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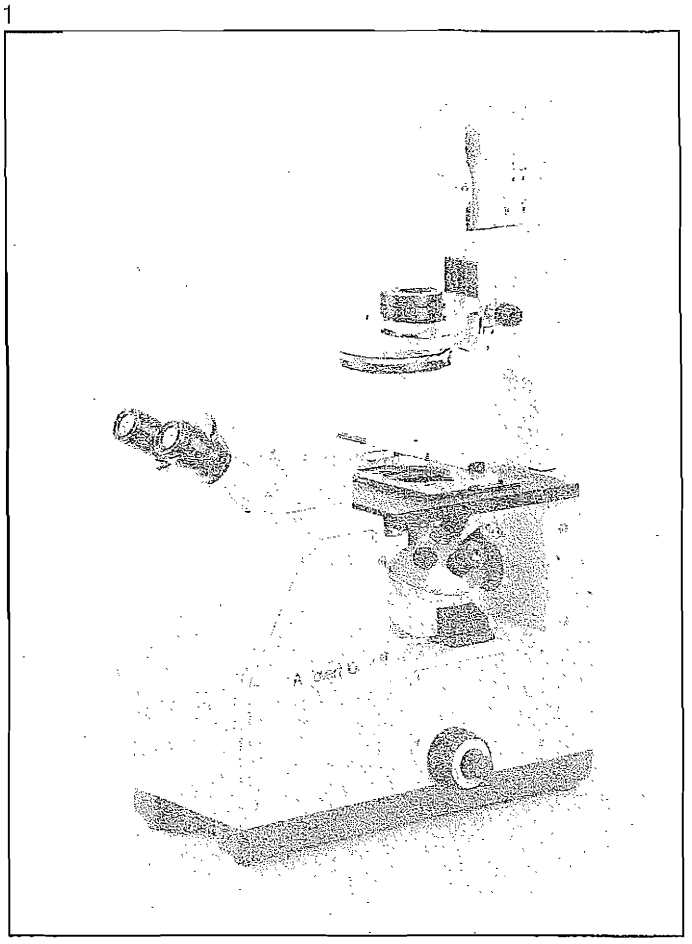

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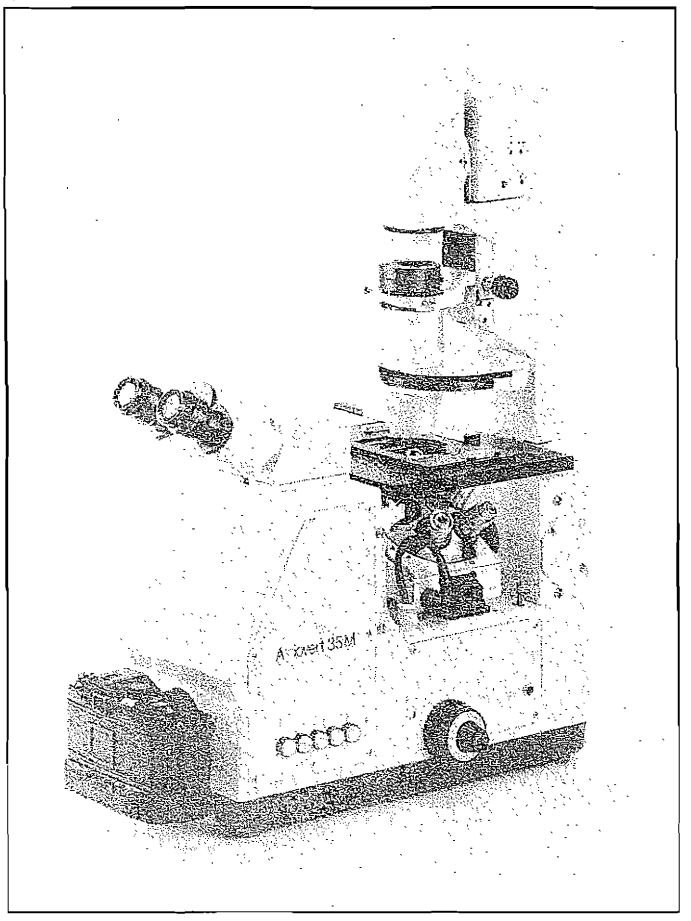

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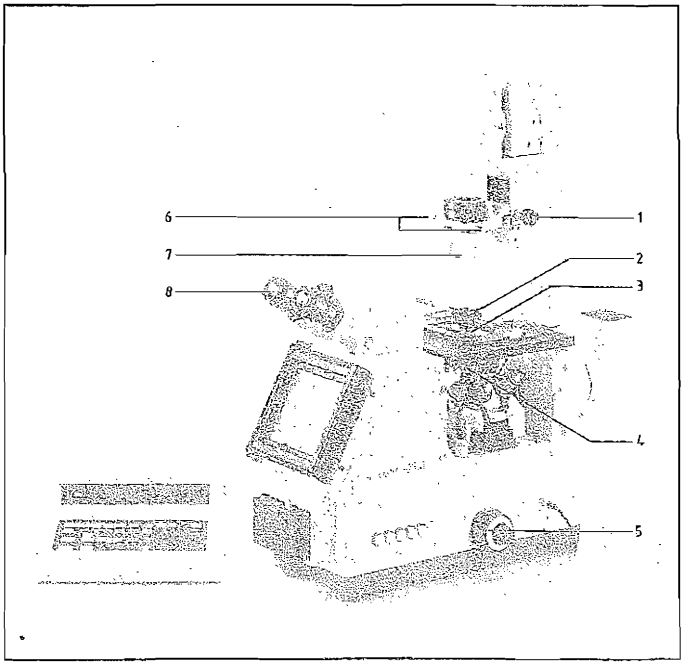


Fig. 1
Axiovert 10
equipped for long illumination distance

Fig. 2
Axiovert 35 M
equipped for high illuminating aperture

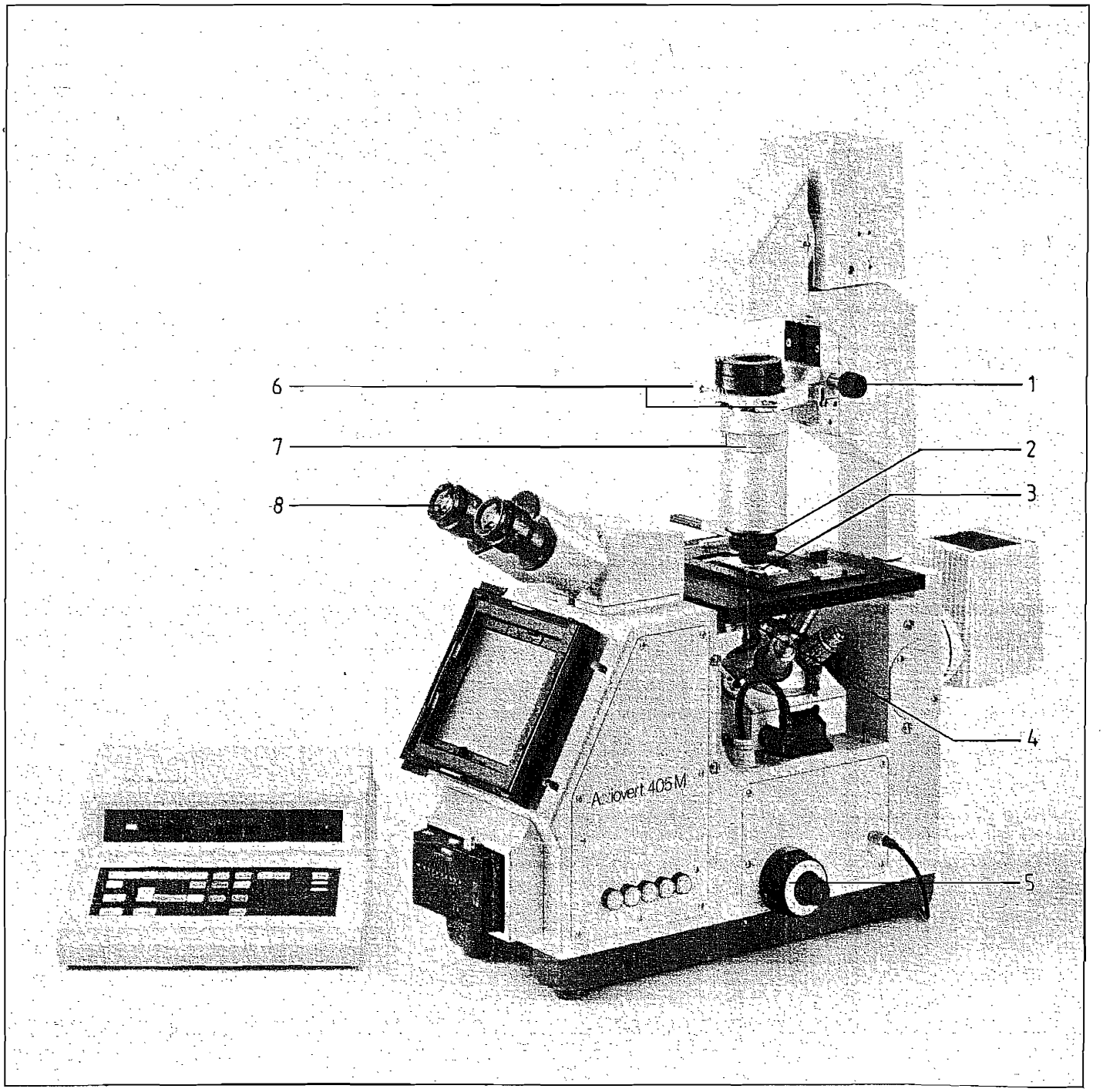
Fig. 3
Axiovert 405 M
equipped for incident-light fluorescence

Contents

Special notes

- The 6- to 10-digit numbers, e.g. 451700, are ordering numbers of instruments or instrument components.
- The instruments shall be changed and/or repaired only by the manufacturer or his authorized representative.
- Specifications subject to change.

4



Microscope adjustment in brief (Brightfield)

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

Described is the microscope adjustment with 2 focusing eyepieces, as usual in microscopy with the Axiovert 35, 35 M and 405 M inverted microscopes. For the adjusting procedure with other eyepieces (e.g. with Axiovert 10) see Section **5.0**. The description below applies to an illumination system with condenser system 0.32 Pol. Other condensers and their specific operating controls are described on page 12.

- Check data on label of illumination power supply for coincidence with the line data and connect it to the line.
- Switch on illumination on power supply and set to 3 – 4 V.
- Load a high-contrast specimen (3). If mounted on a specimen slide, the smaller, thinner coverglass must face down.
- Turn in a 10× objective (yellow ring) (4) on the nosepiece, and check the 0 positions on the eyepiece scale. With (1) move the condenser all the way down (front lens not swung out).
- Close diaphragm (2) of condenser system 0.32 Pol about half.

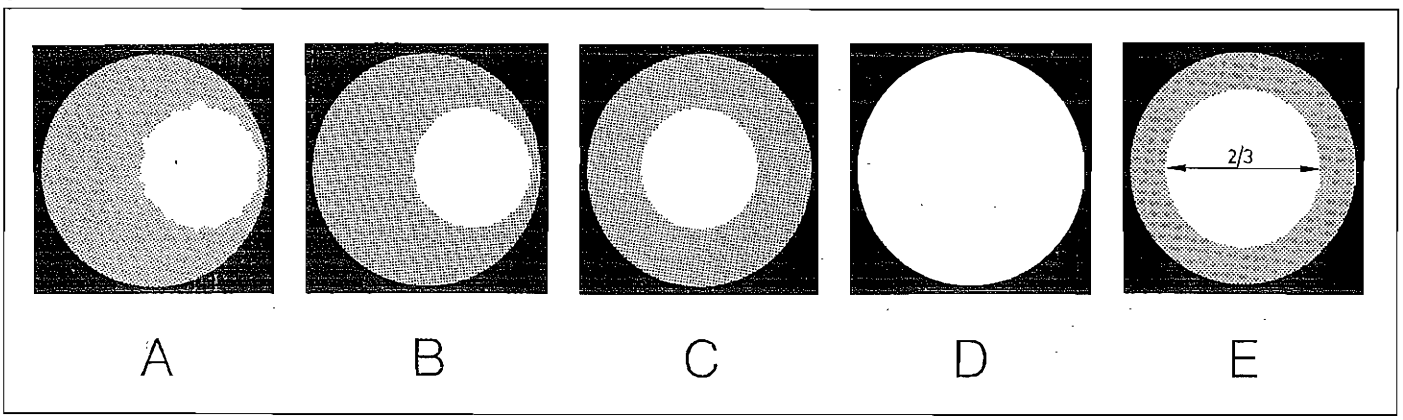
You should now see light spots (the exit pupils) behind the eyepieces. The pushrod of a binocular phototube with sliding prism must be pushed in to achieve this.
Through the tube you will see a bright circle (the eyepiece stop) with each eye. By turning the two eyepiece tubes to your PD you will merge the two circles into one.

Further steps of Köhler illumination adjustment:

- Adjust both eyepieces to the photographic format reticle by turning the eyelens (8).
- Focus on the specimen with coarse/fine focusing control (5).
- Close luminous-field diaphragm (7) moderately; it will become unsharp in the image (A).
- Focus the diaphragm image by slightly lifting the condenser (1) (B).
- With screws (6) move the diaphragm image to the center of the field of view (C), and
- open luminous-field diaphragm (7) until it just disappears from the field of view (D).

The contrast is adjusted for each specimen with the condenser diaphragm (2). If you are not certain how far to stop down: approx. ⅔ of the rear lens element of the objective (it is visible at the bottom of the tube without eyepiece) should be illuminated if the specimen is of moderate contrast (E).
Field of view and objective aperture change, of course, with each objective change so that the last-mentioned steps must be repeated in each specific case. If a low-power objective images more than the condenser can illuminate, swing out the condenser front lens (for a full description see page 14).

5



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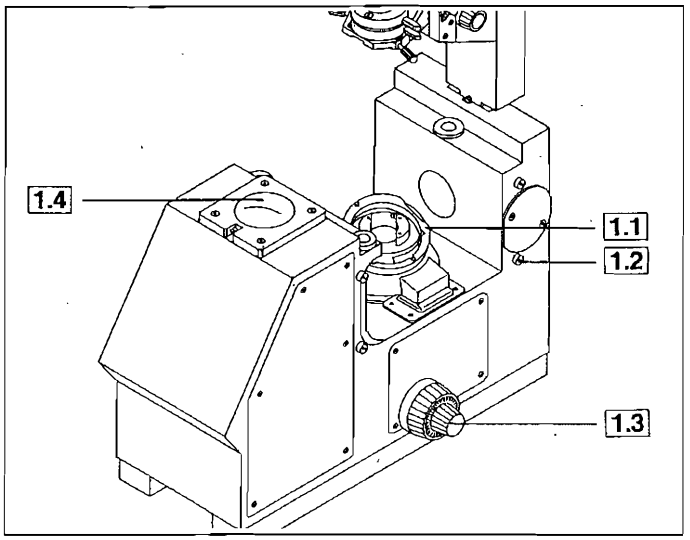

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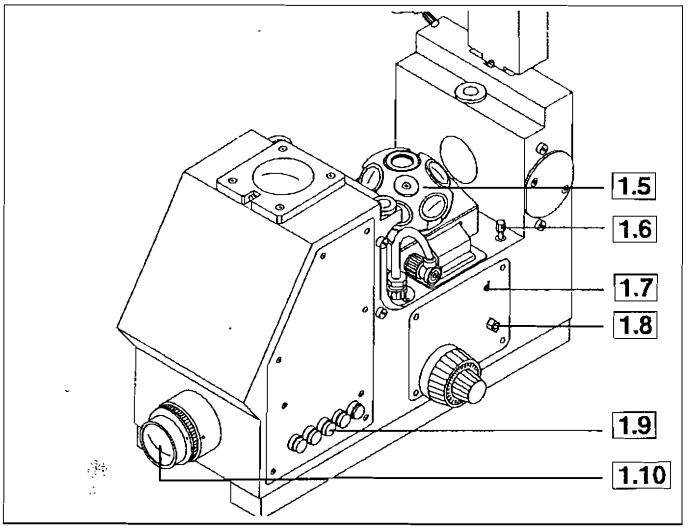

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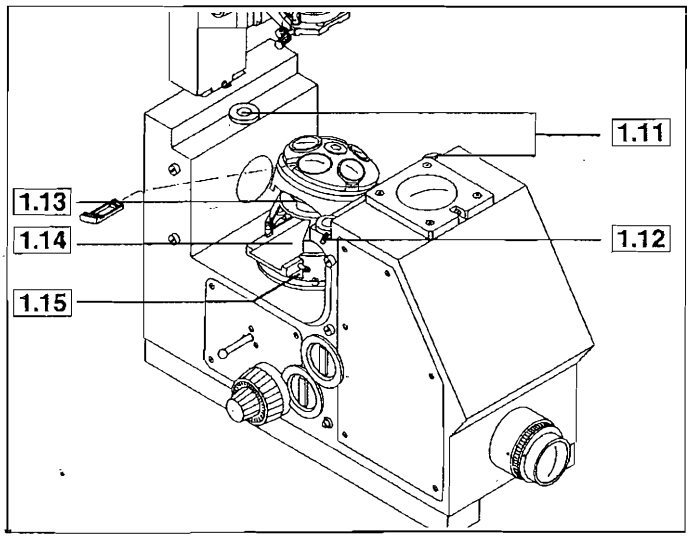


NB:
All relevant screws are socket head cap screws for which the following tools are supplied:
Allen wrench SW 3 with red handle for the assembly of tubes and illuminators, and for diaphragm adjustment. Allen wrench SW 1.5 to center the phase-contrast diaphragms of LD condensers.

1.0 Stands

1.18 Transilluminator carrier, rigidly connected with all transmitted-light Axiovert stands. The carrier is supplied separately and can be mounted aligned with 4 screws. For details see Section 3.0.

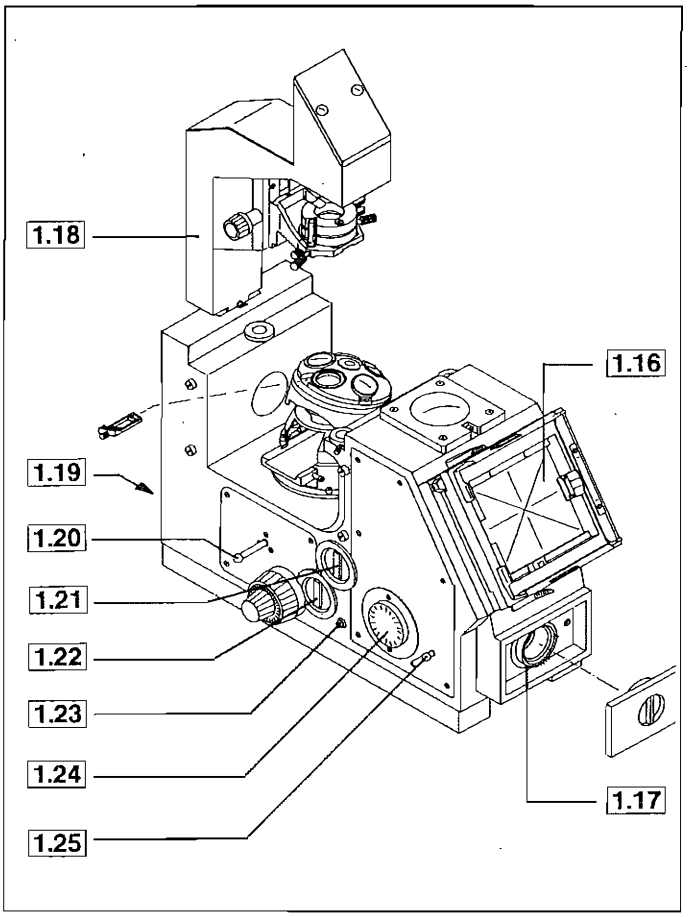
1.3 Coaxial coarse/fine focusing control acting on the nosepiece. Turning the outer part of the knob anticlockwise lowers the nosepiece. Total vertical travel range (including fine focusing control): 5.5 mm. One revolution of the coarse focusing control corresponds to approx. 2 mm travel; gear ratio of fine focusing control: 1:10. The index line on the coarse focusing control can be used to roughly measure the object thickness: 1 scale division corresponds to approx. 1 µm.

1.4 Port with clamping screw for binocular tube.

1.11 Boreholes for the 3 pins of the microscope stage.
1.12 Clamping screw to secure the microscope stage.
1.2 Boreholes (on both sides) to mount micromanipulators.

Axiovert 10 and 35:
1.1 Dovetail mount for nosepiece.
1.5 Rigidly mounted nosepiece 5× H DIC or 5× HD DIC of Axiovert 35 M and 405 M.
1.13 Slot for auxiliary objects, required e.g. for differential interference contrast.
1.14 Slot for reflector sliders, e.g. 2 FL for incident-light fluorescence illumination or Optovar for magnification change.
1.15 Slot for analyzer slider, required for differential interference contrast.
If no sliders are inserted, the slots are closed with covers.

9



Axiovert 35 and 35 M:
1.10 Adapter T 2 to mount a camera, e.g. Contax SLR with cable release.

Axiovert 35, 35 M and 405 M:
1.20 Pushrod of beam splitting prism:
pushed in: 100% of the light for observation
pulled out: 20% of the light for observation,
80% for photography.
1.21 Slot for photographic format reticles on which both focusing eyepieces must be focused. An illuminated reticle is available; it connects directly to a power plug of the Axiovert 35, or to plug **1.23** of Axiovert 35 M or 405 M.
1.22 Slot for a reticle (e.g. a scale) for projection into the photography light path.

The letter M in the designations Axiovert 35 M or 405 M indicates that the rigidly mounted nosepiece is motorized, and that the nosepiece positions can be selected by five illuminated keys.
Required in addition: power plug for reticle illumination and motorized nosepiece, which connects to **1.8**.
Switch **1.7** interrupts the power supply from the power supply unit to the stand.
1.9 Five illuminated keys for selection of the nosepiece positions. An addressed position is indicated by a light in the corresponding key. Which objectives the user choses is up to his requirements. The supplied adhesive tape can be used to mark the objective magnifications on the keys. Depressing either of the keys directly addresses a specific objective and brings it into the light path.
1.6 Knurled screw for stop limiting the height of the motorized nosepiece for objective protection. It is vertically adjusted with the knurled screw: turned clockwise it moves the stop down (the objective on the nosepiece must be lowered), anticlockwise up (smaller objective distance).
If the light in the pushed key flashes during adjustment with the knurled screw, the limit is achieved, and motorized operation of the nosepiece no longer possible. If motorized operation is wanted, the nosepiece must be lowered, or the knurled screw turned anticlockwise, if possible.

Axiovert 405 M inverted camera microscope:
1.25 Control to change between 4×5"/35 mm format, with display and transfer of the adjusted ASA value for either format to the exposure control.
1.17 Rigidly mounted 35 mm film cassette Mot.
1.16 4×5" ground glass and cassette holder.
1.19 Multi-point connector for connection of Axiovert 405 M and automatic exposure control
1.24 ASA setting for 4×5"; the adjusted ASA value is automatically transferred to the exposure control.

2.0 Specimen stages

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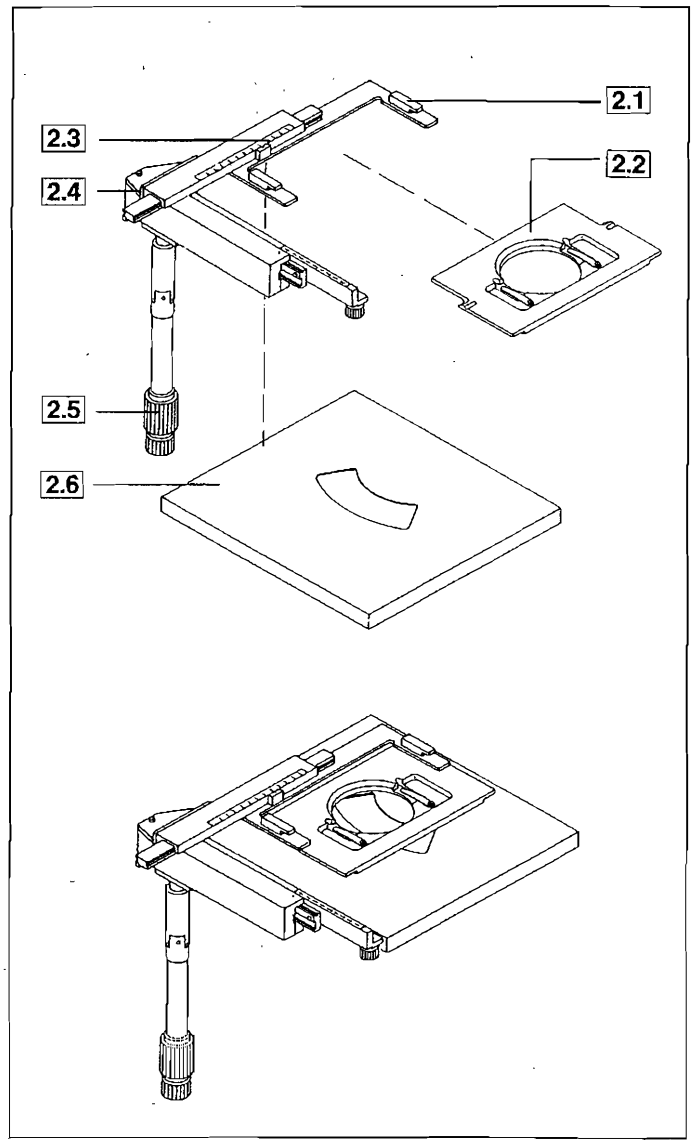

Standard equipment comprises:
2.6 Stage plate 211×230
2.4 Attachable mechanical stage mounted on the left edge of the stage plate and secured with 3 knurled screws. The three pins of the specimen stage engage corresponding boreholes in the microscope stand. A clamping screw secures the stage. The attachable mechanical stage is mounted before the stage plate is attached.
2.5 Coaxial control for movement in X and Y.

For different investigation methods and specimen slides:
2.2 Mounting frames for specimen slides
2.3 Adhesive scales for mounting frames
2.1 Spring clips. The mounting frame is slid from the front under the clips until it snaps in. Stick the scales in the corresponding recesses of the mounting frames.

Mounting frames are available for:
- Microtest plates with 60, 72 or 120 positions (Terasaki), 81.5×56 mm, with adhesive scales
- Microtiter plates with 96 positions, 128.5×86.3 mm, with adhesive scales
- Hamax plates, Möller-Coats plates with 60 positions, 93.5×67.5 mm, with adhesive scales
- Multi dishes, e.g. Costar plates with 24 positions, 133.5×88.5 mm
- Specimen slide 76×26 mm
- Petri dish 36 mm dia.
- Petri dish 54 mm dia.
- Petri dish 65 mm dia.
- Petri dish 88 mm dia.
- Costar flask 125×77 mm
- Corning flask 98.5×52 mm
- Tissue culture plate (made by Rainer), 6×4×16 = 384 positions, 136.5×92.5 mm
- Plankton chambers 42 mm dia. (plankton culture dishes are also offered, e.g. culture tubes with covers, compound and plate chambers).

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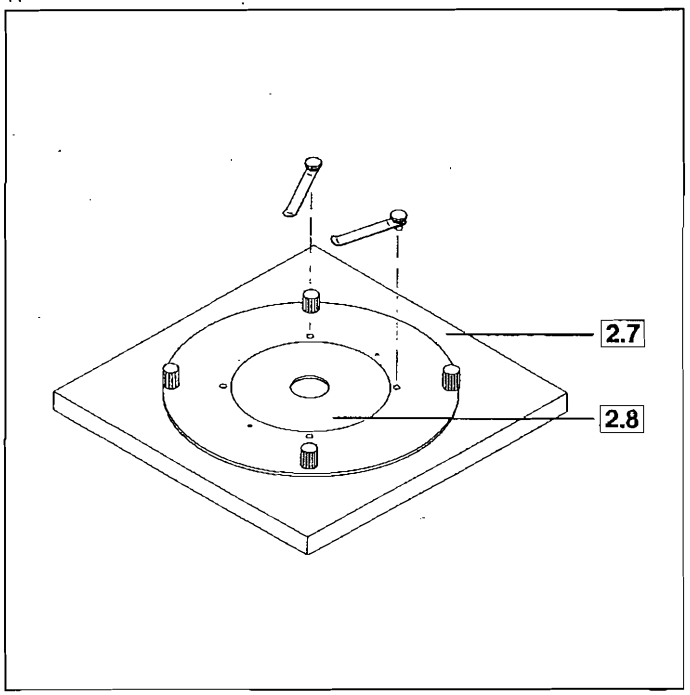

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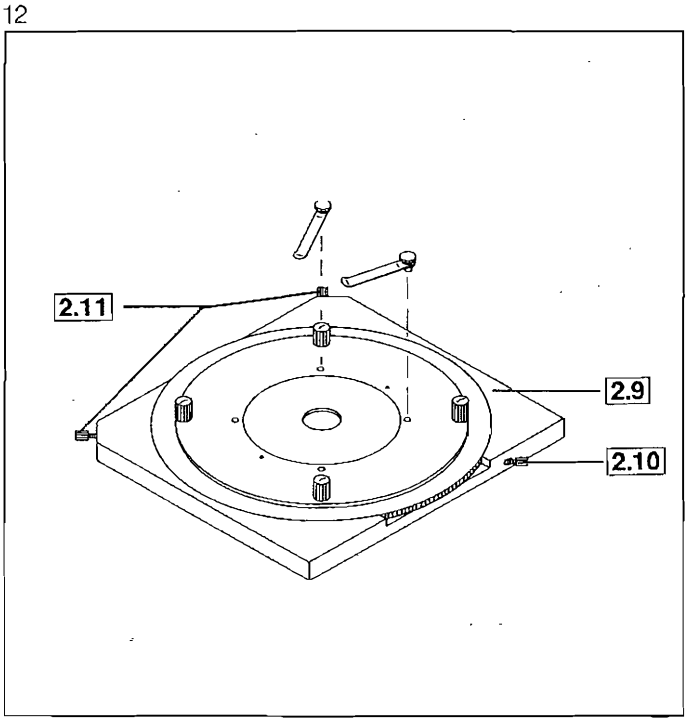


Other stages

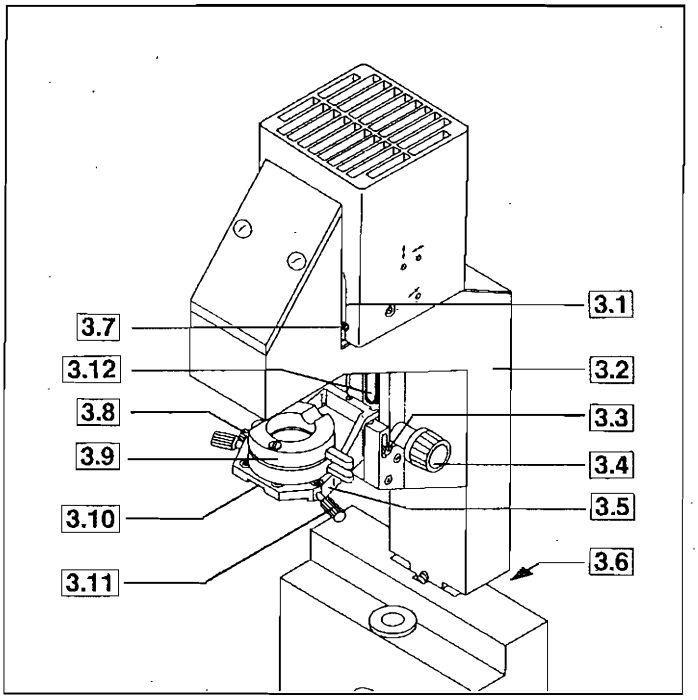
Mounted like the stage plate:
2.7 Gliding stage 10; travelling range in any direction 22 mm.
2.8 Two stage inserts for the above stage; the opening (24 mm and 48 mm dia.) depends on the size of the cover-glasses of the specimens.

2.9 Rotary, centrable gliding stage Z with two stage inserts 24 mm and 48 mm dia.
2.11 Centering screws for stage carrier
2.10 Clamping screw for stage carrier
Travelling range in any direction 22 mm.

For special applications:
Heating stage M (with temperature control TRZ 3700 and hot plate) for temperature adjustment from 3 °C above room temperature to 50 °C.
Scanning stage 100×100 with step widths of 0.25 µm and 0.5 µm (for microscope photometry).

3.0 Transillumination system

13



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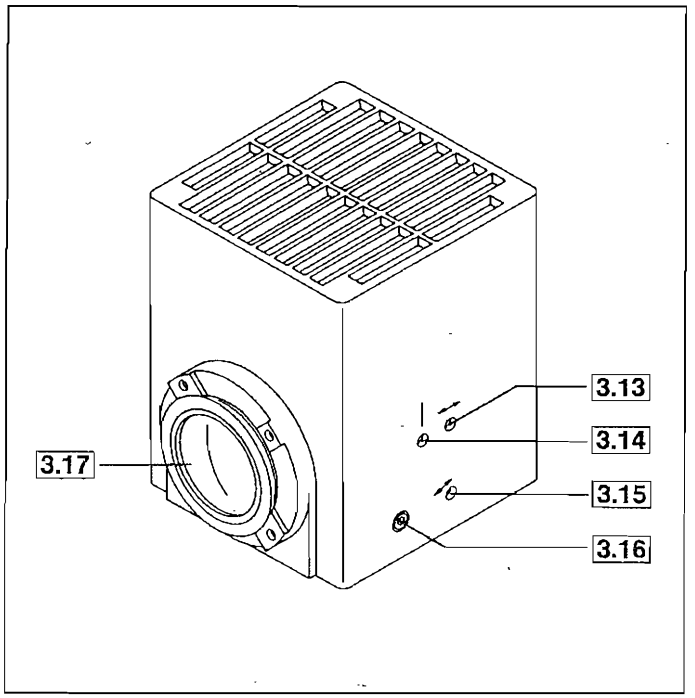


3.2 Transilluminator carrier
3.6 4 screws for mounting on the stand and adjustment

3.1 Mount for illuminator 100 Hal
3.4 Coaxial control for vertical adjustment (approx. 30 mm) of
3.5 condenser carrier. The motion of the carrier is factory-adjusted (re-adjustment shall be made by a service technician).

3.10 Guide notch for condenser
The condenser or the spacer tube with iris of high-aperture condensers is inserted from the front in the dovetail mount; the guide pin must engage the notch.

3.11 Two condenser centering screws. They serve to center the luminous-field-diaphragm image when adjusting the illumination (see page 5).

3.8 Condenser clamping screw; it is needed only for condenser exchange.

3.9 Two swing-out holders for filter or polarizer (lower holder).

A stop of the vertical condenser adjustment prevents the specimen from being damaged. The stop is adjusted as follows:
1. Adjust specimen
2. Form image of luminous-field diaphragm (see page 5); loosen screw with pin 3.3 with supplied wrench.
3. Lower condenser slightly (the diaphragm image becomes unsharp again).
4. Move screw with pin all the way up and tighten it. The specimen is secured.

3.12 Upper limit stop for condenser. To prevent the LD condenser 0.55 from hitting against the carrier, the tongue of the limit stop must point down. It must point to the side for all other condenser types.

The transilluminator can be hinged back for easy specimen exchange.

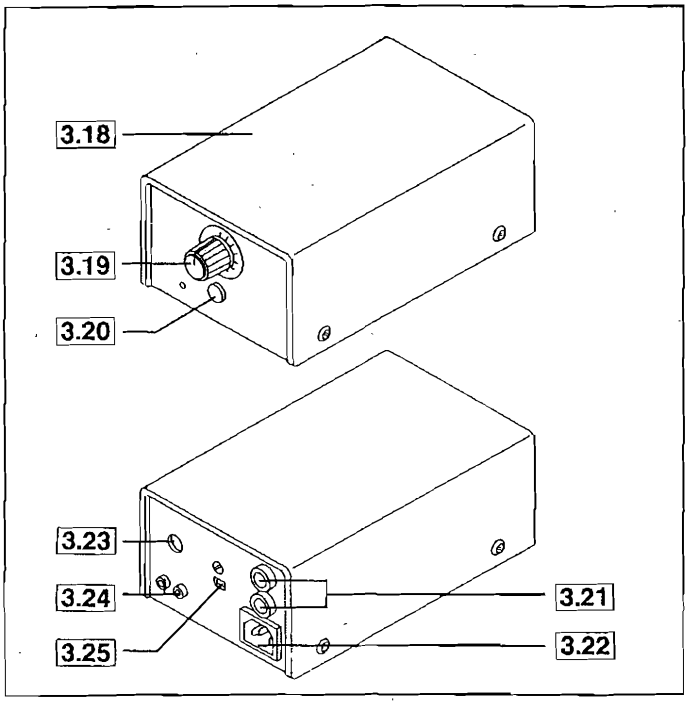
The illuminator 100 Hal comprises a lamp housing 100 Hal with collector and socket and a 12V 100W halogen lamp.

3.18 12V 100W power supply.

Features of illuminator 100 Hal:
3.17 Light exit port with dovetails for mounting on carrier for transilluminator:
- Unscrew screw 3.7 sufficiently
- Attach illuminator dovetails at an angle to port opposite the clamping screw, swing illuminator on seating surface and tighten screw.
- A holder in the light exit port before the collector accepts a 42 mm dia. heat-reflecting filter.

3.16 Clamping screw for lamp socket and collector
3.13 Focusing of lamp coil
3.14 Vertical adjustment of lamp coil
3.15 Lateral adjustment of lamp coil

15



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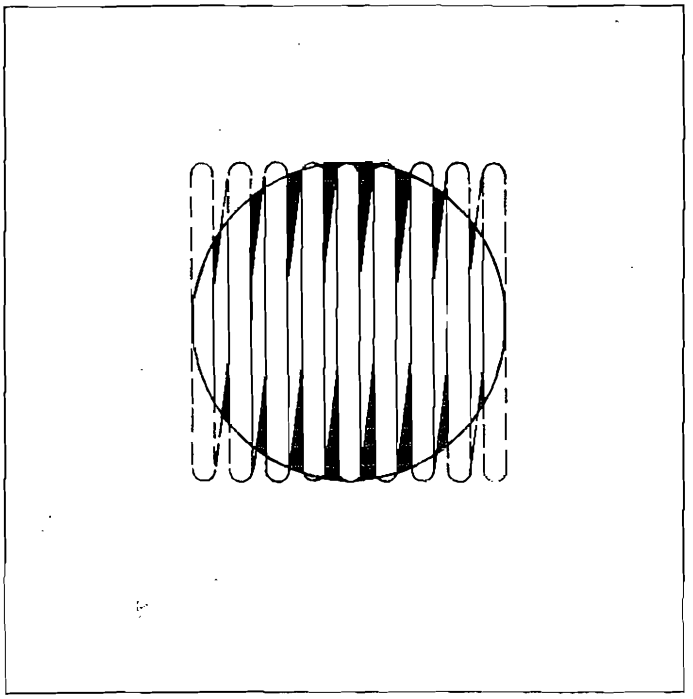
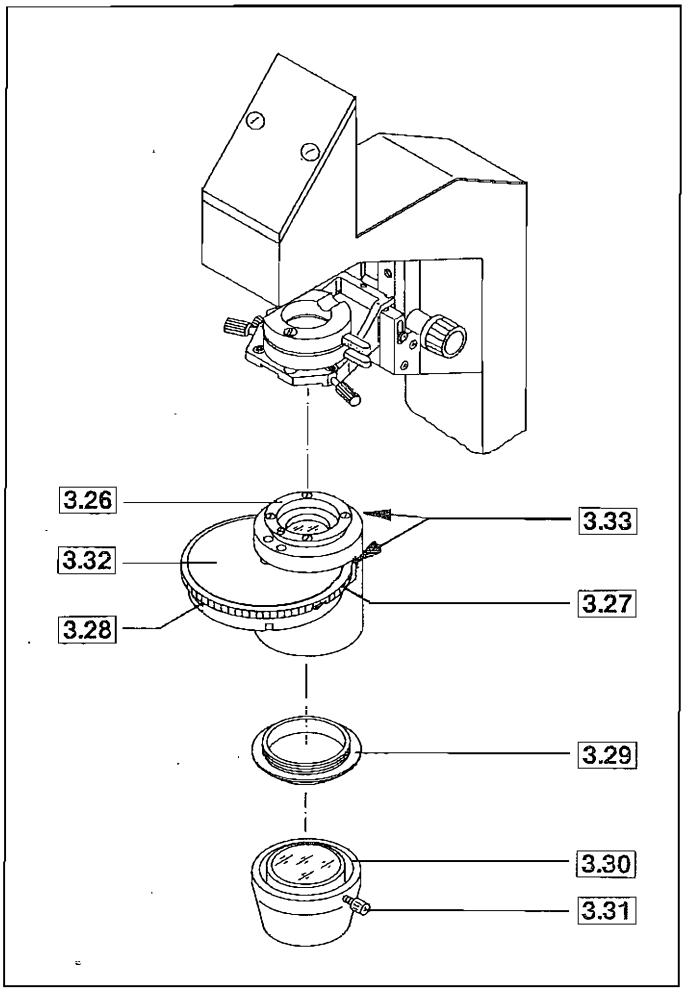


Stabilized 12 V 100 W power supply
Power consumption 100 VA
Frequency 50...60 Hz.
The output voltage is stabilized and adjustable.
The power supply is radio-screened and complies with IEC, VDE, UL and CSA regulations.
The input voltage is adjusted: 100–110–120–127–220–240 V AC and indicated on **3.25**. Before connecting the instrument to the line, check whether the adjusted voltage corresponds to the local line voltage.

Features of the power supply:
3.19 Power switch with potentiometer; turned fully clockwise it supplies 12 V lamp voltage. The index indicates the adjusted voltage. Adjustment to 11.5 V corresponds approximately to the color temperature 3200 K.
3.20 Power signal lamp
3.22 Power socket on instrument
3.21 Two fuse inserts (see page 47)
3.25 Voltage indicator
3.24 Sockets for 12 V 100 W halogen lamp
3.23 Output for a control line to set the lamp to 3200 K independent of the potentiomenter **3.19** position. It facilitates color temperature adjustment for photography. Triggering is possible with automatic exposure control of Axiovert 405 M (see Section **6.0**).

The lamp is factory-centered. Should centering be necessary, proceed as follows:
- Detach lamp from transilluminator carrier, unscrew diffusion disk from holder on carrier.
- Switch on lamp, swing out filter on condenser carrier and adjust specimen with condenser system 0.32 Pol and 40× or similar objective.
- Without eyepiece the pupil with the coil image can be seen at the bottom of the tube. Focus with **3.13**. The coil image should evenly cover the pupil. If the image is other than in the opposite Fig. 16 correct with **3.14** and **3.15**.
- Detach lamp again, screw diffusion disk into holder on carrier, check pupil image again, and optimize adjustment with **3.13**, if necessary.

17



Free space is necessary between specimen plane and illuminator to examine specimens in chambers and flasks. For brightfield and phase-contrast illumination with long working distance use

3.26 Condenser LD 0.3 H, Ph 1,2

It includes

3.27 a centered aperture diaphragm with iris which is used as contrast aperture diaphragm.

3.32 Turret with the positions:

- Brightfield (H)
- Phase contrast 1 (Ph 1)
- Phase contrast 2 (Ph 2)

3.28 Index for turret position

3.33 Centration of phase-contrast diaphragms (with Allen wrench).

With this equipment the working distance is approx. 70 mm in brightfield and phase contrast. Object fields of max. 8 mm dia. can be illuminated (e.g. with 2.5× objectives; to illuminate the object field for 1.25× objectives for photography do not use the condenser).
Objectives with NAs ≦ 0.45 are suitable for brightfield; the condenser in Ph 2 position can be used for objectives with NAs up to 0.75.

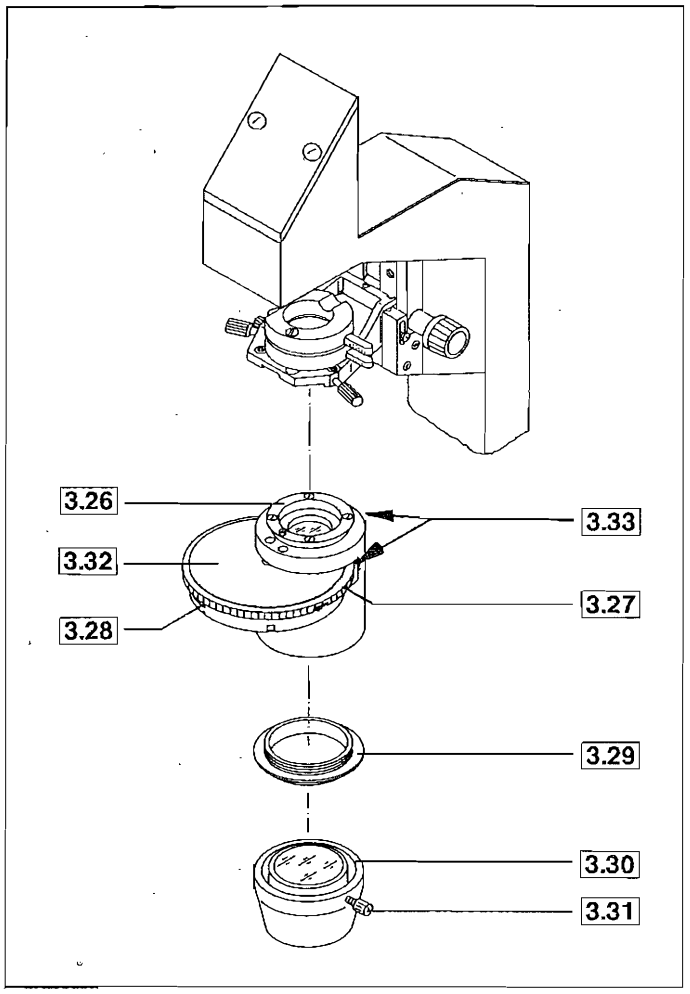
3.30 Front lens 0.55 increases the numerical aperture of the condenser to 0.55.

3.31 Clamping screw to mount it on condenser LD 0.3

3.29 Dovetail mount screwed into the condenser before mounting the front lens.

With the front lens the working distance is approx. 23 mm, the diameter of the illuminated object field 4 mm (with 5× objectives). Only in brightfield is the condenser suitable for objectives with NAs ≦ 0.6 (max. 40× objectives, with the exception of immersion objectives).

17



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With this equipment the working distance is approx. 70 mm in brightfield and phase contrast. Object fields of max. 8 mm dia. can be illuminated (e.g. with 2.5× objectives; to illuminate the object field for 1.25× objectives for photography do not use the condenser).
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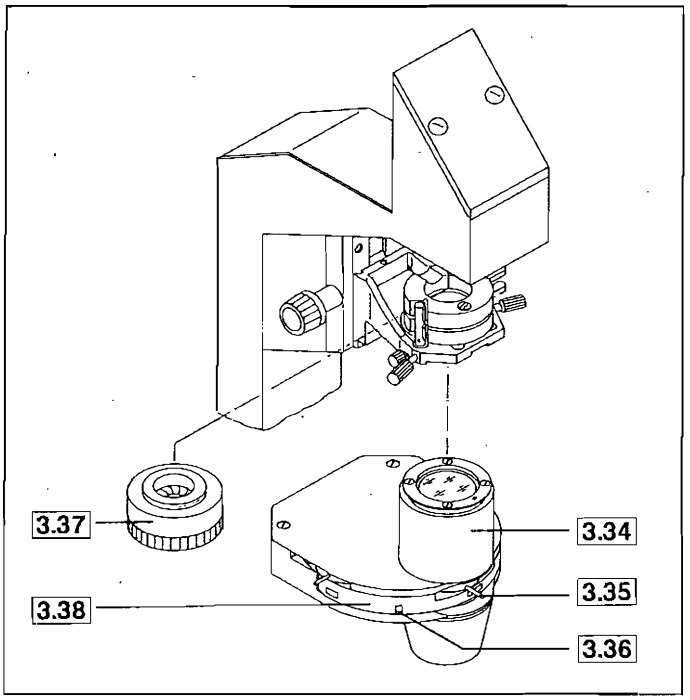

For ample working space and the use of brightfield, phase contrast and DIC:
3.34 LD condenser 0.55 Ph/DIC suitable for adjustment of Köhler illumination.
3.37 Centrable luminous-field diaphragm; the mechanical part with the diaphragm is screwed into the lamp mount from beneath.

The standard equipment of turret
3.38 provides for
- DIC 0.3–0.4 (position I, DIC .3–.4) or brightfield (H), alternatively:
- DIC 0.5–1.3 (position II, DIC .5–1.3) or brightfield (H), alternatively:
- Phase contrast 1 (Ph 1)
- Phase contrast 2 (Ph 2)

3.35 Centered aperture diaphragm which is adjustable in all DIC positions. The selected position is indicated in front of the turret.
3.36 Centering screws for the diaphragms in Ph positions.

This condenser has a working distance of 22 mm for all methods; object fields of max. 4 mm dia. can be illuminated (that is up to 5× objective magnification).
In brightfield the condenser is suitable for objectives up to a numerical aperture of 0.6, in Ph and DIC up to 0.75 (dry objectives up to 40× magnification).

For exchange of DIC prisms see page 43.

The condenser LD 0.55 Ph/DIC is adjusted as follows:
- Screw luminous-field diaphragm unit into illuminator carrier, close diaphragm.
- Center the diaphragm image in the field without condenser and without objective by adjusting the 3 centering screws on **3.37** with 1.3 mm Allen wrench. Secure the adjusted luminous-field diaphragm by tightening the spring screw.
- Insert condenser and objective, focus luminous-field diaphragm with condenser control and center with the centering screws of the condenser mount.
- Center phase-contrast rings with centering telescope (Allen wrench 1.5 mm), see page 34.

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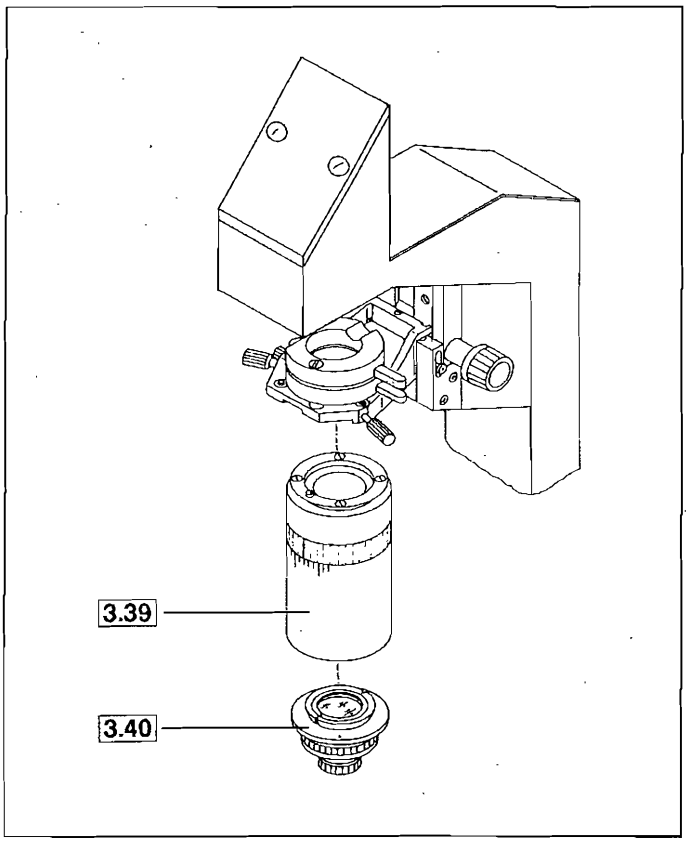

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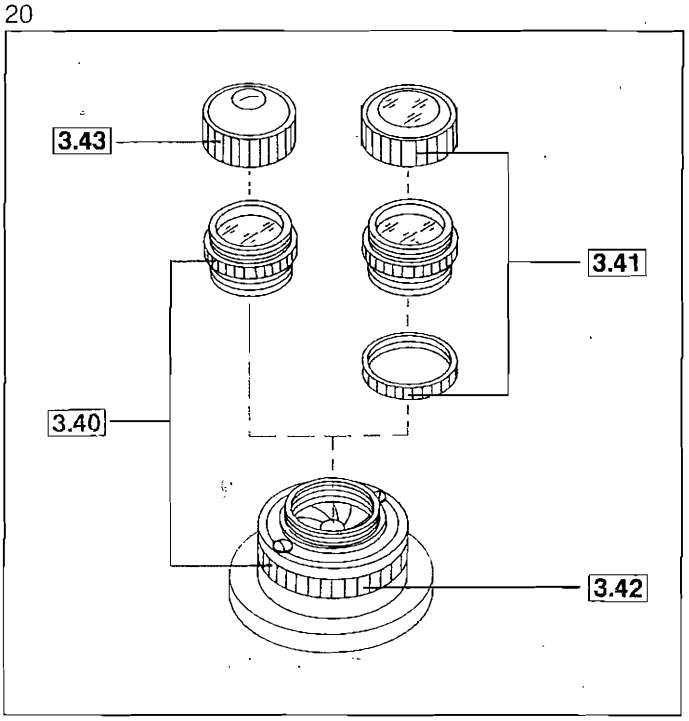


The resolving power of high-power objectives is fully utilized with the following condenser. Köhler illumination provides for a homogeneously illuminated object field, a brilliant image without reflections and glare, and optimum specimen protection.
3.39 Condenser mount with iris (pre-centered luminous-field diaphragm) is required for this condenser.

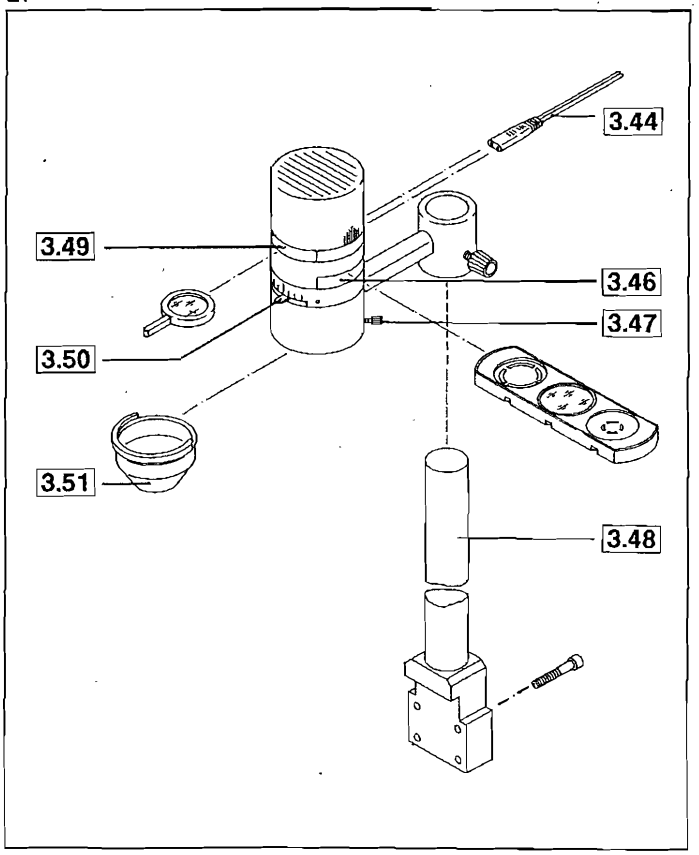
For brightfield or DIC with long back focal distance or high aperture (1.4):
3.40 Condenser system 0.32 Pol (445245), with screw-in optics (aperture 0.32), and
3.42 knurled ring for aperture iris diaphragm.

3.41 Front lens 0.63 Pol for long back focal distance (an additional spacer ring is required), or
3.43 front lens 1.4 Pol
can be screwed on the condenser system 0.32 Pol.

Equipment with front lens 1.4:
Köhler illumination possible with 20× ... 100× objectives, centrable luminous-field and aperture diaphragms. DIC with 20× ... 100× objectives with built-in DIC prism (445294).

Equipment with front lens 0.63:
Köhler illumination possible with 5× ... 40× objectives,
DIC with 5× ... 10× objectives with DIC prism (434410)
DIC with 20× and 40× objectives with DIC prism (445293).
The correction is achromatic-aplanatic.

21



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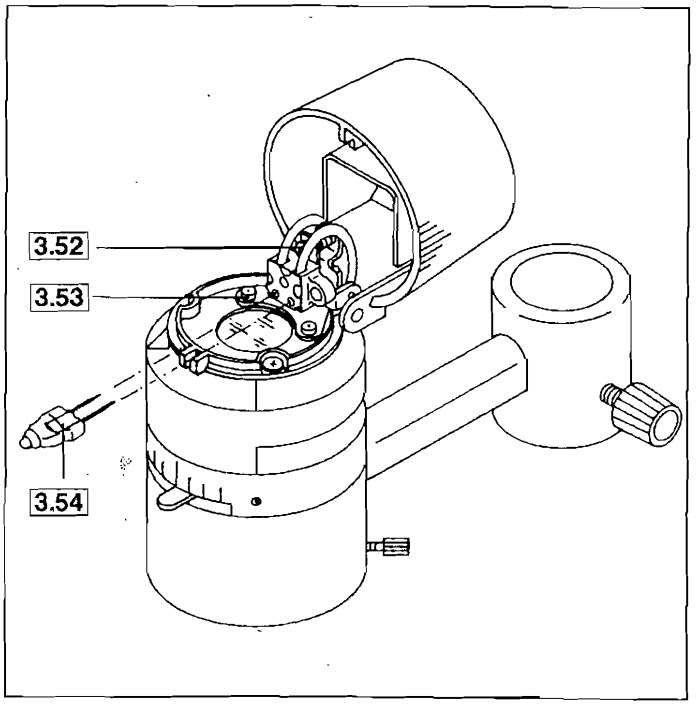


For routine examinations in brightfield and phase contrast and for long working distances:
Simple 6V 20W illumination system, comprising:
3.48 Illuminator carrier with illuminator 20 mounted on top,
3.54 6V 20W halogen lamp (it is visible when the black cover is hinged up and secured by 2 contact pins),
3.44 cable with plug for stabilized 6V 20W power supply, 100–240 V changeable, 50...60 Hz.

From top to bottom:
3.49 Slot for green interference filter, 32 mm dia. (for contrast enhancement in phase contrast)
3.46 Slider with 3 positions: Ph 1 – brightfield (ground glass) – Ph 2
3.50 Aperture diaphragm for brightfield contrast enhancement
3.47 Centering screws for phase-contrast adjustment

The above illumination system has the numerical aperture 0.3 which can be increased to 0.6 by
3.51 front lens 0.6 which is mounted at the bottom of the illuminator carrier.

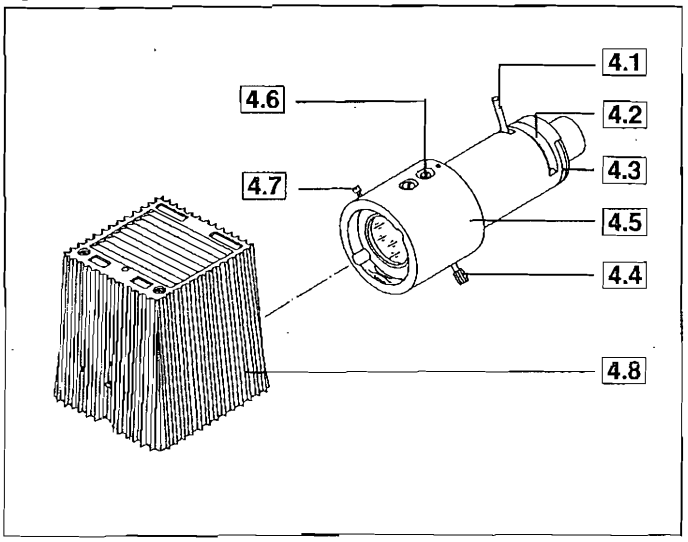
Without front lens this simple illumination system illuminates the object field homogeneously with 1.25× objective.

Adjustment of illuminator 20

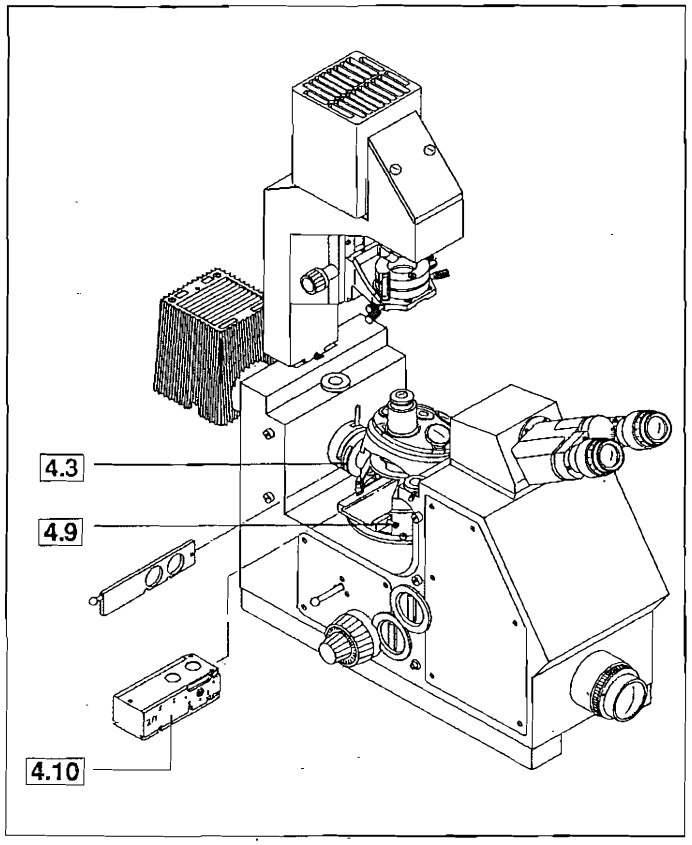
- Clamp illuminator on 32 dia. column so that the distance between front lens mount (without front lens 0.6) and stage plate is approx. 56 mm.
- Set slider to brightfield position and center luminous spot on the stage with reference to the objective by turning the illuminator on the carrier.
- The lamp is usually centered. Field and/or pupil may be inhomogeneously illuminated in case of extreme lamp coil deviations. For adjustment, loosen the two locking screws **3.53** of the lamp socket with a 2 mm Allen wrench so that the socket can be shifted. The vertical lamp adjustment **3.52** can be corrected with the same tool. Adjust to maximum brightness.
- The phase-contrast rings must be controlled in phase contrast (see also page 34). In brightfield control the aperture diaphragm with the centering telescope and center it with the adjustable front lens (knurled screws).
- Plug on front lens 0.6 for objectives with apertures higher than 0.4. Check the pupil illumination with the centering telescope and adjust the illuminator on the column laterally and vertically until homogeneous illumination is achieved with highest aperture.

4.0 Incident-light fluorescence illuminator

23



24

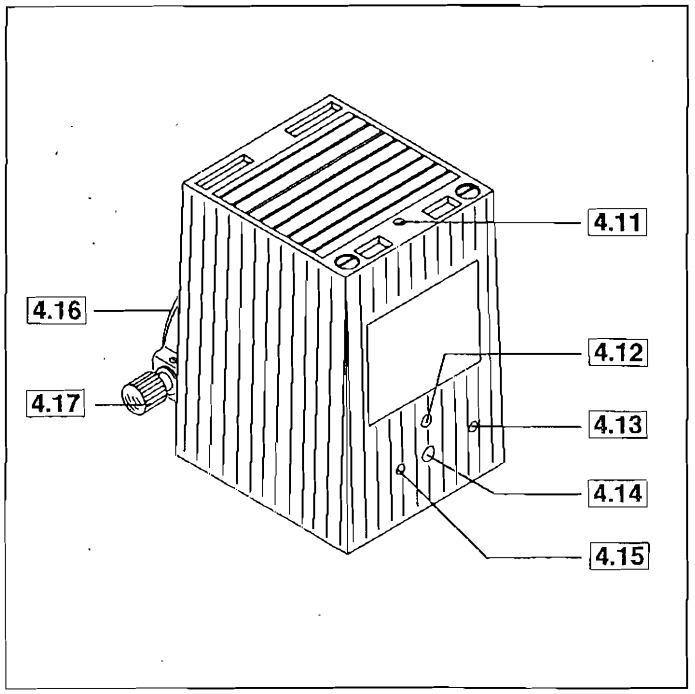


The incident-light fluorescence illuminator converts any Axiovert inverted microscope into a fluorescence microscope with incident-light excitation. It can be combined with either of the transmitted-light illumination systems described in Section 3.0 for specimen observation in brightfield and/or phase contrast.

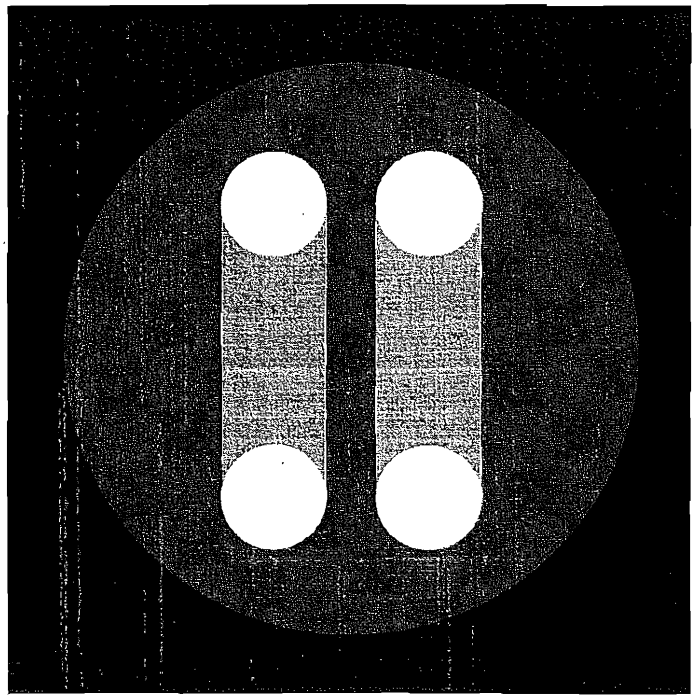
The fluorescence illuminator comprises:
4.8 Lamp housing HBO/XBO with 3-lens collector, socket for HBO 50 lamp and an HBO 50 lamp.
It connects to the line via power supply for HBO 50, 220 – 240 V, 50...60 Hz and power cable.
(For excitation suitable for the FITC method use the 12V 100W Hal illuminator.)
The fluorescence illuminator is secured on
4.5 illuminator adapter with
4.7 clamping screw.
In the light path:
4.6 Slotted screw; when loosened it serves to focus the luminous-field diaphragm.
4.4 Screws for the centration of this diaphragm.
4.1 Lever to close the luminous-field diaphragm.
4.2 Slot for extra, loose exciter filter. The slot also accepts 32 mm dia. filters or 32 mm dia. polarizing filters (for reflection contrast) in a holder.
4.3 Slider with 3 positions:
Position 1 – dark slider blocking the light path
Position 2 – red-attenuating filter BG 38
Position 3 – free light path (25 mm dia. filter holder).

4.10 Reflector slider 2 FL to change, for example, between UV and violet excitation by sliding the reflector slider from one click stop of the guideways to the next. The engraving on the reflector slider must face the user. After loosening clamping screw
4.9 the slider can be exchanged for another one for blue and green excitation.
Both positions of the reflector slider 2 FL can be equipped with sets comprising exciter filter-reflector (45° plane glass plate acting as chromatic beam splitter) and barrier filter, or one position left unoccupied for transmitted-light work, to adjust the specimen in brightfield or phase contrast. For details see page 37.

25



26



The lamp is factory-centered. If re-centering is necessary, you should first of all know the features of the HBO/XBO lamp housing.

4.16 Light exit port with dovetails to mount the incident-light fluorescence illuminator FL.

4.17 Knob for collector adjustment. The collector can be removed when this knob is pulled out (a pin of the knob engages a notch of the collector). A holder at the front in the collector accepts a 42 mm dia. heat-reflecting filter, but should be empty if the illuminator is used for UV blue fluorescence excitation.

The clamping screw for the lamp socket is concealed at the bottom of the housing.

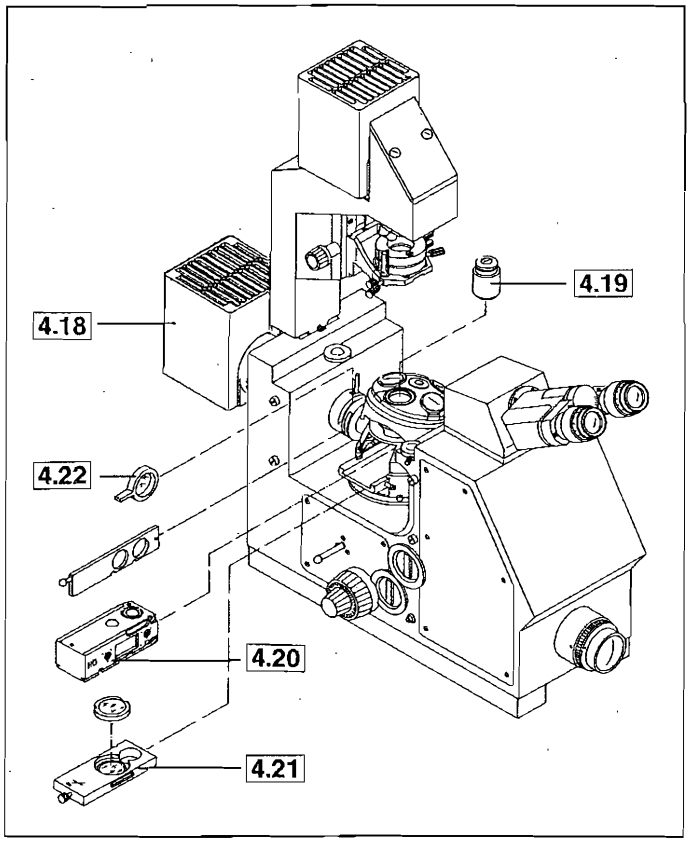
4.11 Vertical lamp adjustment
4.13 Lateral lamp adjustment
4.12 Vertical adjustment of mirror image
4.15 Lateral adjustment of mirror image
4.14 Focusing of mirror image

A heat-reflecting filter KG 1 is built into the illuminator adapter FL **4.5**.

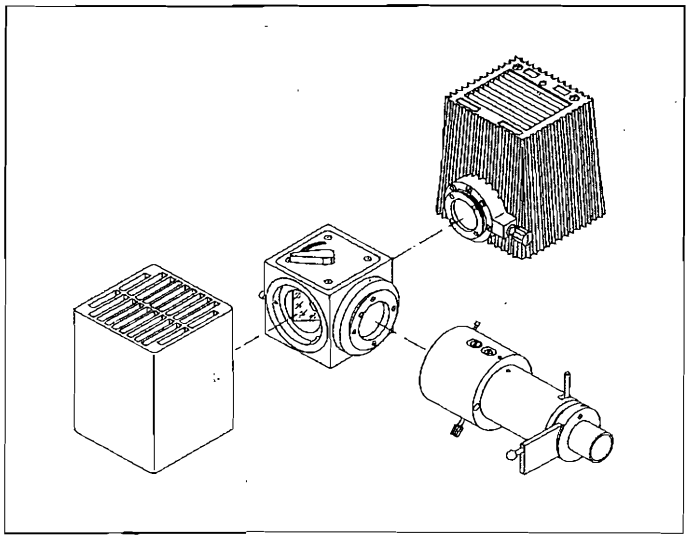
For lamp centration proceed as follows:
1. Detach illuminator from the microscope, switch on lamp and with **4.17** image cathode focal spot and its mirror image (see opposite image) on a suitable surface (e.g. a wall at 1 m distance).
2. If image and mirror image are other than in the opposite Fig. correct with **4.11** to **4.15**.
3. Attach illuminator, set slider **4.3** to free light path and switch reflector slider 2 FL to blue excitation (e.g. with filter set 09).
4. Unscrew objective and check the light source image on a sheet of paper in the object plane (stage plane).
5. If necessary, correct with **4.17** and **4.11** to **4.15**. Screw in objective.

Reflection-contrast illumination system

27



28



Use the Antiflex method for reflection-contrast microscopy.

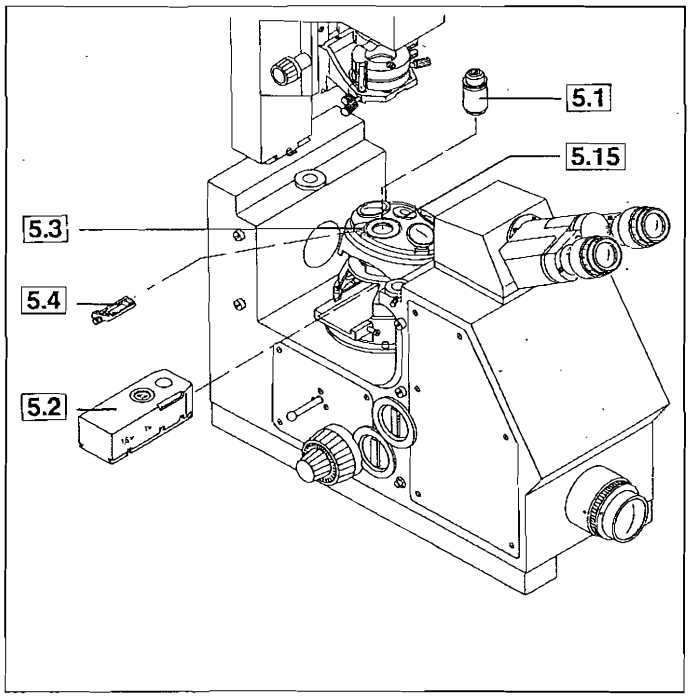
The above-mentioned incident-light fluorescence illuminator FL is used in combination with
4.18 illuminator 100 Hal.
4.20 Reflector slider HD with Smith reflector in the bright-field position replaces the reflector slider 2 FL.
4.22 Polarizer inserted in its slot.
4.21 Fixed-analyzer slider; a lambda plate can be plugged in. This slider can be fixed with a lever.
4.19 Plan-Neofluar objective 63×/1.25 oil Antiflex. The objective front lens is covered by a rotary λ/4 plate which is immersed together with the specimen.
The luminous-field diaphragm of the illuminator FL acts as aperture contrast diaphragm.

If incident-light fluorescence and reflection contrast are used together, a deflecting mirror for 2 illuminators (Hal and HBO/XBO) is mounted on the incident-light fluorescence illuminator FL.

5.0 Image-forming components

5.1 Objectives are the most important elements of the microscope and must be meticulously clean, especially the front lens surfaces. Clean them with a Q-tip after breathing over the surface.
The numbers and symbols engraved on the objective, e.g. Plan-Neofluar 20×/ 0.50; ∞ /0.17 signify: 20× = (individual) magnification, 0.50 = numerical aperture, ∞ = infinite image distance, 0.17 = coverglass thickness in mm, for which the objective is computed.
(Individual) magnification multiplied by the eyepiece magnification (generally 10×) results in the microscope magnification. (The factor 1.6 or 2.5 must be considered if an Optovar slider 5.2 is used).
The numerical aperture multiplied by 1000 (that is 500 in the above example) is the highest useful magnification; no more details will be revealed above this value.

29



The symbol ∞ is to remind the user that the objective cannot be used on microscopes for which objectives engraved with the number 160 are intended.
The correct coverglass thickness 0.17 mm is the more important the higher the numerical aperture of the objective. Some objectives have correction mounts for adjustment to different coverglass thicknesses. Find out, by means of a high-contrast specimen feature, that position of the correction mount which provides for the best sharpness (re-focusing will always be necessary).

The coverglass thickness is irrelevant for immersion objectives.
Because of the short working distance, 20× objectives and those with higher powers have spring mounts to protect the specimen. To prevent specimen contamination by oil when turning the nosepiece, these objectives can be "locked" with the spring mount in topmost position (don't forget to disengage them from "lock-in" position!).

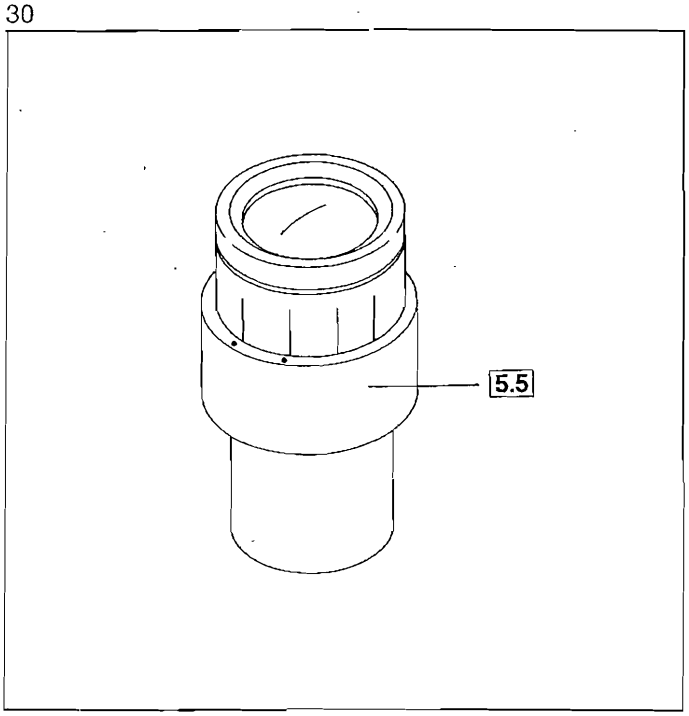
The air between the coverglass and an immersion objective is replaced by a liquid, generally immersion oil. To apply it bubble-free requires some experience. Always control the exit pupil, preferably with the centering telescope to trace bubbles. If the bubbles have not disappeared after turning in the objective several times, clean the specimen and repeat the procedure.

Long distance (LD) objectives have long working distances for observation and manipulation of specimens in culture dishes, microtest and micro titer plates. With coverglass caps some objectives can be adjusted to the thickness of dish or plate bottoms, e.g. the objective LD Achroplan 32×/0.4: without cap it is parfocalized to 1.5 mm ± 0.3 mm, with cap 0.6–1.2 to 0.6–1.2 mm, with cap 0–0.6 to 0–0.6 mm. Objectives in correction mounts, e.g. LD Achroplan 20 or 40 corr. are available for adjustment from 0–2 mm.

5.15 Nosepiece. After loosening the screw and pulling at the same time the spring pin at the end of the screw, the nosepiece can be pulled out (e.g. to check the front lens for cleanliness). Exceptions: the motorized nosepiece H DIC or HD DIC of the Axiovert 35 M and 405 M inverted microscopes which is rigidly connected with the microscope stand.

A microscope equipped for DIC features in the knurled ring of the nosepiece 5×:
5.3 slots for the
5.4 DIC sliders. They must snap in when inserted (designation face down) (see also DIC adjustment on page 35). If you do not use DIC, the DIC sliders can remain in their slots as dust covers, provided the polarizer on top of the condenser is swung out.

30



5.5 Eyepieces – 10× magnification with field-of-view number 20 – produce angular fields of 44°, are equally well suited for eyeglass wearers (Br) and provided with an exchangeable rubber ring to protect eyeglasses (folding eyecups are available under ordering number (444801). Two focusing eyepieces (foc) are used on the Axiovert 35, 35 M and 405 M inverted microscopes. Both must be focused on the photographic format reticle before focusing on the specimen with the microscope focusing control.

The procedure is different for a microscope camera which is mounted on a binocular phototube (this is possible on all microscope models):
A reticle is provided in the eyepiece diaphragm plane of one focusing eyepiece. The slight image displacement it causes is considered by the zero position indicated on the diopter scale by the red instead of the white dot. With the focusing eyelens of this eyepiece focus at first on the reticle, then on the specimen with the microscope focusing control. Now refocus with the other eyepiece until the focus is the same for both eyes.

If you do not photograph with an Axiovert 10 microscope, use one focusing and one fixed eyepiece. If your eyes have different powers or for microscopy without eyeglasses, proceed as follows:
- Focus on the specimen with the less ametropic eye through the fixed eyepiece.
- Leave this microscope adjustment unchanged. With the eyelens of the focusing eyepiece re-adjust the focus for the other more ametropic eye until the sharpness is the same for both eyes. A screw on the eyepiece which engages a notch in the eyepiece tube secures the eyepiece position, which is important for the use of reticles.

Eyeglass wearers who take off their glasses for microscopy may experience defocusing after objective change (objective parfocalization). If your eyeglasses have cylinder power you should wear them for microscopy.

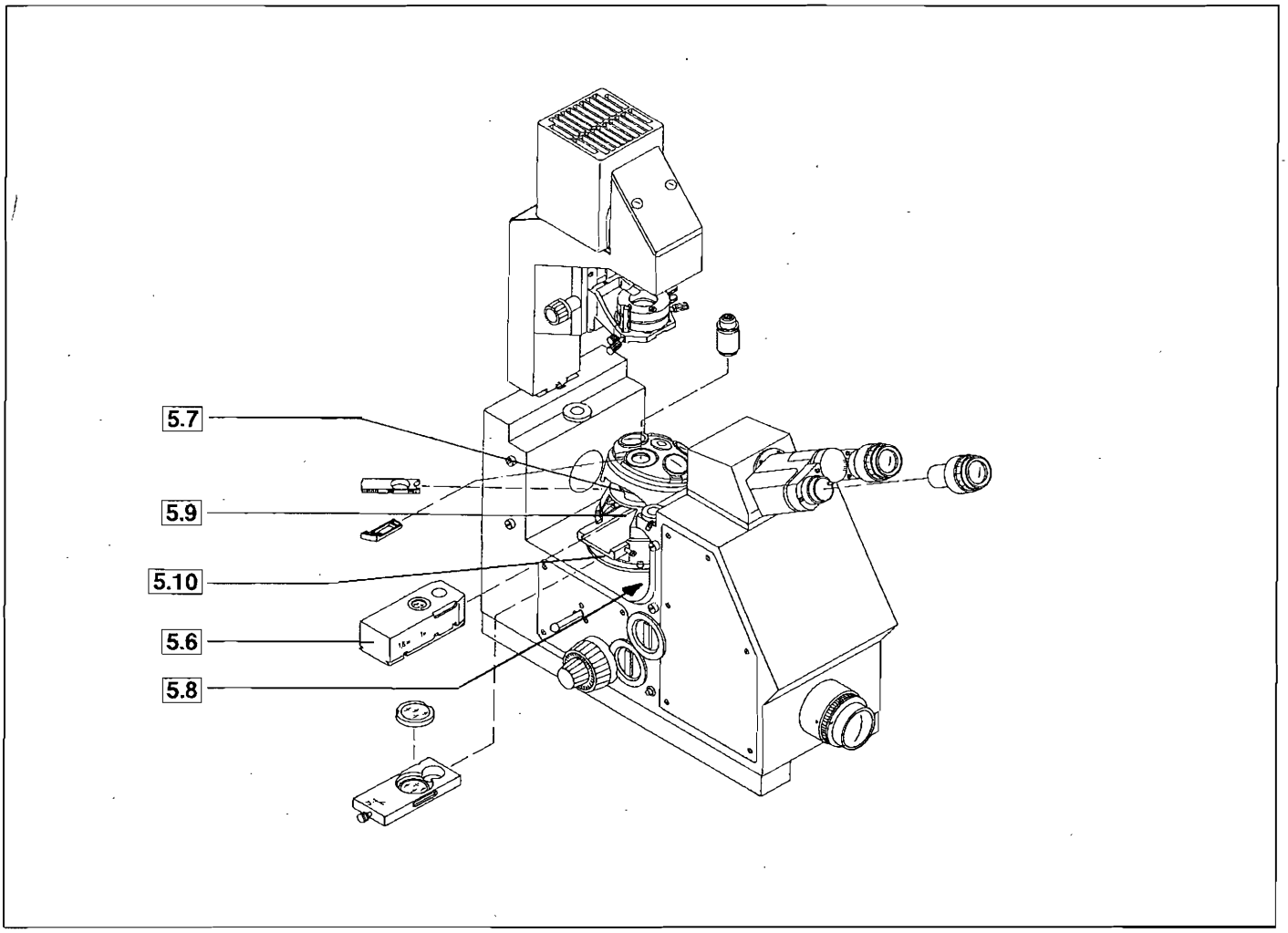
Reticles in the focusing eyepiece should be exchanged only by a specialist because of the high demands on cleanliness and alignment. (The lower eyepiece part can be unscrewed; the scale-bearing surface of the reticle must face down!).

5.6 Optovar slider D (451770) with the factors 1× and 1.6× or (451771) with the factors 1× and 2.5× is inserted in **5.9** with the engraving facing the user.

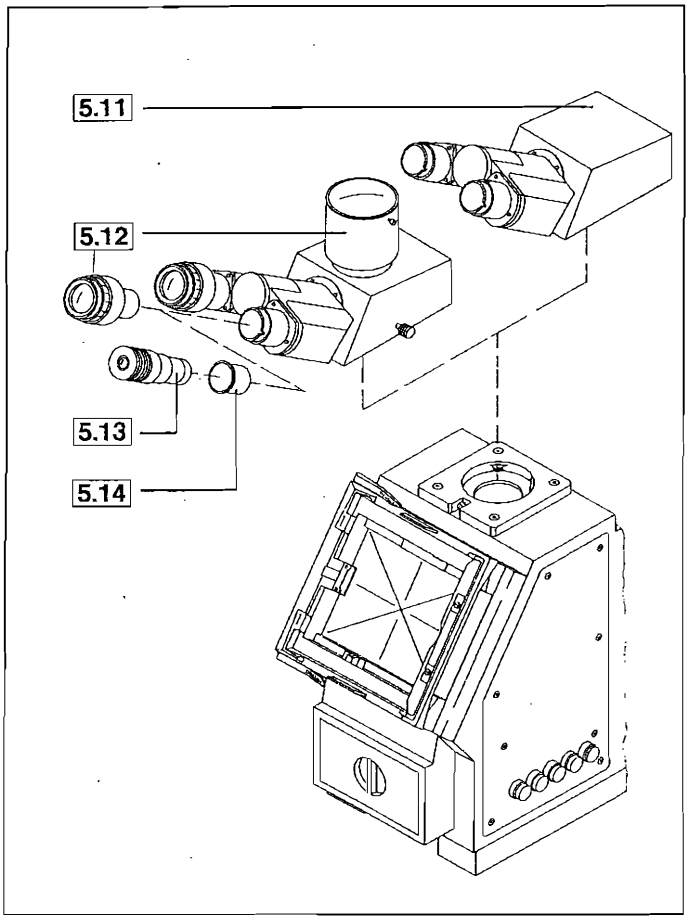
5.7 Slot for auxiliary object lambda (473704), λ/4 (473714) or quartz wedge 0–3 λ (473724) for qualitative polarizing microscopy.

5.10 Slot for fixed-analyzer slider. Besides a blank position it features a position to plug in an oriented analyzer. A lambda plate can be inserted on top of it. White index lines indicate the orientation. To pull out the analyzer slider all the way, press lever **5.8** out of its click-stop position.
The analyzer slider is required for DIC microscopy.

31



32



5.11 Binocular tube. It is the most commonly used, producing an upright, unreversed image of the specimen, which moves synchronously with the specimen in X and Y.

5.12 Binocular phototube with sliding prism. With the knob to the right a pushrod can be set to the following positions:
pushrod pushed in: 100% of the light for observation
pushrod pulled out: 70% of the light for observation,
30% for photography.
The binocular phototube is used
- for photography with the Axiovert 10 or to mount a TV camera on the instrument.
- to mount a 4×5″ camera on the Axiovert 35 or 35 M,
- to mount a TV camera on top instead of at the side of the Axiovert 35, 35 M or 405 M.

Both tubes have viewing angles of 30°, PDs from 55 to 75 mm are adjusted by moving the tube halves in or out. The phototube is provided with a reticle erection (accurate to within 1°).

Attachment, TV and special cameras are mounted on the upper port of the phototube. A special adapter and a photo eyepiece S-PL 10× or S-PL 12.5× are required for the Microscope Camera MC 100. Cine and TV cameras with C mounts fit via standard C adapter (452995) without eyepiece. Both adapters can be adjusted to equal sharpness with the reticle in either of the eyepieces.

Observation of the objective pupil, especially for centering of the phase-contrast rings, is possible with the
5.13 centering telescope (464822-9902) which is inserted with the
5.14 reducing ring 30/23.2 (464911) in one of the tubes instead of an eyepiece.

6.0 Camera module

The binocular phototube 20 (451722) fits to all Axiovert inverted microscopes for mounting of
- SLR camera
- Microscope Camera MC 63 A for 35 mm and 4×5″
- Microscope Camera MC 100

The pushrod of the binocular phototube can be set to these positions:

100% of the light for observation when pushed in
30% of the light for observation and
70% for photography when pulled out.

The use of these photography systems is described in the relevant Operating Instructions.
The above-mentioned equipment is intended for the inverted microscopes Axiovert 10, but on the Axiovert 35 and 35 M only for 4×5″ photography, because these feature integrated 35 mm camera light paths.
The Axiovert 405 M is a full-fledged camera microscope with integrated 4×5″ and 35 mm photography light path.

Axiovert 35 or 35 M inverted microscope

6.2 Pushrod for beam-splitting prism.
Pushed in: 100% of the light for observation
Pulled out: 20% of the light for observation
80% for photography.

6.4 Slot for photographic format reticle. The reticle also serves for focusing with the two focusing eyepieces. It is exchangeable and brought into the observation light path. An illuminated format reticle is also available; it connects directly to a power plug of the Axiovert 35, and to plug 6.3 of Axiovert 35 M.
6.2 Slot for exchangeable reticles, e.g. a scale, which is brought into the photography light path.
6.5 Special adapter T 2 to mount
6.6 Contax SLR camera housing with automatic exposure control and cable release.

The objective magnification multiplied by the camera factor 2.5 results in the magnification on the film. A computer flash can be controlled by the SLR camera (electrically connected with the power supply which is connected with double collector and flash slider in the illumination system; see Operating Instructions for Microflash III).

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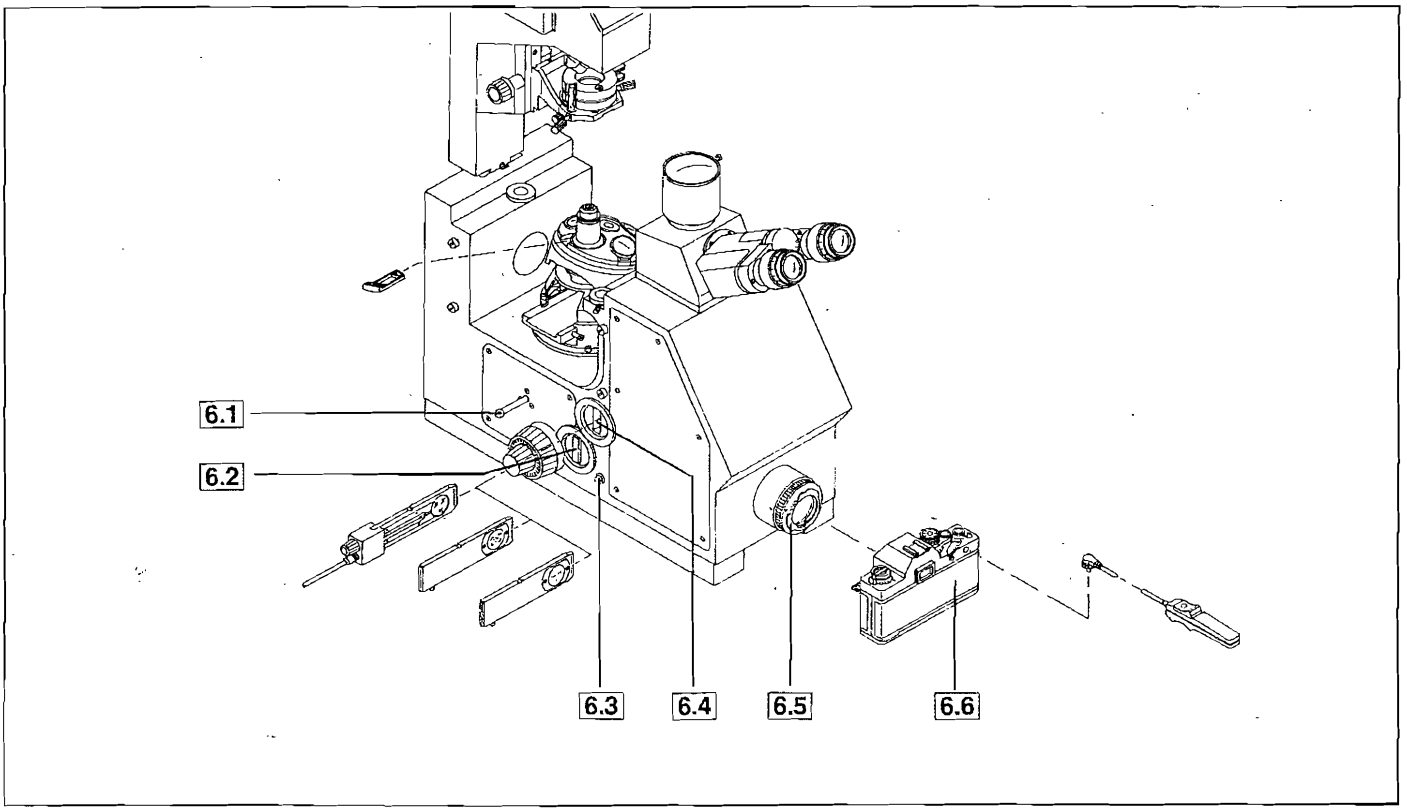

Axiovert 405 M inverted camera microscope

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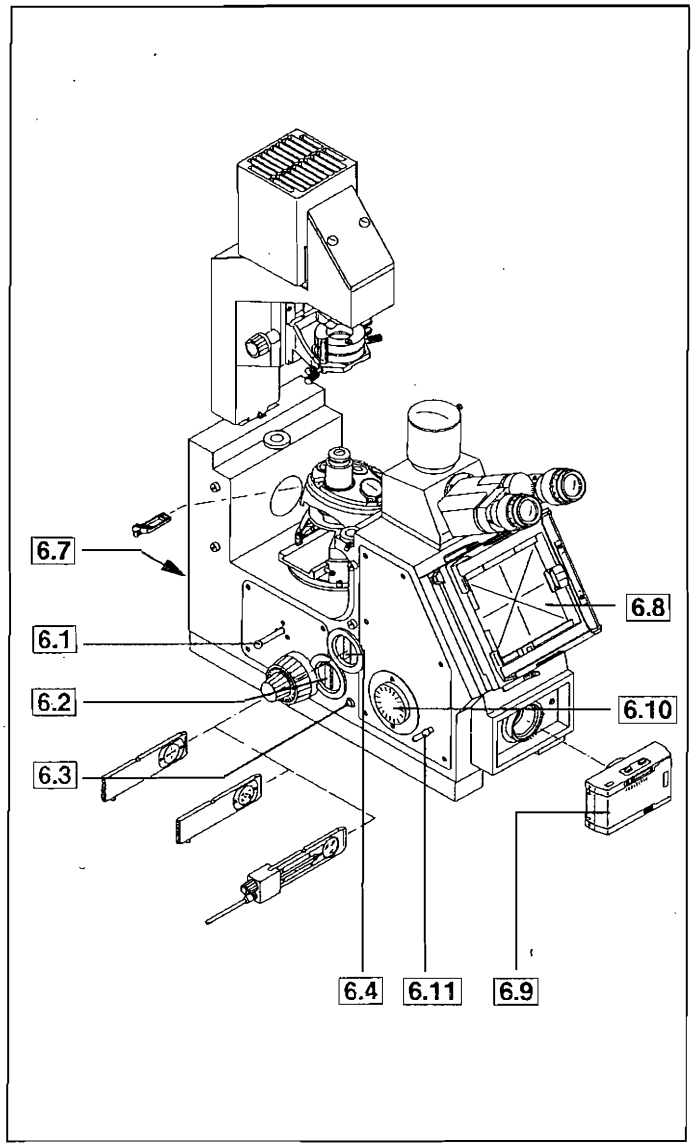


6.1 Pushrod for beam-splitting prism.
Pushed in: 100% of the light for observation
Pulled out: 20% of the light for observation and
80% for photography.

6.4 Slot for photographic format reticle; the reticle also serves for focusing with the two focusing eyepieces. The reticle is exchangeable and brought into the observation light path. An illuminated format reticle is available which connects to plug **6.3**.

6.2 Slot for exchangeable reticle, e.g. a scale, which is brought into the photography light path.

6.10 Dial to set the ASA value for 4×5″ format.

6.11 Change between 4×5″ and 35 mm. The camera factors are 2.5 for 35 mm and 10 for 4×5″. It follows that:
objective magnification multiplied by 2.5 equals image scale on 35 mm format
objective magnification multiplied by 10 equals image scale on 4×5″ format.

6.9 35 mm film cassette Mot

6.8 Ground glass frame (international camera back for
double cassette 9×12 cm/4×5″
Polaroid Land sheet-film cassette 545/4×5″
Polaroid Land film-pack cassette 550/4×5″).

6.7 Multi-point connector for connection of exposure control which controls the camera functions.

Features and options of the camera module of the Axiovert 405 M are described below in the sections
35 mm film cassette Mot
4×5″ ground glass and cassette holder
Exposure control.

35 mm film cassette Mot

Detaching the cassette

Push "Eject" key 6.15 and pull off cassette. Setting the film speed: push 6.16 ; 6.17 can be shifted. The adjusted ASA value is automatically transferred to the exposure control.

Loading the film

Push 6.13 (bottom) in the direction of the arrow, the cartridge is ejected and the camera back can be taken off. Load cartridge in 6.12 ; depress 6.13 ; thread film leader into slot 6.20 of take-up spool, the sprocket teeth must catch the perforation; tighten the film by turning take-up spool 6.19 gently clockwise (possible only if rewind slider 6.14 is set to R); insert camera back on left side (arrow) and press it down on the right side until it snaps in. The mechanical counter 6.18 is set to S (Start).

Mounting the cassette

Attach the cassette to the exit port with the contact pins as guides. With control panel power ON the loaded film is automatically transported to the first picture; the counter is set to 0. The film is advanced after each exposure (and, with Data Back, after each projection of data onto the film), and the frame number indicated by the mechanical counter of the cassette 6.18.
The film advance is switched off when the end of the film is reached; the light in the display END of the automatic exposure control flashes.

Rewinding the film

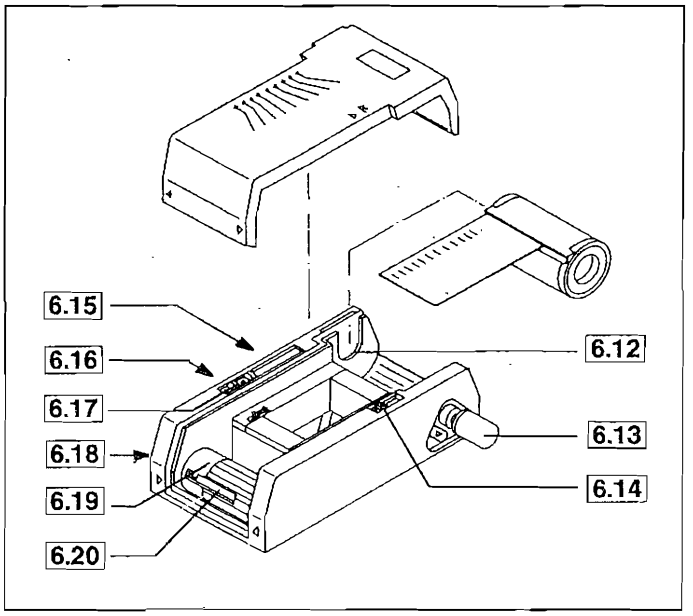
Operation of slider R 6.14 automatically rewinds the film; the light in the display END flashes. Mounting the camera back provides for automatic resetting. When the film is taken out and the camera back attached again, the slider R returns automatically to normal film advance position.

Data Back for 35 mm film cassette Mot

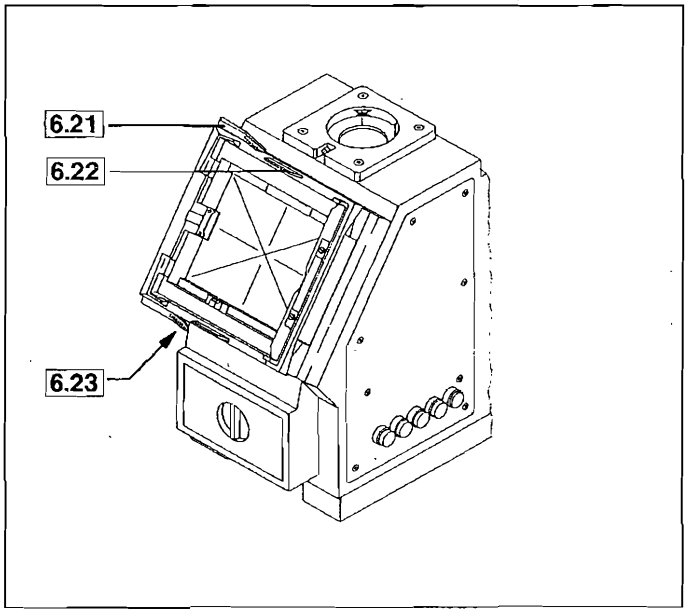
It is mounted like the normal cassette back. With the Data Back six numbers can be projected on film to indicate:
- year, month, day
- day, hour, number
- any 6-digit number
- any 3-digit number from 000 to 399

which is increased by 1 after each exposure. (For details see the Operating Instructions of the Data Back).

35



36



Large-format ground glass and cassette holder

After lifting off the ground glass with lever 6.21 cassettes for the international camera back can be slid behind the ground glass. To take off the ground glass depress 6.22 and slide it to the right; mount the ground glass accordingly. Most cassettes are not held by the ground glass but secured by lock 6.23.
The ground glass frame accepts accessories from the Sinar system (ground glasses, mirrors).

Exposure control for Axiovert 405 M

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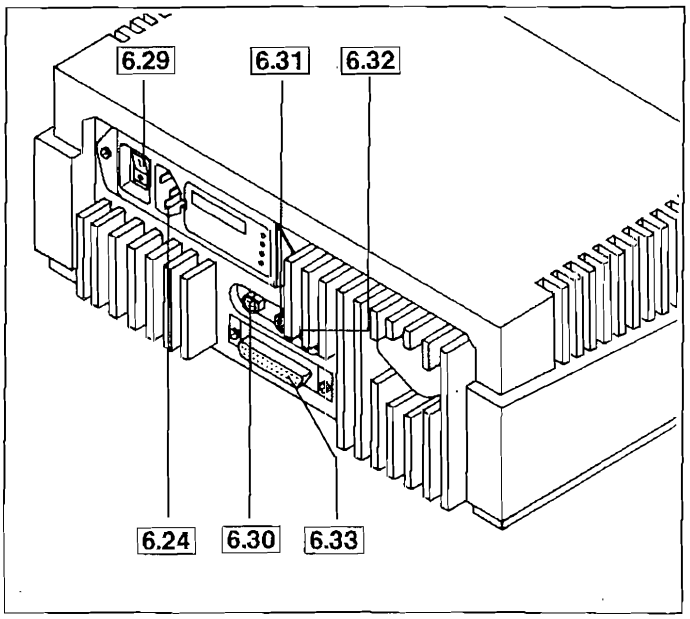
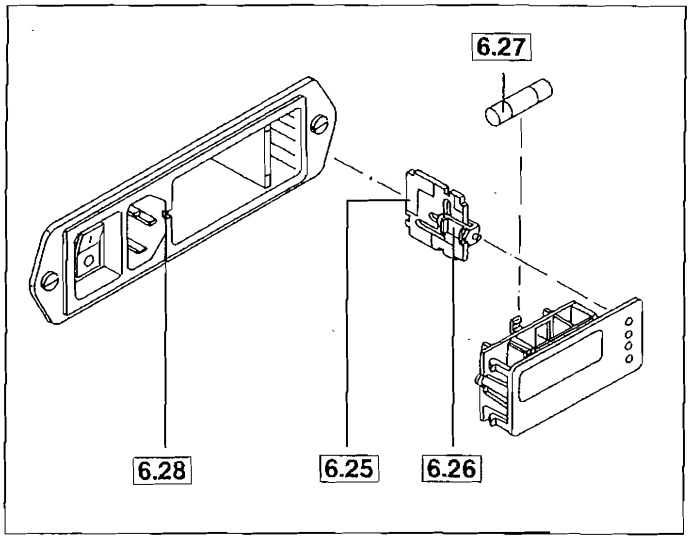


38



Connections

1. Power supply
Power consumption 20 VA.
The instrument connects to the line via **6.24**. It is ready for connection to 100–120–220–240 V. The voltage setting (white dot pointing to either of 4 voltages) must coincide with the local line voltage.
A change is made as follows:
- Insert a small screwdriver or similar tool in the recess **6.28** between cable socket and fuse plate and lift out the plate.
- You can now pull square board **6.25** out of the small slot to the right of the black part. The adjusted voltage is indicated on the board opposite the black plastic part **6.26**. The other adjustable voltages are imprinted on the other three sides.
- Shift the plastic part **6.26** so that it snaps into the recess opposite the voltage you want to adjust.
- Slide the board back into the slot, legend to the left. Put on the fuse plate; the white dot will point to the right voltage.

NB: When you change the voltage the right fuses **6.27** must be inserted:
100...120 V SB 0.63 A, Cat.No. 380127-0180
220...240 V SB 1.315 A, Cat.No. 380127-0150

2. **6.29** Power switch (see also page 27)
3. **6.30** 4-pin socket for microflash
4. **6.31** 2-pin socket for remote control
5. **6.32** 3-pin socket to switch the halogen lamp to 3200 K (control line for power supply, see page 11).
6. **6.33** Connection to multi-point connector of Axiovert 405 M.

The multi-point connector must be plugged into the socket.
Caution: Make any connections or disconnections only with power off!

Keys and displays

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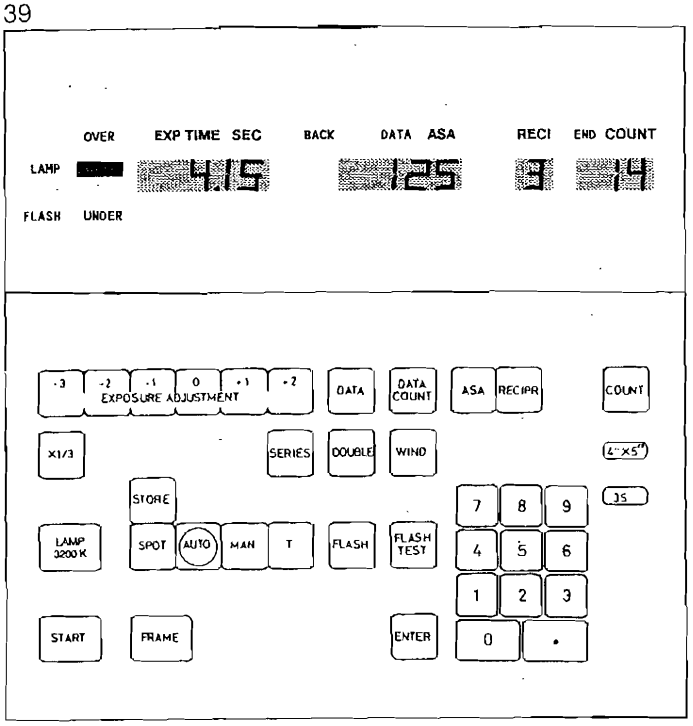


For better recognition and easier differentiation all display designations are capitalized below (e.g. OVER), and all key designations capitalized and framed (e.g. START).
Light in a display or key indicates that a function is operative or in a specific state. A flashing light reminds you of something,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

Basic setting and exposure

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

The exposure control is connected with the Axiovert 405 M, the power supply and the line, and ready after switch-on. The basic setting is displayed as follows:

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

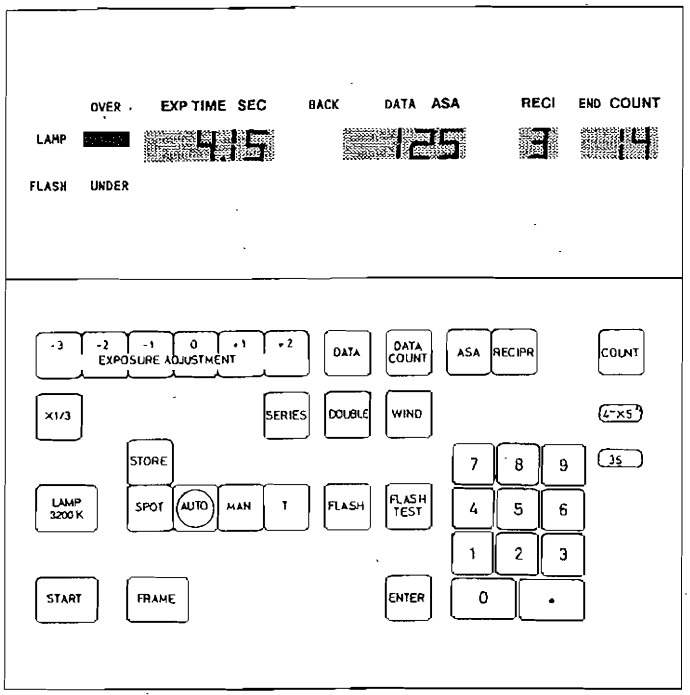
0 lights with EXPOSURE ADJUSTMENT. The exposure adjustment is automatically set to 0.

START

START Release key for exposure.
Film advance follows for 35 mm film.
The time displayed by EXP.TIME counts down to 0.
You cannot release 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: you can release if the light in the key DOUBLE flashes).

If you depress T and release with START the shutter opens and is closed when you depress START again. EXP.TIME will then display in whole seconds the time the shutter is open.

Further keys and displays



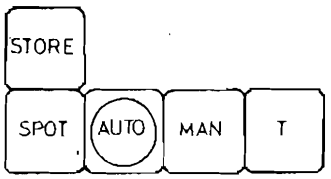
[-3] [-2] [-1] [0] [+1] [+2] EXPOSURE ADJUSTMENT
Exposure adjustment in steps of whole exposure values.
[0] is for medium contrast of the specimen. Adjustment to [0] is, therefore, automatically made with instrument power on.
[+1] the exposure time is extended by 1 exposure value, i.e. factor 2
[+2] = 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. An input exposure step, e.g. [+1] or [+2] is reduced to 1/3, i.e. to +1/3 or +2/3.

Applications:
1. The automatic exposure control converts the brightness of an object into mean brightness of the image, because 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.
It generally applies that:
the exposure time in brightfield should be adjusted with [0] to [+2],
in darkfield and fluorescence with [-1] to [-3].

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

[LAMP 3200 K] The power supply switches the 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 the user from being blinded.
Pushing the key again cancels the 3200 K setting.



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.

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 extended dark areas; integral measurement would then cause too long exposure times (darkfield illumination, polarization and fluorescence). The reverse is also possible.

STORE stores the exposure time.
The value remains stored if T or MAN are used intermittently.

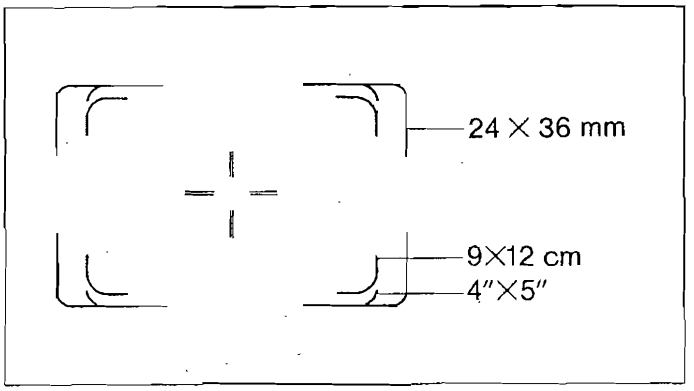
Applications:
1. If for spot measurement with SPOT the object is to be removed from the center during exposure.
2. If extended specimen areas are to be covered by a series of exposures. Were the exposure time not stored, different coverage of the field by the object would cause different exposures and, for example, different brightness of the background.
3. If intensity differences are to be represented by multiple exposures with DOUBLE, e.g. multi-fluorescences.

MAN Manual input of specific exposure times. When the key is pushed the number field will light. You may now select the time and key it in with ENTER.
Any exposure time between 0.01 and 9999 s (2 – ¾ hrs) can be keyed in manually.

T (long-time exposure).
If after pushing T you release with START, the shutter opens, and is closed when you push START again.
EXP.TIME displays in whole seconds the time the shutter is open.

Applications: Long-time exposures and to direct the light to the ground glass of the 4×5″ camera.

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 – the brightness changes continuously; releasing the key – the actual setting is fixed.



DATA For 35 mm format.
Causes the display BACK and prepares the system for the projection of data from Data Back after exposure.

DATA COUNT is functionless

ASA is functionless

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 sensitivity is different for the photographic emulsions, 9 programmed reciprocity code numbers are provided for automatic compensation. Which code number applies to the film you use is said on page 40.
Input of code number: RECIPR; number field lights; key in code number with number key; ENTER.

COUNT Frame counter setting. With newly loaded 35 mm 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 4×5" camera:
COUNT; ENTER.
COUNT will display the number.

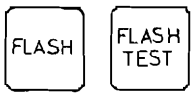
SERIES Exposure series. 35 mm exposures with varying exposure times are taken automatically.

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 detached or WIND 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.

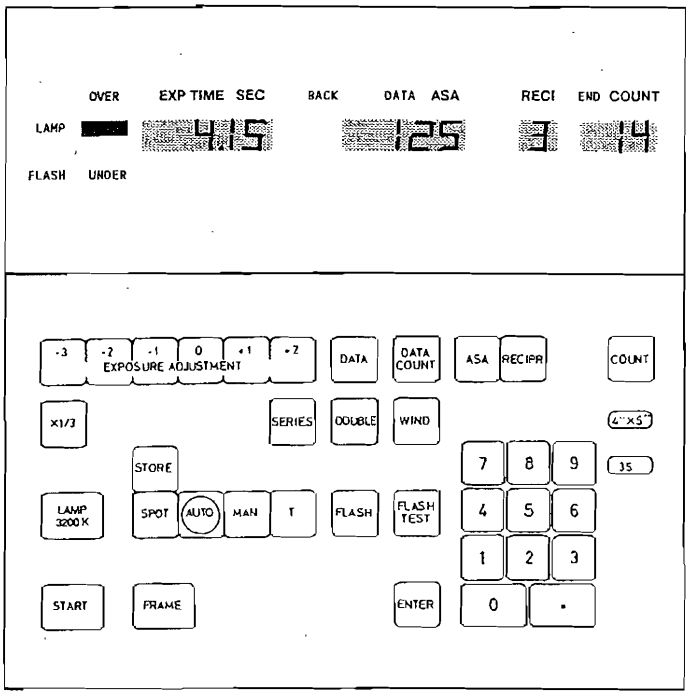
Applications:
1. Multiple exposures of the same film field using different methods of illumination, different fluorescence filters, and similar applications.
2. Multiple exposures to imprint scales, marks, overlay nets, etc.
NB: As the exposures will overlap at least in part, shorten the individual exposures, e.g. by selection of the exposure adjustment value −1.

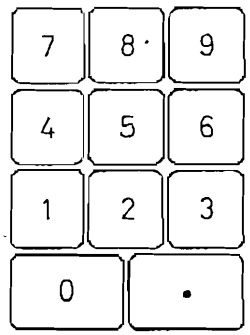
WIND It is used for blank exposures. It also breaks off any running exposure. 35 mm film will be advanced.



FLASH Flash mode with 0.01 s shutter opening time.

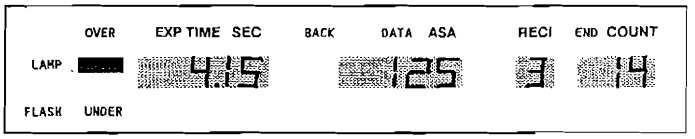
FLASH TEST Release of a test flash without exposure of the film.





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

[ENTER] Input key to select the modes [MAN], [RECIPR], [COUNT], [SERIES] + [EXPOSURE ADJUSTMENT] (with its keys [-3] – [+2] and [X·1/3]).



LAMP lights if the key [Lamp 3200 K] is pushed.

FLASH lights if the connected microflash is ready.

The green signal field lights if the exposure time is within the range of the automatic exposure 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 alternation, the brightness is too low or there is no light in the photography light path. Select exposure time manually.

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] whole seconds are counted as from zero.

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

DATA and ASA: 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 microscope stand is displayed.

RECI: Displayed is the reciprocity code number you 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.

7.0 Mounting of equipment for TV microscopy

With the phototube

- Possible on all Axiovert inverted microscopes.
- Possible for light and heavy TV cameras.
- The image will always be upright and unreversed.

This kind of mounting provides free access for micromanipulation.

The equipment is mounted on the upper port of the phototube 7.1, TV cameras with C mount by means of standard C adapter (452995), without eyepiece. The adapter is adjustable to achieve equal sharpness of the TV image and the reticle in either of the eyepieces.

At the side of the stand

- Possible on the Axiovert 35, 35 M and 405 M inverted microscopes.
- TV cameras weighing max. 1 kg can be mounted at the side of the stand.
- The image will be upright and unreversed if the TV camera is turned through 90° (thread mount of the stand to the rear).

If the TV camera is mounted laterally, the cover at the left side of the stand is replaced by the TV adapter (451775) 7.3.
The two switch positions 7.4 (indicated by arrows) are for:
100% of the light for observation
30% for observation, 70% reflected out at the side (hinged mirror).
This is possible only if the basic instrument is not switched to photography light path.
The camera is mounted with the adapter for TV with C mount (452995) 7.2. Because of the telecentric beam path centering and adjustment are not necessary.
If the TV camera is mounted at the side of the stand, TV and photographic cameras can be used alternatively.

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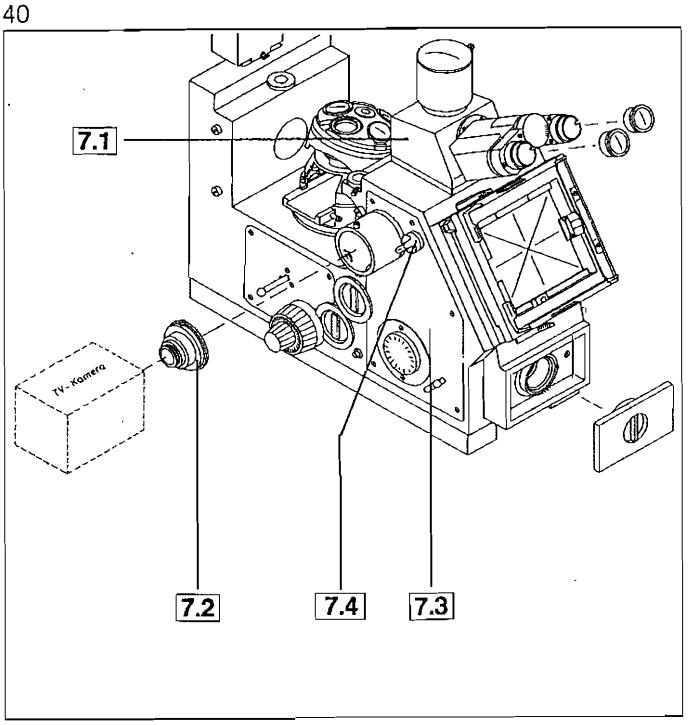

Free space is required between specimen plane and illumination system for the examination of specimens in chambers or microtest plates. It is provided by LD condensers.

In brightfield

Use the condenser LD 0.3 H, Ph 1, 2 which can be supplemented by the front lens 0.55 for microscopy with higher illuminating aperture (e.g. for Achroplan objectives), or the condenser LD 0.55 H, Ph 1, 2 DIC.

For a full description of the microscope adjustment for brightfield see page 5.
Only the operations which are different if you use LD condensers are described below.
The turret of the LD condenser 0.3 H, Ph 1, 2 must be set to position H.
The iris diaphragm acts as contrast (aperture) diaphragm.
Adjustment of the luminous-field diaphragm according to Köhler's rules is, therefore, not necessary.

Set the turret of the LD condenser 0.55 H, Ph 1, 2 DIC to H or either of the two DIC positions without prism.
(1) indicates the luminous-field diaphragm, (2) the aperture diaphragm.

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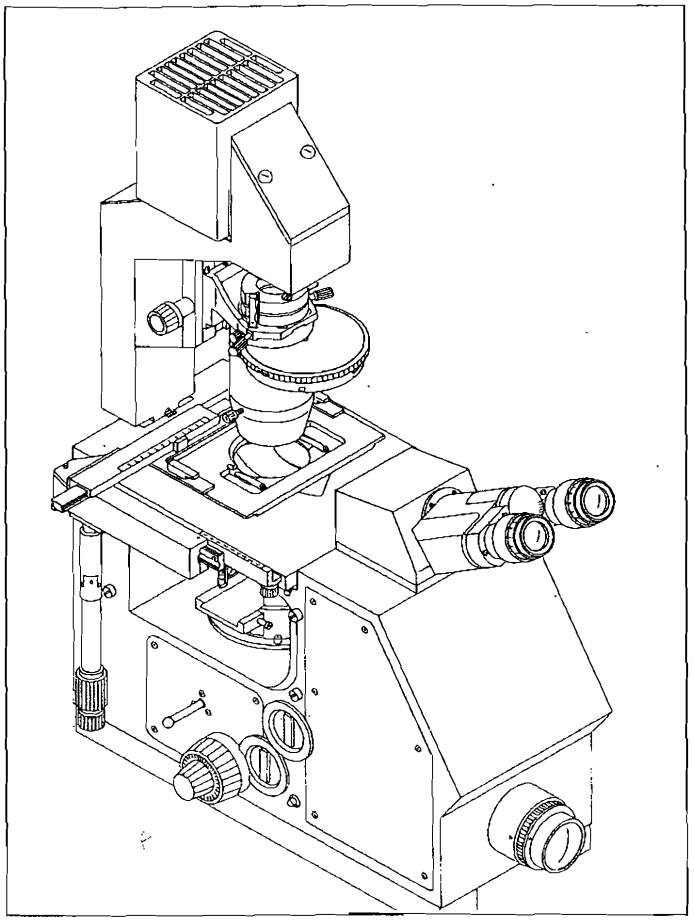

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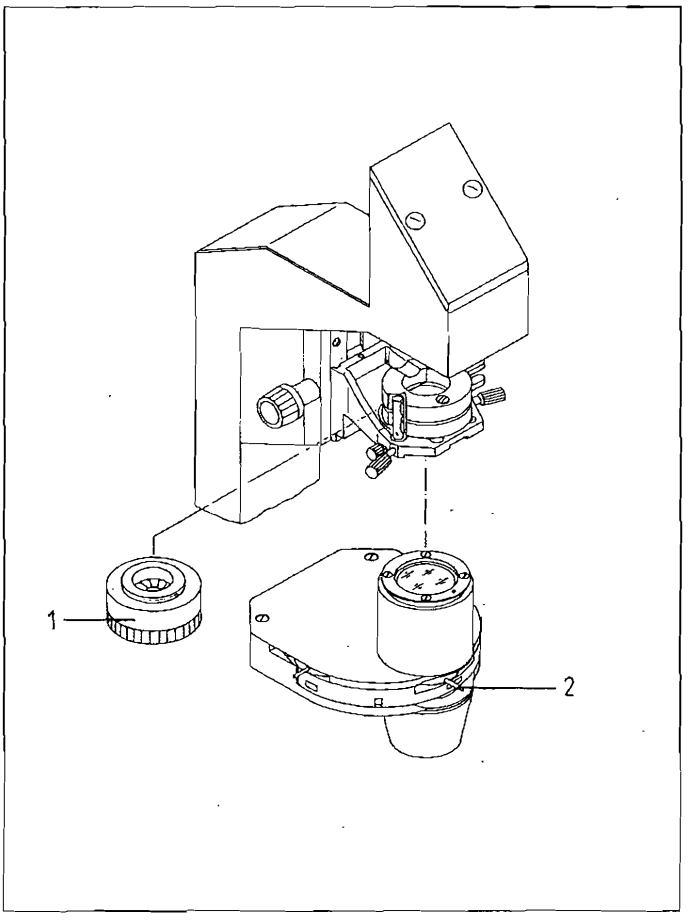
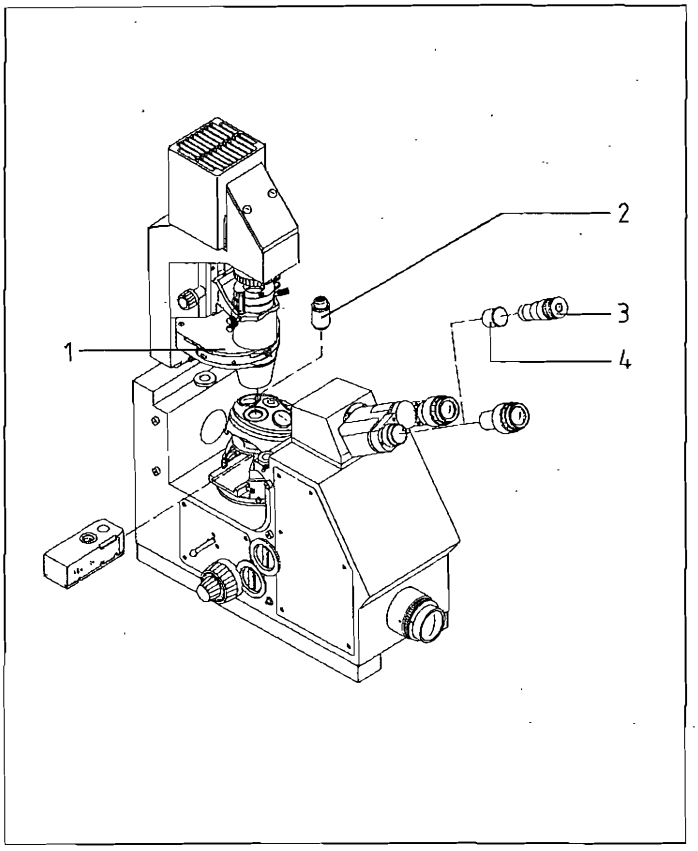
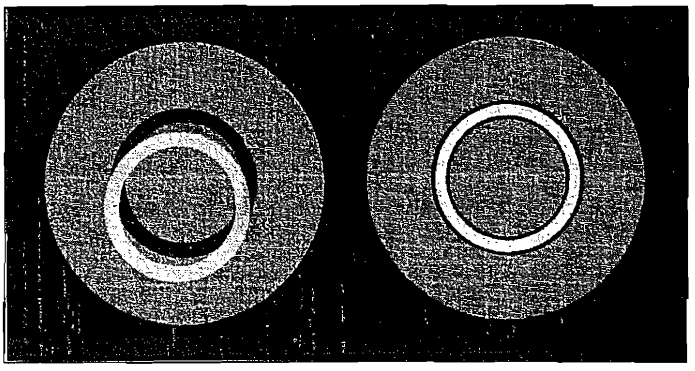


43



44



In phase contrast

Phase contrast is used mainly to enhance the contrast of unstained specimens.

Required equipment for phase contrast
- Objectives (2) designated Ph, which are equally well suited for brightfield.
- For microscopy with long illumination distance the LD condenser 0.3 Ph 1, 2 (the front lens 0.55 cannot be used in phase contrast), or
the LD condenser 0.55 Ph/DIC.
Both condensers have turrets (1) with phase-contrast diaphragms.

The adjustment for phase contrast with the lamp 6V 20W is described on page 15.

The following additional adjustments are necessary in phase contrast:
The phase rings in the objectives have different sizes and are indicated on the objective (2) by Ph 1, Ph 2 and Ph 3. The ring size is indicated on the turret (1) by the engraved numbers 1 and 2 (with some condensers also 3) for selection and combination with the objective. LD condensers have only the positions 1 and 2 because they are primarily used with low-power objectives.

Perfect contrast is only achieved if (dark) ring in the objective and (bright) ring in the condenser exactly coincide (see opposite Fig.). This can be controlled after insertion of the centering telescope (3) with the reducing ring (4) and focusing by moving the eyelens of the centering telescope which must be held on its knurled ring. (Without this attachment the control can be made without eyepiece like the condenser diaphragm control that is described on page 5.)
The phase-contrast diaphragms are centered with the Allen wrench SW 1.5 on **3.33** of the LD condenser 0.3 Ph 1, 2 and on **3.36** of the LD condenser 0.55 Ph/DIC.

Special note
More than brightfield phase contrast requires meticulously clean glass-to-air surfaces of the specimen (fingerprints?). The diaphragm ring of the LD condenser 0.3 Ph 1, 2 is functionless, because no iris diaphragms are provided in the Ph positions.

In DIC (differential interference contrast)

DIC is used, for instance, if a specimen is too thick for phase-contrast examination, so that object sections outside the plane of focus impair the brilliance of the image, or if the halo which is typical of phase contrast, is disturbing for the observation of small features.

Required equipment for DIC
- Objectives Plan-Neofluar, Plan-Neofluar Pol for most exacting demands, and LD Achroplan (20 and 40 corr.) for a long working distance between specimen and objective.
- A special nosepiece (3) with slots (4) to accommodate
- DIC sliders (5) bearing on their bottom surfaces type, magnification and aperture of the objective for which they are intended.
- LD condenser 0.55 Ph/DIC (2) for microscopy with long illumination distance.
- A polarizer (1) which is swung in on top of the condenser.
- An analyzer (7) which fits into (6) and can be provided with a lambda plate for color contrast.

The following additional adjustments are necessary in DIC:
Set the condenser turret to I or II. DIC prisms are provided in both positions, which are required for objectives with the following apertures:

Position I: 0.3 – 0.4
Position II: 0.5 – 1.3

For DIC you use an iris diaphragm which you open at first (this is generally the last step of the adjustment); this diaphragm can be moderately closed for further contrast enhancement.

The polarizers are crossed. They must be brought into crossed (extinction) position before starting to work in DIC. For this adjustment screw the polarizer from above into the lower filter holder and re-adjust it. Remove objectives, DIC slider, eyepieces and condenser from the light path, swing in the illumination system, and turn the polarizer until extinction is achieved.

With the knurled screws of the DIC sliders in the nosepiece you can optimize the contrast adjustment.

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

Because DIC uses polarized light, "optically active" elements between polarizer and analyzer will be disturbing. Such elements may be mica plates which are sometimes used for cytological sections, or plexiglass culture chambers with bottoms of synthetic material (chambers with glass bottoms are available). A possible loss in performance must be taken into consideration if such materials are used.

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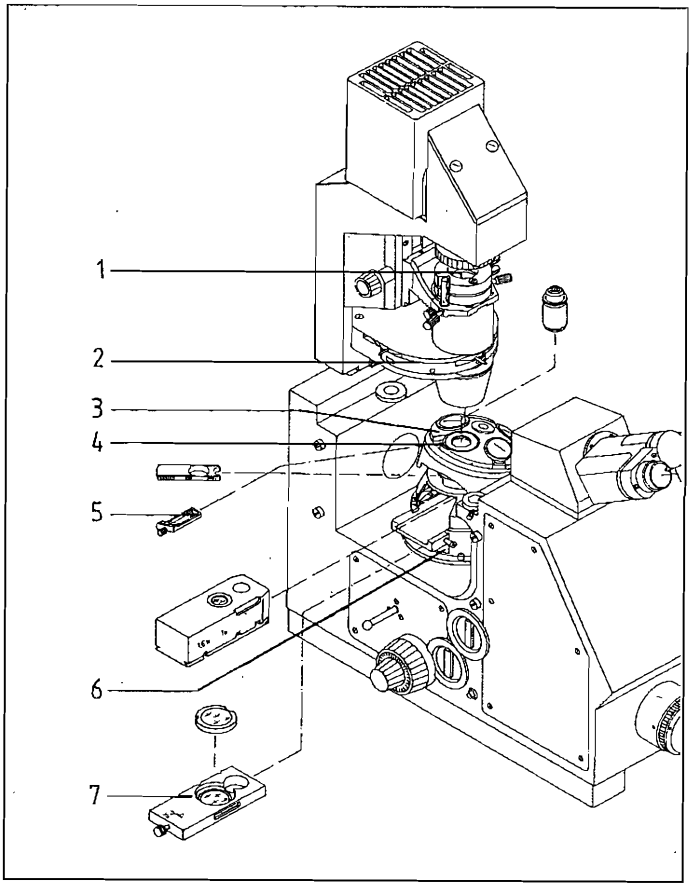
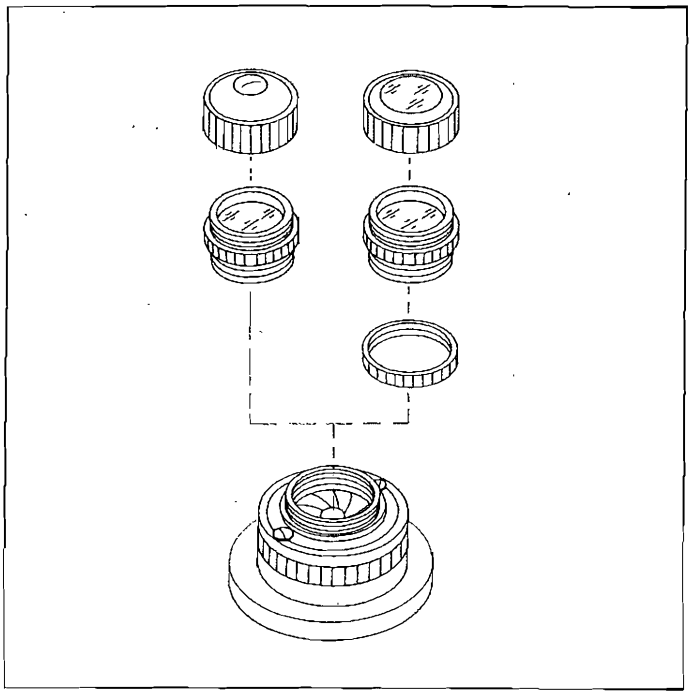


46



Use condensers which allow full utilization of the resolving power of high-power objectives. Köhler illumination will provide for a homogeneously illuminated object field, a brilliant image without reflections or glare and optimum specimen protection.

In brightfield

The adjustments described on page 5 apply here, too.
Use the achromatic-aplanatic condenser system 0.32 Pol* with front lens 0.63 Pol or 1.4 Pol (see description of condensers on page 14).

In DIC (differential interference contrast)

For the adjustments see chapter "DIC" on page 35. Use the condenser system 0.32 Pol* with front lens 0.63 Pol or 1.4 Pol and the DIC prism matched with each front lens, inserted in the condenser in oriented position.
DIC prism (445293) for front lens 0.63 with 20× and 40× objectives.
DIC prism (434410), NA 0.3–0.4, for front lens 0.63 with 5× and 10× objectives.
DIC prism (445294) NA 0.5–1.3 (1.4), with 20×, 40×, 100× objectives.

*These condensers are mounted on the condenser mount with iris (see Fig. 19).

Fluorescence microscopy

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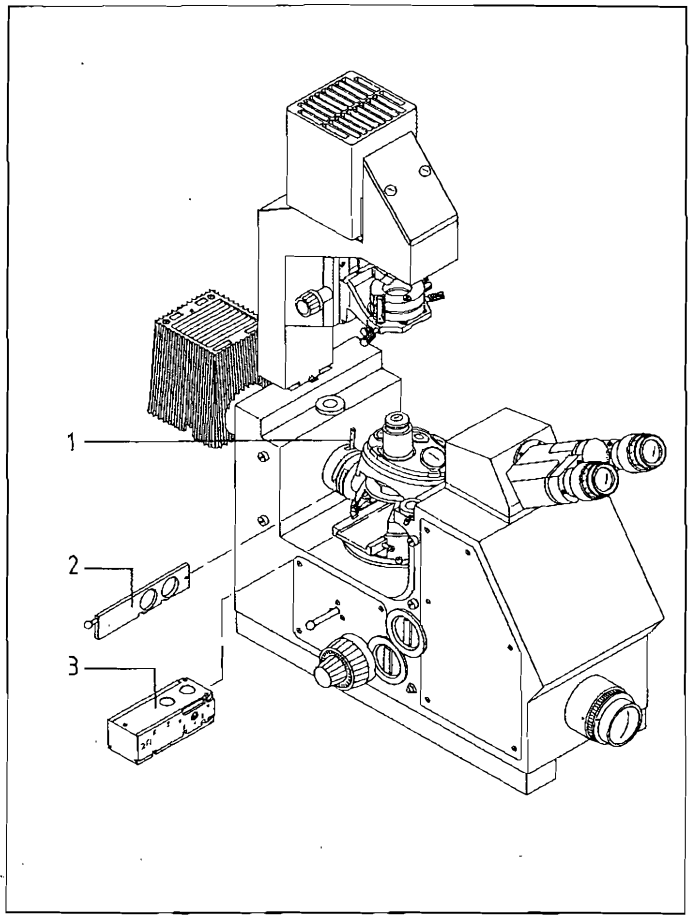

Required equipment
- No special objectives, Plan-Neofluar objectives for UV excitation.
- Special, incident-light illuminator (see page 16).

Procedure:
- Set the reflector slider (3) to a position for free light path. Adjust the selected specimen feature in transmitted-light brightfield or phase contrast, with the upper illuminator with halogen lamp. Switch on the mercury lamp, but block its light path with dark slider (2).
- Switch off the transillumination (or reduce its brightness considerably), select on the reflector slider the position with the desired type of excitation, and free the light path with (2).
- Because an aperture diaphragm in the illumination tube would not influence contrast, etc. in fluorescence observation, there is only a luminous-field diaphragm. With lever (1) close the latter so far that it becomes visible in the image. Focus with **4.6** and center with **4.4**, and open the diaphragm so far that it disappears from the field of view.

Special notes

It is helpful for the first fluorescence adjustment if you begin with a 20× objective and a highly fluorescent specimen. Such specimens are available for demonstration purposes, but you may also prepare them yourself. A specimen of anthracene crystals is quite popular. (If necessary, one may even use a specimen label to control the illumination.)

Filter sets for specific applications are contained in the reflector slider. Each filter set comprises an exciter and a 25 mm dia. barrier filter, with a chromatic beam splitter (26×36 mm) between the two. For details about the filters and their exchange see pages 43 and 47.

If you want to know more about fluorescence microscopy please refer to our brochure K 41-005 "Worthwhile facts about fluorescence microscopy".

Additional exciter filters can be loosely fitted in the filter holder of the incident-light fluorescence illuminator. Use a holding ring (467252) for 32 mm dia. filters (e.g. polarizers). An adapter ring 18/32 mm dia. (467893) must be used in addition for 18 mm dia. filters.

For the FITC method use the 12V 100W Hal illuminator for fluorescence excitation in incident light.
It is possible to retrofit attachments for the use of the dual-wavelength method (e.g. for Ca and pH determinations).

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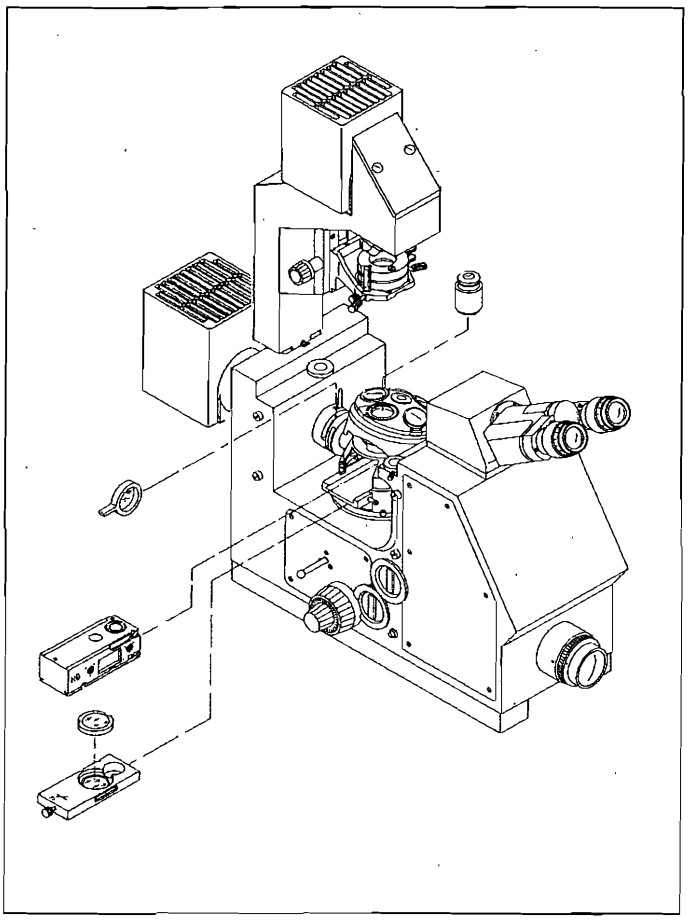

Antiflex (reflection contrast)

With the Antiflex method the image contrast can be enhanced for reflected-light examinations of objects with low reflection. In cell cultures, for example, part of the light is reflected by the bottom of the cell, part by the top surface of the culture dish bottom. If these reflections are superimposed the result will be characteristic interference colors. Growth structures, for example, can be recognized.

Such characteristic interferences can be represented with high contrast only if disturbing reflections by lens surfaces, especially in the objective, are eliminated. This is achieved by objectives with a rotary λ/4 plate between front lens and specimen. Objective and specimen must be arranged between crossed polarizing filters.

Required equipment
- Incident-light fluorescence illuminator
- 32 mm dia. polarizing filter (473600) in filter holder inserted in filter slot
- Double reflector HD (451764) instead of reflector 2 FL
- Objective Plan-Neofluar 63×/1.25 oil Ph 3 Antiflex (440469)
- Analyzer slider, fixed, with plug-in lambda plate (451793)
- Illuminator 100 Hal

Procedure:
Cross the polarizers:
Remove objective and eyepiece from the light path. Turn polarizer in filter holder until max. darkness is achieved. Insert objective and eyepiece again. Remove analyzer from the light path.
Immerse objective and focus on the specimen with coarse/fine control.
Slide analyzer back into light path.
Turn front part of objective (with λ/4 plate) until max. brightness is achieved in the field of view.

For incident-light fluorescence and Antiflex used together, you will need the deflecting mirror for 2 illuminators (447230) with attached illuminator 100 Hal with 12V 100W halogen lamp, and HBO 50 illuminator with HBO 50 mercury lamp (Fig. 28).

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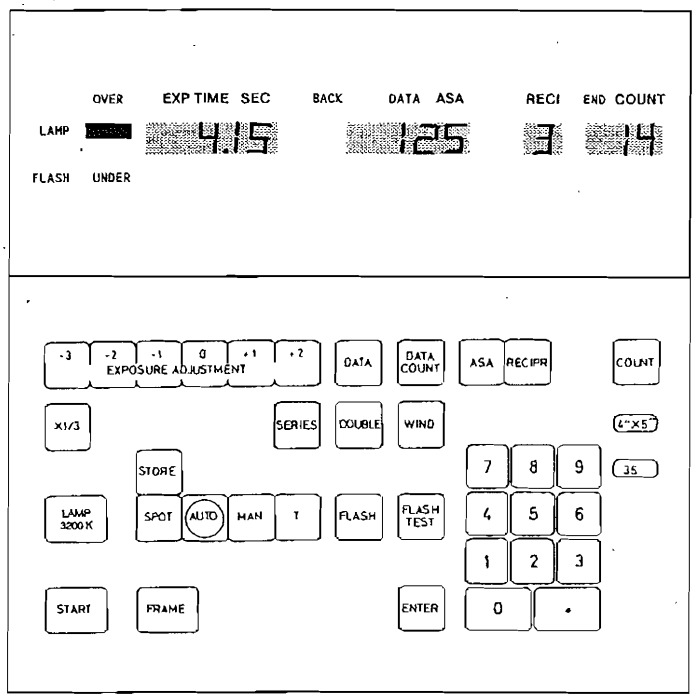


Preliminary remarks

With a phototube, e.g. on the Axiovert 10 for 35 mm and 4×5″ or Axiovert 35 or 35 M for 4×5″, the following systems can be mounted on the tube port:

Microscope Camera MC 100
Microscope Camera MC 63 A
SLR camera

For operation of the three cameras see the relevant Operating Instructions.

With the Axiovert 35 or 35 M with integrated 35 mm camera light path, focus with two focusing eyepieces on the exchangeable format reticle before you adjust the specimen. The pushrod must be set to photography. For a full description see the Operating Instructions of the camera.

The description below refers to the inverted camera microscope Axiovert 405 M (Fig. 4) with exposure control.

Photomicrography with the Axiovert 405 M inverted camera microscope

B/W photography

Both focusing eyepieces are focused on the illuminated format reticle, and the object is adjusted with the focusing control. Use a critical focus telescope for this adjustment at low magnification. Set the pushrod on the stand to photography. Select 35 mm or 4×5″ format with **1.25**.
The right film is loaded in the 4×5″ or 35 mm cassette (see selection of film on page 40), and the film speed set for 4×5″ format **1.24** and 35 mm format **6.17**.

You have connected:
Power supply and lamp housing Hal
Exposure control and stand
Power plug and stand
Power supply and exposure control (control line for adjustment of color temperature 3200 K).
Power supply, exposure control and power plug for motorized nosepiece and for the reticle illumination are connected to the line.

Switch on power supply and exposure control.

On the control panel you have:
- keyed in the RECI value of the selected film indicated on page 40: RECIPR, e.g. 1; ENTER.
- set the frame counter to 0: COUNT; ENTER. (Not necessary with newly loaded film).

Displays:
- The green signal lamp lights; should OVER light, reduce the brightness; if UNDER lights check whether the light path is free.
- The exposure time is displayed (EXP.TIME). (For integral automatic exposure measurement and adjustment value 0).
- The RECI value is displayed.
- The ASA value is displayed.

Exposure: Push START. The following procedure includes: automatic exposure, film advance, the mechanical counter on the cassette and the electrical counter on the control panel count. A green filter can be used for contrast enhancement.

Color photography

For color photography remember in addition to the above: 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 100 K for correct color rendition. If the 12V 100W halogen lamp Hal is set to the color temperature 3200 K, its brightness will be so high that undervoltages will generally be used for observation. The voltage can be easily changed before each color exposure with the key [LAMP] on the control panel. Neutral density filters will sometimes be necessary to attenuate the light; these filters have no influence on the color temperature.

For photomicrography we recommend reversal films for artificial light (3200 K). If daylight film is used, an additional conversion filter must be inserted in the filter holder, to increase the color temperature from 3200 K to 5500 K (flashlight has daylight color temperature).
For more details see our leaflet A 41-400.5 "Film material for photomicrography".

Compensation of reciprocity failure

Use the advantage of the automatic compensation of the reciprocity failure for exposure times which are longer than 1s (see also the description of the key [RECIPR] on page 30). Listed below are the code numbers for some frequently used film types, which you must key in with the key [RECIPR] on the control panel.

You can find the value yourself for film types which are not listed:
Make test exposures (with automatic exposure control) with an exposure time shorter than 1s, then reduce the brightness with neutral density filters or similar means until you achieve an exposure time of several seconds; with this setting take a series of exposures with the RECI numbers 1–9. After development you can find out the long-time exposure which best complies with the first one. The RECI value of this exposure will be the code number for this film type. It is set whenever you use this film type, independent of the exposure time.

You will not need test exposures if the film manufacturer indicates the extension of the exposure time for his films, e.g. "for 10s + 2 light values". +2 light values mean a 4 times longer exposure time, i.e. 40 s. Adjust your microscope so that the automatic system displays 10 s for RECI = 0 (here you may for once use the aperture diaphragm to reduce the brightness). Change the RECI value and you will quickly find the one which best approximates 40 s.
This would be the code number of your film, in the above example.

The film manufacturers often recommend correction by filtering which should be observed for color films.

Code numbers for compensation of reciprocity failure for some frequently used film types

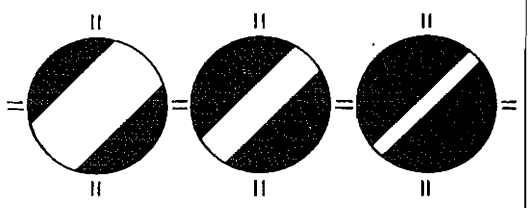
Film	Code number
AGFACHROME RS 50, 100, 200	5
AGFACHROME RS 1000	4
FUJICHROME 50 D, 100 D	3
EKTACHROME 50, artificial light	4
EKTACHROME 64, 400	6
EKTACHROME 160, artificial light	5
EKTACHROME sheet film 6118, artificial light	1
POLACHROME CS	9
POLAROID 58, 668	7
AGFAPAN 25	6
AGFAPAN 100, 200, 400	8
AGFA ORTHO 25	1
ILFORD PAN F	4
ILFORD HP 5	6
KODAK PLUS-X, TRI-X	9
KODAK Technical Pan 2415	3
POLAROID 51	3
POLAROID 52, 552, 55	1

Fluorescence photomicrography

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

- 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, no objections to films of 400 ASA and more.
- Even in case of spot exposure measurements the dark or even black background will represent an essential part of the measuring field used by the automatic exposure control, and 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.

50



Bright/dark approx.	50:50	25:75	10:90
Adjustment:	−1	−2	−3

(If you want to remove a measuring field which is typical of the specimen from the image center for exposure, you can store the exposure time with STORE .)

- The "exposure range" is quite extensive 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 protect the more "valuable" ones.

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.

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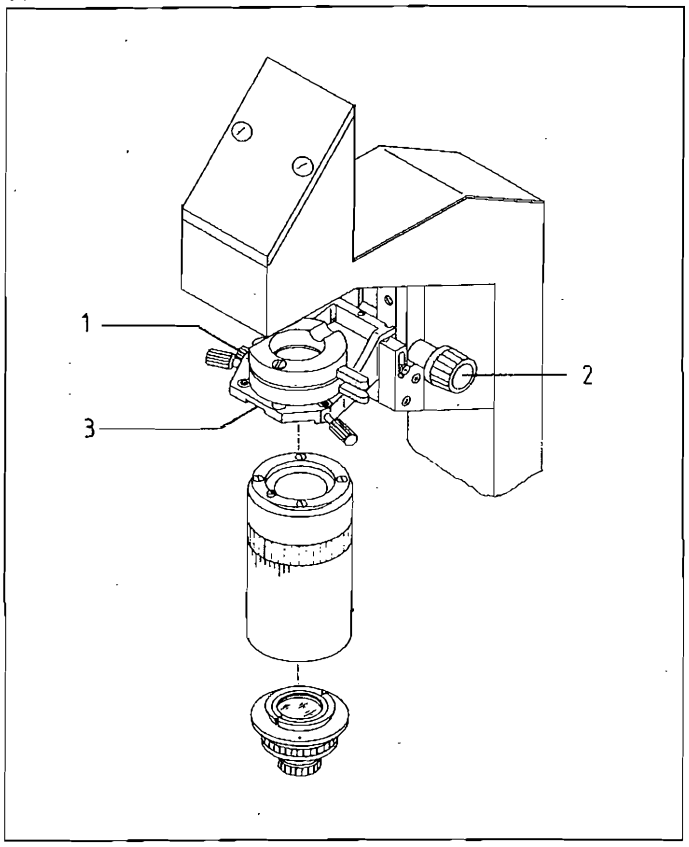
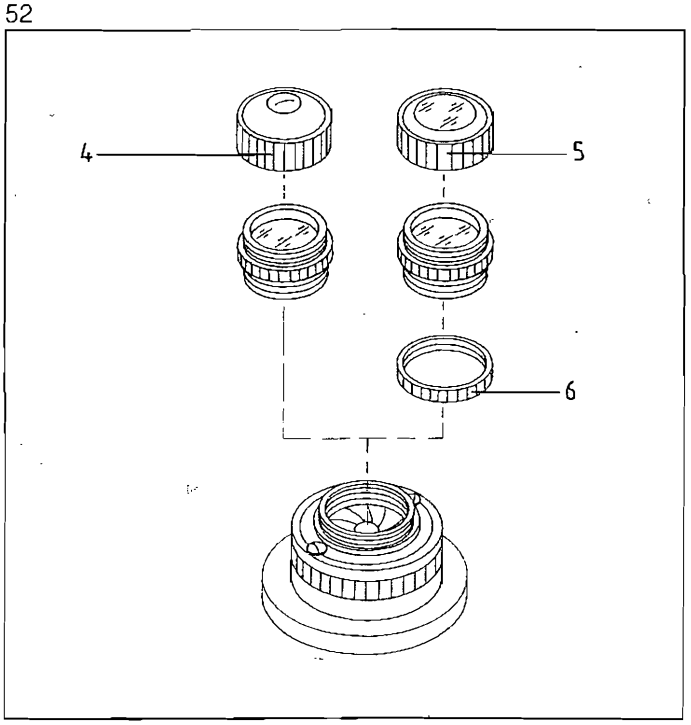


Should minor exchanges be necessary on your microscope and no service technician available, the following hints may be helpful to do the work yourself.

Condenser exchange

To exchange, for example an LD condenser for a condenser system, lift the condenser with (2) as far as possible, loosen screw (1), and pull out the condenser.
Before inserting the condenser system, put in the condenser holder with iris; the condenser itself is then attached to its dovetail mount.
When inserting the LD condenser or the condenser holder with iris, the notch (3) will guarantee exact orientation.

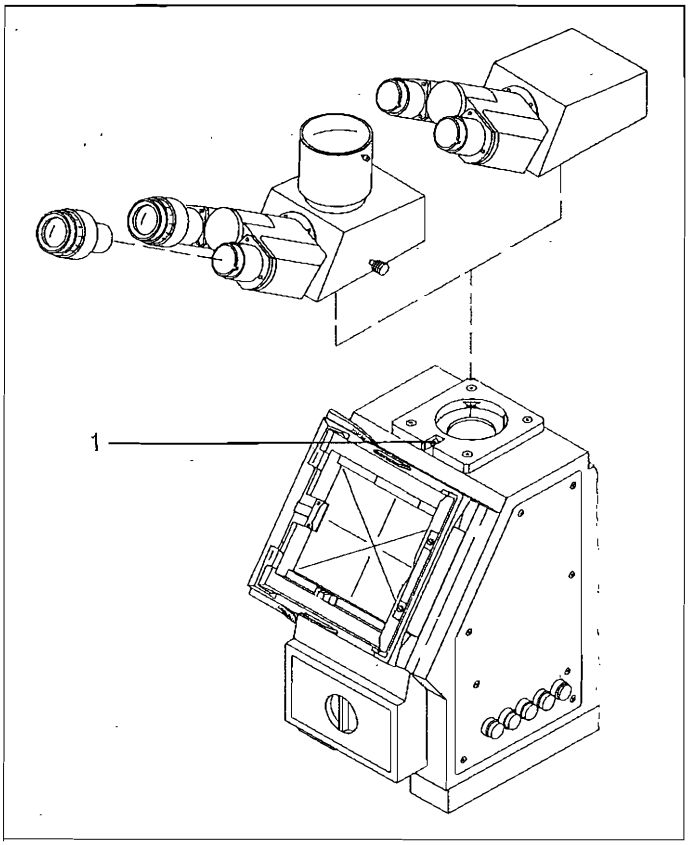
52



Exchange of the condenser front lens

The condenser system 0.32 accepts the front lens 1.4 Pol (4) or 0.63 Pol (5) for long back focal distance (7 mm in air, 11 mm in glass). Both front lenses are screwed on the condenser 0.32. A spacer ring 0.63 (6) between condenser body and condenser part 0.63 is required in addition for front lens 0.63 Pol.

53



Tube

For tube exchange loosen the clamping screw and take off the tube. Insert the new tube and secure it with the screw.

Exchange of DIC prisms on the LD condenser 0.55 Ph/DIC

Turn the condenser turret with the DIC prism you want to exchange through two positions into working position.
The prism is now on top of the cover plate. Lift the plate and remove the retaining ring.
You can insert the prism only if the pin engages the corresponding borehole of the holder. Control after insertion for perfectly flat seating to avoid any kind of mechanical and optical interferences.

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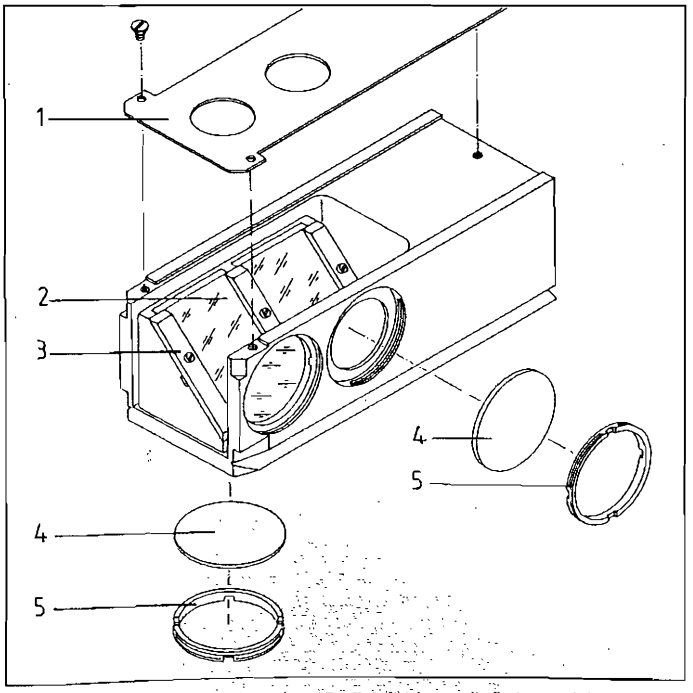

Fluorescence reflector

Exchange of filters (4) and chromatic beam splitters (2): the built-in filter sets can be exchanged after unscrewing the retaining rings (5). The plate carrying the chromatic beam splitters (2) is accessible after removal of plate (1). Plate (2) rests on an elastic sheet-metal mask and should not be touched. For exchange of the beam splitter on the mask jig (3) need normally not be removed; it suffices if it is loosened.

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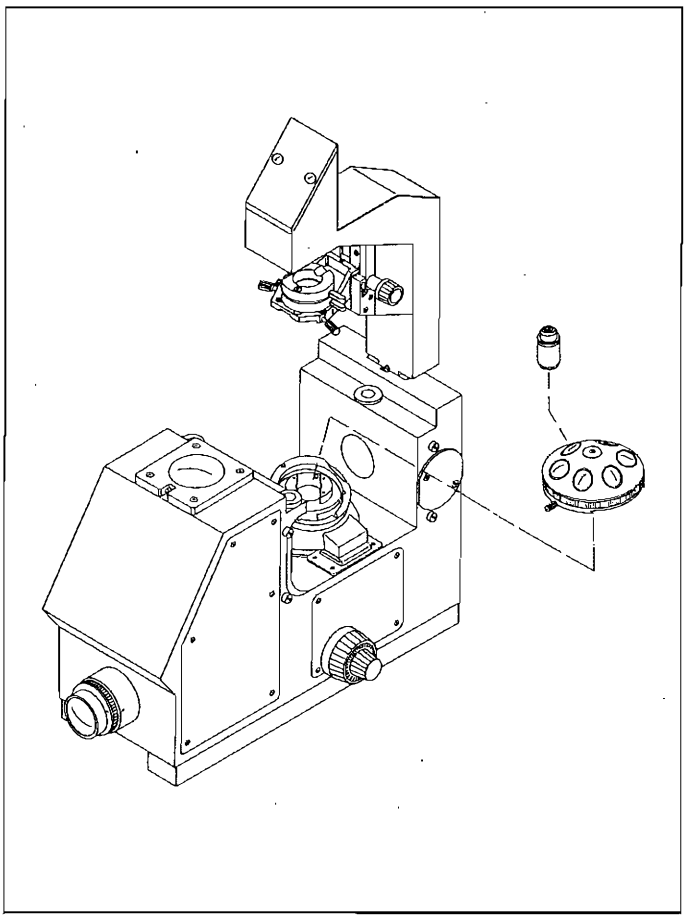
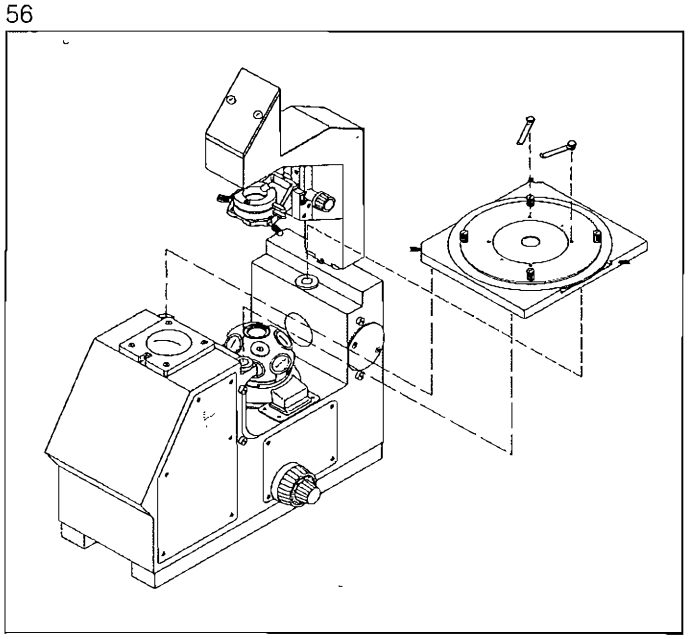


Nosepiece

The non-motorized nosepiece can be exchanged after removal of the microscope stage:
Loosen clamping screw and pull out spring pin at the end of the clamping screw. Move the nosepiece a bit to the right, lift at first the rear and then the front dovetail surface and lift out the nosepiece. Hold the new nosepiece in your hand so that the clamping screw is in front.
Put on the front dovetail surface of the nosepiece and then lower the rear one. The pin which engages a notch will prevent the nosepiece from sliding down.
Move the nosepiece all the way to the left and secure it in this position with the clamping screw.

56



Specimen stage

Loosen clamping screw and lift the three pins of the stage out of the corresponding boreholes of the microscope stand. Mount another stage in the reverse sequence.
Fit an attachable stage on the right edge of the stage plate and secure it with 3 knurled screws, before mounting the stage itself.
A mounting frame is put from the front on the attachable mechanical stage. Then let it snap in. You can now stick scales in the recesses of the attachable mechanical stage.

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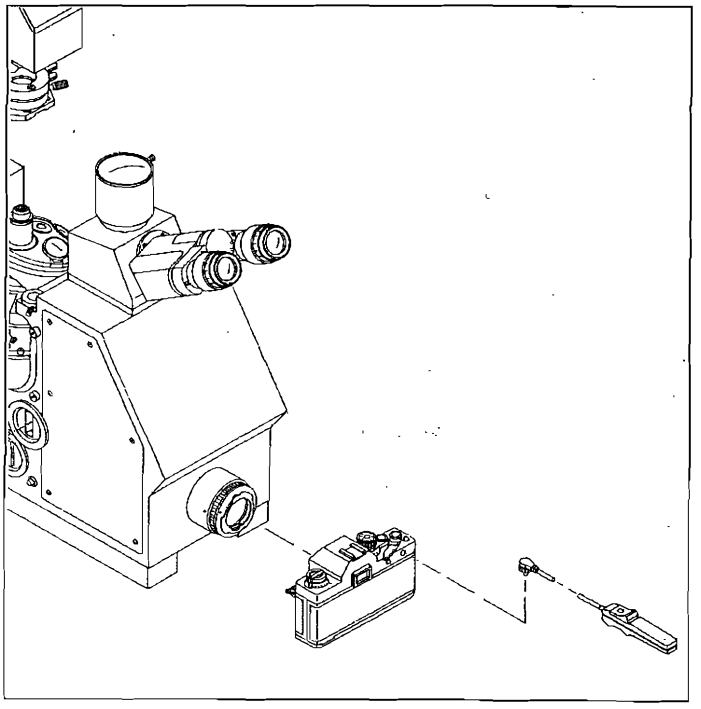

Exchange of the SLR camera on Axiovert 35 or 35 M

Screw adapter T 2 for Contax housing to camera adapter (thread M 42×0.75) of the microscope, unless it is factory-mounted. Attach the camera housing: red dot opposite red dot of the adapter, turn camera to the right and let it snap in. The camera can be aligned after loosening the three screws. Tighten the screws after alignment. Suitable adapters are available for other camera models.

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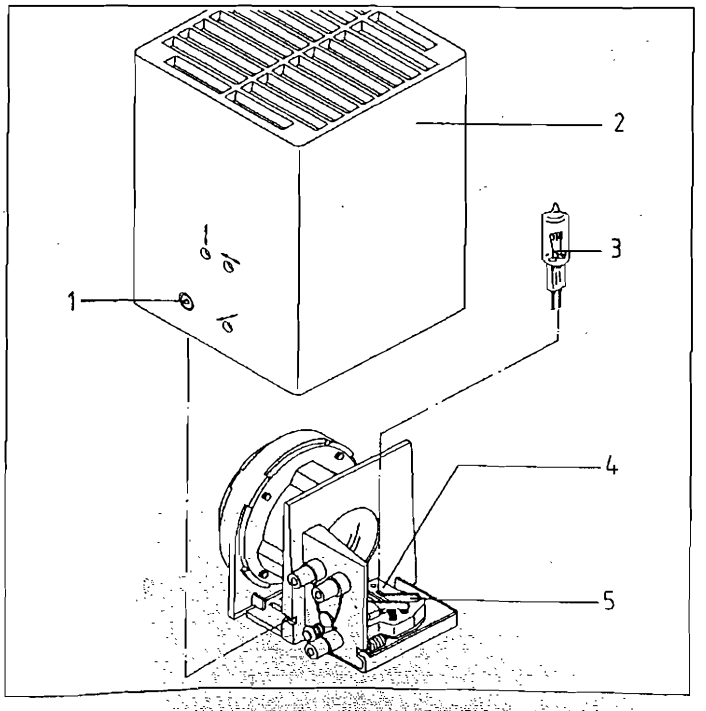

Exchange of the 12V 100W halogen lamp

Pull the plug on the power supply, loosen screw (1) and lift off lamp housing Hal (2). Depress the two spring clips (5), take the defective lamp out of the socket (4) and insert the new one (3).
Put lamp housing (2) back and secure it with screw (1); avoid fingerprints on the lamp bulb (if any, remove them with tissue paper soaked in alcohol).

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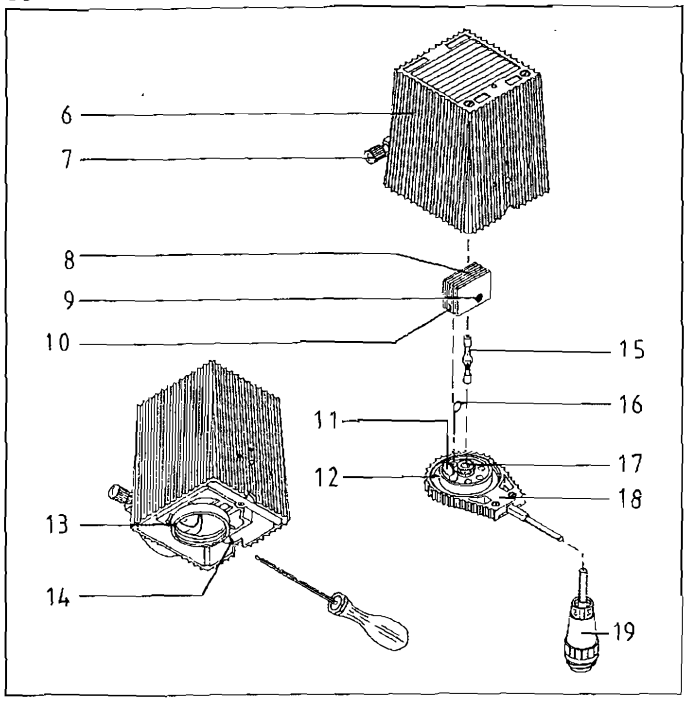

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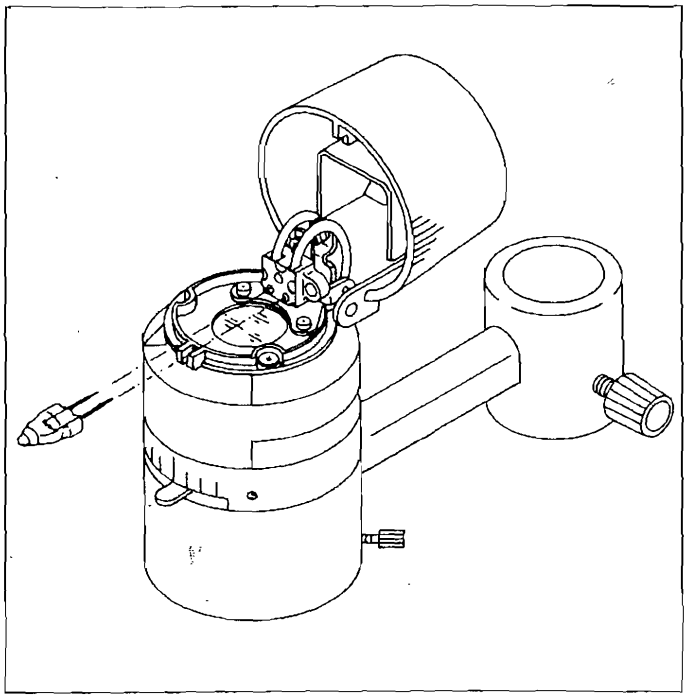

Exchange of the HBO 50 mercury lamp

Pull plug (19) of lamp socket on the power supply and loosen screw **4.7**. Detach lamp housing HBO/XBO (6) from the microscope. With (7) bring collector into frontmost position and loosen clamping screw (14). You can now remove the lamp socket (18) from the housing. Loosen (11) and (17) and pull out lamp (15) and loop (16); loosen (9) and (10) and remove dissipator (8).
Insert new lamp socket with lamp number in the dissipator and clamp with screwdriver; the fuse tip must be aligned parallel with the dissipator.
Plug loop into dissipator, orient it parallel with the latter and secure it.
Insert lamp with dissipator and supply line in the corresponding openings of the lamp socket; secure with hexagon nut (17) and knurled screw (11). The longer side of the dissipator and the supply line contact with loop and fuse tip must be in one line.
Insert the lamp socket (18) with the new lamp in the lamp housing as far as it will go; the pin (13) must engage the notch (12) of the lamp socket. Tighten screw (14). Set the switch on the back of the power supply to the lamp type used (L1 or L2), plug in the lamp plug and connect the instrument to the line. The adjusted voltage is indicated on the dial. The power switch of the lamp is on the front panel of the power supply.
The lamp should be exchanged after about 100 hours (see operating time counter).

Exchange of the lamp in illuminator 20

- Pull plug of illuminator 20 on power supply.
- Fold black cap of illuminator 20 to the left.
- Pull out lamp and slide in new one.

Fuses for power supply 12V 100W	(392585-9901)
primary: 230 V 2× SB 3.15 A	380127-0260
115 V 2× SB 6.3 A	380127-0290
Fuses for power supply 6V 20W	(458415)
230 V 2× SB 0.25 A	380127-0140
115 V 2× SB 0.5 A	380142-2860
Fuses for power plug 230 V	(458405)
SB 125 mA	380127-0110
Fuses for power plug 115 V	(458406)
SB 250 mA	380142-2830
Halogen lamp 12V 100W	380079-9540
Halogen lamp 6V 20W	380143-1350
HBO 50 mercury lamp	381619
Fuses for exposure control	(451749)
for 100...120 V SB 0.63 A	380127-0180
for 220...240 V SB 0.315 A	380127-0150

Filters for photomicrography	**32 mm dia.**
Neutral density filter 0.50 (50% transmission)	46 78 40
Neutral density filter 0.12 (12% transmission)	46 78 41
Neutral density filter 0.03 (3% transmission)	46 78 42
Conversion filter 3200–5500 K	46 78 47
Blue filter CB 6	46 78 51
Blue filter CB 3	46 78 52
Green interference filter	46 78 03

Filter sets

Excitation	Filter set	Exciter filter	Chromatic beam splitter	Barrier filter
UV-G 365	487902	G 365 (447704)	FT 395 (446431)	LP 420 (447731)
Blue-violet G 436	487907	G 436 (447706)	FT 510 (446434)	LP 520 (447737)
UV-H 365	487901	BP 365/12 (447710)	FT 395 (446431)	LP 397 (447330)
Blue-violet H 436	487906	BP 436/10 (447712)	FT 460 (446433)	LP 470 (447753)
Blue H 485	487916	BP 485/20 (447713)	FT 510 (446434)	LP 520 (447737)
Blue H 485 SB	487917	BP 485/20 (447713)	FT 510 (446434)	BP 515-565 (447723)
Green H 546	487915	BP 546/12 (447714)	FT 580 (446435)	LP 590 (447738)
UV-violet 390-420	487918	BP 390-420 (447720)	FT 425 (446432)	LP 540 (447752)
Blue-violet 395-440	487905	BP 395-440 (447721)	FT 460 (446433)	LP 470 (447753)
Blue 450-490	487909	BP 450-490 (447722)	FT 510 (446434)	LP 520 (447737)
Blue 450-490 SB	487910	BP 450-490 (447722)	FT 510 (446434)	BP 515-565 (447723)
Green 510-560	487914	a) LP 510 (447736) b) KP 560 (447765)	FT 580 (446435)	LP 590 (447738)
Green 530-585	487900	BP 530-585 (447724-8001)	FT 600 (446436-0001)	LP 615 (447060-8001)