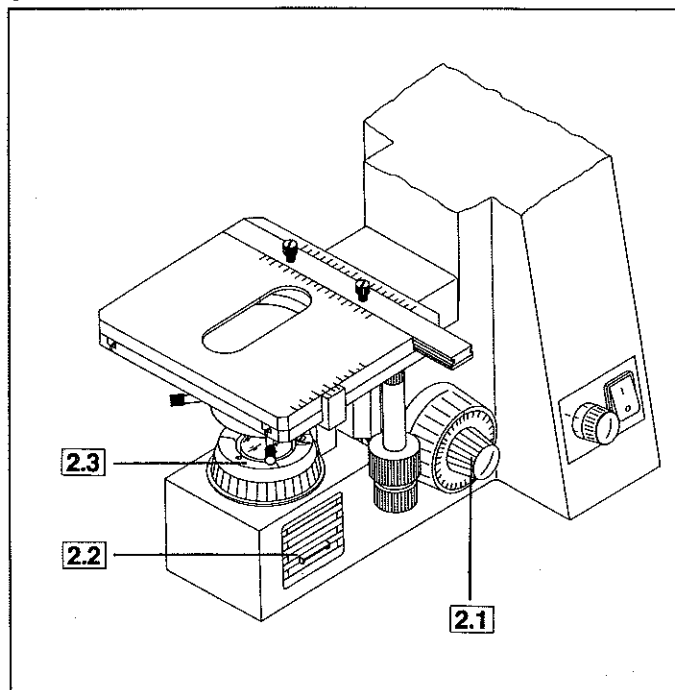
2.3 Luminous-field diaphragm which is adjusted with the knurled ring. A 32 mm dia. filter fits on the removable dust cover glass. This plane is not imaged in the microscope. A 32 mm dia. filter can also be placed on the color-glass carrier (451834) on the condenser carrier.

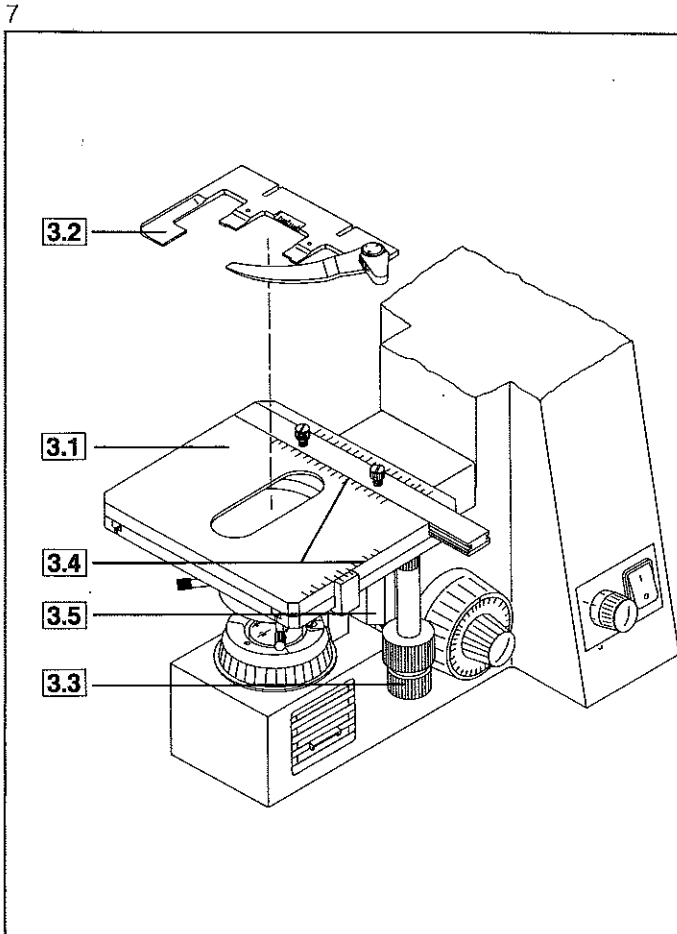
Frequently used filters:

Green interference filter 32×4 (467803) for contrast enhancement in B/W photography of stained sections and in phase contrast.

Conversion filter CB 3 32×2 (467852) converts the incandescent lamp light of 2800 K into artificial light of 3200 K for color photography.

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3.0 Specimen stage

- 3.1** Mechanical stage 75×30 mm with low-mounted controls to the right (45 35 22).
- 3.2** Specimen holder with spring clip for standard (26×76 mm) or 26×45 mm specimen slides.
- 3.3** Coaxial controls for 75×30 mm travelling range in x and y.
- 3.4** Graduations and verniers for the re-location of specimen features.
- 3.5** Stage carrier with firmly mounted specimen stage.

4.0 Condenser

The condenser¹⁾ is mounted in

- 4.1** condenser carrier with the following operating controls:
- 4.2** Knurled knob for vertical adjustment; the stiffness is factory-adjusted and should be changed only by the maintenance service. The vertical condenser adjustment is limited by a stop to prevent a specimen from being pressed out by mistake. The stop is factory-adjusted for standard specimens. The vertical limit stop should be changed only by our maintenance service.

- 4.3** Spring pin for orientation

- 4.4** Two condenser centering screws to center the image of the luminous-field diaphragm during illumination adjustment (see page 5).

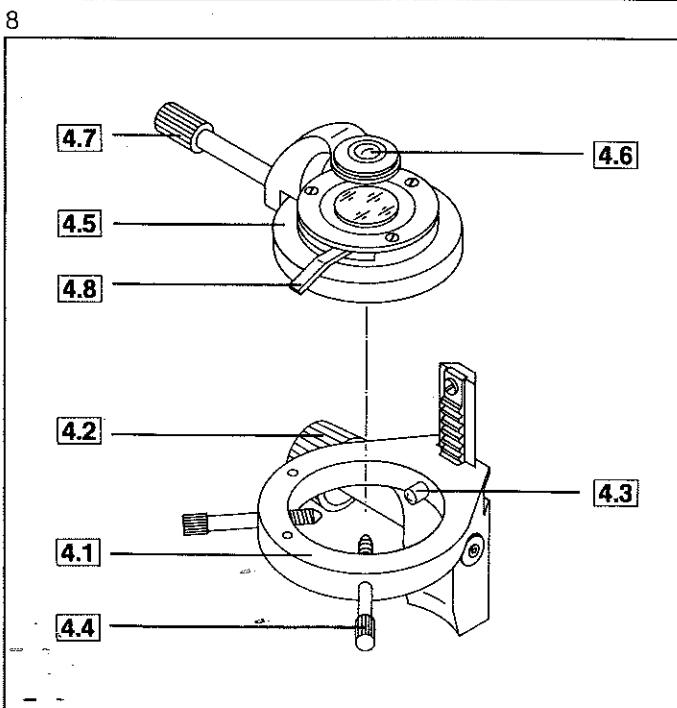
- 4.5** Condenser 0.9 Z with swing-in front lens (44 35 30) for brightfield.

- 4.6** Front lens, aperture 0.9

- 4.7** Lever to swing the front lens in or out (for objectives 2.5× ... 5×)

- 4.8** Lever for aperture iris diaphragm.

Phase contrast is produced with this condenser plus carrier with annular phase-contrast diaphragm Ph 2 (see p. 15).



¹⁾ Inserting the condenser

To insert the condenser in carrier **4.1** turn lever **4.7** for the front lens to the left so that lever **4.8** for the aperture diaphragm can be easily operated from the front. Press the condenser dovetail ring against spring pin **4.3** and let it snap in.

5.0 Image-forming components

5.1 The objectives, the most important elements of a microscope, must be kept meticulously clean, especially the front lens surfaces. (Breathing and wiping over the surface with a Q-tip will generally do for cleaning; for thorough cleaning see the brochure 41-100 "Microscopy from the very beginning".)

The numbers and symbols engraved on the objectives, e.g. Achroplan 40X/0.65; ∞/0.17 signify: 40X = (individual) magnification;

0.65 = numerical aperture;

∞/0.17 = infinite image distance calculated for a cover glass thickness of 0.17 mm.

The (individual) magnification multiplied by the eyepiece magnification (generally 10X) results in the microscope magnification.

The numerical aperture × 1000 (650 in the example) is the highest useful magnification; no more details will be revealed above this value.

∞ is to remind you that these objectives cannot be used on microscopes with objectives bearing the number 160.

Observation of the cover glass thickness 0.17 mm is the more important the higher the numerical aperture of the objective. Certain objectives in correction mounts are adjustable to different cover glass thicknesses: find out, by means of a high-contrast specimen feature, in which position of the correction mount the sharpness is optimized (re-focusing will always be necessary). Immersion objectives are insensitive to differences in the cover glass thickness.

Because of their short working distances 20X and higher-power objectives have spring mounts for specimen protection. To prevent the specimen from being contaminated by oil when turning the nosepiece, immersion objectives can be locked in the upper position of the spring mount by a clockwise turn (don't forget to disengage them from this "lock-in" position!).

The air between cover glass and immersion objective is replaced by immersion oil. Some training is necessary to obtain a bubble-free layer. Some microscopists prefer to turn the objective from the side into the oil drop on the cover glass, others to lower it from the "lock-in" position of the spring mount. The exit pupil should always be controlled, preferably with the centering telescope **5.6**, if any, a procedure which will instantly reveal any bubbles. If the bubbles have not disappeared when the objective was turned in several times, clean the specimen and repeat the procedure.

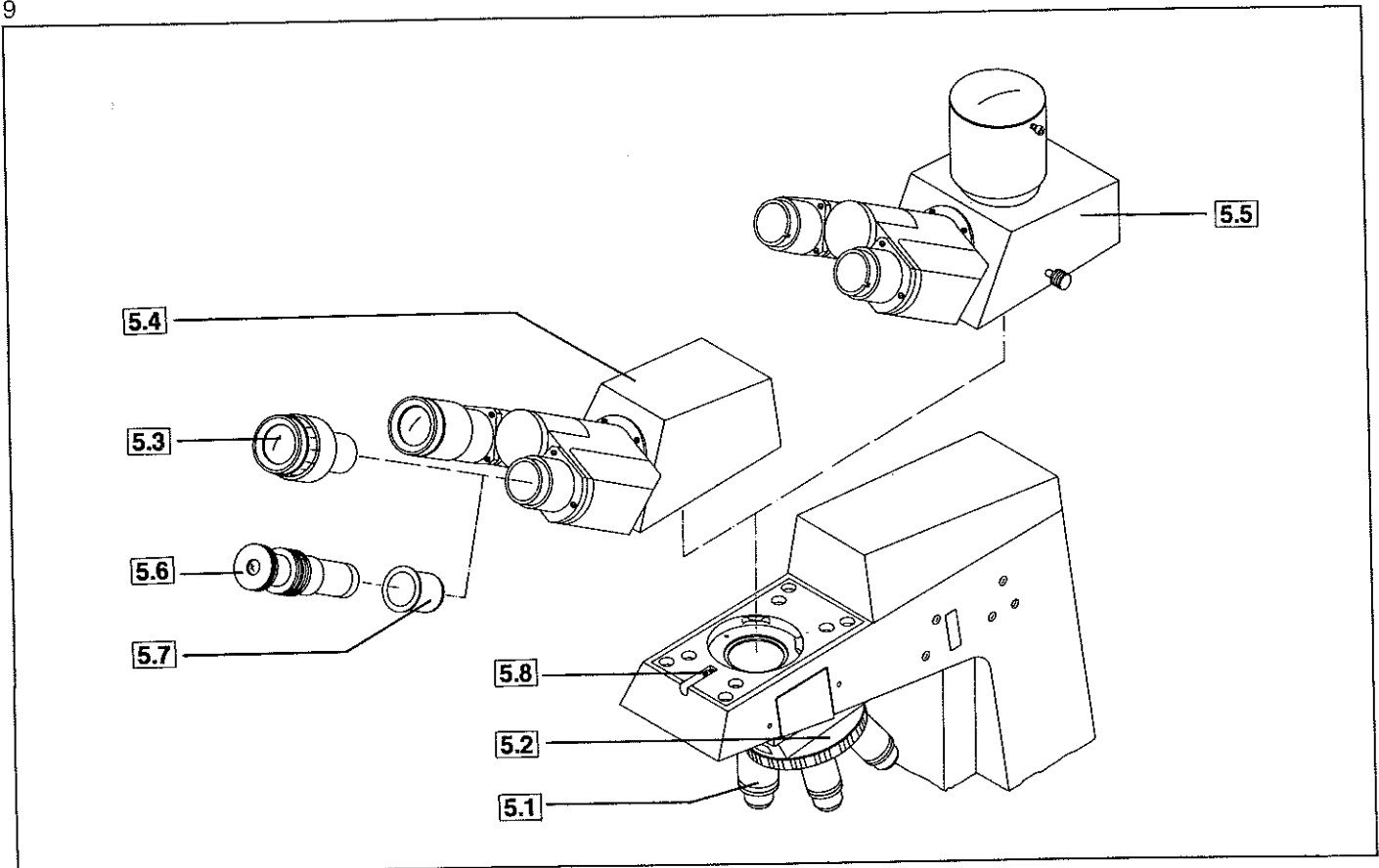
5.2 Nosepiece, firmly connected with the microscope stand.

5.3 Eyepieces with 10X magnification and field-of-view number 20 produce angular fields of 43°, are equally well suited for eyeglass wearers (Br), and provided with exchangeable, soft rubber rings to protect the eyeglasses (folding eyecups are available under ordering number 444801). One eyepiece is a focusing (foc) eyepiece. If your eyes have different powers, or for microscopy without eyeglasses, make the following adjustments:

- Look through the fixed eyepiece with the less emmetropic eye and focus on the specimen.
- Leave the microscope adjustment unchanged, but adjust the focus with the eyelens of the focusing eyepiece for the more emmetropic eye, until the specimen is in focus for both eyes. The diopter value determined once can be adjusted on the diopter scale of the eyepiece.

Eyeglass wearers who take their glasses off for microscopy may experience unsatisfactory parfocalization after objective exchange. Eyeglasses with a "cylinder" power must be kept on for microscopy.

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5.4 Binocular tube (452907)

The viewing angle is invariably 30° , but the folding bridge can be used in two positions. A turn of the binocular body through 180° will vary the viewing height by a few centimeters, depending on the PD. Unless done in the factory, unscrew the pilot screws from focusing eyepieces, because the tubes are not provided with corresponding notches.

Binocular tube and binocular phototube have PDs between 55 mm and 75 mm, adjustable by moving the tube halves in and out, and a tube factor $1\times$.

5.5 Binocular phototube with sliding prism

With the knob to the right of the tube body a pushrod can be moved in or out and set to two positions:

100% of the light for observation (pushrod pushed in), or 30% of the light for observation and 70% relayed to the camera (pushrod pulled out).

The tube has a viewing angle of 30° .

The lateral screw of the focusing eyepiece engages a notch in the tube and fixes the eyepiece in this position. This eyepiece orientation is important also for the use of reticles.

The binocular phototube is provided with a reticle erection which is accurate to within 1° .

The upper tube port accepts a photomicrographic, a TV or a special camera. The adapter for microscope camera and a photo eyepiece S-PI $10\times$ or S-PI $12.5\times$ are required for mounting an MC 63 or MC 100 Microscope Camera. A cine or TV camera with C mount is fitted via standard C adapter (452995) without eyepiece.

Both adapters are parfocalized with the reticle in one of the eyepieces.

5.6 Centering telescope (45 49 20) fitted in either tube instead of the eyepiece via

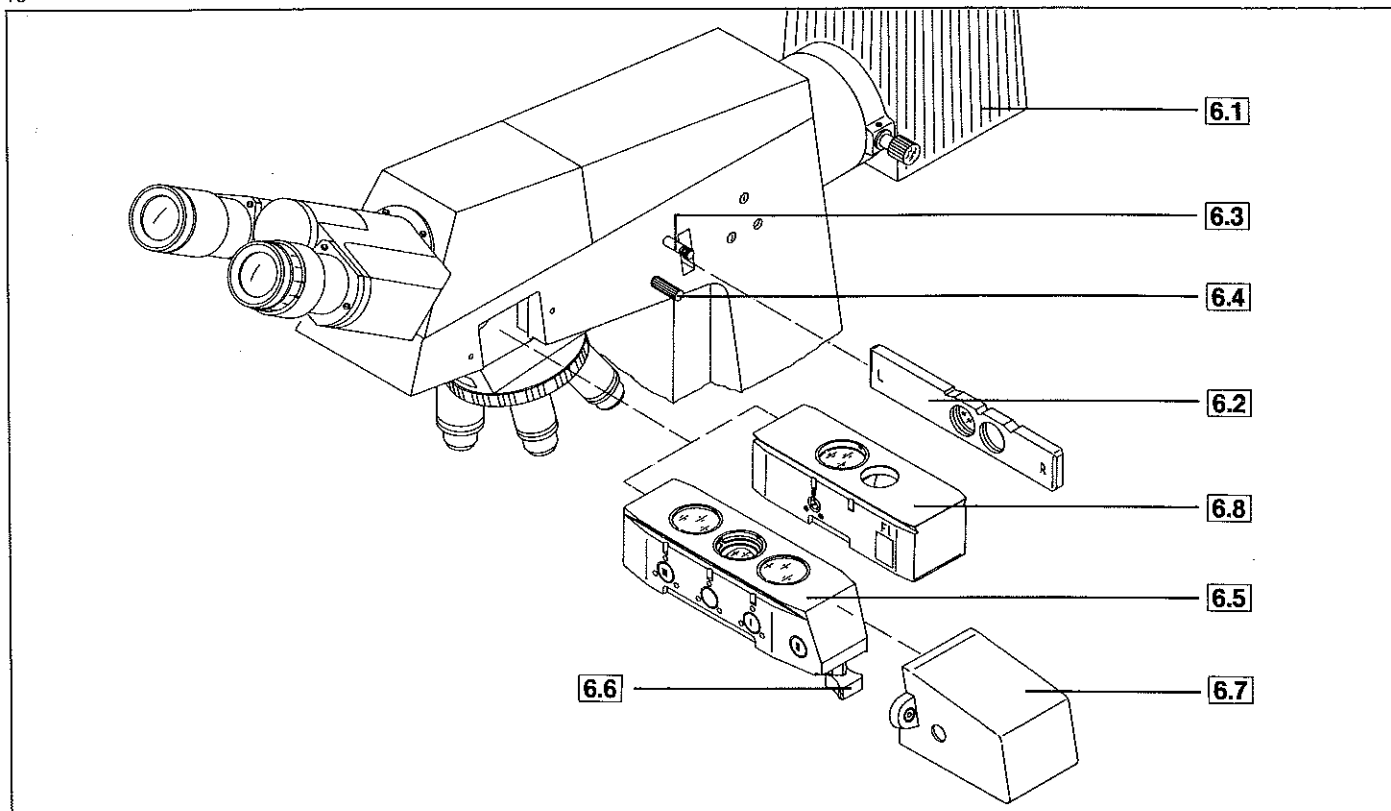
5.7 reducing ring 30/23.2 (464911)

eases the observation of the objective pupil, especially for phase-contrast centration.

Tube exchange

Loosen Allen screw **5.8** and take off the tube. Unscrew the Allen screw so far that it is not visible when looking into the tube. Put on the new tube and secure it with the screw.

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6.0 Fluorescence equipment

6.1 Fluorescence illuminator with aspheric collector and HBO 50 high-pressure mercury lamp which is supplied from its own power supply (392642).

6.2 Slider marked L for left and R for right, which either interrupts the illuminating light path (slid in all the way from the left), brings a red-attenuating filter BG 38 in the light path (middle position), or allows all the light to pass through (slid in all the way from the right). An 18 mm dia. additional exciter filter can be accommodated in the empty filter position.

A heat-reflecting filter KG 1 is invisible from the outside; it has no influence on UV excitation.

6.3 Pushrod for the luminous-field diaphragm

6.4 Centering screws to the left and right on the stand moving the pushrod **6.4**.

6.5 Reflector slider 3 FI fitting into the large opening above the nosepiece. It has three positions. The one in the middle is generally left free for brightfield or phase-contrast observation, the others contain suitable exciter filter/chromatic beam splitter/barrier filter sets (for details see page 14).

6.6 Handle to slide in the filter set; display in the right (positions I and II) or left window (III).

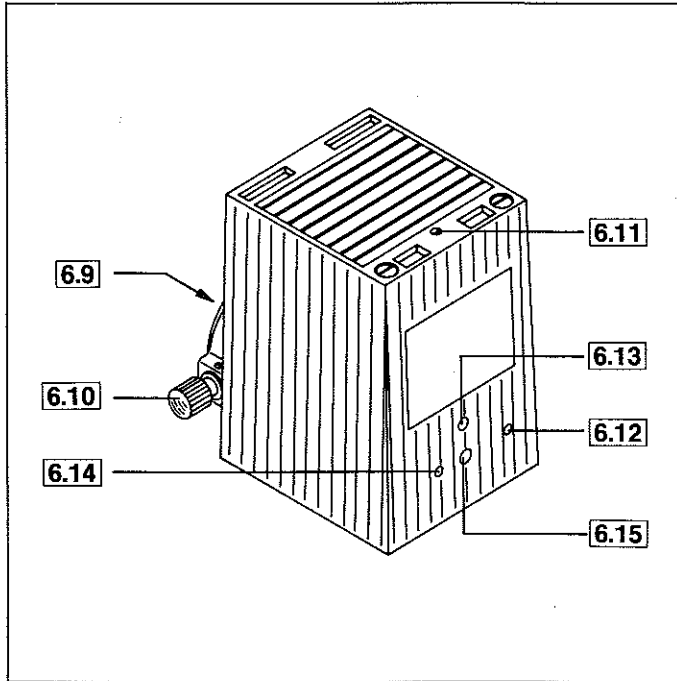
For the reflector 3 FI for fluorescence:

6.7 Covers on both sides of the microscope secured by 2 screws (tighten them alternately). The covers protect projecting reflector positions from dust and fingerprints.

6.8 Reflector slider FITC (487969) with a filter set for blue excitation (left) and a free light path (right) for brightfield or phase-contrast observation. It is inserted instead of the reflector slider 3 FI.

Change between the positions of the reflector slider FITC is made by pushing it from the outside. If the filter set is in the light path, "FI 09" is visible on the outer right side of the slider.

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Fluorescence illuminator

For installation and centration of the HBO 50 high-pressure mercury lamp see page 13.

Features of the HBO/XBO lamp housing:

6.9 Light exit with dovetail ring to mount the illuminator on the microscope.

- Unscrew clamping screw of adapter **1.3** sufficiently.
- Insert the dovetail ring of the lamp housing inclined in the recess opposite the clamping screw, then tilt the illuminator against the contact surface and tighten the screw.

6.10 Knob for collector adjustment. The collector can be taken out when the knob is pulled out (the pin of the knob engages a notch of the collector). In front of the collector there is a holder for a 42 mm dia. heat-reflecting filter; it should be empty if the illuminator is used for UV fluorescence excitation (blue fluorescence).

6.11 Vertical lamp adjustment

6.12 Lateral lamp adjustment

6.13 Vertical adjustment of mirror image (red dot)

6.14 Lateral adjustment of mirror image (red dot)

6.15 Focusing of mirror image

Mounting the HBO 50 mercury lamp in the lamp housing

Pull lamp socket plug [6.28] on the power supply, loosen screw [1.3], and detach the HBO/XBO lamp housing [6.16] from the microscope. Screw the collector to foremost position with [6.10] and loosen clamping screw [6.23]; you can now take lamp socket [6.27] out of the lamp housing. Loosen [6.20] and [6.26] and pull out lamp [6.24] and wire loop [6.25]; loosen [6.18] and [6.19] and remove dissipator [6.17].

Insert the end of the new lamp socket bearing the lamp number in the dissipator and clamp with a screwdriver; the melt tip should be aligned parallel with the dissipator. Plug wire loop into dissipator, align it parallel with the latter and secure it.

Insert the lamp with the dissipator and the wire loop in the corresponding openings of the lamp socket and secure with hexagon nut [6.26] and knurled screw [6.20]. The longitudinal side of the dissipator, the lead end, the wire loop and the melt tip should be in one line.

Insert the lamp socket [6.27] with the new lamp all the way in the lamp housing; pin [6.22] should engage borehole [6.21] of the lamp socket.

Secure lamp socket [6.27] with screwdriver SW 3.

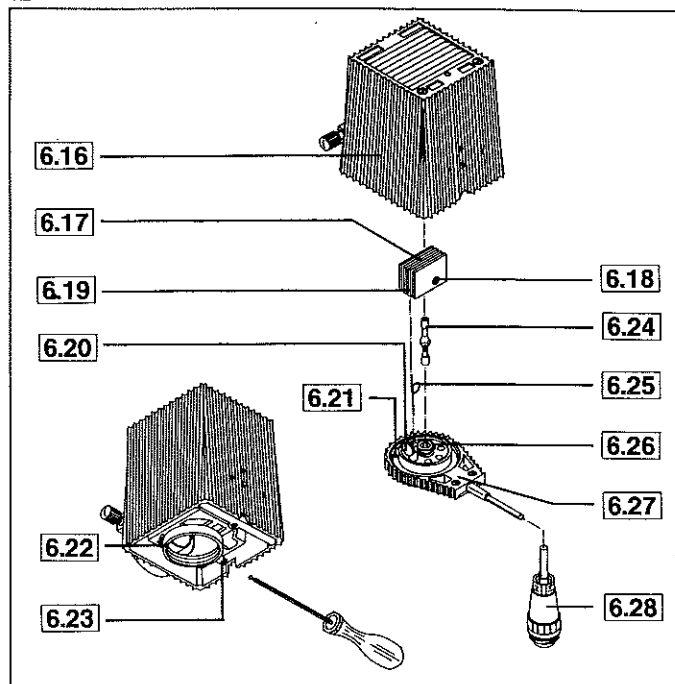
Set the lamp type selector on the power supply to L1 or L2, plug in the lamp plug and connect the instrument to the line. The adjusted voltage is displayed on the dial.

The power switch of the lamp is on the front panel of the power supply.

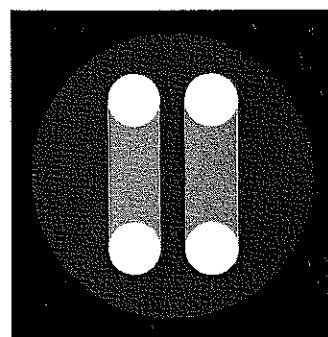
Centering the HBO 50

- Carefully detach ignited illuminator from microscope with screwdriver SW 3.
- Image the brighter of the two cathode focal spots sharply on a white surface approx. 1 m away with knurled knob [6.10] for collector adjustment. Look only shortly on the light spot while handling the ignited lamp, to prevent your eyes from being damaged by UV radiation.
- Center the image of the cathode focal spot with screwdriver SW 3 on the adjusting screws [6.11] and [6.12].
- Focus the unsharp light spot with adjusting screw [6.15]; lamp image and mirror image must be of equal size. With the red adjusting screws [6.13] and [6.14] align the focused mirror image next to the real image (see Fig. 13). The two images should not overlap.

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- Re-mount illuminator and tighten clamping screw [1.3]. Open slider [6.2] and set reflector slider to blue excitation (e.g. filter set 09 for FITC fluorescence).
- Unscrew objective and check the light source image on a white sheet of paper approx. 20 mm beneath the empty nosepiece opening.
- If necessary, correct with collector adjustment knob [6.10] and adjusting screws [6.11] through [6.15]. Screw in objective.

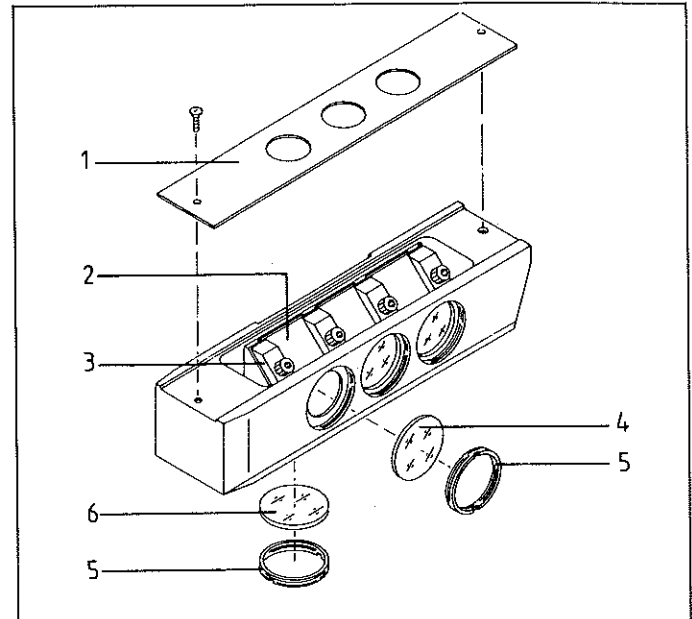
Required are:

- No special objectives; Plan-Neofluar objectives for UV excitation.
- Special incident-light illumination (Fig. 10).

Proceed as follows:

- Adjust the chosen specimen feature in transmitted-light brightfield or phase contrast with the reflector slider 3 FI in middle position or the FITC slider in free-light-path position [6.8]. Use the in-base illuminator with halogen lamp. Switch on the HBO 50 mercury lamp but block the light path with slider [6.2].
- Switch off or at least considerably turn down the transillumination, remove the filter from the transmitted-light path, select the desired type of excitation on reflector slider [6.5] or [6.8] and open the light path with [6.2].
- Closing an aperture diaphragm in the illuminating light path would result in reduced image brightness in fluorescence observation. Only a luminous-field diaphragm is, therefore, provided. Close it with lever [6.3] until it becomes visible in the image. After centering with [6.4] open it until the field of view is free.

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Fluorescence reflector

Exchange of exciter filter (4), barrier filter (6) and chromatic beam splitter (2). The built-in filter sets can be exchanged when the retaining rings (5) are unscrewed. The plate carrying the chromatic beam splitters (2) is accessible when base plate (1) is removed. The latter rests on a spring mask and should not be touched. Generally, the straps (3) need not be removed completely. Loosening them is usually sufficient for exchange of the chromatic beam splitters on the mask. Plate (1) is not symmetrical. Make sure that when mounting the plate it does not cut off the free openings. Filters and chromatic beam splitters in the reflector slider FITC (6.8) are exchanged the same way.

Special notes

Make the initial fluorescence adjustment with a 20× objective and a strongly fluorescent specimen. Suitable specimens are available but you may also prepare them yourself; anthracene crystal diffusing specimens are quite popular. (Sometimes the specimen label will do for a check of the illumination).

Different filters sets in the reflector slider are offered for specific tasks. Each set comprises 25 mm dia. exciter and barrier filters which enclose a 26×26 mm chromatic beam splitter.

Filter sets

Excitation	Filter set	Exciter filter	Beam splitter	Barrier filter
Green H 546	487915	BP 546/12	FT 580	LP 590
Blue 450-490	487909	BP 450-490	FT 510	LP 520

For additional filter sets see p. 28 of G 42-110.

It is applied mainly to enhance the contrast of unstained specimens.

Required are:

- Objectives (5) designated Ph, which are equally well suited for brightfield microscopy.
- Carrier with annular phase-contrast diaphragm Ph 2 (470864) (13)
- Condenser 0.9 Z with swing-out front lens (445230) (8)

Proceed as follows:

- Swing carrier with annular phase-contrast diaphragm (13) out of light path and swing in the condenser front lens with (7).
- Adjust an object in brightfield (e.g. with 10× objective, see p. 5), narrow luminous-field diaphragm, focus, center, and open it.
- Turn in e.g. a 40× objective bearing the designation "Ph 2".

Swing the condenser front lens out of the light path with (7).

Turn in carrier with annular diaphragm Ph 2.

Match lamp brightness to the object.

Open luminous-field diaphragm (6) and condenser diaphragm with (9).

- Perfect phase contrast is obtained if the dark ring in the objective and bright annular diaphragm exactly coincide. Check this without eyepiece, at the bottom of the tube, according to the condenser-diaphragm adjustment (see p. 5) or with the centering telescope (3), if any, with reducing ring (4) and focusing by shifting the eyelens (2) of the centering telescope which must be held on the knurled ring (1).
- In exceptional cases the two centering screws (14) on the carrier with diaphragm Ph 2 may be adjusted with a screwdriver to bring the two rings (Fig. 17) to coincidence.

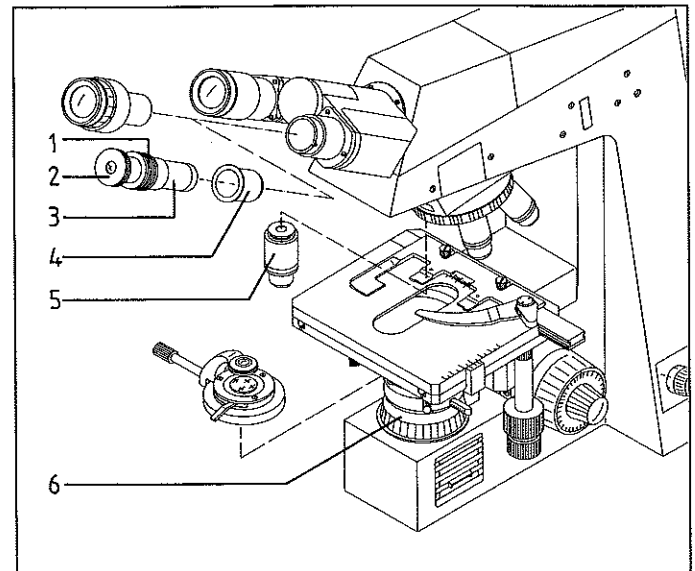
Special notes

More than brightfield phase-contrast microscopy requires meticulously clean glass-to-air surfaces of the specimen (fingerprints?).

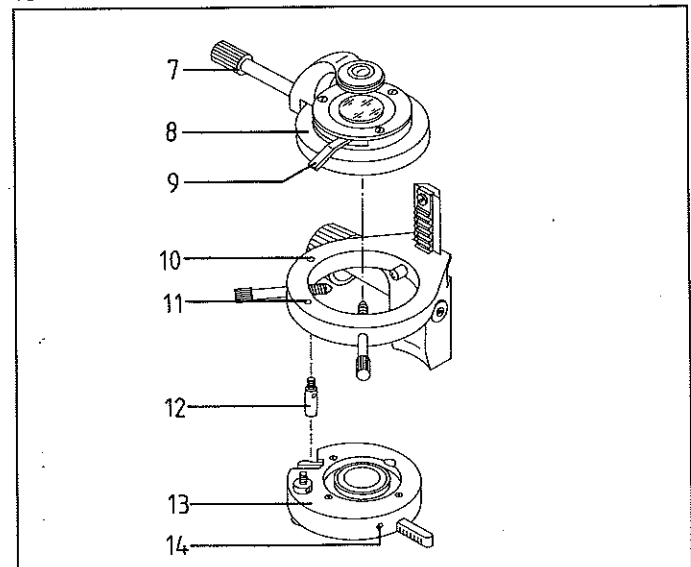
Screwing the carrier with diaphragm Ph 2 on the condenser carrier (retrofitting)

- Screw shaft with annular-diaphragm carrier (13) into front borehole (11) and tighten with socket screw wrench.
- Screw stop pin (12) into rear borehole (10) and tighten with socket screw wrench.
The diaphragm carrier must snap into the stop pin.

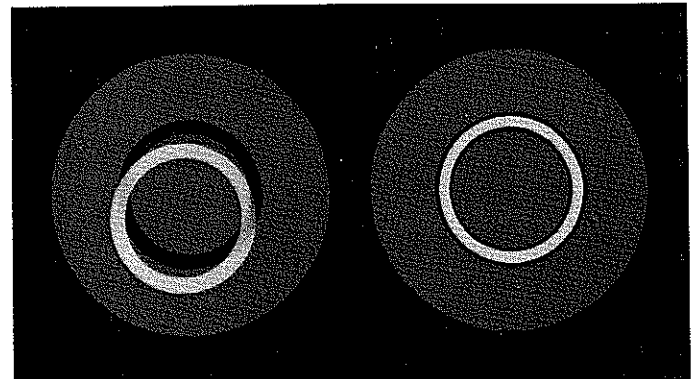
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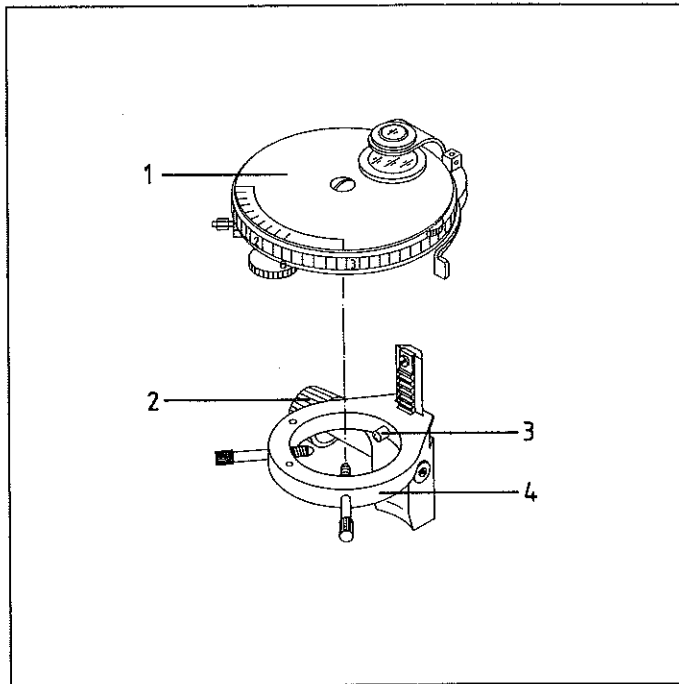
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17



18



Condenser exchange

To exchange, e.g. the condenser 0.9 Z for a phase-contrast condenser II/Z 0.9, lower the condenser with (2) as far as possible, press the condenser against the spring pin of the condenser carrier at rear and at the same time lift it out in front.

Insert the phase-contrast condenser II/Z in the condenser carrier (4) so that it is straightly aligned with the front. Insert the spring pin (3) in the notch of the condenser which points straightly to the front.

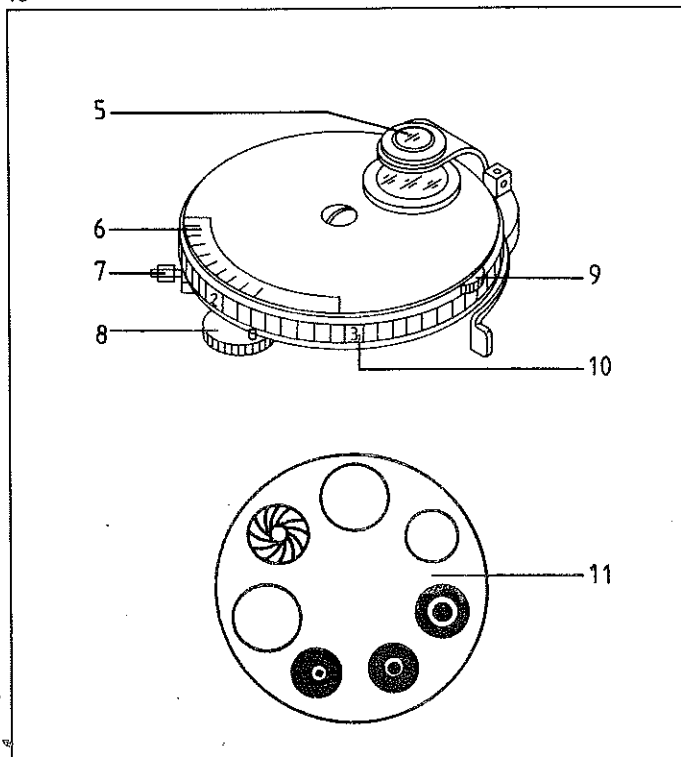
The phase-contrast condenser II/Z (45 17 54) (1) is equipped with a swing-in front lens 0.9 (5).

The standard outfit of the turret (11) with 7 click stops includes equipment for:

- brightfield "J"
- phase contrast 1 "1"
- phase contrast 2 "2"
- phase contrast 3 "3"

The phase-contrast diaphragms are centered at (7) and (8) with a lockable lever and a knob.

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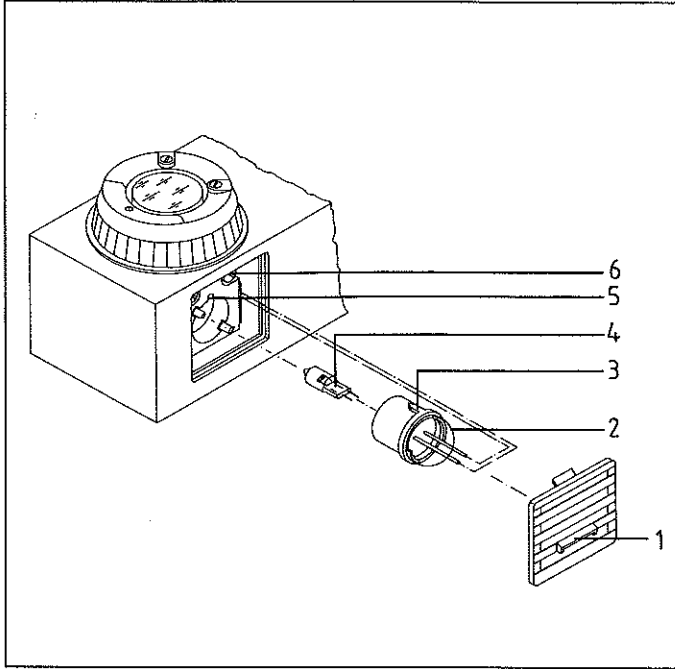
The aperture diaphragm is operated with (9) and the aperture position can be read on (6). Top view of the equipment in the click stop positions of the turret (11).

The phase rings in the objectives are of different size and indicated on the objective (15.5) by Ph 1, Ph 2 and Ph 3. The numbers 1, 2, and 3 engraved on the turret (10) refer to the phase ring size for selection and combination with the objective.

For the phase-contrast condenser II/Z (45 17 54) the coincidence of the two rings (centration) must be corrected with the lockable lever (7) and knob (8). See page 15 for a description of how to check this coincidence.

The phase-contrast condenser acts as darkfield condenser if the inner illuminating aperture of the annular phase-contrast diaphragm is larger than the objective aperture. The inner aperture is 0.44 for Ph 3.

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Exchange of 6V 20W halogen lamp

- Remove ventilation grid on the right side of the stand base by holding it on its grip (1) and pulling it off.
- Swing out bracket (2) with the fingertip. Pull lamp socket out on the bracket.
- Take defective lamp out of the socket. Hold new 6V 20W lamp (4) on the cover foil or with a clean tissue paper and slide the contact pins all the way into the lamp socket. Use only a 6V 20W halogen lamp (38 00 79-9690). Other commercially available lamp types do not feature flat core coils and have different coil positions, which will result in difficulties.
- Plug the socket with the halogen lamp into the opening. The pilot tongue (3) on the socket must engage notch (5). Then slide in the socket as far as it will go. Screws (6) are enamelled and must be left unchanged.
- Insert the claw of the ventilation grid on top, then press it down at the bottom until both catches snap in.

Some spares which may need exchange are listed below with the numbers and in the order as they appear in the description of the instrument starting on p. 6.

1.2 Fuses for the power supply integrated in the stand, for
230 V: 0.25A SB (380127-0140)
115 V: 0.5 A SB (380142-2860)

5.1 PCP-free immersion oil:
Plastic oiler containing 50 cm³ immersion oil (462958)

6.2 Red-attenuating filter BG, 18 mm dia. (467991-9902)

HBO 50 mercury lamp (381619) see p. 13

6V 20W halogen lamp (380079-9690) see p. 17.