ZEISS

Germany

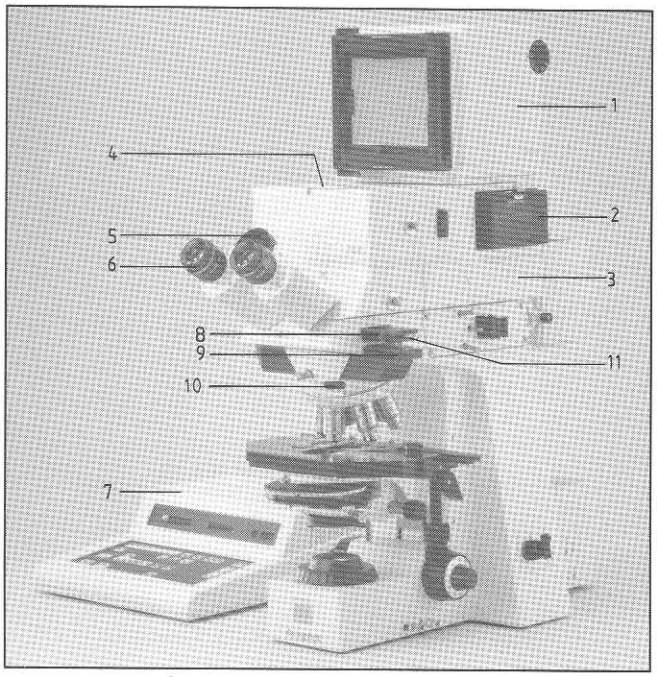
Carl Zeiss
Geschäftsbereich
Mikroskopie
7082 Oberkochen

Axiophot
Photomicroscope

Transmitted light and incident-light fluorescence

Operating instructions

1



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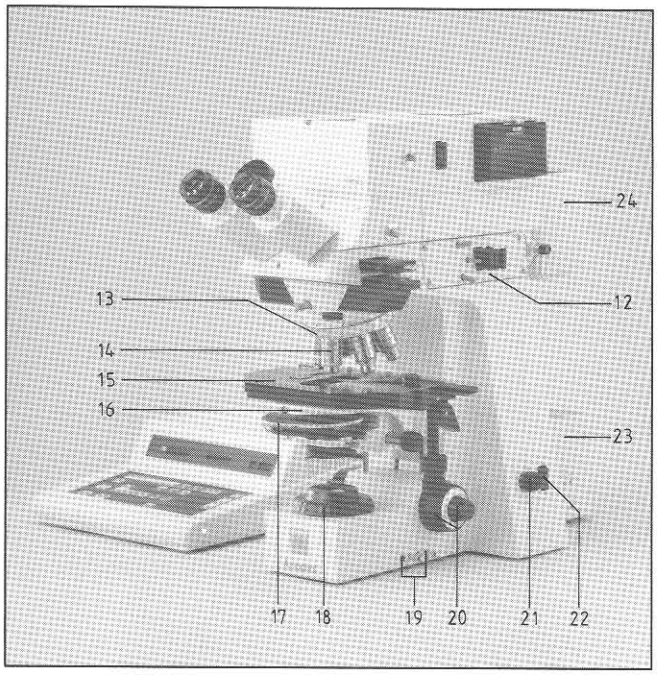

Camera components

1 4" x 5" camera
2 35 mm film cassette Mot
3 Camera system
4 Port for TV camera
5 Binocular tube
6 Eyepieces
7 Camera control panel

Stand head, stand head carrier with incident-light system FI

8 Slot for analyzer and Bertrand lens slider
9 Slot for Optovar slider and reflector slider FL
10 Slot for auxiliary objects
11 Slot for reflected-light polarizer
12 Incident-light system FI (for incident-light fluorescence only

Nosepiece, objectives

13 Nosepiece
14 Objectives

Stage components

15 Specimen stage
16 Stage carrier with condenser carrier
17 Condenser

Stand base

18 Luminous field diaphragm
19 Filter magazine
20 Coaxial coarse/fine focusing control

Lamp power supply and illuminators

21 Potentiometer for Hal illuminator
22 Power switch and signal lamp
23 Transilluminator
24 Incident-light fluorescence illuminator

Microscope stand, camera system and control panel are adapted to each other. All components bear equal numbers and are not interchangeable with the components of other instruments.

Contents

Special notes :

* The 6- to 10-digit numbers, e.g. 45 14 04, are ordering numbers of instruments or instrument components.
* Changes and/or repairs of the instruments should be carried out only by the manufacturer or his authorized representative.
* Specifications subject to change
* CAUTION: Do not operate the instruments in explosion-risk areas.

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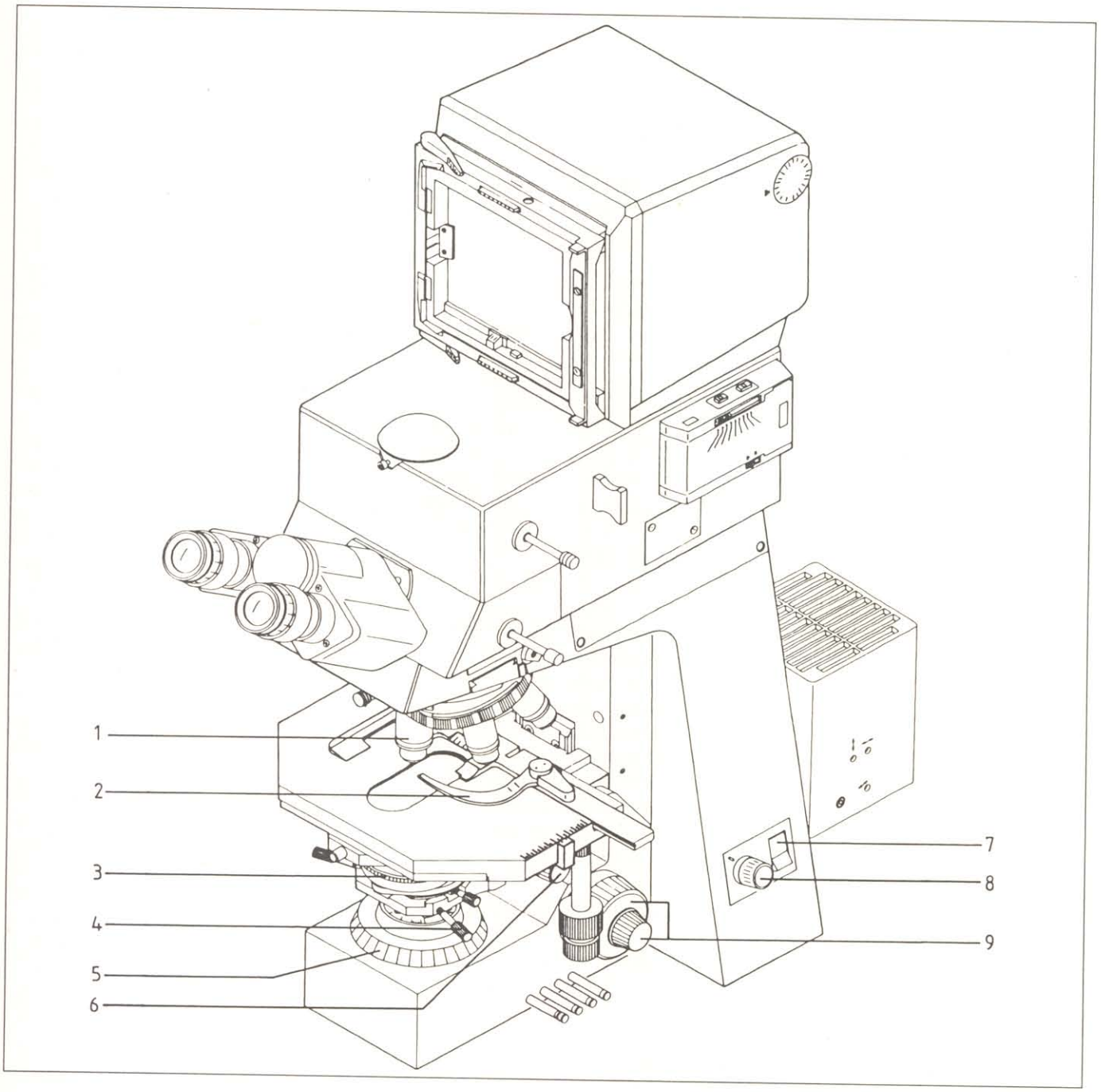

Note: Numbers like **1.1** refer to the description of the instrument starting on page 6.

- Check voltage on red display window (instrument back) and line voltage for coincidence. Plug in microscope and camera control panel power cables, select lower (or only) illuminator, switch on with (7) and set potentiometer (8) to 3 - 4 V.
- Load a high-contrast specimen on stage (smaller, thin coverglass face up!).
- Turn in 10x objective (yellow ring) (1) on nosepiece, check 0-positions on the eyepiece scale. Use (6) to move condenser up to the specimen carrier (front lens not swung out).
- Set index of condenser turret to H (brightfield) and close the diaphragm about half using (3).

You should now see light spots (the exit pupils) behind the eyepieces. (If not, beam splitter I is perhaps set to "all light directed to beam splitter II" with **8.1** or **8.2**).
When you look into the tube you will see a bright circle (the eyepiece stop) with each eye. Setting the two eyepiece tubes to your PD will merge the two circles into 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 → **6.6**).
- Close luminous field diaphragm (5) moderately; it will become unsharp (A).
- Focus the diaphragm image by lowering the condenser slightly with (6) (B).
- Move the diaphragm image to the center of the field of view with screws (4) (C).
- Open luminous field diaphragm (5) until it just disappears from the field of view (D).

The diameter of the luminous-field diaphragm can be read off the white scale (5) and reproducibly set using the white index mark on the knurled ring.
Dependent on what specimen is used, the contrast is adjusted using the condenser diaphragm (3). The setting of the aperture diaphragm can be read off the condenser scale.
- If you are not certain how far to stop down: approx. 2/3 of the rear element of the objective (visible at the tube bottom without eyepiece in the tube) should be illuminated if a specimen is of moderate contrast (E).

Field of view and objective aperture change, of course, with each objective exchange, so that the last-mentioned steps must be repeated.
As soon as a low-power objective images more than the condenser can illuminate, the condenser front lens must be swung out, either automatically by lowering the condenser, or with a lever. For a full description of the procedure see page 10.

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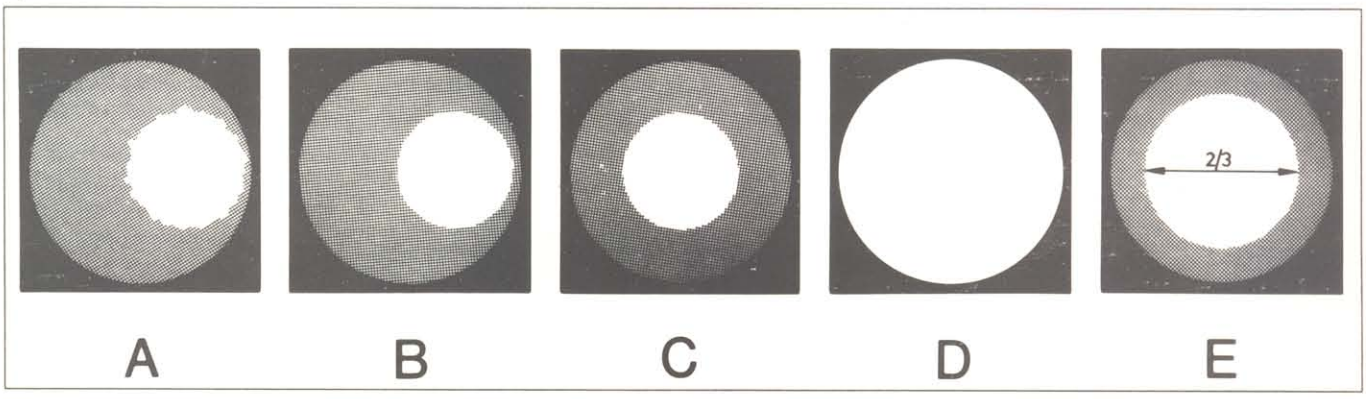

Special note:
Almost all screws you need are SW 3.0 mm or SW 1.5 mm Allen screws for which the tools with the red handles are supplied.

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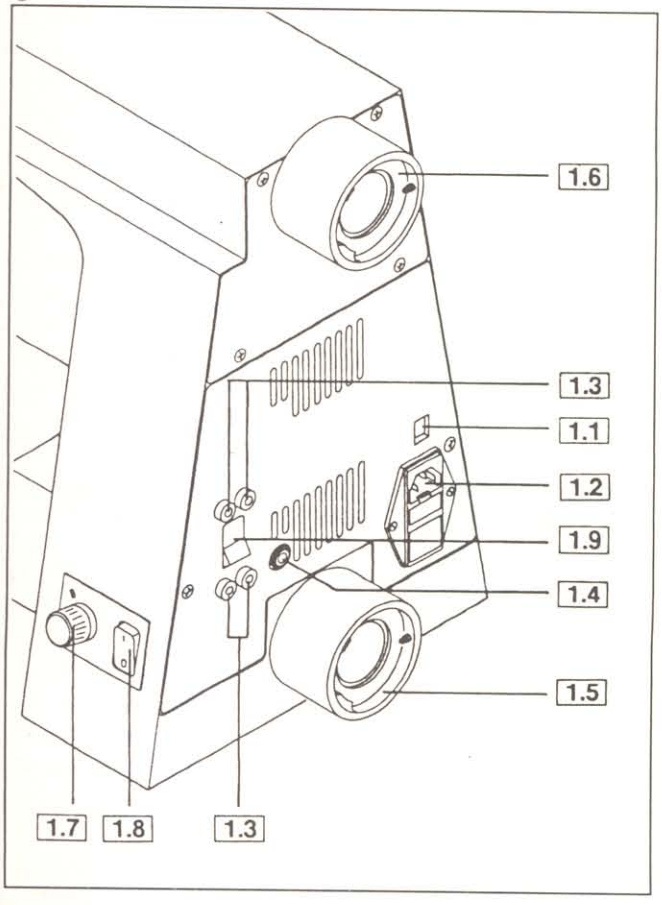

1.0 Lamp power supply

The power supply (45 18 89) for the Hal illuminator is integrated in the stand (45 18 10).

1.1 Data on display window: 230 V for 220. . .240 V AC or 115 V for 110 . . . 127 V AC.
If the local voltage does not coincide with the set voltage, the switch can be readjusted vertically using a screwdriver (switch off instrument first and pull cable out of socket).
The power supply is highly stabilized to power fluctuations, provides DC, variable from 3 . . . 12V and is suitable for demanding photometry. Power consumption max. 200VA.
The instrument is radio-screened and complies with VDE, IEC, CSA and UL regulations. It is categorized as a safety class I, type B instrument.

1.2 Power socket and below it, an insert with 2 fuses. Use a screw-driver to pull out the insert and change fuses.
Spare fuses:
230 V: T 2 A (No. 127.024)
115 V: T 4 A (No. 144.060)

1.3 Sockets for connection of 12V 100W halogen lamp; arrows indicate transmitted and reflected light.

1.4 Socket for control line of the camera control panel **9.3** to set the lamp to 3200 K (color temperature in color photography) independent of the position of potentiometer **1.7**.

1.5 Transilluminator port
It contains a tube with heat-reflecting filter and diffusion disk for uniform illumination. This tube can be removed to observe the lamp coil and reflector image in the pupil for lamp centration.

1.6 Reflected-light illuminator port. Holder for 42 mm dia. heat-reflecting filters is empty in fluorescence microscopy.

1.7 Lamp voltage setting potentiometer supplies 12V AC in stop position. The adjusted voltage can be read off on the index. Should any malfunction occur during operation of the 12V 100W halogen lamp, the lamp is automatically switched off electronically. Set lowest voltage and switch off the lamp using toggle switch **1.8**. Then switch the lamp on again and set the desired lamp voltage.

1.8 Power switch with signal lamp.

1.9 Toggle switch to change between transmitted and reflected-light illumination according to the arrows.
After the switch-over it takes a few seconds before the lamp lights.

2.0 Illuminator Hal

The standard equipment includes the Hal lamp housing (44 72 17- 9901) with reflector, collector, heat-reflecting filter and mount, and a 12V 100W halogen lamp (38 00 79-9540). Connection to sockets **1.3** as indicated by the arrow.

2.1 Light exit. Dovetail ring for mounting on the microscope:
- Unscrew screw at **1.5** or **1.6** sufficiently.
- Attach dovetail ring of illuminator in recess opposite the clamping screw in an inclined position, lower illuminator on to the seating surface and tighten the screw.

A holder in the light exit in front of the collector accepts a 42 mm dia. heat-reflecting filter; the holder must be empty if the illuminator is used for UV blue fluorescence excitation.

2.2 Clamping screw to secure the lamp housing.
2.3 Focusing of lamp coil.
2.4 Vertical adjustment of lamp coil.
2.5 Horizontal adjustment of lamp coil.

Adjustment of halogen lamp with reflector

The exchange of the lamp is described on page 42.

- Remove illuminator Hal from microscope.
- Switch on halogen lamp via power supply.
- Use screw **2.3** to project coil image on a wall or similar object approx. 3 m away.
- Use screw **2.4** for vertical adjustment of the coil image and **2.5** for horizontal adjustment. Move the coil image in such a way that the gaps of the reflector image are covered (see Fig.7). The fixed reflector need not be adjusted, since it lies exactly in the optical axis.

Fine adjustment:
- Remove internal tube with diffusion disk **1.5** and filter, if used, from light path. Attach microscope illuminator and focus on specimen using an objective with magnification 40x or higher. Find a vacant specimen position.
- When the eyepiece is removed or the Bertrand optics swung in, check whether coil image and reflector image are in center position. If not, make correction using the adjusting screws mentioned above.
- Replace tube **1.5**. Check homogeneous illumination of the pupil image and correct it, if required, using screw **2.3**.

The lamp housing HBO/XBO with HBO 50 mercury short arc lamp, which is described on page 17, is required for incident-light fluorescence.

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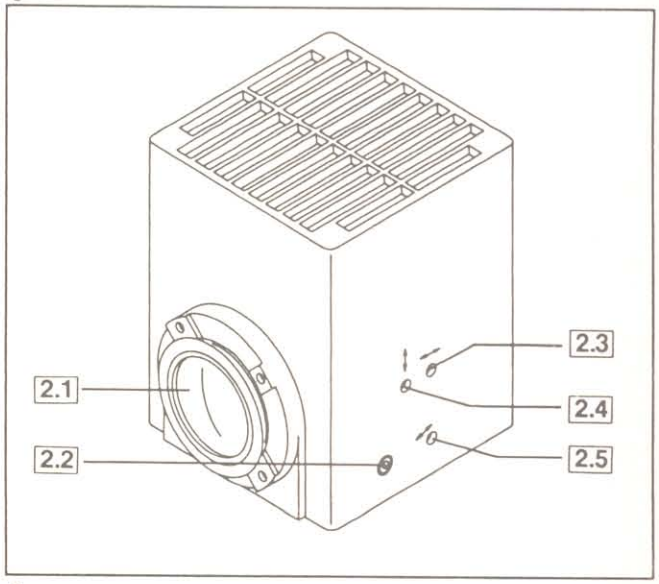

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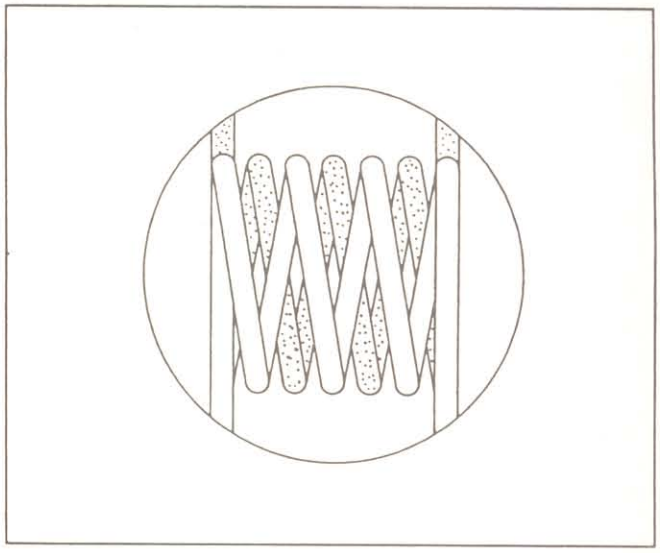

Technical data of the halogen lamp	(38 00 79-9540)
Lamp voltage, max.	12 V
Power	100 W
Color temperature at 11.5 V*	3200 K
Luminous flux	3100 lm
Mean life at 12 V	50 h
Luminous surface	3.2x3.2 mm

* 3200°K for artificial-light color reversal film is set automatically by pushing the key <LAMP/3200K> of the camera control panel.

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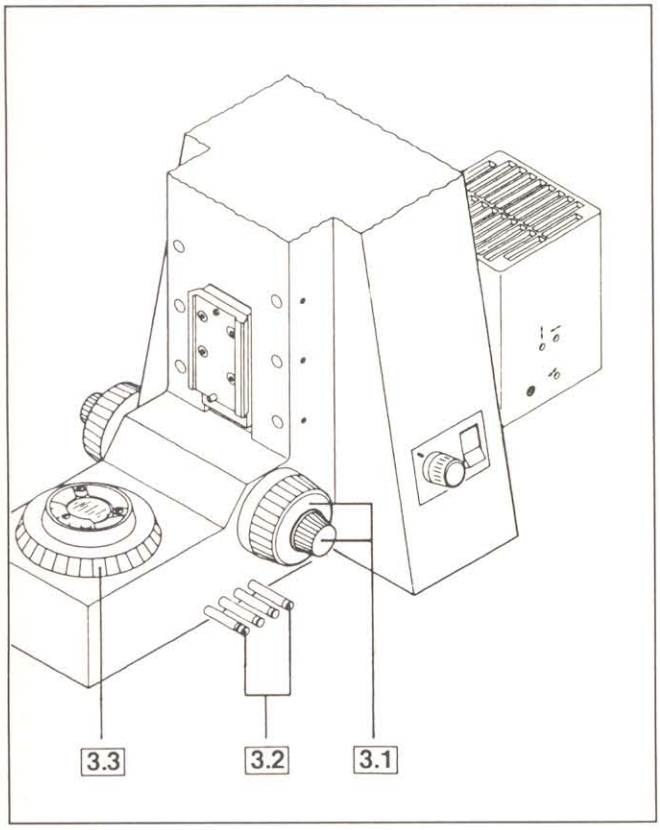

3.0 Stand base

The coaxial coarse/fine focusing controls **3.1** act on a plate with dovetails to which the stage carrier (with condenser carrier in transmitted light) is attached. Turning the outer part of the control anticlockwise lowers the stage. Total travel range (including fine focusing control): 25 mm.
- One revolution of the coarse focusing control corresponds to approx. 2mm travel; gear ratio of fine focusing control: 1:10. The index line of the coarse focusing control can be used for a rough measurement of the object thickness:
1 interval corresponds to approx. 2 μm.

3.2 Filter magazine in the illuminating beam path with four pushbuttons, from front to back:
- black and dark-gray ring: 32mm dia. neutral density filter 0.015
- dark-gray ring: 32 mm dia. neutral density filter 0.06
- light-gray ring: neutral density filter 0.25
- blue ring: conversion filter 3200/5500 K

The brightness is variable in steps via the neutral density filters, used singly or in sets. The transmittance of a filter set is determined by multiplication (e.g. 0.06 x 0.25 = 0.015, i.e. 1.5% transmittance).

The conversion filter converts artifical light of 3200 K into daylight of 5500 K.

If several filters are to be used at a time, the corresponding pushbuttons must be pressed simultaneously. Pressing the foremost button removes all filters from the beam path.

Exchange of filters in the magazine should be made by the maintenance service. (The bottom plate is removed. A filter - secured by a retaining ring - is accessible if all others are swung in).

The luminous field diaphragm **3.3** is adjusted by a knurled ring. The (removable) dust coverglass accepts a 32 mm dia. filter. This plane is not imaged.

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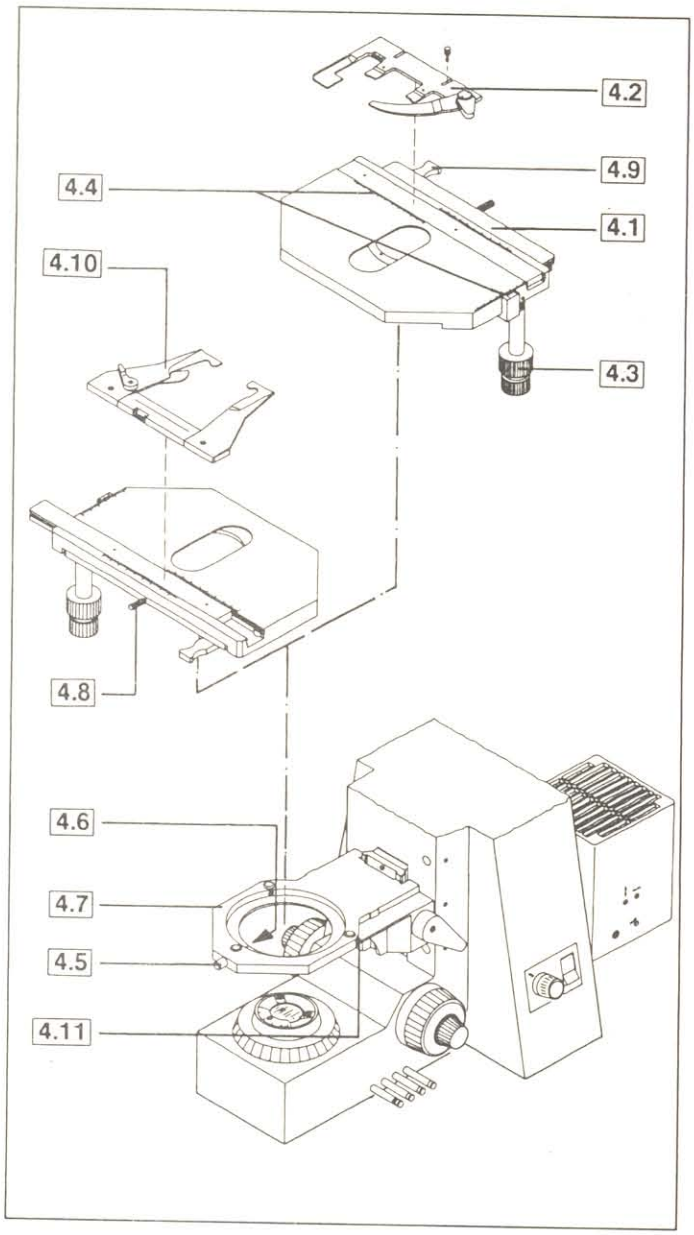


4.0 Specimen stage

Standard outfit includes:

4.1 Rotary centerable mechanical stage 75 x 50R/140° (45 35 02), with controls to the rear right.

4.2 Specimen holder with spring clip R (45 35 35) for standard (76 x 26 mm) or 45 x 26 mm specimen slides.

4.3 Coaxial controls for x,y movement with 75 x 50 mm motion range.

4.4 Graduations and verniers for the relocation of specimen areas.

To use the mechanical stage as rotary stage (to turn a specimen for photography, to optimize contrast in DIC, etc.), mount it in stage carrier **4.7** turned through 180°. Loosen screw cap **4.5**, pull stage forward (against spring pin **4.6**) and (starting at the back) take it out of stage carrier **4.7**.
Turn stage through 180° and remount it: press back spring pin **4.6** with the notch in the dovetails at the stage bottom; you can now put the stage down also at the back.
Tighten screw cap **4.5**.
If clamping screw **4.8** is loosened and the marked screw at the bottom of the stage removed, the stage can be turned through 140°.

4.9 Handle to turn the stage without accidentally moving the specimen.

4.10 This special specimen holder (45 35 37) is recommended for the mechanical stage (45 35 02) turned through 180° before mounting. Standard specimen slides can be slid into this holder from the front.

The stage is centered, which means that a feature once adjusted remains in the image center when the stage is turned. Should it be necessary to recenter the stage, proceed as follows: loosen screw cap **4.5** and plug small Allen key into

4.11. Correct migrations during stage rotation until the stage is correctly centered. Tighten **4.5**.

Other stages: stage carrier **4.7** also accepts a special circular stage for polarizing microscopy.
Scanning stages are firmly mounted on carriers of their own.

5.0 Condensers

5.1 Condenser carrier with:

5.2 Controls on both sides for vertical adjustment (max. 34 mm). The stiffness is factory-adjusted and should be changed only by the maintenance service.

5.3 Orientation notch for condenser.

5.4 Clamping screw for condenser (used only for condenser exchange).

5.5 Two screws to center the luminous field diaphragm image when the illumination is adjusted (see page 5).

To prevent the specimen from being pressed out by the condenser, the vertical condenser movement is limited by stop screw **5.6** which is adjusted as follows:
1. Adjust specimen (use a thick specimen slide).
2. Image the luminous field diaphragm (see page 5).
3. Move the condenser up by a small amount (diaphragm image becomes unsharp).
4. Loosen stop screw **5.6** with red Allan key - the stop pin will fall down - tighten it again. Now the specimen is protected from being touched by the condenser.

The available condenser systems meet the high demands made on the versatility of a large research microscope.
Standard condenser is
5.7 Condenser system (44 53 50) with brightfield insert.

5.8 Front lens, aperture 0.6 or 0.9.

5.9 Lever (on both sides) to swing the front lens in or out (for objectives 2.5x and 5x).

5.10 Knurled ring for aperture iris diaphragm with aperture scale below.

5.11 Fixing screw: loosen and lift it to allow the brightfield or any other insert to be removed. When inserting the condenser turret, press it slightly back and down so that the knurled screw can be turned in.

Illumination of large object fields (objectives 2.5x and 5x):
Without imaging of the luminous field diaphragm (Köhler illumination), the **5.12** diffusion disk can be plugged on the front lens (pointing to the left). If the front lens is swung out and the diffusion disk swung in, you need not lower the condenser. The aperture diaphragm should be fully open, which is of advantage for routine work requiring quick change between low-power and detail investigations.

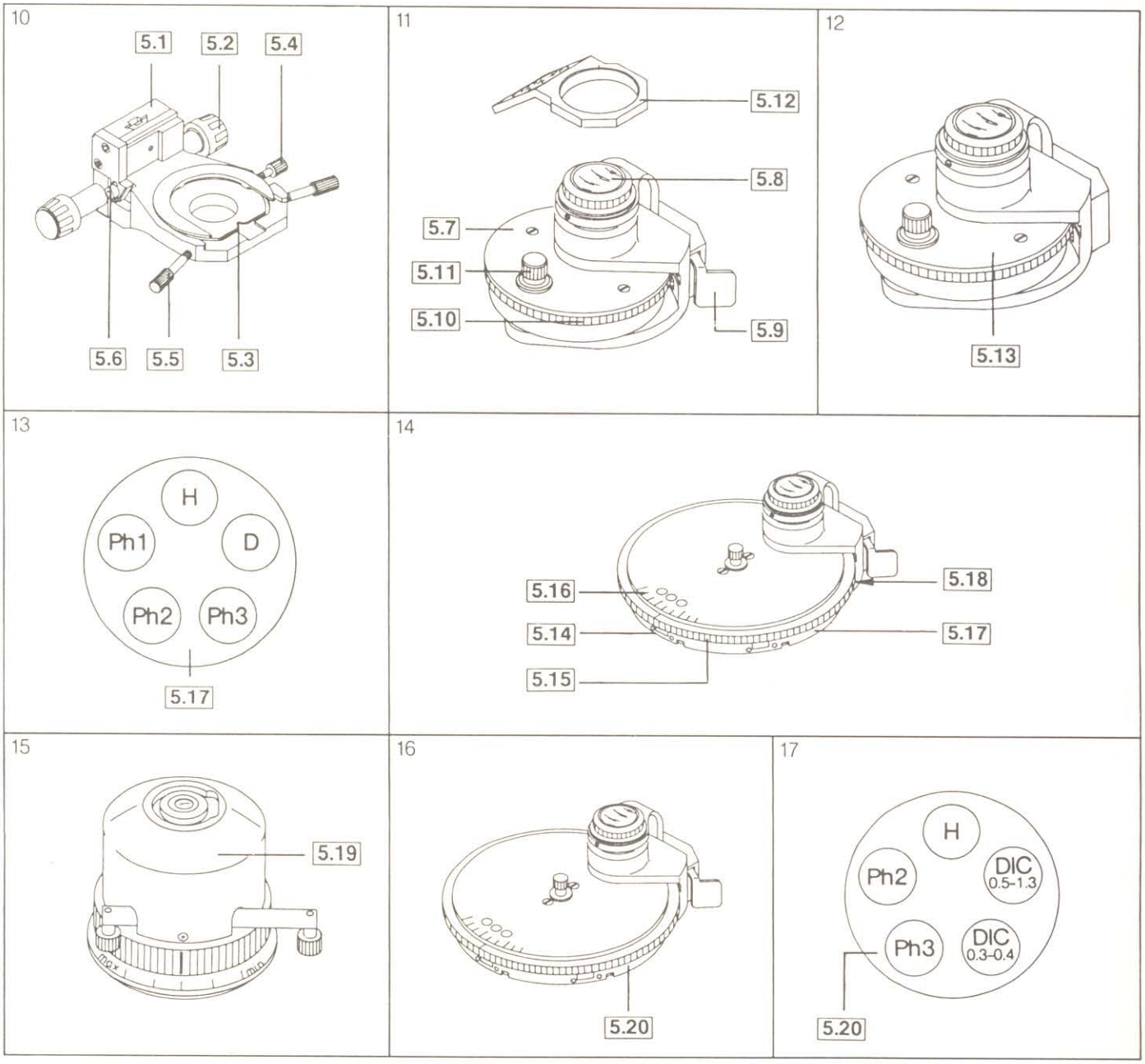
5.13 Condenser system (44 53 51). It corresponds to **5.7**, but the front lens is automatically swung out when the condenser is lowered. Lowering it further swings the front lens in again; it remains in this position when the condenser is moved up.

5.14 Index indicates the 5 positions of the condenser turrets allowing the quick change between different illumination and contrast-enhancement techniques.
5.15 Control for aperture diaphragm and
5.16 aperture display.

5.17 Condenser turret H D Ph (44 53 66), usually provided for
- brightfield (H)
- phase contrast 1 (Ph 1)
- phase contrast 2 (Ph 2)
- phase contrast 3 (and darkfield, if required) (Ph 3, D)

Centering of the annular phase and darkfield diaphragms is made with the supplied 2 keys through the
5.18 centering openings.
(The condenser system (44 53 50) has these openings before the right and behind the left lever **5.9**, when the front lens is swung in).

5.19 Achromatic-aplanatic switch condenser 0.5/ dia. 20 mm (44 53 40)
- Brightfield condenser for 1.25x to 40x objectives
- Numerical aperture 0.5 with swung-in front lens
- Diameter of maximum illuminated field: 20 mm
- Homogeneous illumination of field of view of objectives 1.25x . . . 5x with swung-out front lens and open aperture diaphragm.
- Köhler illumination for 5x . . . 40x objectives with swung-in front lens
- Ideal for large specimens, e.g. in pathology.



5.20 Condenser turret H D Ph DIC (44 53 65) (Fig. 17) with standard equipment for:

- brightfield (H)
- phase contrast (two annular phase stops, as required: Ph 1, Ph 2 or Ph 3)
- DIC 0.3 - 0.4
- DIC 0.5 - 1.4)

The operating controls are like **5.17**.

The turret Ph DIC (44 53 67) features 3 centerable positions, equipped with the annular phase stops Ph1, Ph2, Ph3, and 2 positions equipped with iris diaphragms for the corresponding prisms DIC .3 - .4 and DIC .5 - 1.4. Brightfield is produced in both DIC positions if the analyzer or the polarizer is removed from the beam path (see page 28).

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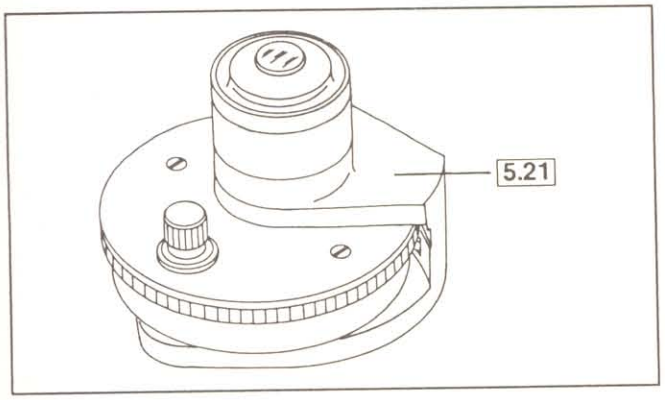

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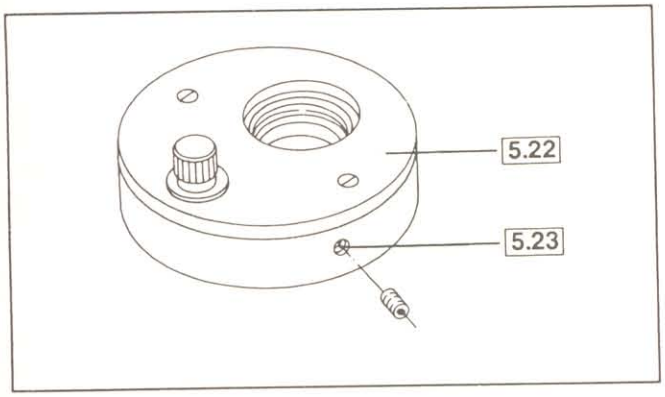

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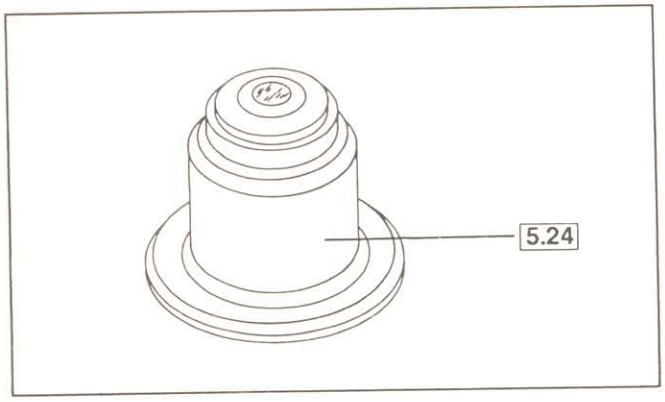

5.21 Condenser system (44 53 53 + 44 53 57) for maximum illuminating aperture (1.4). There must always be oil between its front lens and the bottom of the specimen slide. The front lens cannot be swung out, but can be unscrewed to illuminate the fields of objectives 2.5x, 5x and 10x (the aperture will then be 0.24). This condenser system is suitable either for brightfield or for brightfield and DIC.

5.22 Darkfield insert (44 53 63) contains the darkfield diaphragm 0.75/0.9 (44 53 99). It fits in all condensers for condenser turrets with front lens 0.9. It has no iris diaphragm, but can be centered with **5.23**.

5.24 Ultra darkfield condenser 1.2/1.4 oil (44 53 15) for maximum apertures in darkfield. The objective aperture should be lower than 1.2, or stopped down with the objective iris diaphragm.

Concerning DIC: Optical components with ordering numbers or aperture values in red are ideal for DIC, since they are virtually strain-free (Pol equipment).

Optical condenser data

Without front lens (swung out or unscrewed), all condensers have

- numerical aperture (NA) 0.24
- working distance of 23 mm
- luminous field of max. 11 mm dia.
- and are suitable for objectives 2.5x . . . 10x.

The values in the table below apply to condensers with front lens:

NA	Working distance	Object field	for objectives
0.6	6.8 mm	Ø 4 mm	10x . . .100x
0.9	0.8 mm	Ø 2.8 mm	10x . . .100x
oil 1.4 (air 0.9)	0.4 mm	Ø 1.9 mm	20x . . .100x

With 10x objective and in critical illumination, the exit pupil of the objective and the field of view can be optimally illuminated by focusing **2.3** and centering of the lamp.
As an alternative you may work without front lens, however, at the expense of a slight loss in aperture.

For the equipment of the condenser with various diaphragms (phase contrast, etc.) see the table on page 39.

6.0 Image-forming components

6.1 Objectives, the most important elements of a microscope, must be kept meticulously clean, especially their front lens surfaces.
The numbers and figures engraved on the objectives, e.g. Plan-Neofluar 20x/0.50 /0.17 signify:

20x (individual) magnification
0.50 numerical aperture (NA)
∞ image distance
0.17 computed for coverglass thickness 0.17 mm

(Individual) magification multiplied by the eyepiece magnification (generally 10x) results in the microscope magnification.
The numerical aperture multiplied by 1000 (500 in the above example) is the highest useful magnification; no more details will be revealed above this value. The numerical aperture is important in darkfield illumination for the choice of the darkfield diaphragms.

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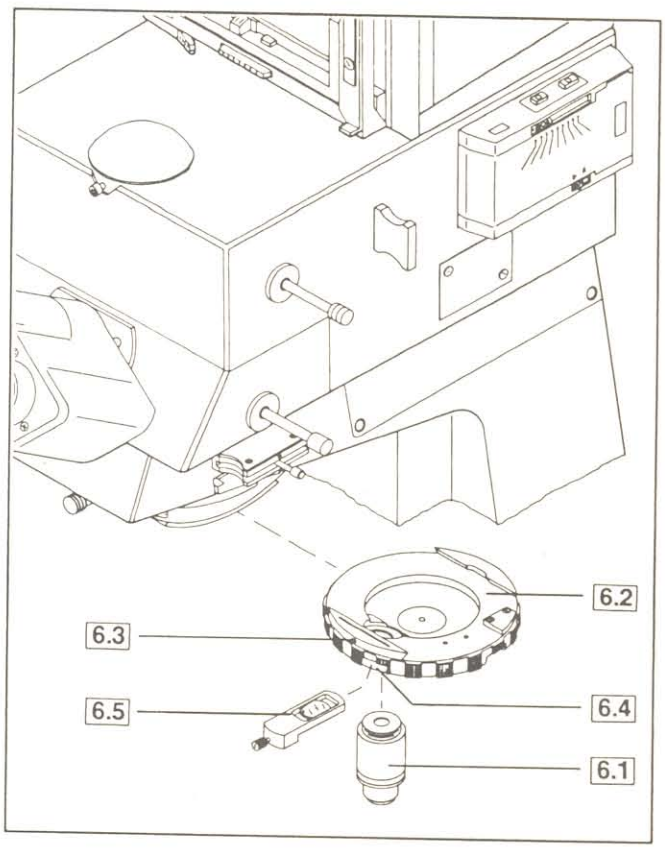

Symbol ∞ is to remind the user that these objectives cannot be used on microscopes with objectives bearing the number 160.

The coverglass thickness 0.17 mm for transmitted-light specimens must be the more observed the higher the numerical aperture of the objective. Certain objectives can therefore be adjusted to different coverglass thicknesses (correction mounts). Find out, by means of a high-contrast specimen feature, in which position of the correction mount optimum sharpness is achieved (refocusing will always be necessary).
Immersion objectives are insensitive to differences in the coverglass thickness.
Objectives 20x and higher have resilient mounts to protect the specimen. To prevent specimens from being contaminated by oil if the nosepiece with immersion objectives is turned, the objectives can be locked in the upper position by turning the spring mount to the right (don't forget to disengage them from "lock-in" position).

The air between the coverglass and an immersion objective is replaced by a liquid, generally immersion oil. Some experience is required to achieve a bubble-free layer. Some microscopists prefer to turn the objective from the side into the oil drop on the coverglass, others recommend to lower the objective from "lock-in" position of the spring mount.
Constant checking of the exit pupil, preferably using Bertrand lens **6.12** or by taking out the eyepiece (as described on page 5) is recommended, since this procedure instantly reveals any bubbles. If the bubbles do not disappear even if the objective is swung in several times, clean the specimen and repeat the procedure.

6.2 Nosepiece.
After loosening screw **6.3** it can be moved to the right and taken off (e.g. to check the front lens for cleanliness). (It cannot be detached if **6.17** is occupied.
If your microscope is also equipped for DIC, the knurled ring of the nosepiece 5x features
6.4 slots for the **6.5** DIC sliders. They must snap in when inserted (designation face up). (See also DIC adjustment on page 28). Even if you are not working in DIC, you may leave the DIC sliders in their slots (dust protection!), provided the polarizer beneath the condenser is swung out.

The sliders for neutral density filters N 0.08, N 0.15, N 0.30 and N 0.50 can be inserted in slots **6.4**. This allows the compensation of brightness differences within one series of objectives, e.g. in TV microscopy or microflash photography (see table on page 43).

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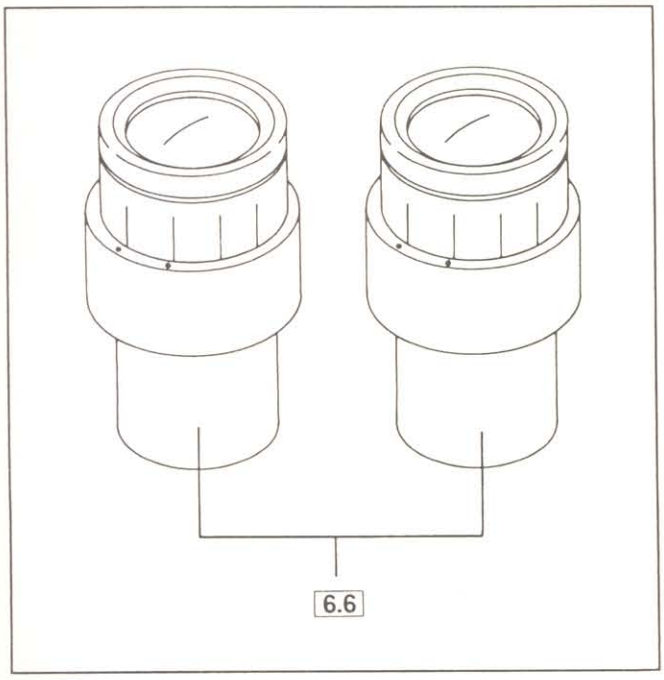
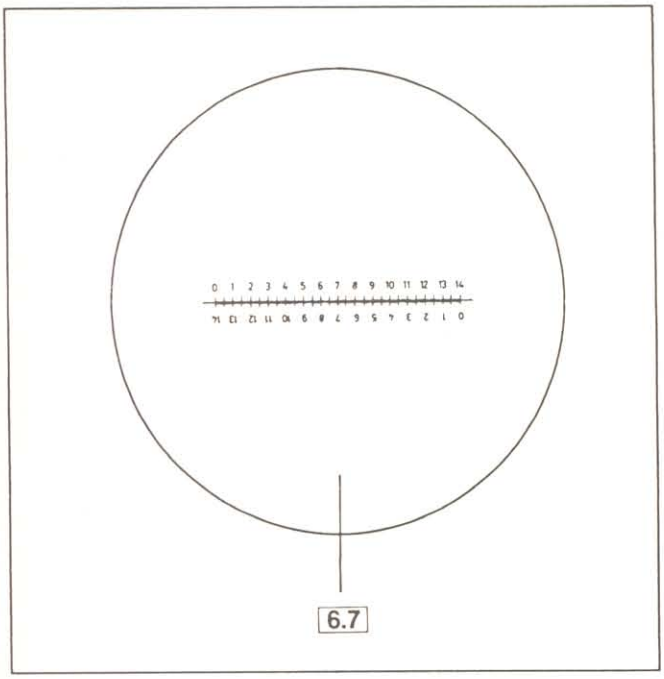


6.6

6.6 Eyepieces with 10x magnification and field-of-view number 25 or 16x magnification and field-of-view number 16 produce angular fields of 54°, are ideal for eyeglass wearers (Br), and feature a removable rubber ring to protect eyeglasses (folding eyecups - (44 48 01) - are also available). Both are focusing eyepieces (foc).

The eyepiece position is secured by a screw which engages a notch in the eyepiece tube. This is important especially for reticles. The diopter scale of the eye lens is set to 0 (white dot) for emmetropic users and eyeglass wearers.
If your eyes have different powers or if you want to work without eyeglasses, turn in the camera focusing reticle with key FRAME and adjust the focus for each eye.
Eyeglasses with a cylinder power must be kept on for microscopic work.
For critical work, especially at low magnification, it is recommended to plug the telescope attachment 3 x 12 B (52 20 12) on the eyepiece to focus on the camera focusing reticle and the specimen. The telescope attachment must be set to infinity for your eyes.

23



6.7

6.7 Reticles in the eyepiece diaphragm plane are used for measurement. They fit only in focusing (foc) eyepieces. The slight displacement of the image they cause is considered by the zero position on the diopter scale indicated by the red dot. Exchange of reticles should be left to specialists because of the high demands made on cleanliness and exact alignment. (The lower part of the eyepiece can be unscrewed; the scale-bearing surface of the reticle must face down).

6.8 Optovar slider D (45 19 90) with the factors 1x (middle), 1.25 and 1.6x, for quick magnification change fits into **6.9** if spring pin **6.10** was pulled out, which provides for the stops at either end and prevents the Optovar slider from being pulled out by mistake.
The adjusted magnification factor (1.25x or 1.6x) of the Optovar is displayed on the top surface of the handle, next to the slider port.

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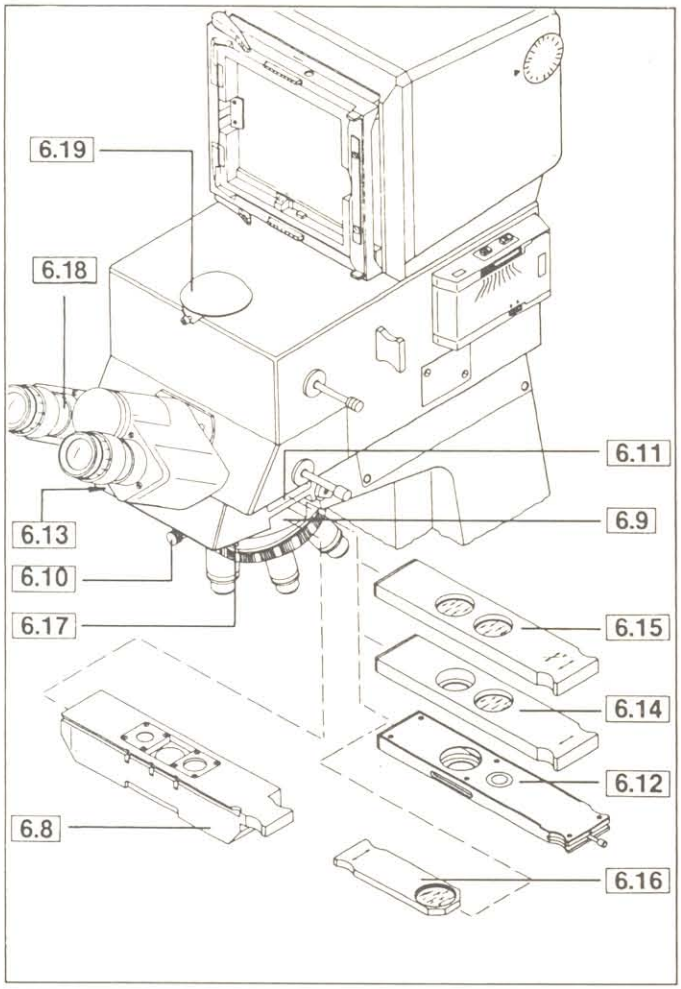

6.11 Slot for
6.12 Bertrand lens slider (45 36 70) for convenient observation of the objective pupil, especially for phase-contrast diaphragm centering. Loosen screw **6.13** visible on the front of the stand so far that the slider can be inserted, and tighten it so far that it moves smoothly between the stops. The Bertrand lens, which is focused with a lever, is brought into the beam path when the slider is moved to the left.

Accessories for DIC
For brightfield/phase contrast microscopy, slot **6.11** accepts the Bertrand lens slider **6.12** and for DIC it accepts the **6.14** analyzer (45 36 55) or a slider with
6.15 additional analyzer with lambda plate (45 36 56). White symbols ensure correct orientation of both components.
For the fast change between Bertrand lens (Ph) and analyzer (DIC), we offer both in a set: **6.12** (45 36 70) and **6.16** (45 36 65); the analyzer in the Bertrand lens slider can be swung in and out to the left, offering:

- free aperture (sliders pulled out to the left and right)
- analyzer in beam path (left slider pushed in)
- pupil observation (right slider pushed in)

6.17 Slot for auxiliary objects and compensators.

6.18 Binocular tube 25 firmly mounted on the camera system. PDs between 55 and 75 mm are adjusted by turning the tube halves in and out. Write down your PD if the tube is used by several persons.

6.19 Port for TV or special camera. TV and cine cameras with C-thread can be mounted on this port using TV adapter (45 29 95). This adapter can be parfocalized with the built-in reticle.
TV Cameras with ENG bayonets can be attached using adapter 1x for 3T CTV (45 29 94).
For the mounting of a second TV camera, see page 37.

7.0 Fluorescence equipment

The fluorescence version of your microscope includes reflected-light equipment Fl (44 64 40). Further components are:
7.1 Fluorescence illuminator with collector and HBO 50 mercury short-arc lamp supplied from a separate power supply (39 26 42).

7.2 Three filter slots for sliders on the side of the microscope. The rearmost slot is generally used for a slider which either interrupts the illumination beam path (fully inserted), brings a red-attenuating filter BG 38 into the beam path (middle position, eliminates disturbing IR light) or provides free aperture. The other two slots accept the filter slider A with one filter position (dia. 18 mm) for an additional exciter filter. A heat-reflecting filter KG 1, which does not affect UV excitation, is invisible from the outside.

7.3 Lever for luminous field diaphragm.
7.4 Centering screws to adjust luminous field diaphragm.

7.5 Reflector slider 3 Fl fits like Optovar magnification changer **6.8** in transmitted-light brightfield and, like **6.8**, has three positions: the middle position is left free for brightfield or phase contrast, the others accept suitable exciter filter/chromatic beam splitter/barrier filter sets.
Stick-on lables in fields I, II and III on the top surface of the reflector slider show the designations of the built-in filter sets. For further details please see page 41. If your microscope is retrofitted for fluorescence microscopy, the reflector slider 3 Fl will be supplied with two **7.6** sheet-metal covers which protect the fluorescence filters from dust; these covers must be inserted in the analyzer slot.

The barrier-filter slider (45 19 81) accepts additional dia. 25 mm barrier filters for specific fluorescence methods. It is inserted in slot **6.11**.

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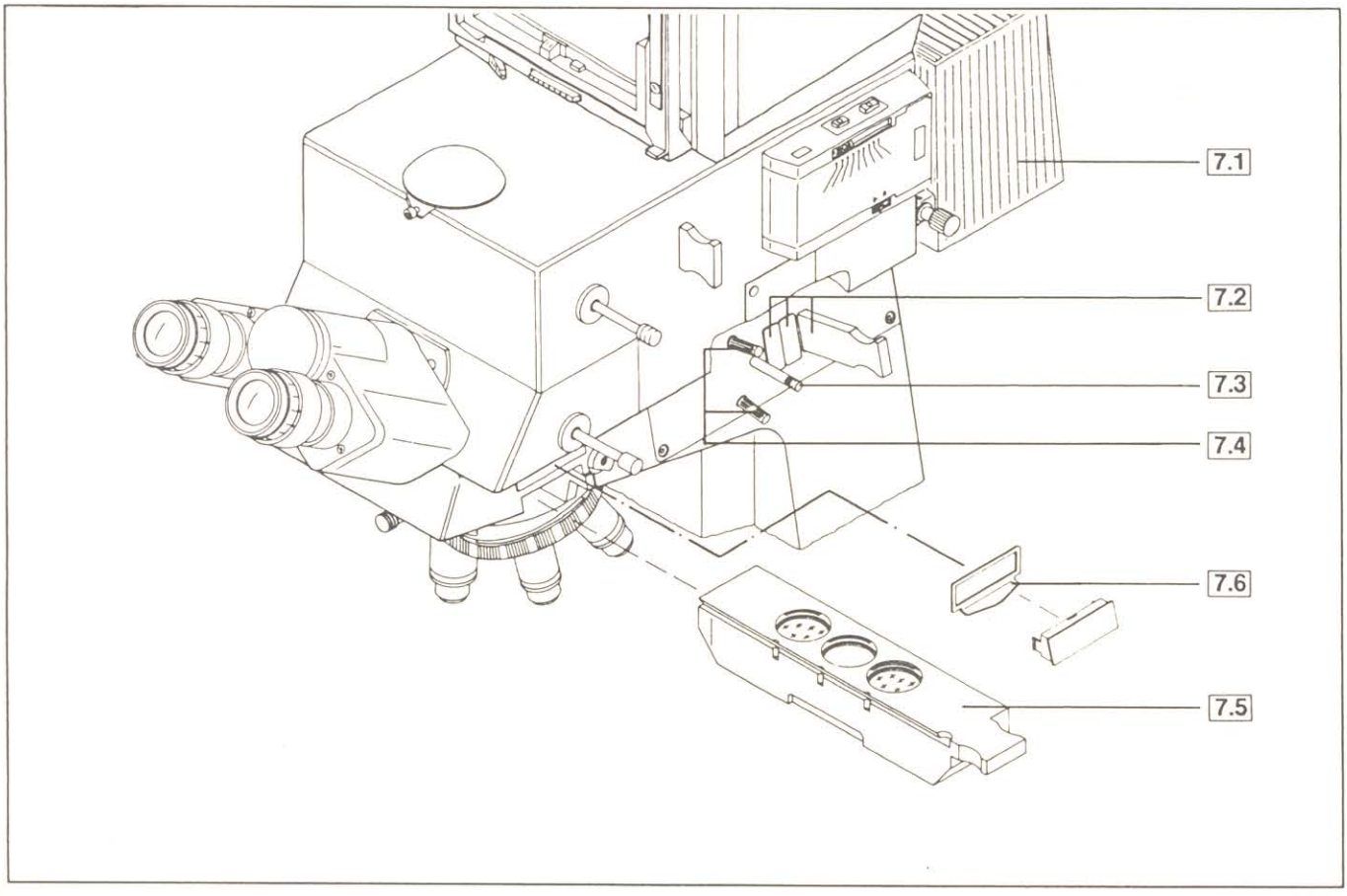

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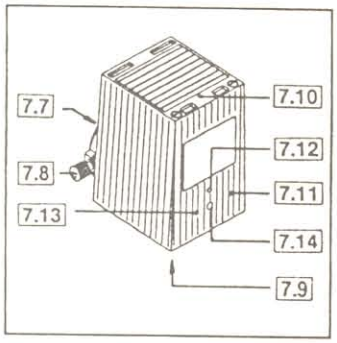

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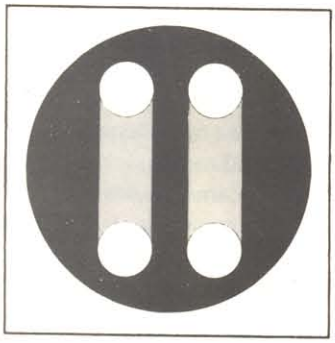

Fluorescence illuminator

The exchange of the HBO 50 mercury short arc lamp is described on page 42. The HBO/XBO lamp housing has the following features:

7.7 Light exit with dovetail ring to mount the illuminator on the microscope. Loosen screw for attachment of illuminator by a few turns.
Insert dovetail ring of illuminator in the mount opposite the clamping screw, then lower illuminator on to the contact surface and tighten the screw.

7.8 Knob for collector adjustment.
7.9 Clamping screw for lamp socket (concealed at the bottom of the housing).
7.10 Vertical lamp adjustment.
7.11 Lateral lamp adjustment.
7.12 Vertical adjustment of reflector image.
7.13 Lateral adjustment of reflector image.
7.14 Focusing of reflector image.

To center the HBO 50, proceed as follows:

- Use screwdriver SW 3 to remove switched-on illuminator carefully from the microscope.
- Use knob **7.8** for collector adjustment to image the brighter of the two light arc images in focus on a wall approx. 3m away. To prevent damage to the eye by UV radiation, avoid looking into the light spot of the ignited lamp for too long a period.
- Use screwdriver SW 3 to set the light arc image to the center via adjusting screws **7.10** and **7.11**.
- Bring the light spot in focus using adjusting screw **7.14**; take care that lamp image and reflector image have the same size. Then use adjusting screws **7.12** and **7.13** (marked red) to adjust the reflector image and the real image side by side. The distance between the lamp image and the reflector image should approximately correspond to the width of the light arc (see Fig. 27).
- Attach illuminator again and tighten clamping screw **1.6**. Open Slider **7.2** and set reflector slider to blue excitation (e.g. filter set (48 79 09) for FITC fluorescence).
- Unscrew an objective and check the image of the light source on a sheet of paper placed approx. 20 mm below the empty orifice of the nosepiece.
- Make corrections with knob **7.8** and adjusting screws **7.10** to **7.14**. Screw in objective again.

The HBO 50 mercury short arc lamp must be exchanged after expiration of the mean lifetime of 100 hrs. Its illuminance decreases in the course of many hours of use so that homogeneous illumination of the object field can no longer be guaranteed. There is also a danger of explosion. The remaining lifetime can be read off on the power supply unit.

Technical data of the HBO 50W/AC mercury short arc lamp

HBO 50W/AC	38 16 19
Lamp voltage	L1: 39. . .45 V / L2: 34. . .39 V
Lamp current	L1: 1.30 A / L2: 1.45 A
Power	50 W
Line spectrum	
Mean luminous flux	2 000 lm
Mean life	100 hrs
Luminous area	0.3x1 mm^2

L1, L2: HBO 50W/AC lamps are produced in two versions. The lamp type is given on the enclosed label. Set power supply to lamp type L1 or L2.

For more details and specially the important safety provisions we refer to the manual
G 42-160 Microscope lamp HBO 50 for fluorescence

8.0 Camera system

The integrated camera system is the most outstanding feature of the Axiophot.

The most important technical features:

- Two 35 mm and one large-format camera with automatic exposure control, and (with 35 mm cameras): automatic film advance, automatic trailing of newly loaded film and automatic rewinding.
- Decimal display of exposure time, down counting during exposure.
- Exposure automatically extended for longtime exposures (compensation of reciprocity failure) in 9 steps.
- Spot or integral measurement.
- The automatically determined exposure time can be stored for repetition.
- Multiple exposures.
- Exposure adjustment within a range of max. 3 shorter and 2 longer exposure values, with a minimum interval of 1/3 exposure value.
- Automatic exposure series with varying exposure times, for calibrations, etc..
- Binoculary visible luminous frame of variable brightness.
- Each negative can be imprinted with data and scale bars.

The 35 mm film cassette Mot uses normal cartridges 135 (visible in window), the 4" x 5" camera back accepts sheet film cassettes, Polaroid sheet film and film pack cassettes (545 and 550) as well as roll film cassettes on plate for universal camera back.
Image scale on film = objective magnificaton multiplied by:

2.5 for 35 mm film
10 for large format 9 x 12 cm/4" x 5".

Attachment of the **Axiophot** camera system to the microscope

- Screw out both pins (4) using a screwdriver.
- Screw out retaining screw (1) until it is no longer visible in the dovetail mount.
- Lift camera system by the sides with both hands.

Insert camera system in the tube mount via the dovetail and align it parallel to the stand edge.

- Move camera system until the thread (3) in the stand becomes visible.
- Insert screw and washer in the camera system and the thread of the stand, but do not tighten it fully.
- Tighten tube retaining screw (1).
- Tighten back screw (2).
- Plug the connecting cable into the camera system and into the camera control panel, then screw the connectors tightly in position.

Caution: Plug in or remove cable only if instruments are disconnected from the power line.

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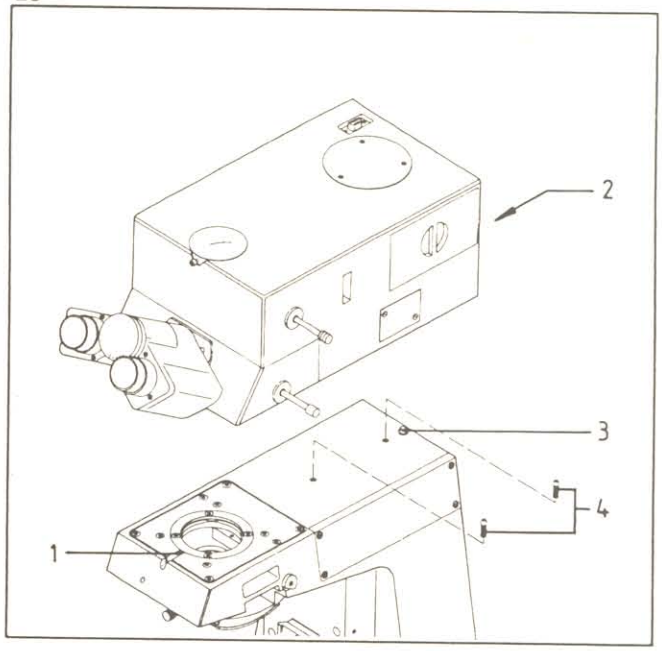
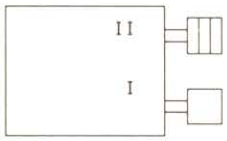


8.1 Beam splitter I
Pushrod pushed in: observation only
(100% of the light to the tube).
Pushrod pulled out to 1st stop:
20% of the light for observation
80% to beam splitter II.
Pushrod pulled out all the way:
No observation through the tube,
100% of the light to beam splitter II.
Light shutter on the eyepiece side prevents straylight from entering.

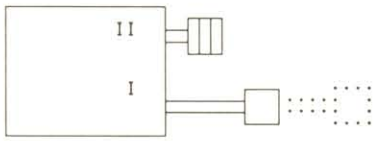
8.2 Beam splitter II
Pushrod pushed in: all the light relayed to the cameras
Pushrod pulled out to 1st stop: 50% to the cameras
50% upwards (e.g. TV)
Pushrod pulled out all the way:
all the light relayed upwards (for TV, etc.)

Standard positions:

Observation only



Observation and photography:



8.3 Slider with scale bars

The slider **8.3** has two stop positions.
Outer stop position (symbol visible):
reference distance is available for exposure;
Slider pushed in: reference distance not available for exposure.

The slider with reference scale (45 19 55) is part of the delivery package of the Axiophot camera system. The reference distance of 1000 µm can be imaged on the film together with the object. Its length in the object is calculated as follows:

$$\text{reference distance} = \frac{1000}{V_{Obj} \times F_{Optovar}} \; [\mu m]$$

Example: Plan-Apochromat objective 20x/0.60 and Optovar 1.25x

$$\text{reference distance} = \frac{1.000}{20 \times 1.25} = 40 \; [\mu m]$$

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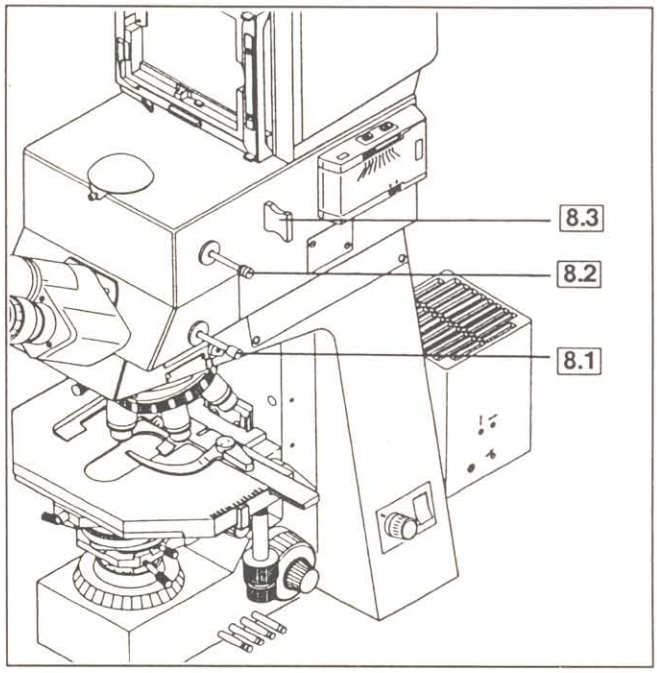

Sliders with scales for special objective magnifications
The reference distance with length indication is imaged on the film together with the object. The length in the object can be seen directly on the photograph. The slider is marked with the reference distance in µm and the objective magnification (valid only for Optovar factor 1x).
Sliders with reference scale are available for the following objectives:

5x/200 µm	45 19 52	40x/25 µm	45 19 56
10x/100 µm	45 19 53	63x/16 µm	45 19 58
20x/ 50 µm	45 19 54	100x/10 µm	45 19 59

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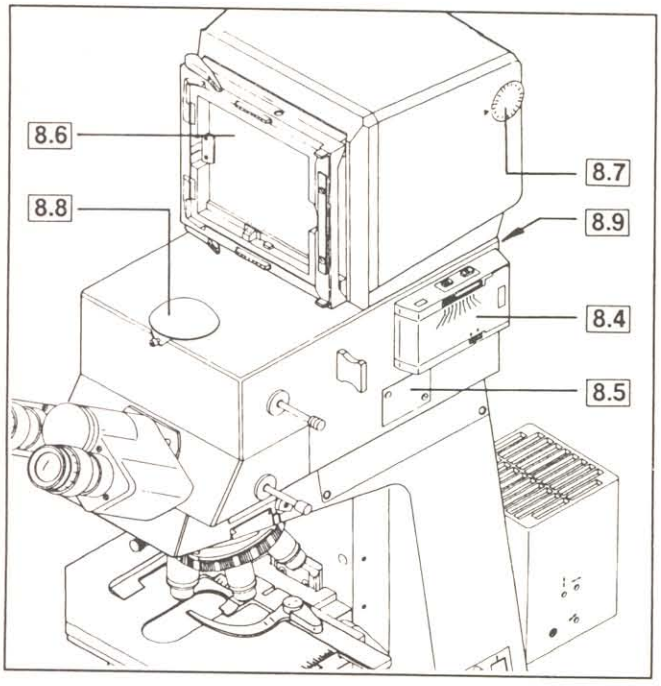

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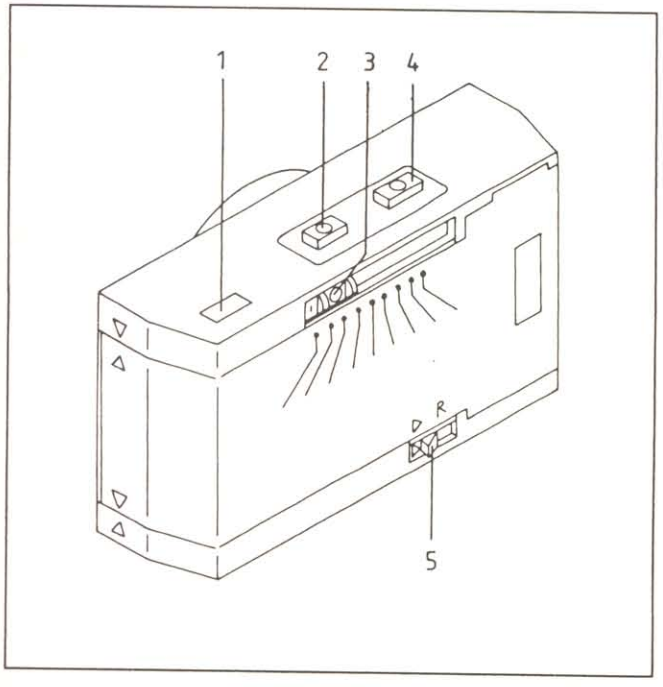

8.4 35 mm film cassette Mot.

Detaching the cassette: To detach it from the instrument, press key (4) (Eject) and pull off the cassette. Setting the film speed: (3) can be shifted when (2) is pressed. The adjusted ASA value is automatically transferred to the control panel.

Loading the film: push lock (9) (bottom) in the direction of the arrow. The cartridge is ejected, the back can be removed. Remove any particles in the film cassette with a soft brush. Load cartridge in (8), press down (9), insert film leader in slot (7); the sprocket teeth must catch the perforation; tighten film by turning the take-up spool (6) outward (possible only if rewind slider (5) is set to R; let left side of camera back engage (arrows) and lock in its right side. Mechanical counter (1) is set to S (Start).

Mounting the cassette: hold the cassette on the sides and press it on the exit port. The contact pins serve to guide the cassette into the port. With control panel switched on (camera selector to this specific port), the leader is advanced automatically; the counter is set to 0.
The ASA value is digitally displayed on the exposure control while the trailer is being advanced and for 3 s afterwards. The instrument then goes to the previously adjusted operating mode. After exposure (and data imprint if a data back is used) the film is advanced, and the frame number displayed on the mechanical counter (1) of the cassette.
When the end of the film is reached, the film advance is turned off, and the display *END* on the exposure control flashes.

Rewinding the film: the film is automatically rewound when the slider R (5) is operated; the display *END* flashes.
Automatic reset when the camera back is attached. When the film is unloaded while the camera back is re-attached, slider R springs automatically to normal film advance position.

Data back (45 60 74) for 35mm film cassette Mot :
Like the normal cassette back, it is mounted on the right side of the camera module.
Year/month/day/hour/minute are set. (For details see G 42-402, operating instructions for the data back.)

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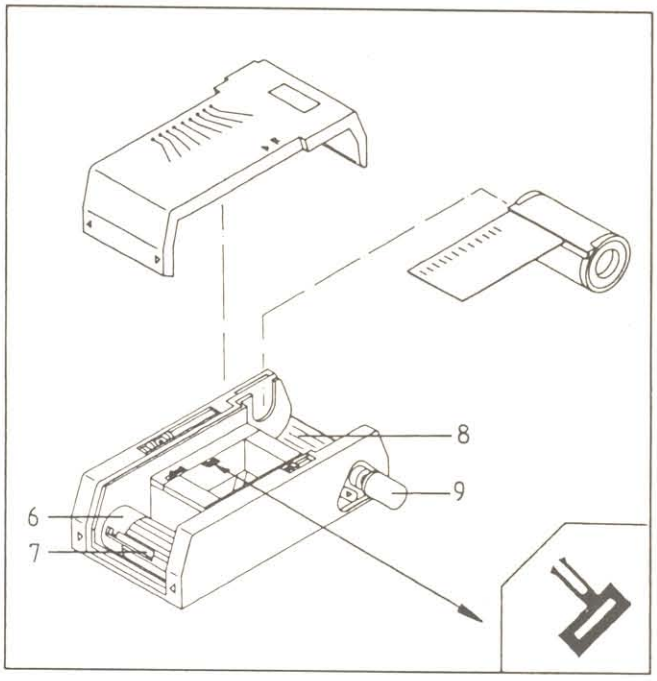

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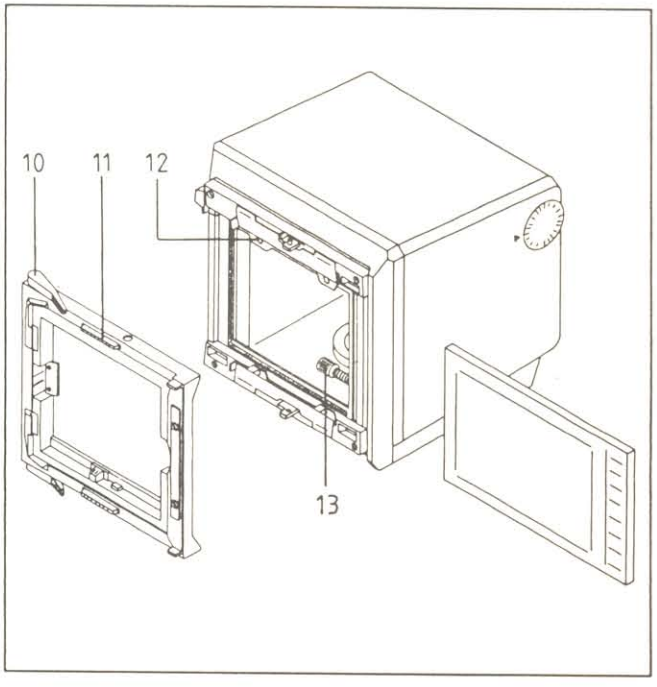

Mask for data projection
A mask with a reference bar can be projected on the film when the 35 mm film cassette Mot is used. The data from the data back (45 60 74) (if available) is projected on a dark background directly above the bar.

Removal
The mask can be easily removed, if not required:
- open film cassette (back)
- pull out mask carefully in the direction of the arrow (see sketch)

Insertion
- open film cassette (back)
- insert mask carefully in the slot as far as it will go, with the longer side facing the film advance spool, as shown in the sketch.

Reference scale
The mask contains a 5 mm long, bright window as a reference scale, which is projected on the film together with the object. The relevant magnification, with usual camera factor 2.5x, is obtained as follows:

$$M = \frac{2000}{M_{Obj} \times M_{Optovar}} [\mu m]$$

M_{obj} and $M_{Optovar}$ are the magnifications of the objective used and of a possibly used Optovar system.

8.5 Replacement of filament lamp for luminous frame illumination

8.6 Large-format groundglass and cassette holder.
Cassettes for universal camera backs are slid behind the large-format groundglass wihich can be lifted with lever (10). To take off the groundglass: press (11) and move it to the right; mounting is made accordingly. Most cassettes are not held by the groundglass but clamped with bolts (12).
Microprojection for small audiences:
Push 4"x5", T and START on the control panel to open the shutter for observation; pushing START again closes the shutter.

8.7 Film speed setting of large-format camera:if 4"x5" lights, the adjusted ASA value is displayed on the control panel.

Knurled screw (13) for the positioning of data on different film formats becomes accessible on the lower right after removal of groundglass **8.6**. Control of positioning on the groundglass (ASA 25: lights up for 1 s).

8.8 Port for TV camera, see **6.19**.

8.9 Connection of camera control panel, see page 22.

9.0 Camera control panel

9.1 Power input. The voltage setting (white pin pointing to one of 4 voltages) must coincide with the local line voltage.
A change is made as follows:

- Pull the power cable.
- Put a small screwdriver or similar tool in the recess (1) between jack and fuse plate and lift out plate.
- To the right in the small slot at the black part you can now pull out a square board (2). The adjusted voltage is indicated on the board opposite the black plastic part (3).
  The other available voltages are imprinted on the remaining 3 sides.
- Shift the plastic part (3) so that it engages the recess opposite the required voltage.
- Slide the board into the slot, legend facing to the left. Put on the fuse plate; the white pin will now indicate the correct voltage.
  The correct fuse must be inserted (see page 43).

9.2 Power switch.
Settings when switching on:

- Selection of camera last used
- The corresponding values of counter (COUNT), ASA, reciprocity code number (RECIPR) are stored or automatically set (ASA, COUNT).
- Automatic exposure control (averaging) AUTO .
  0 is set automatically with EXPOSURE ADJUSTMENT.

9.3 3-pin socket for control line to **1.4** for halogen lamp setting to 3200 K.

9.4 2-pin socket for remote control (see page 43).

9.5 4-pin socket for flash synchronization.

9.6 Connection to camera system **8.9**. Insert connector into socket and tighten screws.
Plug or pull bridging connector only when the instruments are disconnected from the line.

9.7 Camera selectors:
35 R 35 mm cassette to the right
35 L 35 mm cassette to the left
4" x 5" large-format camera 9x12 cm/4"x5".
The following values remain stored for each camera port:

- ASA (film speed setting)
- RECI (reciprocity code number)
- COUNT (frame counter setting)

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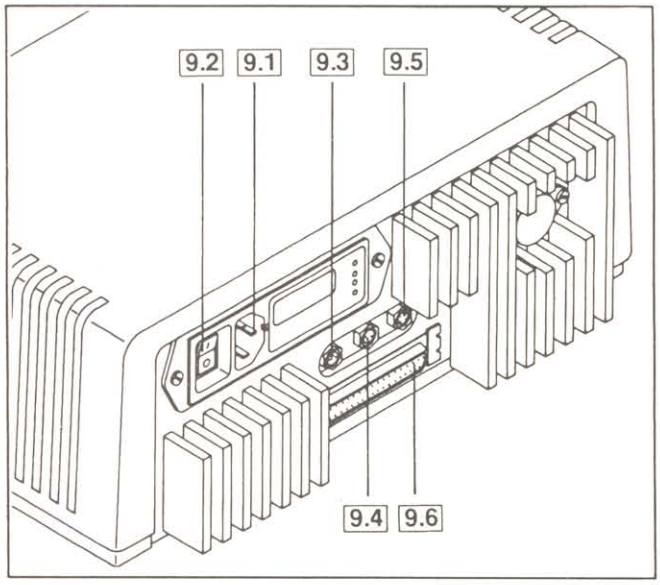

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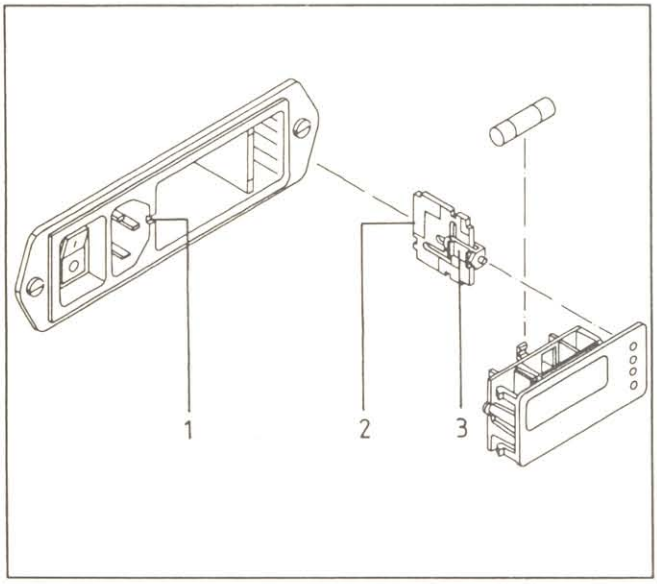
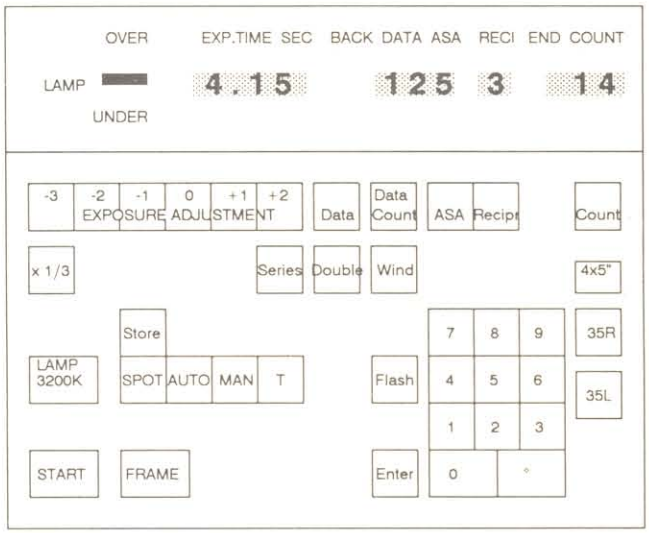


Keys and displays



For better recognition and easier differentiation all key designations are capitalized below (e.g. START), and all display designations capitalized and italicized (e.g. *OVER*).
Light in a display or key indicates that a function has been activated or is in a specific state. A flashing light reminds you of something, to make or end an input, or similar operations.

Before we come to a full description of the use of keys and displays, here are the two most impotant operating controls:
- Power switch of exposure control **"I"** ON provides for basic setting.
- Operation of key **START** : exposure.

The basic setting is displayed as follows:

AUTO lights: the automatic exposure control for integral measurement is ready. *EXP.TIME* gives the exposure time.

0 lights for EXPOSURE ADJUSTMENT.
The exposure adjustment is automatically set to 0.

START

Release key for exposure.
Film advance follows for 35 mm film.
The time displayed by *EXP.TIME* counts down to 0.
Exposures are not possible if:
- no film is loaded,
- the end of the film is reached,
- the film is advanced or rewound,
- an input has been started,
- the light in any of the keys flashes because a setting is not terminated (exception: exposure is possible even if the light in the key DOUBLE flashes).

If START is pressed after T , the shutter opens and is closed after pressing START again.
EXP.TIME displays the time the shutter is open in full seconds.

LAMP 3200K

The power supply switches the 12 V 100 W halogen lamp to the color temperature 3200 K required for color photomicrography.
(An additional conversion filter is required for daylight film; 3200 K → 5500 K .) A neutral density filter should be used to prevent glare.
Pushing the key again cancels the 3200 K setting.

FRAME

The illuminated format reticle is visible as luminous frame. The light goes out automatically during exposure. The brightness is adjustable to the image brightness: holding the key down changes the brightness continuously; releasing the key fixes the actual setting.

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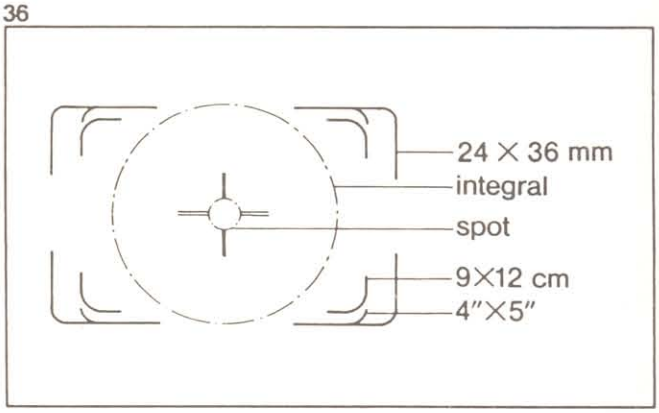
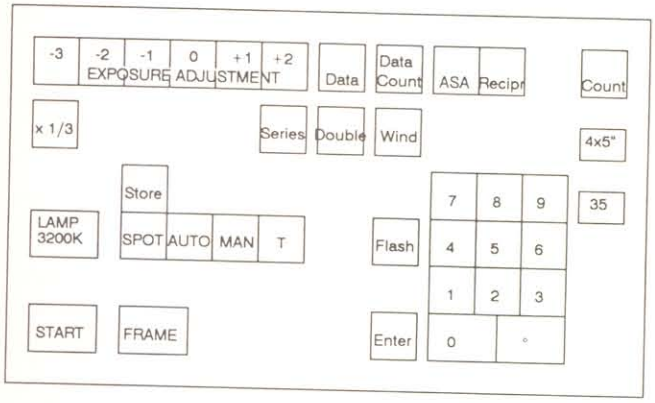
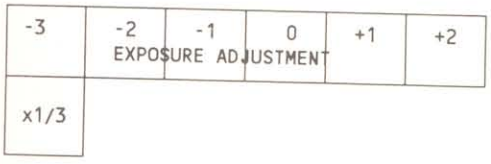


Further keys and displays





-3, -2, -1, 0, +1, +2 EXPOSURE ADJUSTMENT
Exposure adjustment in steps of whole exposure values.
0 is for medium contrast of the specimen. Adjustment to 0 is, therefore, automatically made when the instrument is switched on.

+1 the exposure time is extended by 1 exposure value, i.e factor 2
+2 = factor 4
-1 = factor 0.5
-2 = factor 0.25,
-3 = factor 0.125.

Example: +1 means that the exposure will be 1 exposure value longer than recommended by the automatic exposure control (the time is doubled, the density of the negative will be higher, positives and Polaroid pictures brighter).

x1/3 Exposure adjustment in steps of 1/3 exposure values. For the fine adjustment of exposure times for film of very high gradation (phase-contrast exposures).

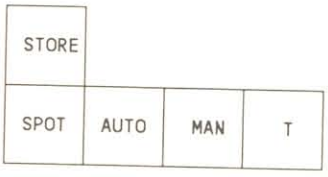
Applications:
1. General rule for **exposure correction** with average metering in the following contrasting techniques :

Brightfield:	**+ 2 . . . + 1**
Phase contrast, DIC:	**0**
Fluorescence, darkfield:	**- 2 . . . - 1**

2. If you are not sure whether critical object features will be optimally projected or printed, use also longer or shorter exposure times in addition to the one automatically measured. This can be easily made with SERIES (see the description of this key) for automatic exposure series.

3. The automatic exposure control converts the brightness of an object into mean brightness of the image. The exposure adjustment informs the instrument whether the object field is very bright or very dark, as the instrument cannot identify an object as exceptionally bright or dark. The exposure adjustment prevents, for example, a bright object from becoming too "gray" in the slide because of underexposure. If the exposure time is doubled with the exposure adjustment +1 , a bright object will be bright also in the image. Corresponding minus values must be adjusted for dark objects.

In general,the exposure time
- in brightfield should be adjusted with 0 to +2 ,
- in darkfield and fluorescence with -1 to -3 .



STORE stores the exposure time.
Applications:
1. If, after SPOT measurement, the object is to be removed from the center for exposure.
2. If large specimen areas are to be covered by a series of exposures. Without storage of the exposure time, there would be differences in exposures and, for example, the brightness of the background - dependend on the area coverage of the object.
3. If intensity differences in multiple exposure with DOUBLE , e.g. multi-fluorescence, are to be represented.

SPOT Automatic exposure measurement; spot measurement: The field used for measurement corresponds to 1% of the integral measurement area, and to the circle left blank in the center of the reticle crosslines.
Used if an object is surrounded by large dark areas; integral measurement would then cause too long exposure times (darkfield illumination, polarization and fluorescence).
The reverse is also possible.

AUTO Automatic exposure measurement and display of the exposure which will be used. Integral measurement. It is automatically provided with instrument power ON, because it is optimal in most cases.
The display *END* will light at the same time if no film is loaded in the 35 mm film cassette.

MAN Manual input of specific exposure times. When the key is pushed, the digit field will light. You may now select the time and key it in with ENTER .
An exposure time between 0.01 and 9999 s (2 - 3/4 hrs) can be keyed in manually.

T (long-time exposure).
The shutter opens, when you push T and release with START . It is closed when you push START again.
EXP.TIME displays, in full seconds, the time the shutter is open.
Applications: Long-time exposure and to direct the light to the ground glass of the 4x5" camera.

DATA for the 4"x5" camera. A number or sequence of numbers input by pressing this key, followed by ENTER , is imprinted on the large-format negative (see also **8.7** !)
If the DATA key is pressed for the 35 mm camera, BACK is displayed, which means that the data from the data back can be imprinted after exposure.

DATA COUNT like **DATA** , but 1 is added to each input number for consecutive numbering of exposure series.
(Not operative with 35 mm film cassette Mot.)

ASA for the 4"x5" camera. Change to ASA display instead of DATA.

RECIPR Compensation of reciprocity failure. The sensitivity of photographic emulsions decreases if the illumination intensity drops to values which require exposure times of 1 s or more (reciprocity failure). Without compensation of this effect, long exposure times will produce underexposed pictures. The exposure control makes the compensation automatically. Because the decrease in sensititvity is not the same for all photographic emulsions, 9 programmed reciprocity code numbers are provided for automatic compensation. Which code number applies to the film you use is mentioned on page 33.
Input of code number: RECIPR ; digit field lights; key in code number with digit key; ENTER .

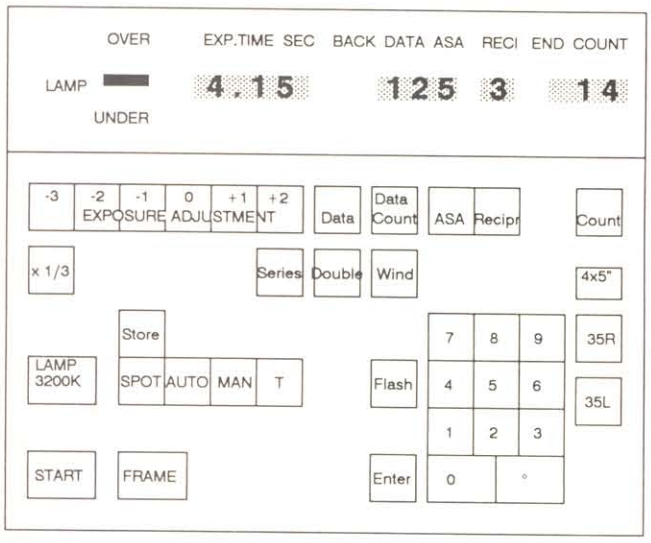
COUNT Frame counter setting. With newly loaded 35mm film it sets automatically to 0; it counts parallel with the mechanical counter of the cassette.
Setting to 0 in cases other than start of film and for the 4"x5" camera:
COUNT ; ENTER .
COUNT will display the number.

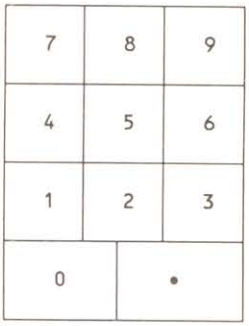
SERIES Exposure series. 35 mm exposures with varying exposure times are taken automatically.
Push SERIES , select the values for adjustment in the desired sequence on EXPOSURE ADJUSTMENT and key them in with ENTER . The exposure series runs automatically after pushing START .
If the end of the film is reached, the film cassette is detached or WIND is pushed, the series is interrupted. Interruption of a series requires a new input.

DOUBLE Double-exposure key.
To override the automatic film advance after exposure, push DOUBLE before releasing with START .
Applications:
1. Multiple exposures of the same film field using different methods of illumination, different fluorescence filters, and similar applications.
2. Multiple exposure to imprint scales, marks, overlay nets, etc.
NB: As the exposures will overlap at least in part, shorten the individual exposure times, e.g. by selection of the exposure adjustment value -1 .

WIND It is used for blank exposures. It also breaks off any running exposure. 35mm film will be advanced.

Flash mode with 0.01 s shutter opening time.
Flash synchronisation via 4-pin socket **9.5**.





Digit field. It lights if either of the keys MAN , RECIPR , COUNT and DATA COUNT is pushed to select specific values. Input of numbers with ENTER .

ENTER Input key to select the modes MAN , RECIPR , COUNT , DATA COUNT , SERIES + EXPOSURE ADJUSTMENT (with key -3 to +2 and x1/3).

Display field

LAMP lights if the key LAMP/3200K is pushed.

The green signal field lights if the exposure time is within the range of the automatic exposue control.

OVER lights in case of excessive brightness. Release of the exposure with START is impossible.
Use a neutral density filter to dim the brightness.

If *UNDER* and the green signal field flash in alternately, the brightness is too low. (Open light path!). The brightness can no longer be covered by the operating range of the automatic exposure control, i.e. the exposure time must be keyed in with MAN.

EXP.TIME and *SEC*: Displays the exposure time measured by the automatic system in decimal notation, with all adjustments, and any number keyed in manually or stored. The display counts down to 0 during exposure. If exposure is made with T , full seconds are counted from zero on.

BACK lights if DATA is pushed. This indicates that data from the Data Back are projected on the film.

DATA and *ASA*: Only for 4"x5".
Displays the ASA value of the activated camera.
If DATA is pushed and a number keyed in, the number is displayed. When ASA is pushed, the ASA value adjusted on the 4"x5" camera is displayed.

RECI. Displayes the reciprocity code number selected for the camera in use and keyed in on the keyboard.

END flashes if the end of the film is reached or the film is rewound. It lights continuously if a new film must be loaded in the cassette.

COUNT counts the frames continuously for the 35 mm camera.

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

Required equipment

- Objectives (1) designated Ph. They may be used in brightfield as well.
- A condenser (5) with turret (2) with Ph positions.

Additional adjustment:

The phase rings in the objectives are of different size, and marked Ph1, Ph2 and Ph3 on the objective (1). The turret (2) bears the same designations - Ph1, etc. - for combination with the suitable objective. Condenser turrets with 2 or 3 Ph positions are available **5.17** and **5.20** .

Perfect phase contrast is achieved only if the dark ring in the objective and the bright ring in the condenser exactly coincide; this is controlled with swung-in Bertrand lens (6) and focusing with lever (7) to the right. (Without Bertrand lens, control is made like that of the condenser diaphragm, described on page 5).
If the two rings do not exactly coincide (centration), correction is possible using the centering screws which are accessible through openings (4) (Fig. 38).

The centration is maintained when the condenser turret is turned or exchanged.
To enhance the contrast, a green filter is brought into the beam path either via magazine **3.2**, or inserted in the color filter holder or put on the luminous field diaphragm.

Special notes

More than in brightfield, meticulously clean glass-to-air surfaces of the specimen (fingerprints!) are necessary in phase contrast.
The diaphragm ring (3) of the condenser is without function, since the Ph openings do not contain iris diaphragms.
The diaphragms in the Ph positions of the condenser are part of the front lens of a specific condenser; they must be exchanged if the front lens is exchanged (see table on page 39).

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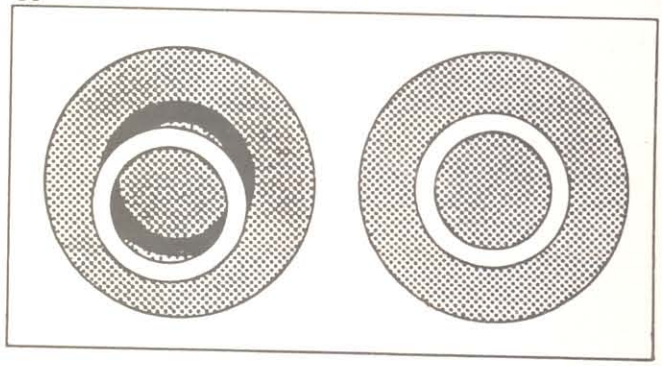
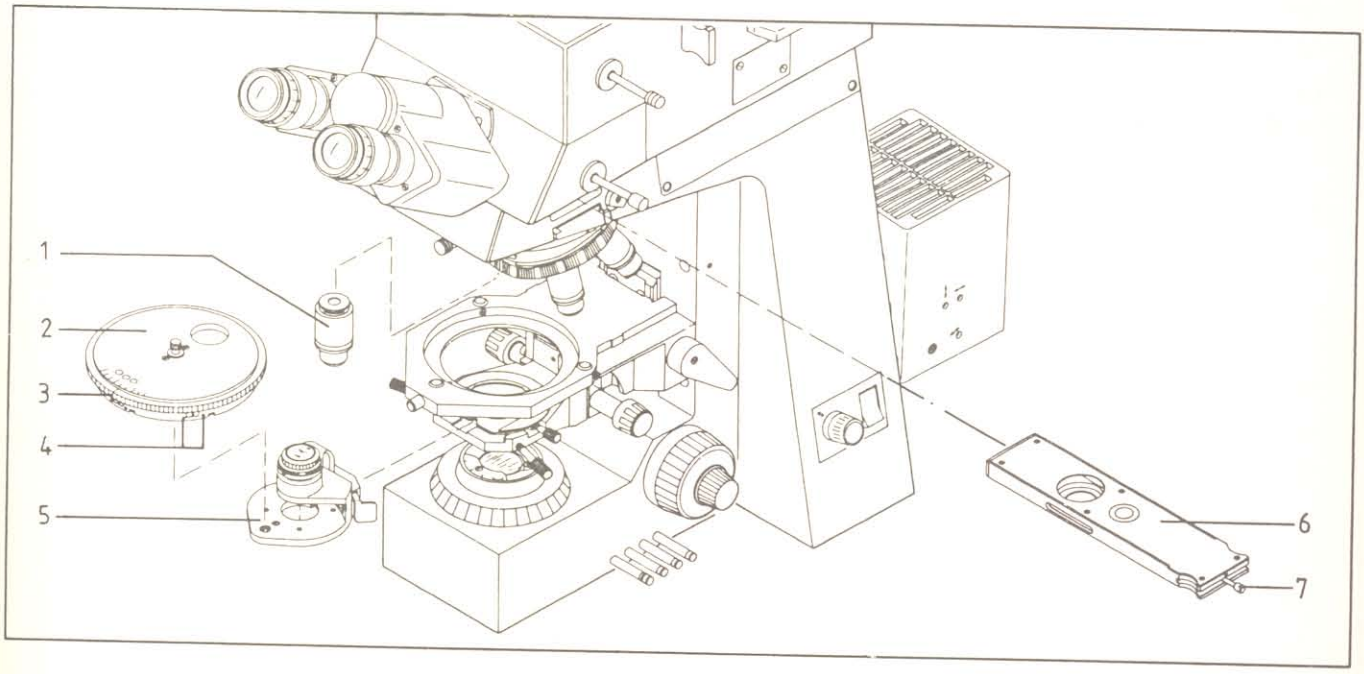


37



It is applied, for instance, if a specimen is too thick for phase-contrast examination so that specimen layers outside the focal plane impair the brilliance of the image, or if the halo which is typical of phase contrast impairs the observation of small features.

Required equipment

- Normally Plan-Neofluar objectives,
- A special nosepiece (1) with slots (2) for
- DIC slider (3) bearing on its top surface magnification and aperture of the objective for which it is intended. Insert DIC slider in slot (2) until click stop.
- A condenser turret (4) with DIC positions
- A polarizer (5) which is swung in beneath the condenser
- An analyzer (10) which is slid into (8).

Additional adjustment

Similar to the 3 (or 2) Ph positions of the condenser there are 2 DIC positions, one for objective apertures 0.3 . . . 0.4, the other for apertures from 0.5 . . . 1.4.

This permits the following combinations:

- objective 10x/0.30 and condenser position DIC .3 - .4
- objective 20x/0.50
- objective 40x/0.75
- objective 100x/1.30

} condenser position DIC .5 - 1.4

Unlike the Ph positions, the DIC positions are provided with iris diaphragms. Open them completely at first. To enhance the contrast, they can be slightly closed, which is generally the last step of the adjustment.

Optimum contrast is adjusted with the knurled screw of the DIC sliders (3) in the nosepiece.

Special notes

The contrast in DIC is produced by a (pseudo) relief. The contrast of linear structures therefore depends on the orientation of these structures: in "light-shadow" direction it will be low, but highest in a direction at right angles to this direction. The possibility of specimen rotation is therefore (almost) absolutely required for adjustment. We would remind you of the possibility of attaching the mechanical stage in such a way that it can be used as rotary stage (page 9).

To ensure reflex-free illumination, luminous field and aperture diaphragms should not be opened wider than for Köhler illumination (see page 5).

As DIC uses polarized light, "optically active elements" between polarizer and analyzer will interfere, e.g. mica plates which are sometimes used for histological sections, or plexiglass culture dishes with plastic bottom (dishes with glass bottom are available).

Analyzer with lambda plate (9) (45 36 56) or auxiliary object lambda (7) (47 37 04) in slot (6) instead of normal analyzer (10) (45 36 55) will generate color DIC.

For combined DIC/phase contrast work, exchange the normal Bertrand lens slider for the one with analyzer (11).

The DIC prisms in the DIC positions of the condenser are part of the front lens of a specific condenser; they must be exchanged when the front lens is exchanged (see table on page 39).

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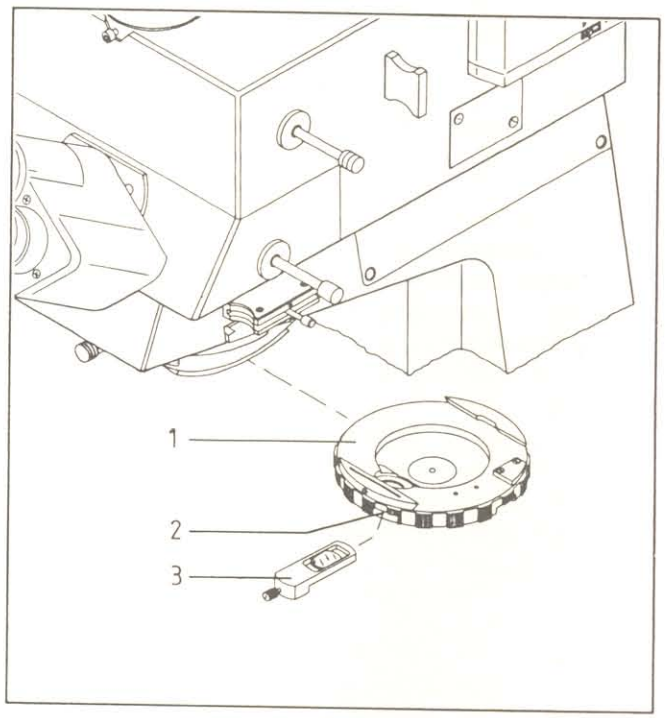

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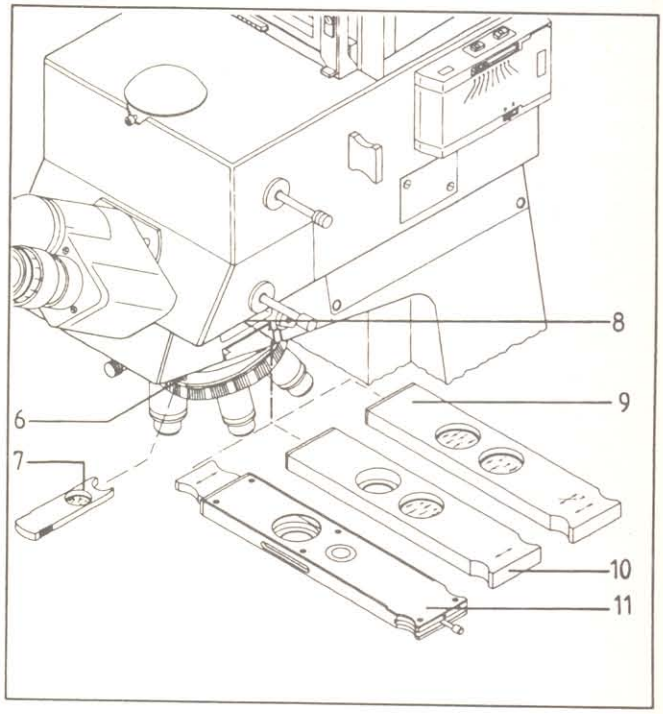
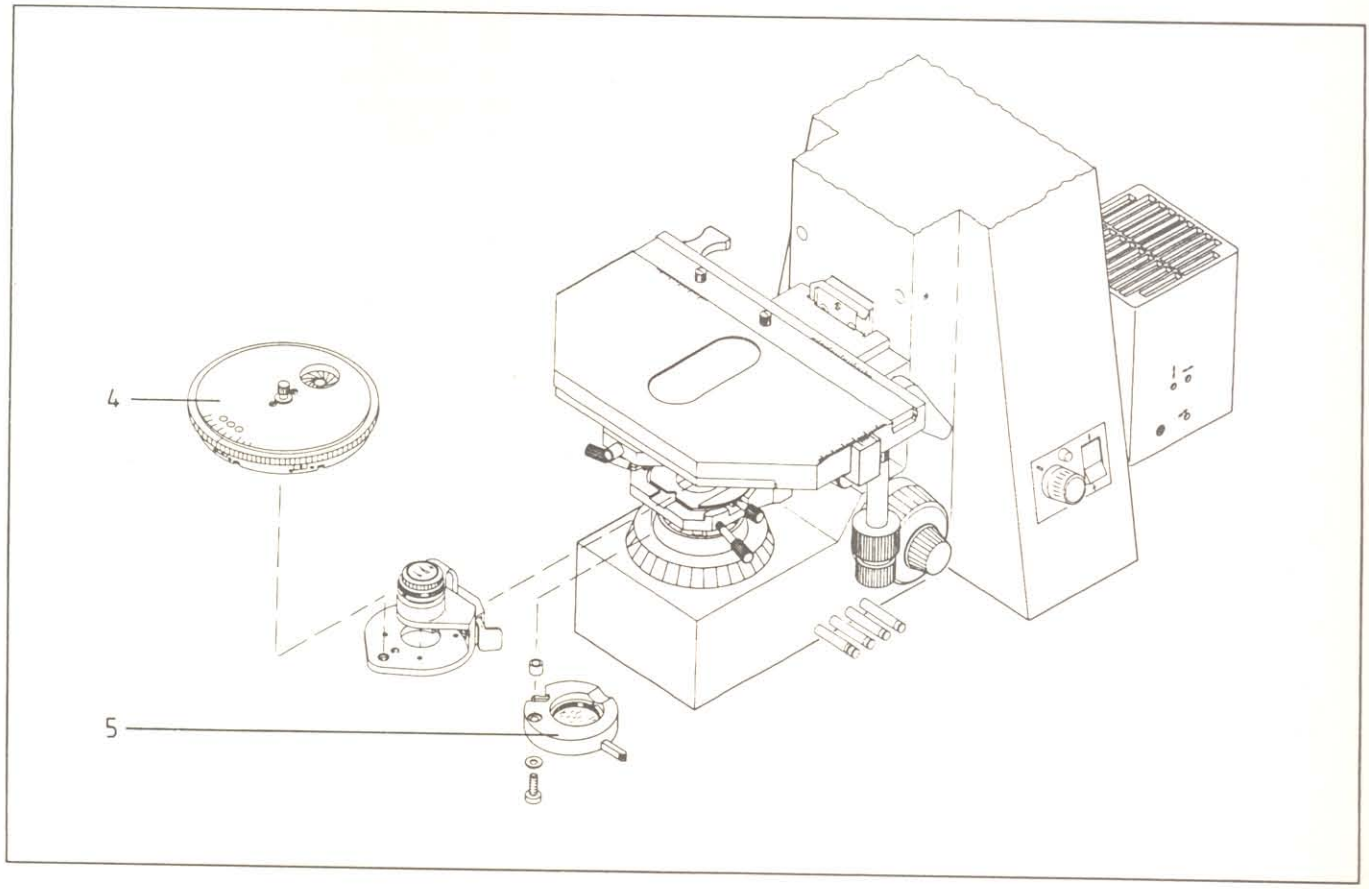


40



Required equipment

- No special objectives; Plan-Neofluar objectives for UV excitation.
- Special reflected-light illuminator (Fig. 42).

Procedure

- Adjust the selected specimen feature in transmitted-light brightfield or phase contrast using reflector slider (4) in middle position (free light path) and lower illuminator with halogen lamp. Switch on the HBO 50 mercury short arc lamp via power supply unit (39 26 42), but block its light path with slider (1).
- Switch off transmitted-light illuminator (or reduce at least its brightness considerably), remove all filters in magazine in the stand base from the beam path, select the left or right position of the reflector slider depending on the type of excitation, and remove slider (1) from the beam path.
- Since a narrowed aperture diaphragm would reduce brightness in incident-light fluorescence. Only a luminous field diaphragm is, therefore, provided. Use lever (2) to close it so far that it becomes visible in the image. Then center with (3) and open the diaphragm until the field of view is free.

Special notes

The first fluorescence adjustment is made easy with a 20x objective and a strongly fluorescent specimen. Demonstration specimens can be supplied, but you can also prepare them yourself; a specimen of spread anthracene crystals is quite popular. (A specimen label can be used to check the illumination).

The reflector slider contains several filter sets for different tasks. Each set comprises one exciter filter, one barrier filter (dia. 25 mm) and a chromatic beam splitter (26 x 26mm) in-between. For more information on the various components please see page 41.

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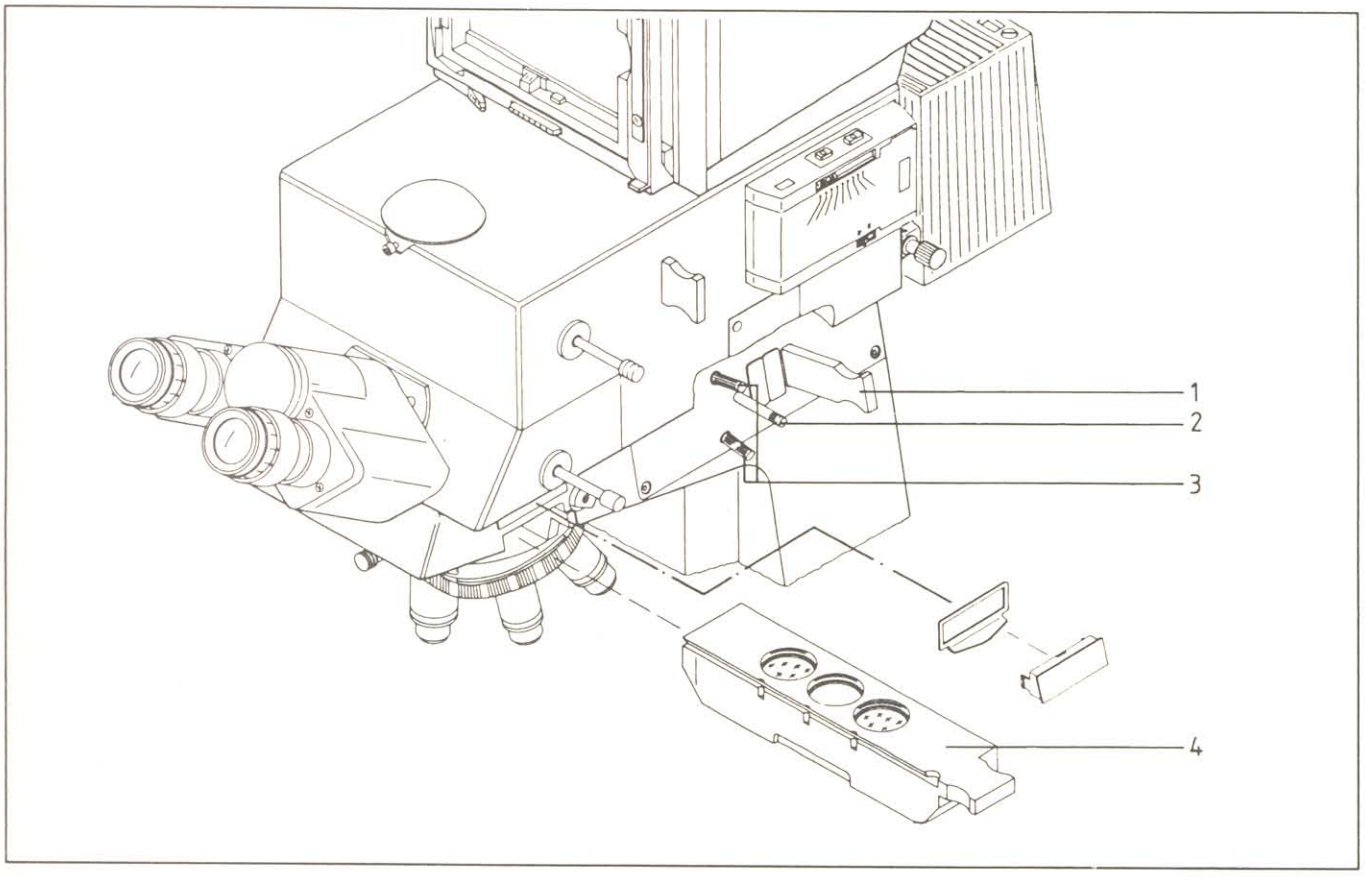

It is applied

- to examine exceptionally small objects or object features, such as treponemas, spirochaetae, flagella, bacteria, etc., or emulsions, if phase contrast is insufficient;
- if the specific colors of natural, i.e. unstained, objects (living organisms in water, like algae, unicellular organisms, lower animals) are well visible.

Required equipment

- Special objectives with integral iris diaphragm only for higher magnifications, but
- a condenser with central stop whose NA is higher than that of the objective used.

Further details are given below.

Necessary adjustments

- The illumination is adjusted as in brightfield. The luminous field diaphragm must be imaged and centered. If the height adjustment of the condenser is correct, a virtually sharp image will be obtained of the luminous field diaphragm.

With a dry darkfield condenser, adjustment can be made in two steps:

1. center the luminous field diaphragm without darkfield insert (brightfield). Set height stop **5.6**, lower condenser and take out brightfield insert.
2. put in darkfield insert and correct with **5.22**.

- Check the objective pupil for complete extinction. With the ultra darkfield condenser there may be a light ring in the pupil which is eliminated with the iris diaphragm of the objective. The background of the eyepiece image must be absolutely dark; this is influenced by the position of the luminous field diaphragm, especially at the edge of the field of view.

Special notes

Darkfield requires cleaner specimens than other methods; especially grease films (fingerprints) will lighten the background.

The critical adjustment of the ultra darkfield condenser is facilitated by precentering with a low-power objective. Use a specimen with uniform feature distribution, e.g. blood smear, for initial adjustment; the luminous field becomes visible only where particles light up, but the darkfield specimen ultimately examined may be "empty" over wide areas.

Darkfield illumination with selected objectives

Plan-Neofluar	Plan-Apochromat	Illumination
10/0.30	10/0.32	Ph stop 3 ≥ 0.44
20/0.50 40/0.75	20/0.60	darkfield stop 0.76 - 0.90
	40/1.0 oil iris	ultra darkfield condenser 1.2 - 1.4 oil
100/1.3 oil iris	100/1.3 oil iris	

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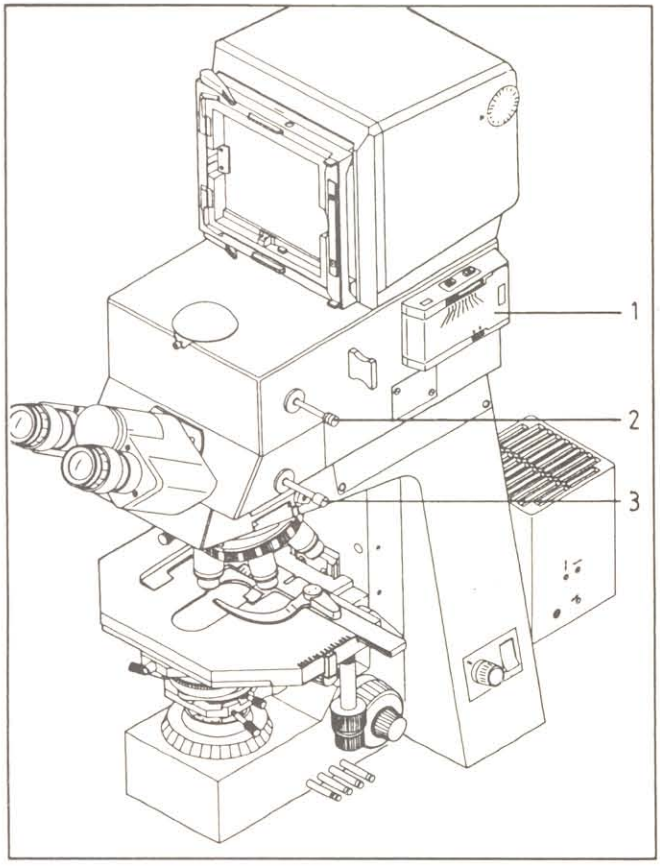

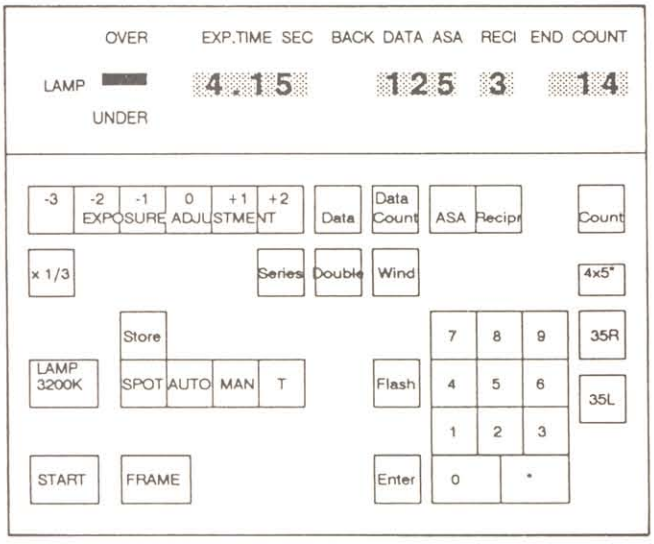

B/W photography

The Axiophot is adjusted for observation (see pages 5). Beam splitters (2) and (3) are set to observation and photography. Cassette (1) is loaded with film (film types see on page 33) and mounted, and the film speed set on the cassette or on the 4"x5" camera.

On the control panel you have

- input the camera port: 35 L, 35 R or 4" x 5".
- input the reciprocity value of the chosen film type (see page 33): RECIPR; e.g. 1 ; ENTER .
- set the frame counter to 0: COUNT ; ENTER . (Not necessary with newly loaded film)
- switched on the luminous frame: FRAME ,both outlines and object are in focus; for low magnifications use a telescope attachment 3x12B to make this adjustment
- For contrast enhancement use a green interference filter

Displays on the control panel

- The green signal lamp lights;
  if OVER lights instead - reduce brightness
  if UNDER lights - is light path free ?
  UNDER also lights if the brightness is extremely low.
- Exposure time (*EXP.TIME*) (integral for automatic exposure measurement and exposure adjustment 0).
- Reciprocity code number.
- ASA value

Exposure: Press START. The luminous frame disappears, automatic exposure is made, the luminous frame is visible again, film advance, mechanical (cassette) and electrical counter (control panel) continue counting.

Color photography

In addition to the above, remember for color photography: Color reversal films (slide films) are available for daylight (5500 K) and artificial light (3200 K). The color temperature values must be accurate to within approx. 100 K for correct color rendition. If the 12 V 100 W halogen filament lamp is set to color temperatur 3200 K, its brightness is so high that undervoltages will be used for observation. The key LAMP 3200K on the control panel is used to change the voltage before each color exposure. One or several neutral density filters must be in the beam path to attenuate the light; they have no influence on the color temperature.

Reversal films for artificial light (3200 K) are generally recommended for photographhy. If daylight film is used, swing in the conversion filter at **3.2** which increases the color temperature from 3200 K to 5500 K. (Flashlight has daylight color temperature).

Compensation of reciprocity failure

Use the advantage of this compensation for exposure times which are longer than 1 s. (For an explanation see RECIPR on page 25). The code numbers for the most frequently used film types, which you must enter with RECIPR on the control panel are listed below.

You can find this value yourself for film types which are not listed:
Use the automatic exposure control to make a series of test exposures with an exposure time shorter than 1 s, reduce the brightness with neutral density filters or similar means until you arrive at several seconds exposure time. Take a number of exposures with the RECIPR settings 1 to 9. After development you can find out the longtime exposure which best complies with the first one. Its reciprocity value will be the code numer for this specific film type. It will be set whenever you use this film type, independent of the exposure time.

No test exposures are necessary if the film manufacturer indicates the extension of the exposure time, e.g. "+2 values for 10 s". +2 values signify a 4x longer exposure time, i.e. 40 s. Adjust your microscope so that the automatic system indicates 10 s with RECIPR set to 0 (here, you may for once use the aperture diaphragm to reduce the brightness).
When changing the reciprocity values you will quickly find the one which best approximates 40 s. This would then be th code number for your film, which is 8 in the above example.

Compensation of reciprocity failure for some frequently used film types:

Film		Code
Agfachrome Professional	50 RS, 100 RS, 200 RS	5
	1000 RS	4
Fujichrome Professional	50 D (RFP), 100 D (RDP)	3
	400 D (RHP)	1
	1600 D	4
	64 T (RTP), artificial light	2
Kodachrome Prof.	25 (PKM), 64 (PKR), 200 (PKL)	8
Kodak Ektachrome Professional	64 T (EPY), artificial light	1
	160 (EPT), artificial light	5
	64 (EPR), 100 (EPN),	6
	100 PLUS (EPP), 200 (EPD)	6
	400 (EL), P800/1600 (EES)	6
Konica Chrome	100	1
Polachrome	CS	9
Polaroid	58	7
Polaroid Prof.Chr.	4x5" 64 T, artificial light	2
Scotch Chrome	100	6
	400, 640 artificial light	5
	1000	4
	800/3200 P	0
Agfapan Prof.	100, 200, 400	8
Agfaortho Prof.	25	1
Ilford	Pan F (50), FP4 (125)	4
	HP5 (400)	6
Kodak Professional	T-MAX 100 (TMX)	4
	TMAX 400 (TMY), P 3200 (TMZ)	5
	Tri-X-Pan (TX)	9
Kodak	Technical Pan (TP)	3
Polaroid	52, 53, 55, 552, 553	1
	57	4

Fluorescence photomicrography

The following information is useful also for darkfield and polarization.
The following features are different compared with normal photomicrography :

- Fluorescence light is neither daylight nor artificial light but originates from the specimen itself. Experience has shown that better color photographs are produced on daylight films.
- The brightness which is generally low requires longer exposure times and thus high-speed film. The graininess of high-speed films is rarely disturbing because it becomes obvious mainly in the range of medium luminance which hardly exists in fluorescence images. In these images one has either a dark background or brilliant, bright features.

Therefore, there is no objection to using films of 400 ASA and higher.
To eliminate disturbing IR light, insert red-attanuating filter BG 38 into the beam path with slider **7.2**.

- Even in case of spot exposure measurements, the dark or even black background will often be an essential part of the measuring field of the automatic exposure control, which may cause overexposures. Here, you should use EXPOSURE ADJUSTMENT. In the field used for spot measurement with SPOT , the ratio of bright to dark areas can be easily estimated. (If a typical measuring field of the specimen is to be removed from the image center for exposure, you can store the exposure time with STORE.)
- There is a wide range of exposure possibilities thanks to the high contrast, because even with different exposure times bright structures stand out (more or less) clearly on dark background. If, however, the exact rendition of the fluorescence colors is important, you should take a series of exposures at different exposure times.
- Fluorescence colors tend to bleach, especially if the exciting radiation is of high intensity and energy. The intensity of the exciting radiation can be reduced at least temporarily by neutral density filters to protect the specimen. (The darker the room the better you will see at low light level, a fact which is often forgotten.) Don't forget the dark slider if you interrupt your work. Last but not least make all preparations on those specimen features in the field of view which are not photographed to prevent "valuable" ones from being damaged.

Under the above-mentioned conditions an exactly adjusted luminous-field diaphragm is important insofar as it prevents energy from reaching specimen features outside the field of view or photographic field.

The Axiophot permits the following longest exposure times in 35 mm photography, with exposure adjustment 0:

Film speed	longest exposure time
100 ASA	120 s
400 ASA	30 s
1600 ASA	8 s

Correction of the color balance of color reversal films

The color balance of color reversal films of the same type can differ from one batch to another.
Both these differences and chromatic changes caused by the optics can be compensated by the use of Color Compensating (CC) filters.
The filter density is indicated by a two-digit number and the color by its initials. Examples: 05-B (Blue), 10-G (Green), 20-R (Red)

Assessment of the color balance:

- Put slides on a standard light box whose light source has the correct luminance and the spectral energy distribution of 5000 K.
- Make test photographs - in transmitted-light brightfield - of a special specimen feature with a background that should be as clear as possible.
- The clear background of one exposure series should be color-neutral: dark grey - medium grey - light grey to white.

Correction of the color balance:

- Put a CC filter of the complementary color of the color tinge on the slide to be corrected.

Color tinge	Color of the CC filter
blue	yellow Y
green	magenta M
red	cyan C
yellow	blue B
magenta	green G
cyan	red R

If, for example, a filter with density 10 shows the desired color balance on viewing, a CC filter of half the density of 10, i.e. 05, should be used for the exposure. Filters of density CC-05 to CC-10 are normally sufficient for correction.
Note: It is absolutely necessary for the perfect correction of color exposures that the microscope adjustment, the developing procedure and the film batches remain unchanged.

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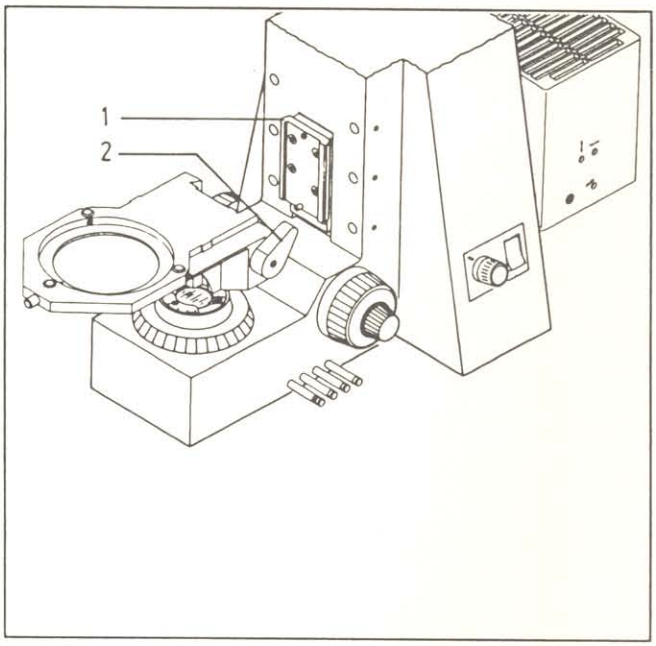
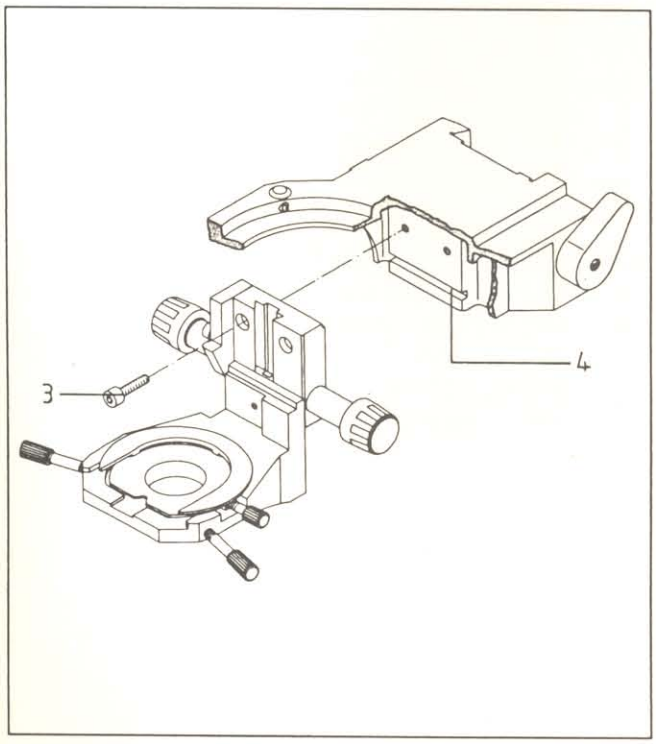


45



Should minor changes of your microscope be necessary and no service technician be available, the following hints may be helpful.

Stage components

Detachment from mounting plate: flick up lever (2) (right) and turn off the entire unit about the left edge (1) of the plate. Attachment: position stage carrier to stop screw on top, attach it to the left edge and - lever up - press down right side. The spring pin is pressed down and fixed when the lever is flicked down. The condenser carrier (transmitted-light equipment) can be removed after loosening 2 screws (3) on the front. When remounting the carrier, make sure that the two orientation pins engage notch (4) and then tighten the screws.

Intermediate tubes

The following intermediate tubes can be attached between camera system and stand (45 18 10-9901).

Zoom intermediate tube (45 19 02) (Fig. 46)
The zoom intermediate tube (10) continuously extends the magnification range of the microscope from 1x to 4x. The requested magnification can be set with knob (11) and the zoom factors can be read off on the scale. A measuring scale with bar distance 1mm (1,000 µm) is integrated. This defined measuring distance is imaged on the film, if it is within the photo reticle with which it will appear on the film.
The measuring scale can be superimposed on the object image using pushrod (2).
The length of the imaged measuring distance is dependent only on the objective magnification; it is not influenced by additional magnifications. The distance between two bars is computed as follows:

1000 / objective magnification in µm,

i.e. 100 µm for the 10x objective.

Assembly

- Remove camera system Axiophot (1) from microscope.

Unscrew screw (12) in back of the stand using a screwdriver and turn in clamping screw (6) until it is no longer visible.

- **Unscrew tube lens (5) from the camera system**

by using cover of case (45 95 11); store tube lens in the case. If the microscope is used without intermediate tube, the tube lens must be screwed into the binocular tube again.

- Attach zoom intermediate tube (10) to the stand so that its dovetail is positioned securely on the tube mount. Align the intermediate tube parallel to both stand edges.
- Push zoom intermediate tube to the back until the screw with both washers can be tightened into (12).
- Tighten clamping screw (6) at the front, then screw at the back (12).
- Remove both pins (9) before mounting the camera system on the intermediate tube.
- Lift camera system by the sides with both hands. Insert it in the intermediate tube mount (3) via the dovetail.
- Align the camera system parallel to the intermediate tube, push it to the back so that the screw with both washers can be screwed into thread (8). Tighten clamping srew (4) at the front, then screw (7) at the back.
- Connecting camera system and camera control panel with cable and tighten screws of plug-in unit.

Caution: plug in or remove cable only if instruments are disconnected from power line.

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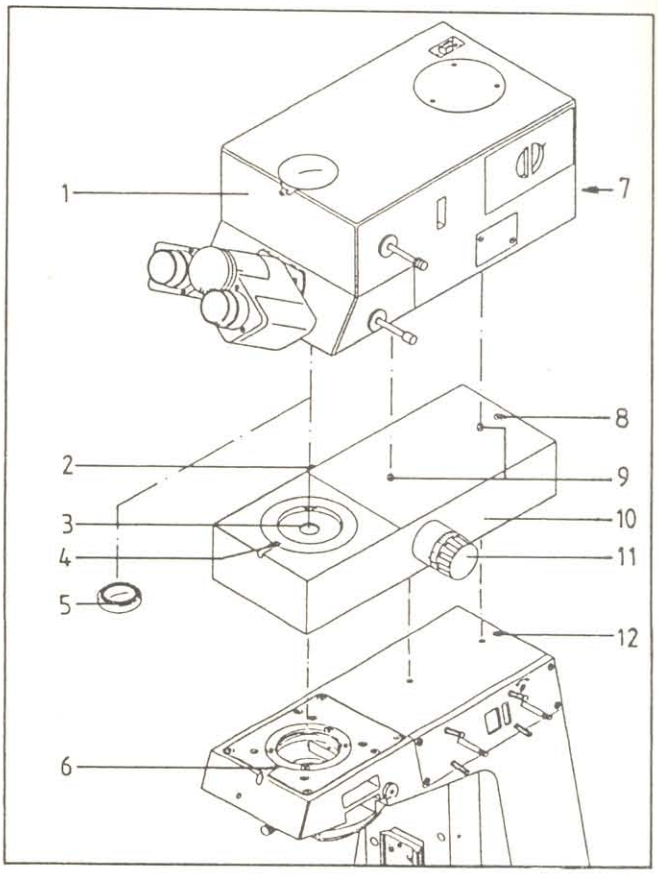

The Optovar intermediate tube 1x - 1.25x - 1.6x - 2x (45 19 03) can be mounted between stand and camera system in the same way as the Zoom intermediate tube.

Co-observation bridge with light pointer (45 19 15)
See operating instructions G 42-404.

Intermediate tube for image projection (45 14 65)
See operating instructions G 42-403.

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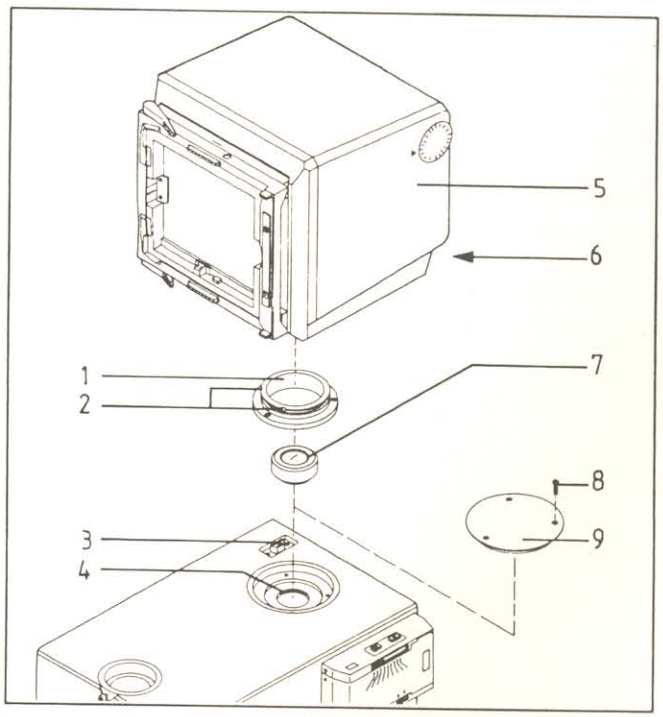


4" x 5" camera

To mount the 4" x 5" camera (5) on the Axiophot microscope:

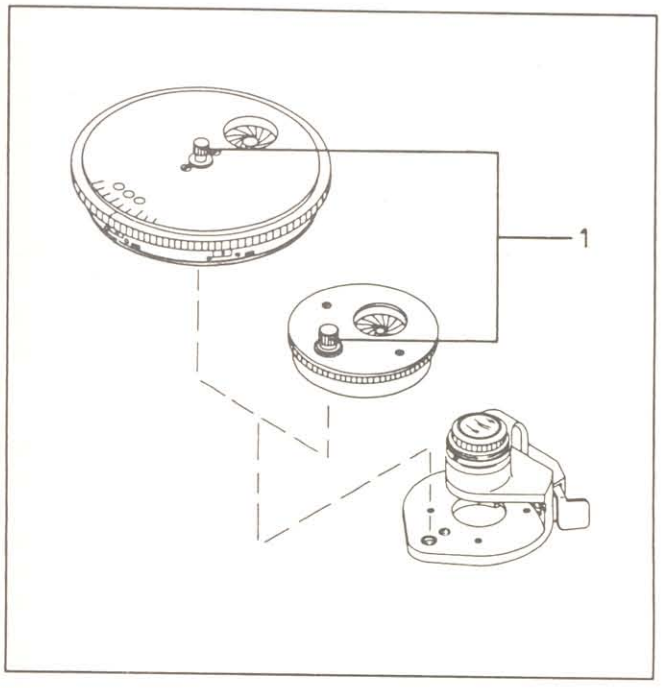
- Unscrew 3 screws (8) with screwdriver and remove lid (9).
- Screw supplied large-format optics (7) into port.
- Insert holding ring (1) for 4" x 5" camera in port with the two notches (2) showing to the user and secure with 3 available screws (8).
- Lossen clamping screw at (6) on 4" x 5" camera and turn it to the red point.
- Mount 4" x 5" camera; make sure that plug at (3) and optics are correctly fitted in the respective ports.
- Turn clamping screw at (6) clockwise and tighten.
- Connect cable to camera system and control panel and secure with screws.

Second TV camera

To mount a second TV camera instead of the 4" x 5" camera:

- Unscrew 3 screws (8) and remove lid (9).
- Fix CTV mount (45 19 30) with 3 screws.
- Plug supplied code plug into camera system at (3).
- Mount TV camera via C mount adapter (45 29 95) or 3T CTV camera via adapter (45 29 94) and secure.
- Select keys T and 4" x 5" on control panel and press START.
- The factor for the TV camera is 2.5x.

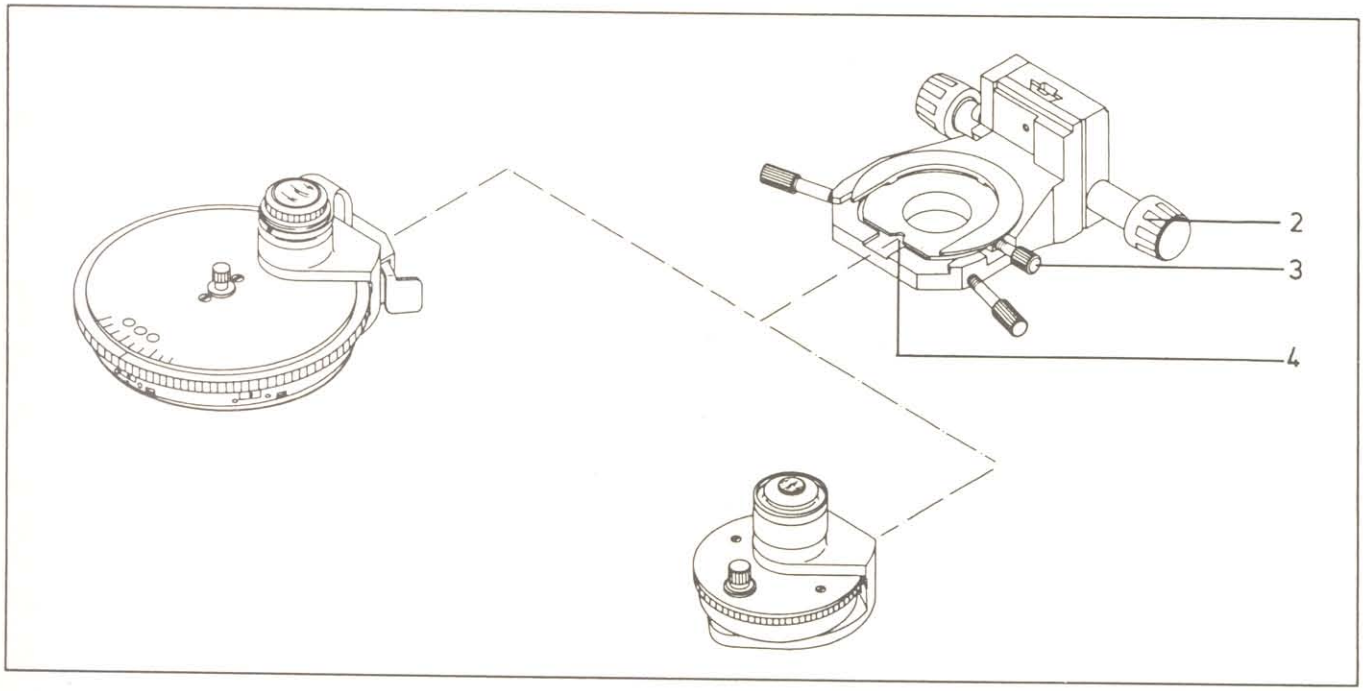
48



Condenser exchange

To convert a brightfield condenser into a phase contrast, DIC or darkfield condenser, only inserts must be exchanged, but not the entire condenser. For exchange, slacken and raise locking screws (1).
The entire condenser must be exchanged for an ultradarkfield condenser or the condenser system 1.4. The condenser must then be lowered as far as possible with (2). Slacken clamping screw (3) and pull out the condenser in a forward direction. The new condenser is inserted accordingly; notch (4) ensures correct insertion. A pin on the condenser carrier serves as lower stop and prevents the condenser from hitting the luminous field diaphragm insert.

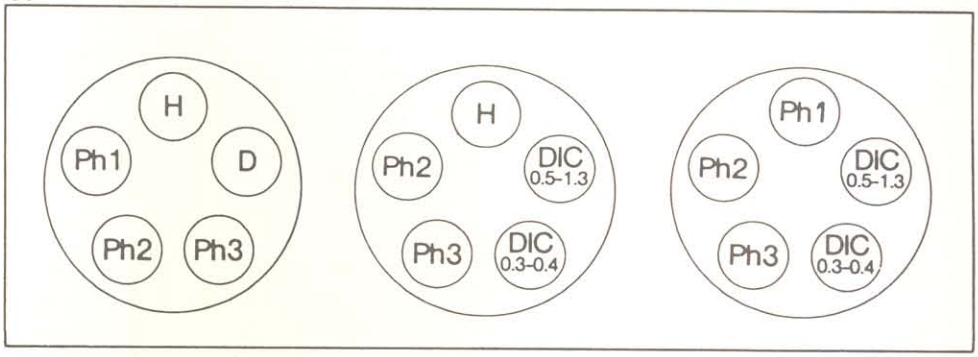
49



Exchanging phase stops and/or DIC prisms in condenser turrets

Fit phase stops only in centerable openings and DIC prisms only in openings with iris diaphragm. For further details please see Fig. 50. The equipment of the condenser turret is listed in the table below.

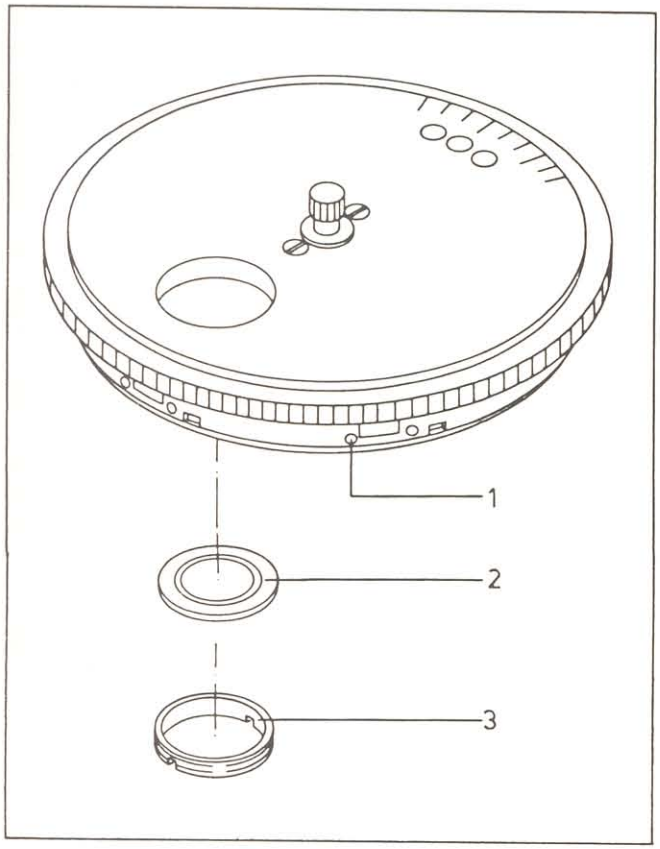
50



Possible equipment of the condenser system

Insert, Turret	Mount for diaphragms,etc	with front lens	Possible equipment
Brightfield insert (44 53 64)	1 plug mount with iris	0.6 (44 53 55)	brightfield
		0.9 (44 53 56)	brightfield DIC 0.3 - 0.4/0.9 (44 53 73) DIC 0.5 - 1.3/0.9 (44 53 74)
		1.4 (44 53 57)	brightfield DIC 0.5 - 1.4/1.4 (44 53 89)
Darkfield insert (44 53 63)	1 centerable mount	0.9	D 0.75 - 0.9 (44 53 99)
Turret H D Ph (44 53 66)	plug mount with iris 4 centerable mounts	0.6	brightfield,
		0.9	brightfield, DIC 0.3 - 0.4/0.9 (44 53 73) DIC 0.5 - 1.4/0.9 (44 53 74) Ph 1/0.9 (44 53 69) Ph 2/0.9 (44 53 70) Ph 3/0.9 (44 53 71) D 0.75 - 0.9 (44 53 99)
		1.4	brightfield, Ph 3/1.4 (44 53 86) DIC 0.5 - 1.4/1.4 (44 53 89)
Turret H D Ph DIC (44 53 65)	3 plug mounts with iris 2 centerable mounts	like turret H D Ph	like turret H D Ph
Turret Ph DIC (44 53 67)	2 plug mounts with iris 3 centerable mounts	like turret H D Ph	like turret H D Ph

51



Annular phase stops or darkfield diaphragms are exchanged from beneath the turret. Unscrew retaining rings (3) and insert the diaphragms (2) with their polished glass surfaces facing down. If you cannot secure the diaphragms with the retaining rings due to movement of the diaphragm mounts, secure them with the centering screws.
The diaphragm in the darkfield insert is exchanged accordingly.

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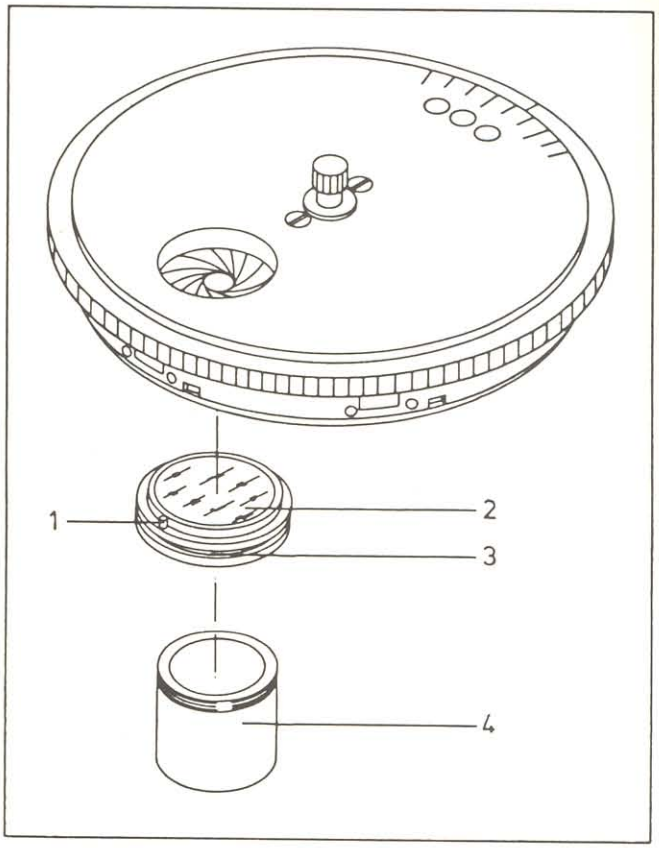
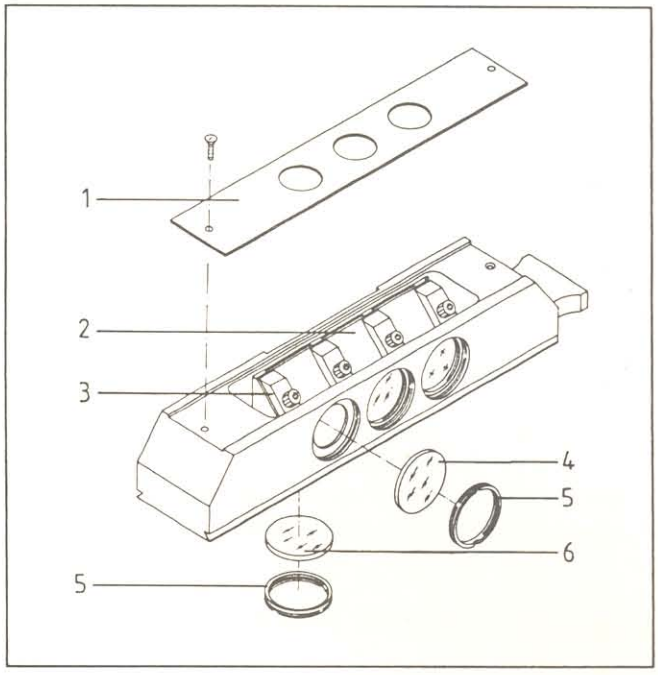


DIC prism exchange: screw tool (4) into prism mount (2) forming a handle to take the prism out. (A wire ring in the holder engages a notch (3) in the prism mount).
Pin (1) must be in the correct borehole of the holder to enable insertion of the prism. Check for absolutely flat seating to prevent mechanical or optical malfunctions.

After exchange, be sure to also exchange the labels which must be on the turret oppopsite the opening.
Insertion of the DIC prism in the brightfield insert is the same as in the condenser turret.

53



Fluorescence reflector

Exchange of exciter filter (4), barrier filter (6) and chromatic beam splitter (2).

- The built-in filter sets can be exchanged when retaining rings (5) have been removed. Remove plate (1); the plate carrying the chromatic beam splitters (2) is now accessible. It is mounted on a spring mask and must not be touched. Generally, straps need not be taken out, but must only be slackened so that the chromatic beam splitter can be exchanged on the mask.

Insert chromatic beam splitter in such a way that its layer faces to the exciter filter.

Plate (1) is not symmetrical; when mounting it, make sure that the free apertures are not cut off.

Fluorescence filter sets

Excitation	Filter set	Exciter filter	Chromatic beam splitter	Barrier filter
UV-G 365	48 79 02	G 365	FT 395	LP 420
Blue-violet G 436	48 79 07	G 436	FT 510	LP 520
UV-H 365	48 79 01	BP 365/12	FT 395	LP 397
Blue-violet H 436	48 79 06	BP 436/10	FT 460	LP 470
Blue H 485	48 79 16	BP 485/20	FT 510	LP 520
Blue H 485 SB	48 79 17	BP 485/20	FT 510	BP 515-565
Green H 546	48 79 15	BP 546/12	FT 580	LP 590
UV-violet 390-420	48 79 18	BP 390-420	FT 425	LP 540

Excitation	Filter set	Exciter filter	Chromatic beam splitter	Barrier filter
Blue-violet 395-440	48 79 05	BP 395-440	FT 460	LP 470
Blue 450-490	48 79 09	BP 450-490	FT 510	LP 520
Blue 450-490 SB	48 79 10	BP 450-490	FT 510	BP 515-565
Green 510-560	48 79 14	LP 510-KP560	FT 580	LP 590
Green 530-585	48 79 00	BP 530-585	FT 600	LP 615
Green H 546	48 79 20	BP 546/12	FT 560	BP 575-640
FURA-2 UV 340+380	48 79 21	BP 340/10* BP 380/10*	FT 395	BP 500-530

* Filter with 18 mm dia.

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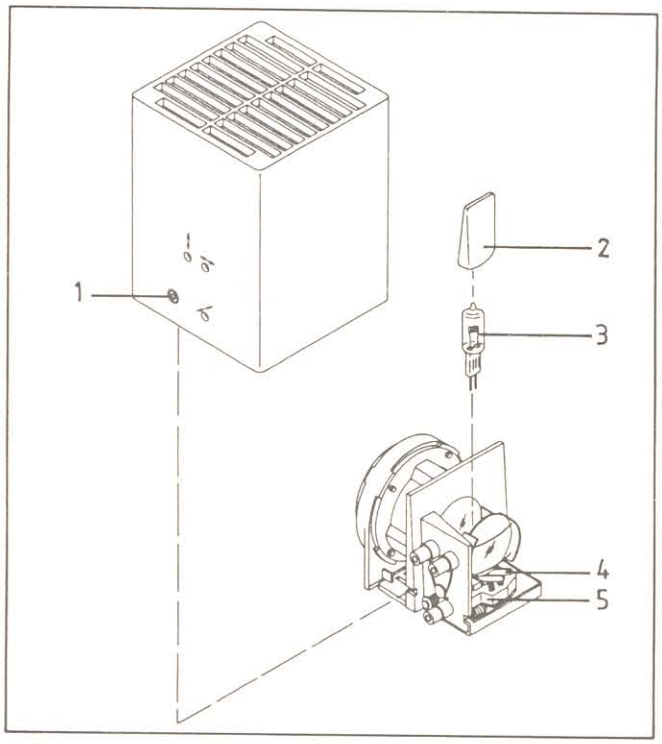
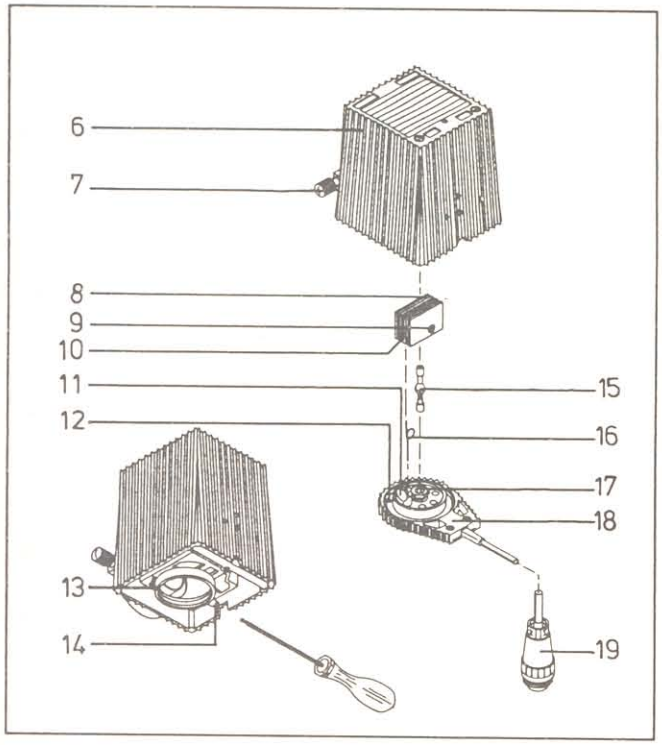


55



Exchange of 12V 100W halogen lamp (38 00 79-9540)

- Pull plug on stand back.
- Remove microscope illuminator (44 72 17-9901) from stand, loosen screw (1) and lift off Hal lamp housing.
- Take defective lamp bulb out of lamp socket (5) by pressing down the two springs (4). Turn the illuminator upside-down and let the lamp fall out.

Insert new lamp (3) using protective sleeve as follows:

- Pull lamp out of protective sleeve until pins are fully visible.

Hold top of lamp using protective sleeve and insert it into the socket, while the springs (4) are pressed down. Press down springs again to enable automatic positioning of the halogen lamp.

- Fix adhesive label supplied with the halogen lamp to the frame at the back of the Hal lamp housing.
- Attach lamp housing again and fix it using screw (1).

For adjustment of the Hal illuminator, please see page 7.

Exchange of the HBO 50W mercury vapor short arc lamp

Pull plug (19) on lamp power supply, loosen screw **1.6** and take off HBO/XBO lamp housing from the microscope.

Move collector to the foremost position with (7), loosen clamping screw (14): you can now remove lamp socket (18) from the lamp housing.

Loosen (11) and (17) and pull out lamp (15) and wire loop (16); loosen (9) and (10) and remove dissipator (8).

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

Insert lamp with dissipator and contact wire in the corresponding openings of the lamp socket and secure with hexagon nut (17) and knurled screw (11). Make sure that the longitudinal side of the dissipator, the contact wire, the wire loop and the melt tip lie in one line. Insert lamp socket (18) with the new lamp in the lamp housing as far as it will go; orienting pin (13) must engage borehole (12) of the lamp socket. Tighten screw (14).

Set switch on back of power supply to lamp type L1 or L2, plug in lamp plug and, if the adjusted voltage (indicated on the dial) complies with the line voltage, connect the unit to the line.

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

The spare parts are listed below as they appear in the description of the instrument starting on page 6.

1.2 Fuses

220. . .240V: T2A	(INR 127.024)
100. . .127V: T4A	(INR 144.060)

1.5 The 42 mm dia. heat-reflecting filter (46 78 28) is inserted in such a way that the surface with the higher reflection faces the light source.
44 mm dia. diffusion disk (45 18 51-0003): the retaining rings are removed with a small screwdriver; insertion accordingly.

2.0 Use only 12V 100W halogen lamp (38 00 79-9540). Other commercially available lamps will cause optical and perhaps also mechanical malfunctions. (Do not handle the lamps with bare hands to prevent fingerprints.)

6.1 PCB-free immersion oil:
oiler (plastic) containing 50 cc immersion oil (46 29 58)

7.1 Mercury short arc lamp HBO 50W (38 16 19)
(for full description see page 42)

7.2 Red-attenuating filter BG 38, dia. 18 mm (46 79 91-9901)
The catalogue number of the KG 1 heat-reflecting filter, dia. 18 mm, integrated in filter slider A is 46 79 90.

8.5 6V 5W spare filament lamp (38 00 29-7200

9.1 Spare fuses:

110 . . . 120 V	0.63 A SB	38 01 27-0180
220 . . . 240 V	0.315A SB	38 0127-0150

9.4 Plug for connection of remote control
(ordering number 141.820)

Slider for neutral density filters to compensate the brightness within one series of objectives
(for nosepiece with slots **6.4**, see page 13)

Neutral density filter N 0.08	44 44 90
Neutral density filter N 0.15	44 44 91
Neutral density filter N 0.30	44 44 92
Neutral density filter N 0.50	44 44 93

Condenser	Plan-Neofluar	Neutral filter slider
brightfield condensers 44 53 50 + 44 53 56	2.5x/0.075 5x/0.15 10x/0.30 20x/0.50 40x/0.75 100x/1.30 Oil	N 0.08 N 0.08 N 0.08 N 0.15 N 0.15 -
brightfield condenser 44 53 40	1.25/0.035 2.5x/0.075 5x/0.15 10x/0.30 20x/0.50 40x/0.75	N 0.15 N 0.15 N 0.15 N 0.15 N 0.30 -