ZEISS

Axiovert 100

Axiovert 135 and 135 M

Transmitted light and Incident-light fluorescence

Operating Instructions

Axiovert 100 equipped for long illumination distance

Axiovert 135 equipped for incident-light fluorescence

Axiovert 135 equipped for higher illuminating aperture

Contents

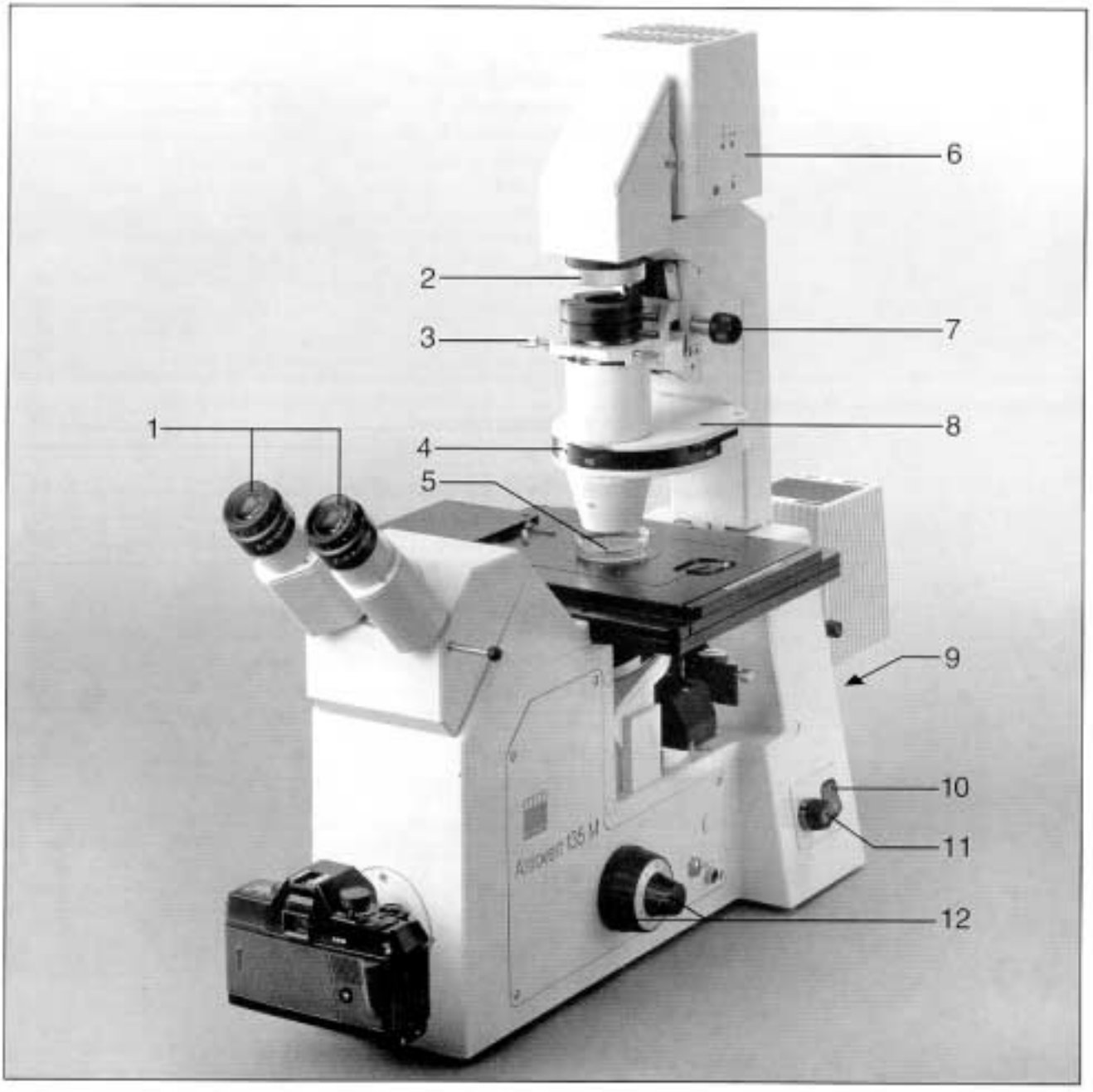
Special notes:

* Please attend to the notes on safety on p. 37!
* The 6- or 10-digit numbers, e.g. 457465, are ordering numbers of instruments or components.

Axiovert inverted microscopes offer ample space to screen specimens in various types of tissue culture dishes and microtest plates on the large specimen stage. Besides binocular observation and the capability to use all standard microscopic methods, the inverted microscopes may be equipped for photomicrography, TV microscopy and microscope photometry.

The line of Axiovert microscopes includes three base stands for the setup of versatile configurations:
Axiovert 100, the efficient base stand.
Axiovert 135 with integrated light path for a 35mm SLR camera.
Axiovert 135 M with additional motorized nosepiece for quick, convenient change between objectives.

1



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

Described is the microscope adjustment which uses 2 focusing eyepieces, as usual in microscopy with the Axiovert 135 and 135 M. For other eyepiece adjustments (e.g. with Axiovert 100) see Section **5.0** of these instructions.
It is assumed for the description below that the illumination system is equipped with a condenser 0.55 Ph/DIC (with pre-centered luminous field diaphragm unit (2)). For a full description of the specific operating elements of other condensers see page 12 ff.

- Check the voltage adjusted on the instrument in window at (9), s. also **3.15**, page 11 on the microscope back. If it complies with the local line voltage, connect the microscope power cable to the line.
- Connect microscope illuminator 100 Hal (6) switch it on with (10) and set to approx. 3 - 4V with potentiometer (11).
- Load a high-contrast specimen (5). If it is mounted on a specimen slide the smaller, thinner cover glass must face down.
- Turn in 10x objective (yellow ring) on nosepiece, check 0 positions on eyepiece scale, and with (7) move condenser (8) all the way down.
- Close diaphragm of condenser at (4) about half.

Behind the eyepieces you should now see light spots (the exit pupils). The left pushrod of a binocular phototube with sliding prism or on the Axiovert 135 should be pushed in.
Through the eyepiece you will see with each eye a bright circle (the eyepiece stop). Merge the two circles into one by turning the two eyepiece tubes to your PD.
Further steps of Köhler illumination adjustment:
- Adjust both eyepieces to the photo reticle by turning eyepiece scale rings (1). (Axiovert 100: focus on the specimen while observing through the fixed eyepiece, then by turning the scale ring of the focusing eyepiece.)
- Focus the specimen using coarse/fine focusing control (12).
- Moderately close luminous field diaphragm (2); it is displayed unsharp in the image (A).
- Focus the diaphragm image by slightly raising or lowering the condenser with (7) (B).
- Center the diaphragm image in the field of view using screws (3) (C), and
- open luminous field diaphragm (2) so far that it just disappears from the field of view (D).

The contrast should now be adjusted for each specimen using the aperture diaphragm (4).
With condenser LD 0.3 **3.26** (page 12) only the aperture diaphragm **3.30** need be adjusted.
If you are not certain how much to stop down, remember that if the specimen is of moderate contrast, about two third of the rear lens element of the objective should be illuminated (visible at the tube bottom with the eyepiece removed, or swung-in Bertrand optics of the Axiovert 135) (E).
Field of view and objective aperture change with each objective change, so that the last-mentioned steps must be repeated in each individual case.

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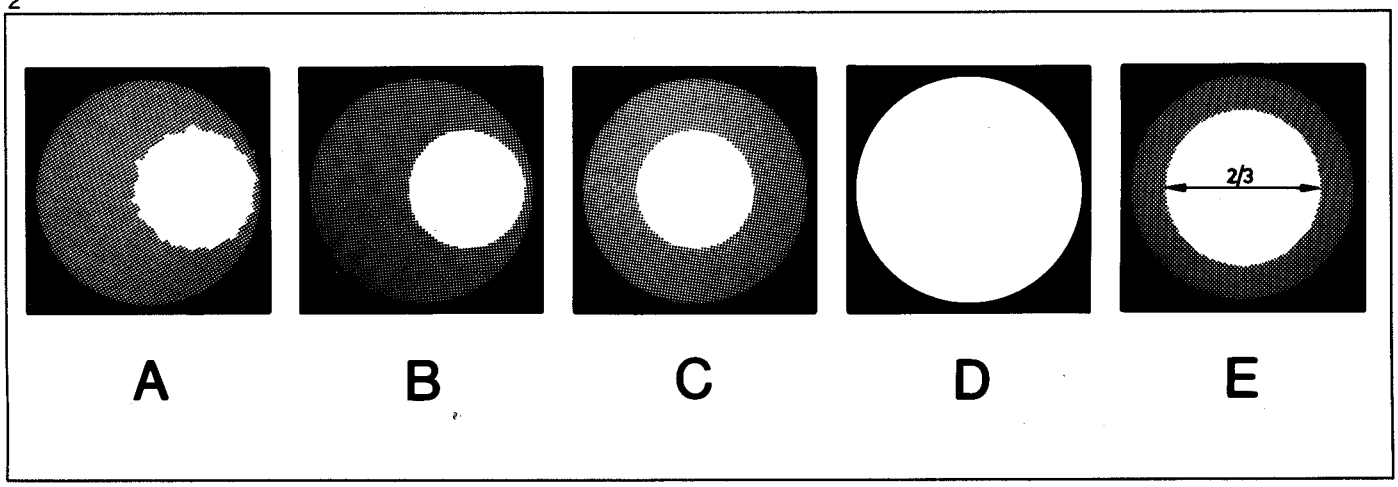

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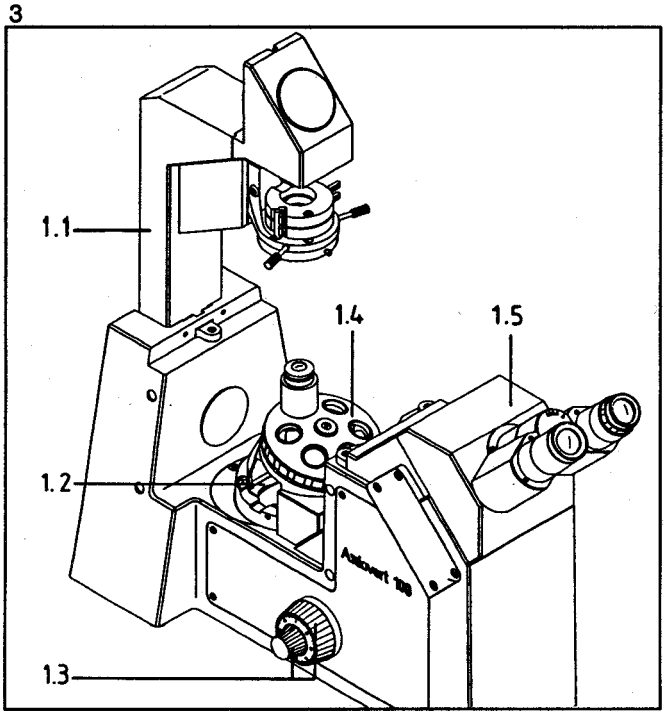

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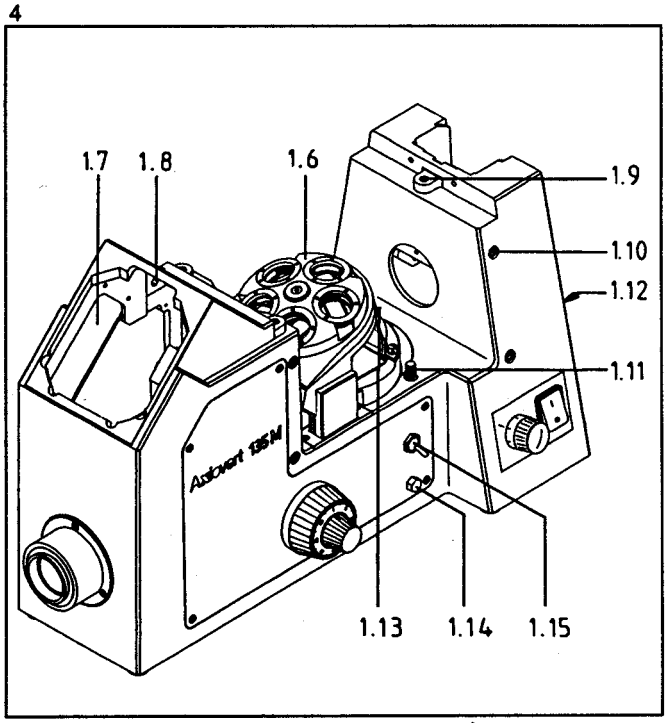

Special note:
All relevant screws are Allen screws for which the following tools are supplied:

- 1 SW 3 Allen wrench with red handle to assemble stages and illuminators, adjust diaphragms, etc.
- 1 SW 2 Allen wrench for tube assembly
- 2 SW 1.5 Allen wrenches to center the phase stops of the LD condenser 0.55 Ph/DIC.

1.0 Stands

Stand of Axiovert 100 and 135:
1.4 Nosepiece 6x H or 5x H DIC.
Stand of Axiovert 135 M:
1.6 Motorized rotatable rigidly mounted H DIC nosepiece 5x.
1.13 Slot for auxiliary objects, required, e.g. for differential interference contrast (DIC).
1.2 Slot for reflector sliders, e.g.: Reflector slider 3 FL or Reflector slider Antiflex/2 FL for incident-light fluorescence illumination, or
Optovar slider for magnification change.

Stand of Axiovert 135 and 135 M:
1.16 Nosepiece 4x equipped with Bertrand optics for phase-contrast adjustment; focused by turning the knurled disk on the projecting knurled knobs. Optovar inserts 1.6x and 2.5x optional.

1.17 Slot for the analyzer slider required for differential interference contrast.

1.3 Coaxial coarse/fine focusing control acting on the nosepiece. Turning the outer part of the knob anticlockwise lowers the nosepiece. Total vertical travelling range, including the fine focusing control: 9mm. One revolution of the coarse focusing control corresponds to approx. 2mm vertical travel; gear ratio of fine focusing control approx. 1:10. The index line on the coarse focusing control may be used to roughly measure the object thickness. 1 scale division corresponds to approx 2 µm.

1.9 3 boreholes to mount the microscope stage with patented three-point support.
1.10 Sockets (on both sides) to mount micromanipulators.
1.5 Binocular tube 45°/20, exchangeable for binocular phototubes. Secure tube in its receptacle **1.7** using
1.8 SW 2 Allen screw.
Required for all Axiovert stands for micoscopy in transmitted8 light:
1.1 Carrier for transmitted-light illumination system. It is supplied separately and mounted aligned by 4 screws. For details see Section **3.0** on transmitted-light illumination system. The power supply **1.12** for the 12V 100W microscope illuminator Hal is built into the back of the stand at.

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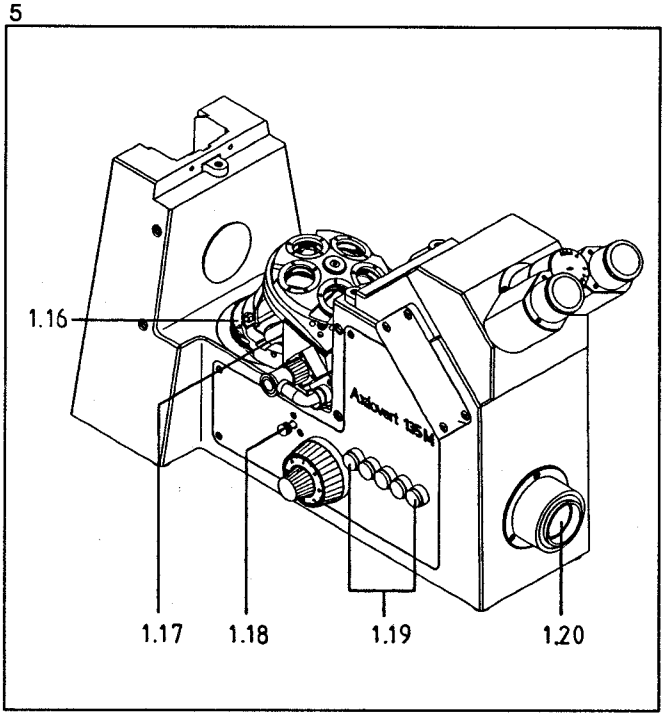

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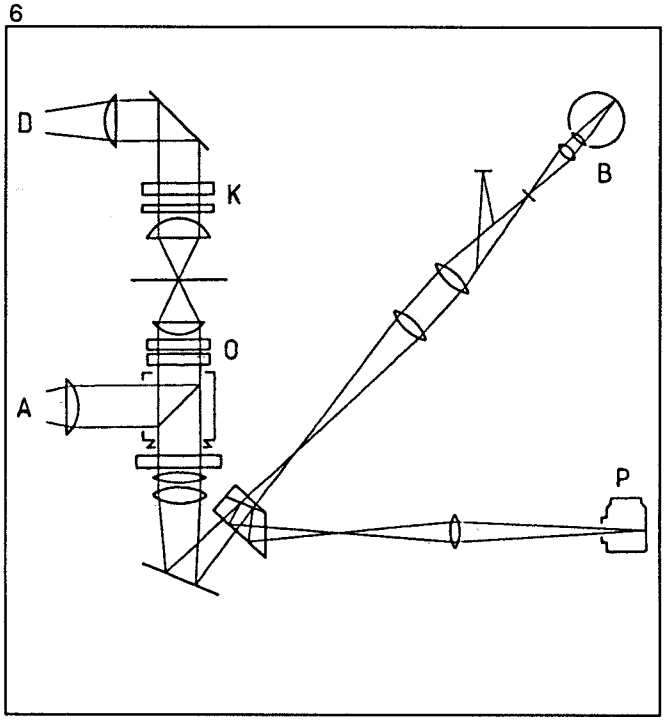

On Axiovert 135 and 135 M:
1.20 Camera port to mount an SLR camera housing by a T2 adapter, e.g. Contax 167 MT with cable release.

1.18 Pushrod for beam splitting prism.
Pushed in: 100% of the light for observation
Pulled out: 30% of the light for observation, 70% to the camera; a photo reticle becomes visible in the light path.

In the designation of the Axiovert 135 M the letter M indicates that the nosepiece is motorized and the individual positions may be addressed by
1.19 five illuminated keys.
The addressed position is displayed by a light in the key. The selection of objectives is at the user's choice.

Required for motorized nosepiece: power plug 7.5V/0.75A which connects to socket **1.14**. Circuit breaker **1.15** interrupts voltage supply from the power supply to the microscope stand.
1.11 Knurled screw to limit vertical movement of nosepiece and prevent the objective from hitting the stage and protect it against contamination by immersion oil. Knurled screw turned clockwise: larger objective distance, turned anticlockwise: smaller objective distance.
If an activated key flashes during focusing with the coarse/fine focusing control, the limit is attained; motorized switching of the nosepiece is impossible. To override the stop, lower the nosepiece or, if possible, turn the knurled screw anticlockwise.

Fig. 6: Beam path of Axiovert 135 microscope, schematic

D	Transmitted light
A	Reflected light
B	Observation beam path
P	Photo beam path
K	Condenser
O	Objective

2.0 Specimen stages

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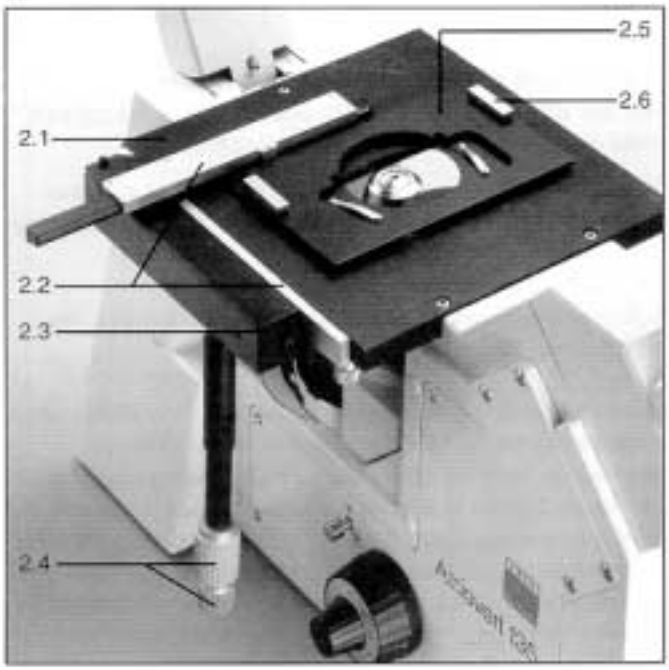

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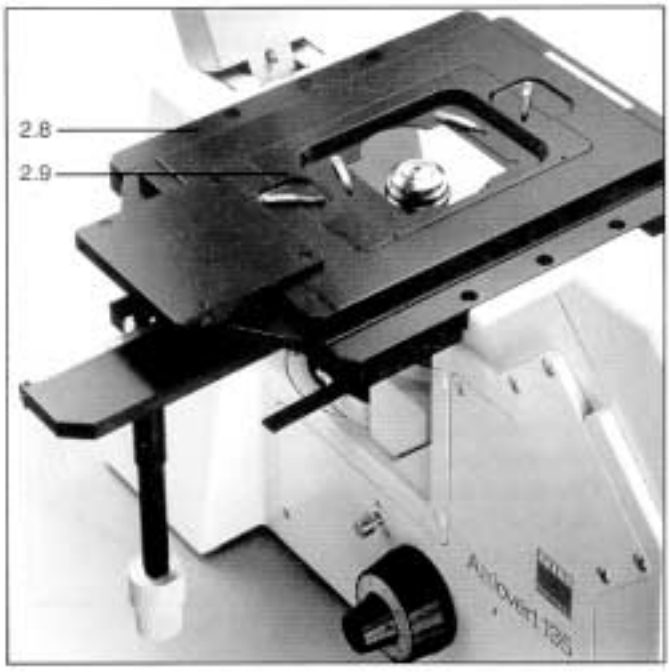

Base equipment:
2.1 Specimen stage 211x230 (45 13 35), usually with
2.3 attachable mechanical stage (45 13 36) mounted on the left edge of the stage plate and secured by three knurled screws (mounting on the right side is also possible). The SW 3 three Allen screws of the stage fit in the corresponding boreholes of the microscope stand. The attachable mechanical stage is mounted before the stage plate is attached.
2.4 Coaxial controls for movement in x and y (125x85mm).

As an alternative: mechanical stage (45 13 39) **2.8**, travelling range 130x85mm.
2.9 Mounting frames K mentioned below are fitted in the mechanical stage as follows: insert corner with red dot in corner with red mark, press down diagonally opposite corners and secure by two spring clips.
If the focus of a specimen in a flask, Petri dish, etc. varies, the flatness of the specimen may be corrected for focusing using the supplied Allen screwdriver SW 1.5.

Available for the above stage and different examination methods and specimen slides:
2.5 Mounting frames for specimen slides, including
2.2 scale stickers. Mounting frame M is fitted in
2.3 attachable mechanical stage (47 17 36)
by sliding it from the front under
2.6 spring clips until they snap in.
Stick the scales in the corresponding recesses of the attachable mechanical stage.

Mounting frames type M and K for:
- Microtest plates with 60, 72 or 120 positions (Terasaki): M and K including scale stickers, sized 81.5x56mm.
- Microtiter plates with 96 positions: M and K, including scale stickers, sized 128.5x86.3mm.
- Hamax plates, Möller-Coates plates with 60 positions: M and K, including scale stickers, sized 93.5x67.5mm.
- Multi dishes, e.g. Coastar plate with 24 positions: M, sized 133.5x88.5mm.
- Specimen slide 76x26mm: M and K
- Petri dish 36mm dia.: M and K
- Petri dish 54mm dia.: M and K
- Petri dish 65mm dia.: M and K
- Petri dish 88mm dia.: M and K
- Costar flasks: M, sized 125x77mm.
- Corning flasks: M, sized 98.5x52mm.
- Tissue culture plate by Greiner, 6x4x16 = 384 positions: M, sized 136.5x92.5mm.
- Plankton chambers 42mm dia.: M

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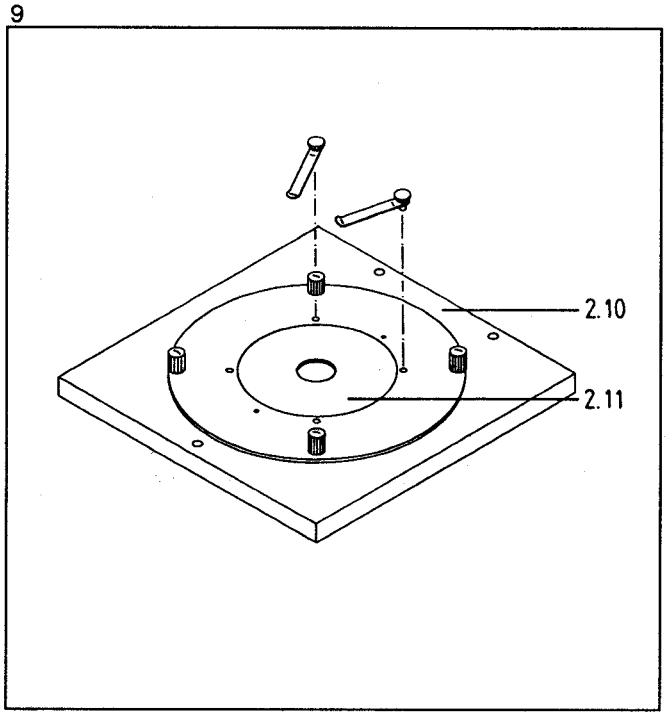

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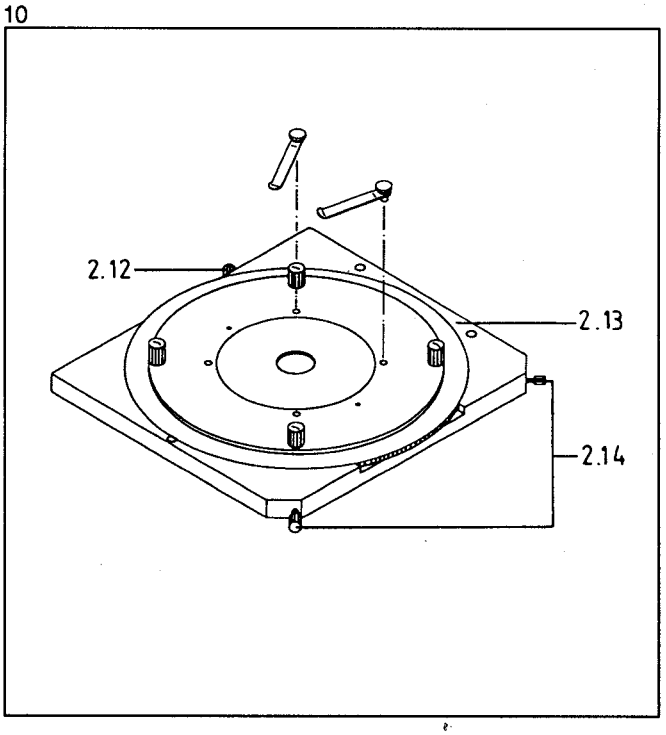

Other stages

Mounted like the stage plate are:
2.10 Gliding stage 10 (47 17 20-9903). **2.11** Two stage inserts for the above stage; the opening (24mm or 48mm dia.) depends on the size of the specimen cover glasses.
20mm travelling range in either direction.

2.13 Rotary, centrable gliding stage Z (47 17 22-9901) with two 24mm and 48mm dia. stage inserts.
2.14 Centering screws and
2.12 clamping screw for stage rotation.
20mm travelling range in either direction.

For special applications:
Heating stage M (47 18 20),
hot plate, and TRZ 3700 temperature control (adjusting range from 3°C above room temperature to 50°C),
MR/ML mot. micromanipulator with control panel (G 42-525),
MMJ manual micromanipulator (G 42-524),
PMZ 20 piezo translator (G 42-523),
environmental chamber/incubator (G 42-526).
Scanning stage 100x90mm (45 17 40) with step widths of 0.25 μm for microscope photometry.

3.0 Transmitted-light illumination system

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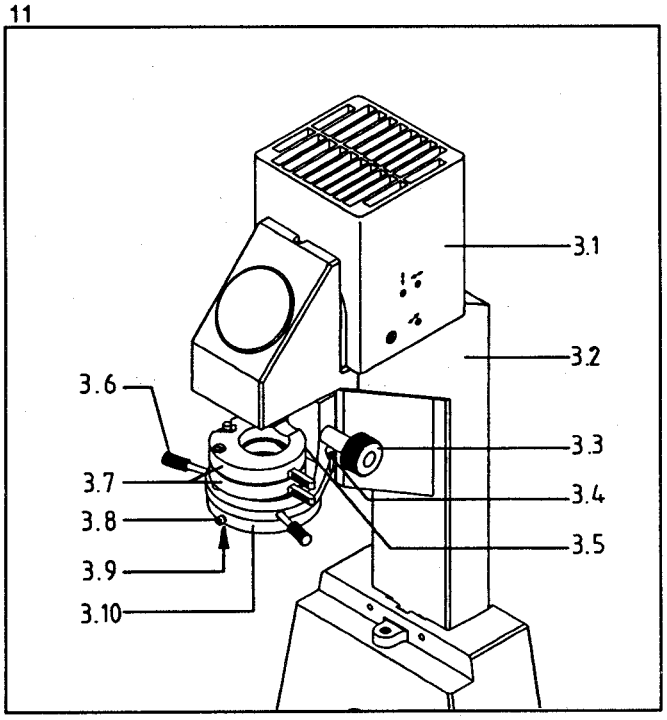

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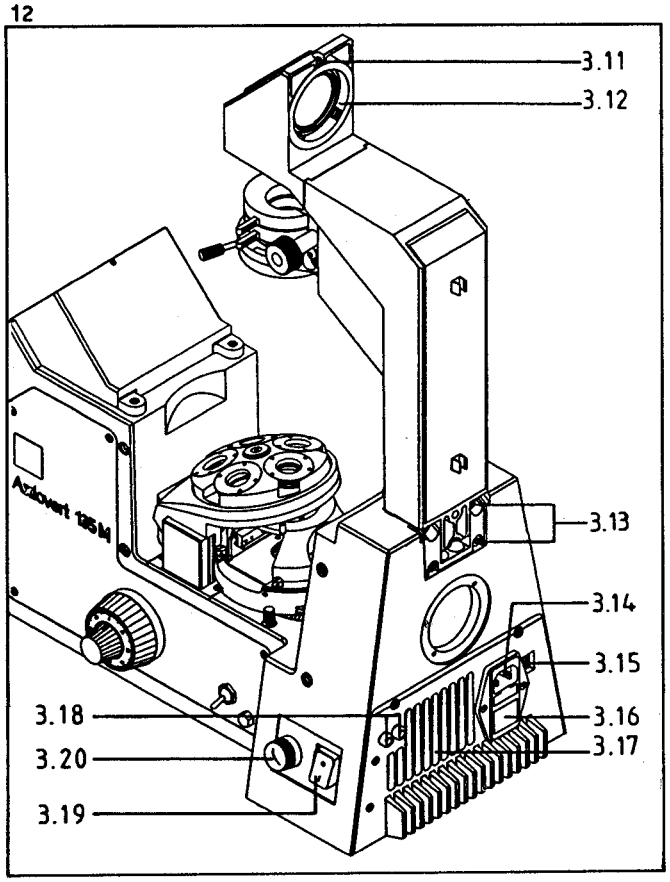

3.2 Carrier for transmitted-light illuminator fixed on stand by **3.13** four screws and adjusted.
3.12 Mount for attachment of **3.1** microscope illuminator 100 Hal
3.3 Control for approx. 30mm vertical adjustment of **3.10** condenser carrier. The motion of the carrier is factory-adjusted and and the control knob **3.3** should be changed only by our maintenance service.
3.9 Guide notch for alignment pin on condenser.
Condenser or spacer tube with iris (45 13 55) of high-aperture condenser is inserted from the front in the dovetail mount; the alignment pin should engage the notch. Insert the condenser dovetail mount all the way in the holder.
3.8 Clamping screw secures the condenser.
3.6 Two condenser centering screws to center the luminous field diaphragm image during illumination adjustment (see page 5).
3.7 Two swing-out holders for filter or polarizer (lower holder).
A limit stop for the vertical condenser motion which prevents the specimen from being damaged is adjusted as follows:
1. Focus specimen.
2. Form image of luminous field diaphragm (see page 5); loosen screw with pin **3.4** using supplied SW 3 Allen wrench.
3. Lower condenser slightly (diaphragm image becomes unsharp).
4. Move screw with pin all the way up and tighten. The specimen is secured.
3.5 Upper limit stop for condenser. The tongue of the limit stop must point in a vertical position to prevent the LD condenser 0.55 from hitting against the carrier. The tongue must point in a horizontal position for all other condensers.
For easy specimen exchange the transmitted-light illuminator may be hinged back.
The microscope illuminator 100 Hal comprises lamp housing with reflector, collector and lamp socket to fit the 12V 100W halogen lamp. It connects to **3.18** built-in power supply. At the back of the illuminator carrier 2 clamps hold the illuminator cable.
Features of microscope illuminator 100 Hal:
3.21 Light exit with dovetail mount for attachment to carrier for transmitted-light illuminator:
- Slacken screw **3.11** sufficiently.
- Attach illuminator dovetail mount inclined to port opposite the clamping screw, lower illuminator on to seating surface and tighten screw.
- A holder in the light exit before the collector contains a 42mm dia. heat-reflecting filter. This filter is inserted in such a way that the surface with the higher reflection faces the light source.

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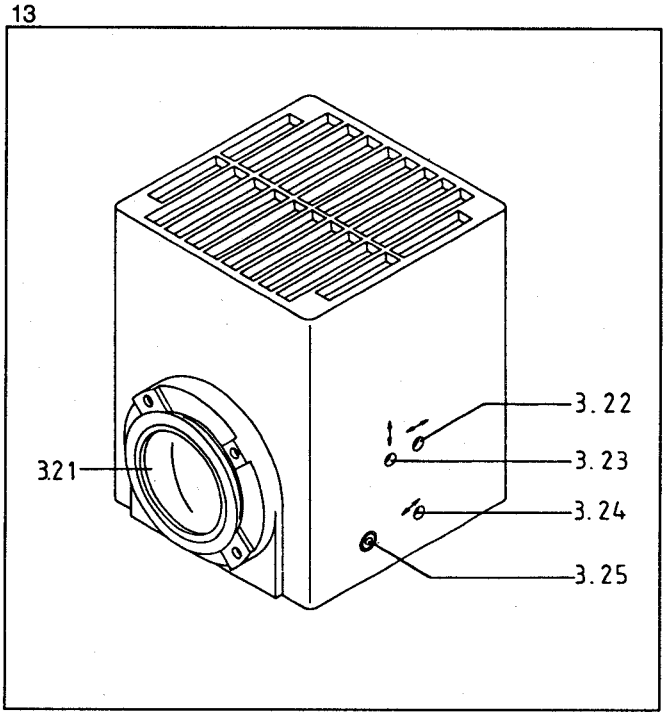

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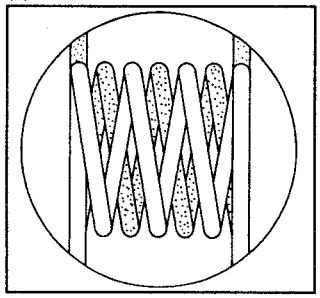

3.25 Clamping screw for lamp socket and collector
3.22 Lamp coil focusing
3.23 Vertical adjustment of lamp coil
3.24 Lateral adjustment of lamp coil

3.17 Power supply for microscope illuminator 100 Hal integrated in stand.
Technical data:

Power consumption	200 VA
Frequency	50 . . . 60 Hz
Input voltage adjustable to	230V for 220 . . . 240V ~
	or to 115V for 110 . . . 127V ~

If the local line voltage does not comply with the adjusted voltage range, switch off the instrument and pull the power cable. Then switch **3.15** may be vertically adjusted with a screwdriver. The power supply is radio-screened and complies with VDE, IEC, CSA and UL regulations. It is categorized as a protection class I, type B instrument.

The power supply is highly stabilized against line fluctuations. It supplies DC voltage, adjustable from 3 . . . 12V and suitable for applications in photometry.

3.19 ON/OFF switch with built-in signal lamp
3.20 Potentiometer for lamp voltage adjustment supplies 12V when turned fully clockwise. The index displays the adjusted voltage. 3200 K color temperature is obtained with adjustment to 11.5V.
In case of trouble during switch-on of the halogen lamp, an electronic fuse will automatically turn off the power supply. The potentiometer should then be turned to lowest value and the lamp switched off. Switch on the lamp again and select the desired voltage.
3.14 Instrument power plug.
3.16 Insert with 2 fuses.
Spare fuses for 230V: SB 2A, for 115V: SB 4A.
Fuse exchange: pull power plug on microscope. Pull out insert with fuses and replace fuses.
Special note: should a fuse fail again, please call our maintenance service.
3.18 Sockets for connection of microscope illuminator 100 Hal.
For lamp exchange see page 33.

Adjustment of microscope illuminator Hal with reflector
- Detach illuminator Hal from microscope.
- Switch on halogen lamp with power switch and select brightness on potentiometer.
- Produce a sharp image of the lamp coil on a wall at approx. 3m distance or similar object, using screw **3.22**.
- Adjust lamp coil image vertically using screw **3.23** and laterally with screw **3.24**. Shift the coil image so that it fills the gaps of the reflector image (see Fig. 14).

The rigidly mounted reflector is arranged exactly in the optical axis of the collector and need not be adjusted.
Fine adjustment:
- With the supplied tool unscrew diffusing screen from the illuminator port of the carrier and remove filter from the light path.
- Attach microscope illuminator to carrier and adjust specimen using 40x or higher-power objective. Look for a blank in the specimen. Completely open aperture diaphragm in condenser.
- With removed eyepiece, inserted centering telescope or Betrand optics observe the pupil with lamp coil and reflector images. If both images are decentered, correct with the above-mentioned adjusting screws.
- Screw diffusing screen into carier, control pupil image again and, if necessary, optimize by adjustment with **3.22**.

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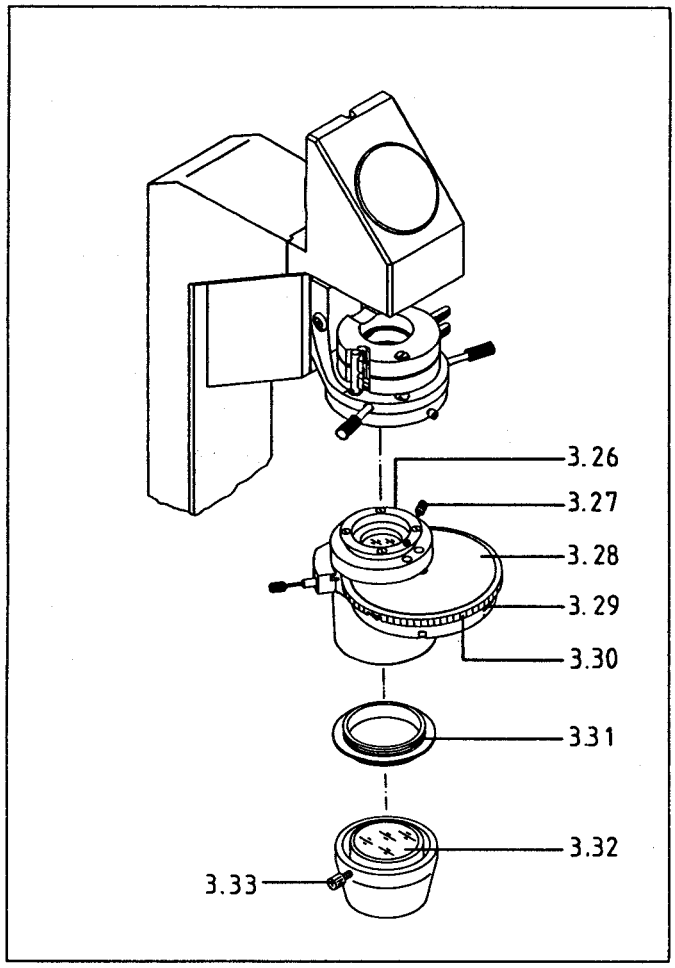

The examination of specimens in chambers and flasks requires free space between specimen plane and illumination system.
3.26 Condenser LD 0.3 H, Ph 1, 2 (45 17 56) for brightfield and phase-contrast illumination with long working distance.
It features at
3.30 a pre-centered aperture diaphragm with iris which serves as contrast aperture diaphragm.
3.28 Turret with the positions:
- Brightfield (H)
- Phase contrast 1 (Ph 1)
- Phase contrast 2 (Ph 2).

3.29 displays the chosen position.
3.27 Knurled screws which when pressed down and turned center the phase-contrast stops for the corresponding phase-contrast objectives (Ph 1 or Ph 2), see p. 26.

With the above equipment the working distance is approx. 70mm in brightfield and phase contrast. Object fields of max. 8mm dia. may be illuminated with 2.5x objectives. For illumination of the object field for 1.25x objectives for photography do not use the condenser.
Objectives with NAs ≤ 0.45 are suitable for brightfield, in Ph 2 position the condenser for phase-contrast objectives with max. NAs of 0.75 may be used.

3.32 Front lens attachment 0.55 H (45 17 57) increases the NA of the condenser to 0.55.
3.33 Clamping screw for mounting on condenser LD 0.3 previously equipped with
3.31 screw-in dovetail mount.
Working distance with front lens attachment approx. 23mm, diameter of illuminated object field 4mm (objectives with magnifications as from 5x). Condenser LD 0.3 with front lens attachment 0.55 is suitable only in brightfield for objectives with NAs ≤ 0.6 (max. 40x, except for immersion objectives).

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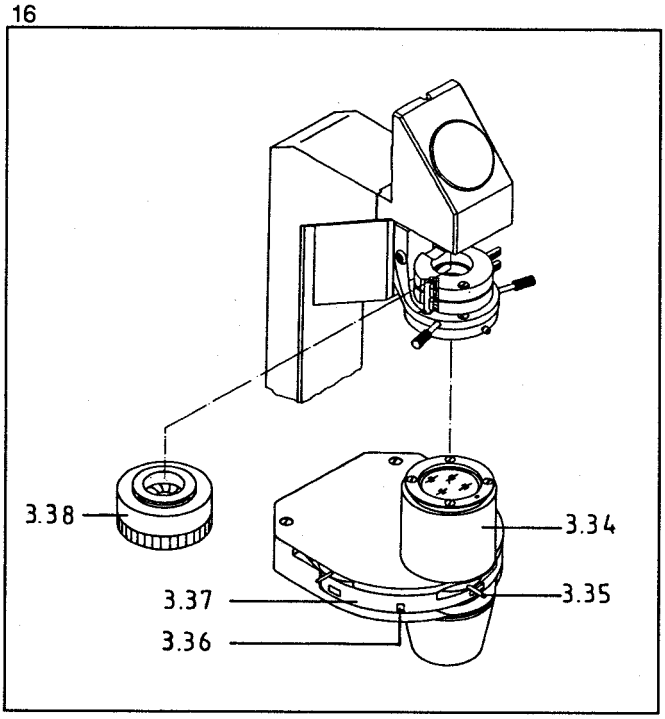

For ample working space and the use of DIC besides brightfield and phase contrast:

3.34 LD condenser 0.55 H, Ph 1, Ph 2, DIC (45 17 59)
LD condenser 0.55 H, Ph 2, Ph 3, DIC (45 17 53)
It is possible to adjust Köhler illumination with these condensers. The working distance is 22mm.

3.38 Centrable luminous field diaphragm. The luminous field diaphragm unit is screwed from below into the light exit opening of the illumination system. Centration of luminous field diaphragm see below.

3.37 Turret with standard equipment for:
- DIC 0.3 - 0.4/0.55 (44 13 95) (position DIC .3 - .4) or brightfield (H) alternatively
- DIC 0.5 - 1.3/0.55 (44 13 96) (position DIC .5 -1.3) or brightfield (H) alternatively
- Phase contrast 1 (Ph 1)
- Phase contrast 2 (Ph 2)

Other than this one the LD condenser 0.55 H, Ph 2, Ph 3, DIC is equipped with annular phase stops Ph 2 and Ph 3.

3.35 Pre-centered aperture diaphragm adjustable in all DIC positions.
The selected position is displayed in front on the turret.

3.36 Centering screws for annular phase stop.
Centration of annular phase stops and phase-contrast objectives using the supplied screwdrivers SW 1.5. The centration may be observed using the centering telescope or the Bertrand optics, see p. 26.

The working distance of the LD condenser 0.55 is 22mm for all methods, object fields of max. 4mm dia. may be illuminated (that is as from 5x objective magnification). In brightfield the condenser is suitable for objectives up to NAs of 0.6, in phase contrast and DIC up to 0.75 (dry objectives up to 40x magnification).

The exchange of DIC prisms is described on page 32.

The LD condenser 0.55 H/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 luminous diaphragm unit **3.38** using 1.3mm Allen wrench. Then secure the luminous field diaphragm by tightening the spring screw.
- Fit condenser and objective, focus luminous field diaphragm using condenser control and center in the field using the centering screws of the condenser holder.

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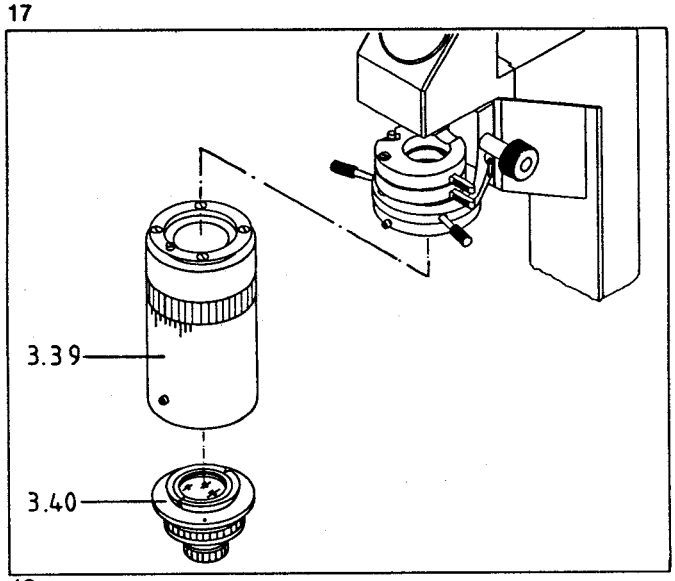

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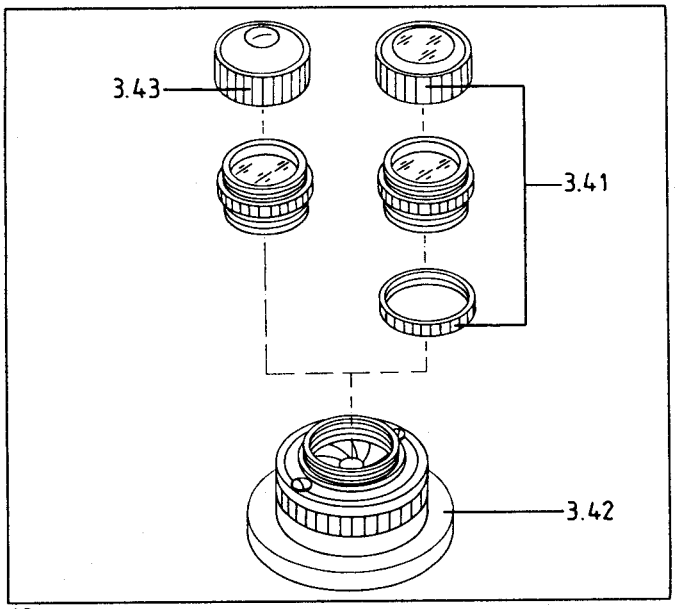

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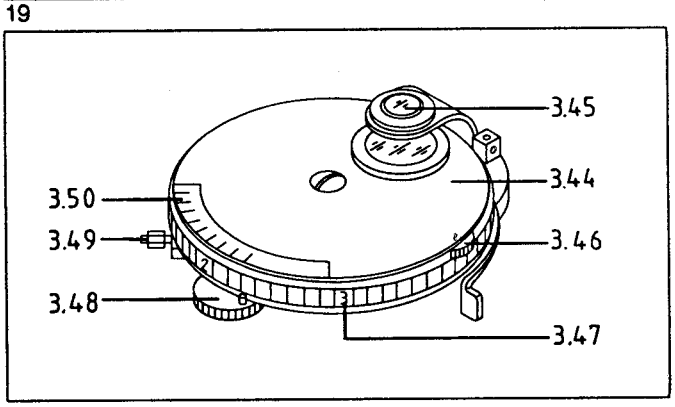


The resolving power even of high-power objectives is fully utilized by the following condensers. Köhler illumination provides for a homogeneously illuminated object field, a brilliant image without reflections and glare, and optimum specimen protection.
3.39 Condenser mount with iris (45 17 55) (pre-centered luminous field diaphragm) is required for this condenser.

For brightfield or differential interference contrast (DIC) with long back focal distance or high aperture (1.4):
3.40 Achromatic-aplanatic condenser system 0.32 Pol (44 52 45) with optics (aperture 0.32) and
3.42 knurled ring for aperture iris diaphragm.

3.41 Front lens 0.63 (46 52 65) Pol for condenser system 0.32 Pol (for long back focal distance; use the extra supplied spacer ring) or
3.43 front lens 1.4 (46 52 68) Pol may be screwed on to condenser system 0.32 Pol.

Equipment with front lens 1.4:
Köhler illumination possible with 20x . . . 100x objectives,
DIC with 20x . . . 100x objectives
with DIC prism 0.5 - 1.4/1.4 (44 52 94).

Equipment with front lens 0.63
Köhler illumination possible with 5x . . . 40x objectives,
DIC with 10x objectives with DIC prism 0.3/0.63 (43 44 10).

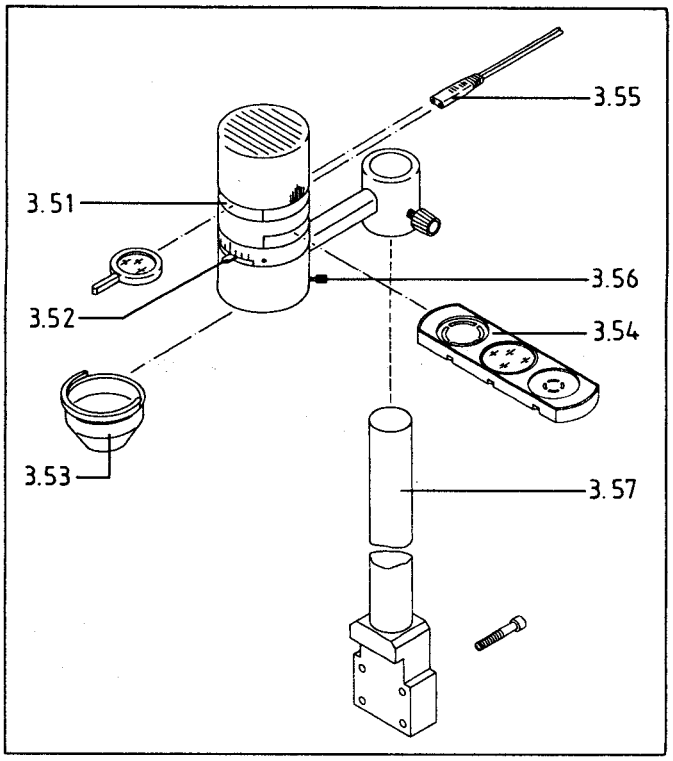
For quick change between brightfield and phase contrast with aperture 0.9:
3.44 Phase-contrast condenser II Z 0.9 Ph 1, 2, 3 (45 17 54) with
3.45 swing-in front lens.
Click stops of condenser turret:

- Brightfield (J) with aperture iris diaphragm
  with 10x . . .100x objectives with swung-in front lens,
  with 2.5x . . . 5x objectives with swung-out front lens.
- Phase contrast 1 with objectives Ph 1
- Phase contrast 2 with objectives Ph 2
- Phase contrast 3 with objectives Ph 3

3.48 and **3.49** Clamping lever and knob to center annular phase stops.
3.46 Control for aperture operation
3.50 Aperture display.
3.47 Display of operative illumination method.

6V 20W illumination system for brightfield and phase contrast and for long working distances.

20



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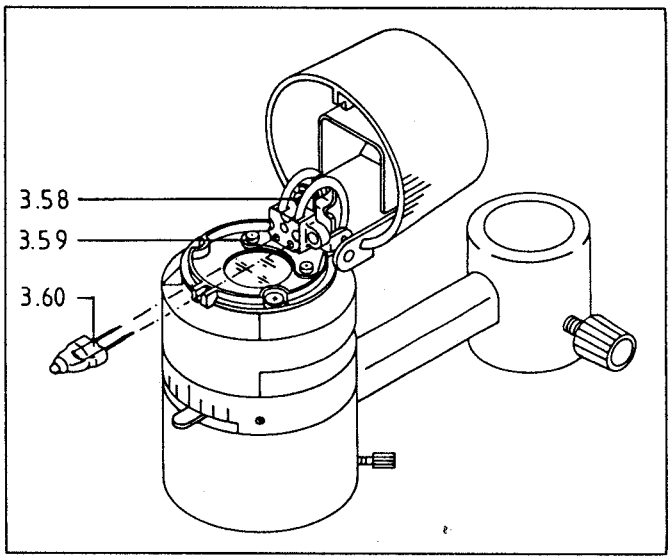


It comprises:

3.57 illuminator carrier (45 17 51) which is screwed to the stand by 4 screws. It carries on top illuminator 20 with LD condenser 0.3 H, Ph 1, 2 (47 12 07).

3.60 6V 20W halogen lamp (visible when black illuminator lid is hinged back), fixed by 2 contact pins, and

3.55 connecting cable. The 6V 20W illumination system connects to built-in power supply at the back of the stand.

The power supply is similar to that described on page 11.

The following performance data are different:

Stabilized DC adjustable from 2.6 . . . 6V. A color temperature of approx. 3200 K is obtained at 6V.

Operating elements from top to bottom:

3.51 Filter slot for 32mm dia. green interference filter for contrast enhancement in phase contrast.

3.54 3-position slider: Ph 1, brightfield, Ph 2.

3.52 Aperture diaphragm for contrast enhancement in brightfield.

3.56 Centering screws for phase contrast adjustment. The numerical aperture of the illumination system is 0.3. It may be increased to 0.6 by

3.53 front lens attachment 0.6 (47 12 08) which is mounted at the bottom of the illuminator.

The system without front lens attachment homogeneously illuminates the object field of the 1.25x objective.

Adjustment of illuminator 20 (47 12 07)

- Clamp illuminator on 32mm dia. column so that the distance between front lens mount (without front lens attachment 0.6) and stage plate is approx. 56mm.
- Set slider to brightfield position, center luminous spot on specimen stage to objective by turning the illuminator on the carrier.
- The lamp is pre-centered. In case of strongly deviating coil position field or pupil may be inhomogeneously illuminated. If this is the case, loosen **3.59** two locking screws using SW 2 Allen wrench so that the socket may be shifted.

With the small tool the vertical adjustment of the lamp may be corrected at **3.58**. Adjust to maximum brightness.

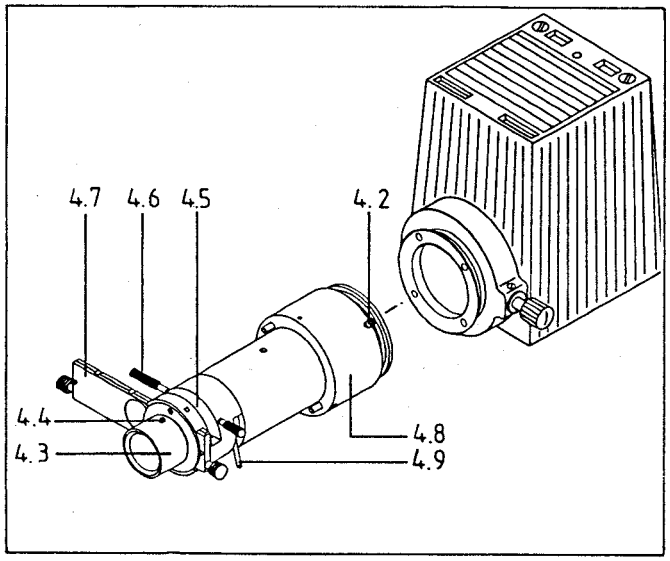
- Observe the annular phase stop Ph in phase contrast, or the aperture diaphragm in brightfield using the centering telescope or Bertrand optics, and center with knurled screws **3.56**.

Observe illumination of pupil in brightfield and, if necessary, correct the lateral and vertical adjustment of the illuminator on the round column so that the highest aperture is homogeneously illuminated.

- In brightfield plug on front lens attachment 0.6 for objectives with apertures $\geq$0.4.

4.0 Incident-light fluorescence illumination system

22



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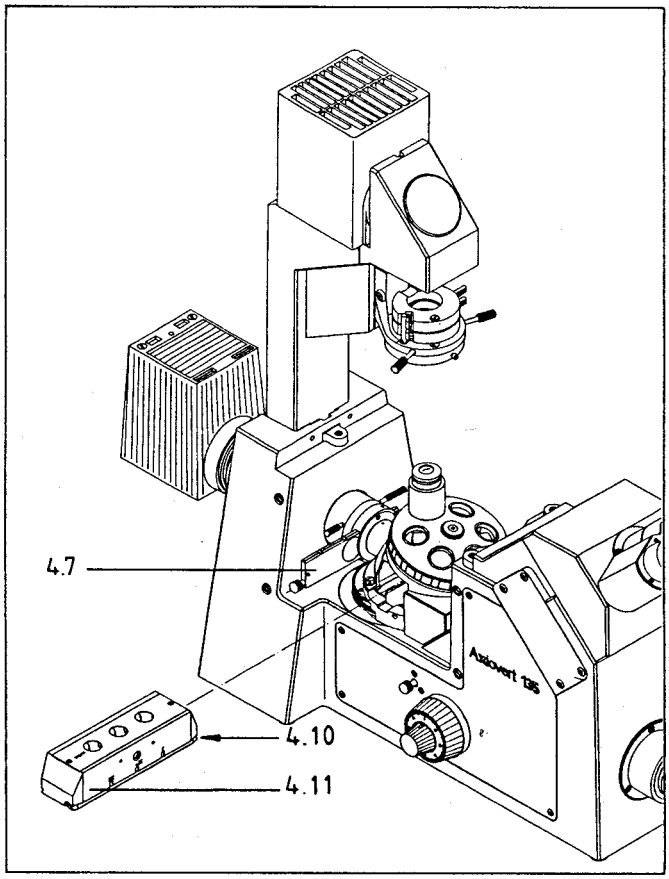


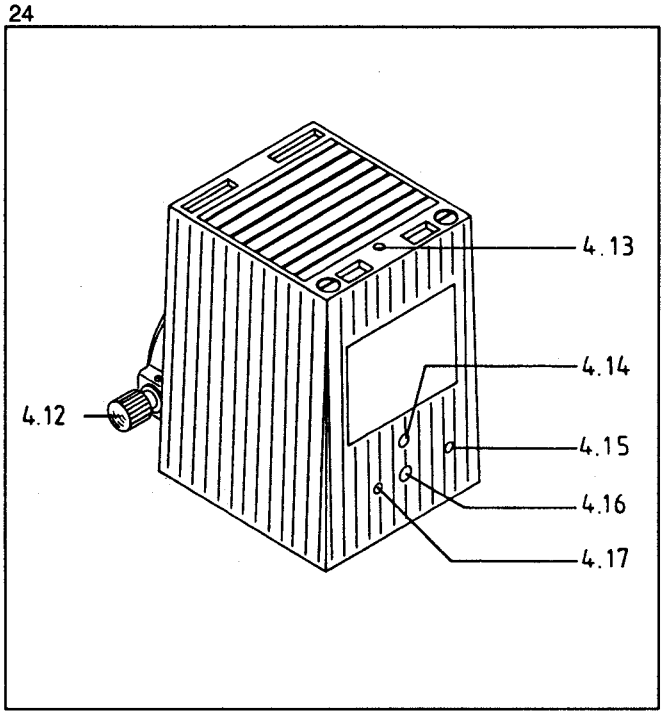
The incident-light fluorescence illuminator upgrades any Axiovert microscope to a fluorescence microscope with incident-light excitation. In combination with either of the transmitted-light illumination systems described in Section **3.0** it allows the specimen to be examined first in brightfield or phase contrast.

The fluorescence illuminator comprises:
4.1 Lamp housing HBO/XBO with aspheric collector, HBO 50W mercury short-arc lamp socket and HBO 50W mercury short-arc lamp and connecting cable.
It is connected to the line by power supply (39 26 42) for HBO 50, 220 - 240V 50 . . .60Hz.
The power supply is radio-screened an complies with VDE, IEC, CSA and UL regulations. It is categorized as a protection class I, type B instrument.
4.8 Illuminator adapter and
4.2 clamping screw to mount the fluorescence illuminator.
The luminous field diaphragm is focused by loosening screw **4.4** (supplied screwdriver) and shifting the sleeve **4.3**.
4.6 Centering screws for luminous field diaphragm.
4.9 Lever to adjust the aperture.
4.5 Slot for extra loose exciter filter. 32mm dia. filters may be fitted using a holding ring.

4.7 Slider with 3 positions:
Pos. 1 - dark slider blocking the light path
Pos. 2 - BG 38 red-attenuating filter, which eliminates disturbing IR-light
Pos. 3 - free aperture (25mm dia. filter holder).

4.11 Reflector slider 3 FL (45 13 66) to change, for example, between UV, violet and green excitation by moving the reflector slider from one click-stop position of the guideways to the next. The inscription on the reflector slider must point to the user. The reflector slider is easily exchanged by flicking up **4.10** lever on the right side of the reflector slider.
Sets comprising exciter filter - reflector (45° plane glass plate acting as chromatic beam splitter) - barrier filter may be accommodated in all 3 positions of the reflector slider 3 FL, or one aperture left free for transmitted-light work to adjust the specimen in brightfield or phase contrast.
For details see page 32.

24



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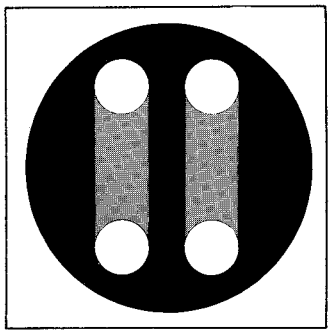

Caution!
The HBO 50W lamp should be replaced after 100hrs. which correspond to its mean life. The longer it is in use the lower the intensity it supplies; the object field will no longer be homogeneously illuminated and there will be a high explosion risk. The operating life of the lamp is displayed on the running time meter of the power supply.

Features of HBO/XBO lamp housing:

Light exit port with dovetail mount for attachment to incident-light system FL.

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

4.12 Knob for collector adjustment.
4.13 Vertical lamp adjustment
4.15 Lateral lamp adjustment
4.14 Vertical adjustment of mirror image (red dot)
4.17 Lateral adjustment of mirror iamge (red dot)
4.16 Focusing of mirror image

Heat-reflecting filter KG 1 is integrated in incident-light system FL (45 13 61) at.

See page 34 for the replacement of the HBO 50W mercury short-arc lamp.

Centration of the HBO 50W lamp:

- Put on goggles, e.g. sunglasses, to prevent your eyes from being damaged by UV radiation when the started lamp is adjusted.
- Loosen clamping screw **4.2** with SW 3 Allen wrench and carefully detach activated illuminator from microscope.
- With knurled knob for collector adjustment **4.12** image the brighter of the two cathode spot images sharply on a white wall at a distance of approx. 3m.
- Center cathode spot image using SW 3 Allen wrench on adjusting screws **4.13** and **4.15** .
- Focus unsharp light spot using adjusting screw **4.16** .

Cathode spot image and mirror image should be of equal size. Place the focused mirror image next to the real image using the red adjusting screws **4.14** and **4.17** (see Fig. 25). The two images should not overlay each other.

- Attach illuminator, set slider **4.7** to free aperture and turn reflector slider 3 FL to blue excitation (using e.g. filter set 48 79 09).
- Unscrew objective and check light source image on a sheet of paper in the specimen plane (on the specimen stage).
- Correct with collector adjustment **4.12** and adjusting means **4.13** to **4.17** . Screw in objective.

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

Reflection-contrast illumination system

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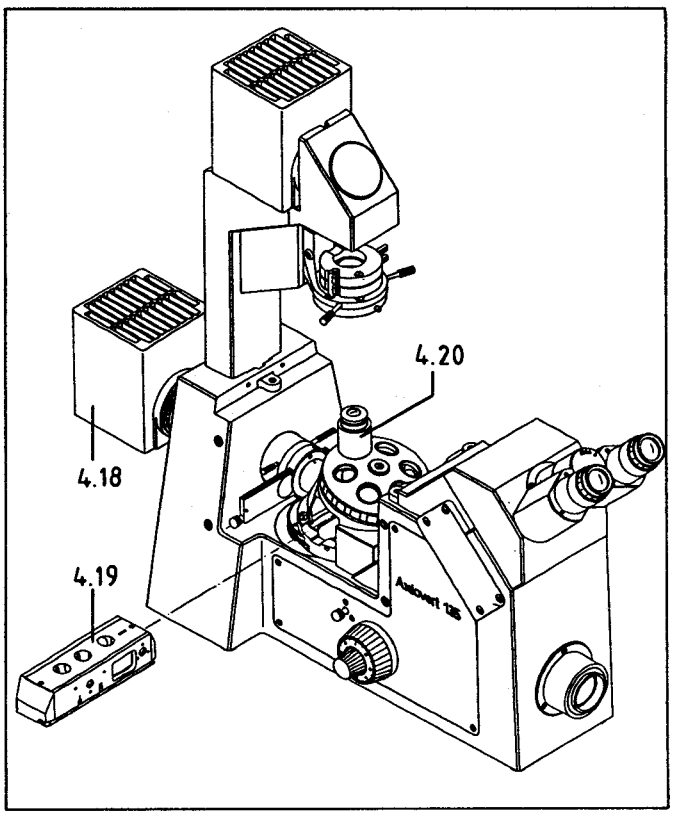

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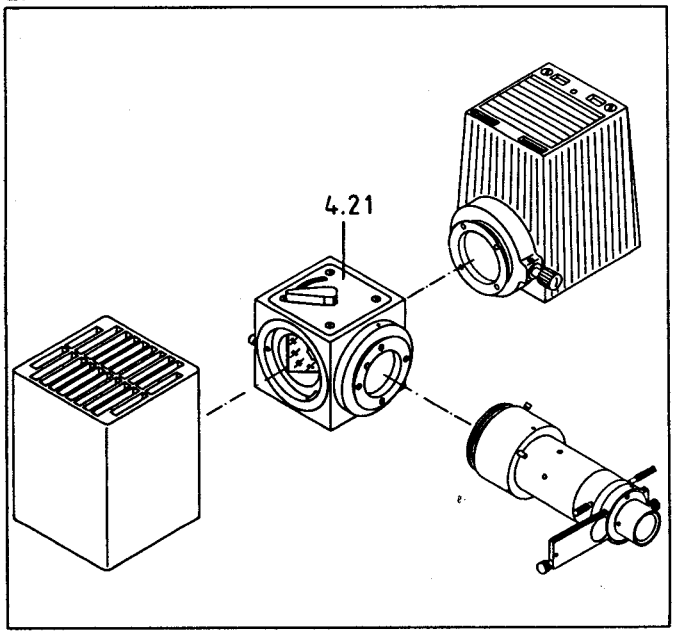

Use of the Antiflex method for reflection-contrast microscopy

The incident-light fluorescence illuminator FL described on page 16 is used in combination with
4.18 microscope illuminator Hal with reflector.
Reflector slider 3 FL is replaced by
4.19 reflector slider Antiflex/2 FL (45 13 67) with built-in polarizer and analyzer; it contains a Smith reflector in the brightfield aperture (H).

4.20 Antiflex Plan-Neofluar objective 63x/1.25 oil Ph 3. The objective front lens is covered by a rotatable $\lambda/4$ plate which is immersed together with the specimen.
The luminous field diaphragm of the incident-light system FL has the function of an aperture contrast diaphragm.

If incident-light fluorescence and reflection contrast are used together, you will need
4.21 deflecting mirror (44 72 30) for 2 illuminators (Hal and HBO/XBO) for attachment to incident-light system FL.

5.0 Image-forming components

5.1 The most important elements of the microscope are the objectives; they should be meticulously clean, especially the front lens surfaces.
The numbers and symbols engraved on the objective, e.g. Plan-Neofluar 20x/0,50; ∞ /0,17
signify: 20x (individual) magnification;
0.50 numerical aperture;
∞ infinite image distance;
0.17 cover glass thickness in mm for which the objective is computed.

(Individual) magnification multiplied by the eyepiece magnficiation (generally 10x) results in the microscope magnification. (The factor 1.6x or 2.5x must be considered if an Optovar system 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.

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 cover glass thickness 0.17mm is the more important the higher the numerical aperture of the objective. Some LD objectives (with long working distances) have correction mounts for adjustment to different cover glass thicknesses. Find out, by means of a high-contrast specimen feature, the position of the correction mount which provides for the best sharpness (re-focusing will always be necessary. Only slight re-focusing is required for the
LD-Achroplan objective 40x/0.6 corr.).

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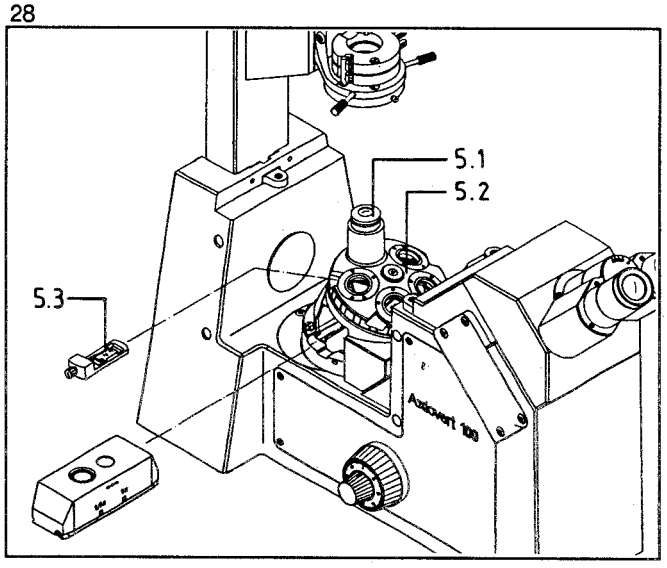

The cover glass thickness is irrelevant for immersion objectives. Objectives with short working distances have spring mounts to protect the specimen. To prevent specimen contamination by oil when turning the nosepiece, these objectives can be "locked" with the spring mount in topmost position (do not forget to disengage them from "lock-in" position!)

The air between the cover glass and an immersion objective is replaced by a liquid, generally immersion oil. Bubble-free application requires some experience. Always control the exit pupil for bubbles, preferably using the centering telescope Bertrand optics. If the bubbles have not disappeared after turning in the objective several times, clean the specimen and repeat the procedure.

LD objectives have long working distances for observaiton and manipulation of specimens in culture dishes, microtest and microtiter plates. With cover glass caps some objectives may be adjusted to the thickness of dish or plate bottoms, e.g. the objective LD Achroplan 32x/0.40 (without cap it is parfocalized to 1.5mm ±0.3mm)
with cap 0.6 - 1.2 (parfocalized to 0.6 - 1.2mm thickness)
with cap 0 - 0.6 (parfocalized to 0 - 0.6mm thickness).
Objectives in correction mounts, e.g. LD Achroplan 20x or 40x corr. are available for parfocalization from 0 - 2mm.

5.2 Nosepiece rigidly mounted on Axiovert microscope. The nosepiece H DIC on the Axiovert 135 M is motorized and the desired nosepiece position addressed using key **1.19**, page 7.
If the microscope is equipped for DIC, the knurled ring of the nosepiece 5x features:
Slots for **5.3** DIC sliders. They must snap in when inserted (designation face down) (see also DIC adjustment on page 27).

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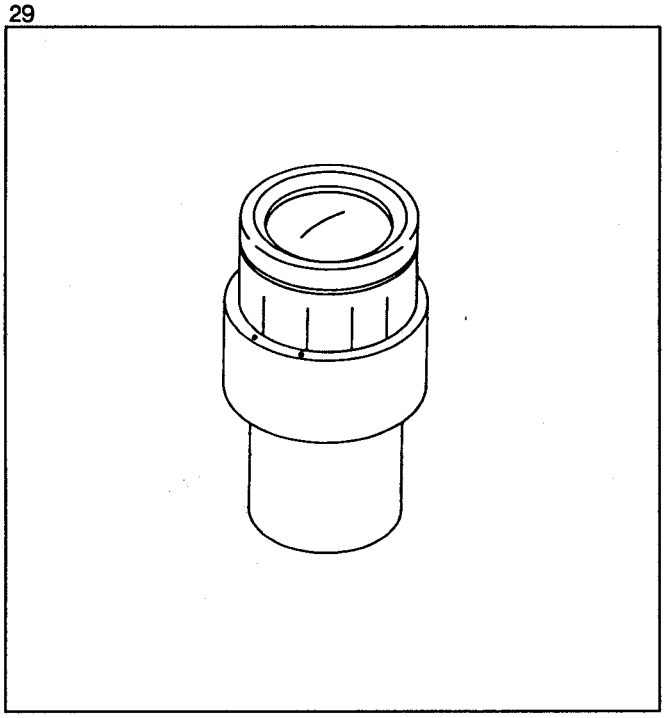

Eyepieces (Fig. 29) - 10x magnification (fig. 29) and field of view number 20 - produce angular fields of 44°, are equally well suited for eyeglass wearers (Br) and provided with exchangeable rubber rings to protect eyeglasses (folding eyecups are available under ordering number 44 48 01).

If you use a binocular phototube and a microscope camera (possible on all stands) you will generally use 2 focusing eyepieces (foc). One focusing eyepiece is provided with a reticle in the plane of the eyepiece stop. The slight image displacement it causes is considered by the zero position marked with the red dot on the diopter scale. 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.

The Axiovert 135 and 135 M microscopes feature integral photo reticles. The reticle is brought into the light path when switching the beam splitting prism. It displays the photographed area on the 24x36mm format. The user must focus on a focusing aid of the reticle.

If you work with an Axiovert 100 microscope but do not photograph, 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 the 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.

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 replaced only by a specialist because of the high demands on cleanliness and alignment. (The lower eyepiece part may be unscrewed, the scale-bearing surface of the reticle must face down!)

Optovar systems for quick magnification change:

5.5 Optovar slider D (45 13 70) with the factors 1x and 1.6x, or (45 13 71) with the factors 1x and 2.5x. It is inserted at
5.6 with the inscription facing the user.
To remove the slider from its holder, flick lock **5.4** upwards and pull out the slider.

The Axiovert 135 and 135 M microscopes feature at
5.7 a 4-aperture turret which may be supplied equipped with Bertrand optics (phase contrast) and Optovar inserts 1.6x (45 13 73) and 2.5x (45 13 74).

A slot beneath the nosepiece accepts **5.9** slider with auxiliary object, e.g. auxiliary object (47 37 04) or auxiliary object $\lambda/4$ (47 37 14) for qualitative polarizing microscopy.
Beneath the Optovar slider:
5.8 Mount slider with aligned analyzer (the aperture on top of it must be free for insertion; see **5.6**). The analyzer slider features click-stop positions. Pushed in it brings the analyzer in the light path, pulled out it provides for free light path.
The analyzer is required for DIC adjustment, see page 27.

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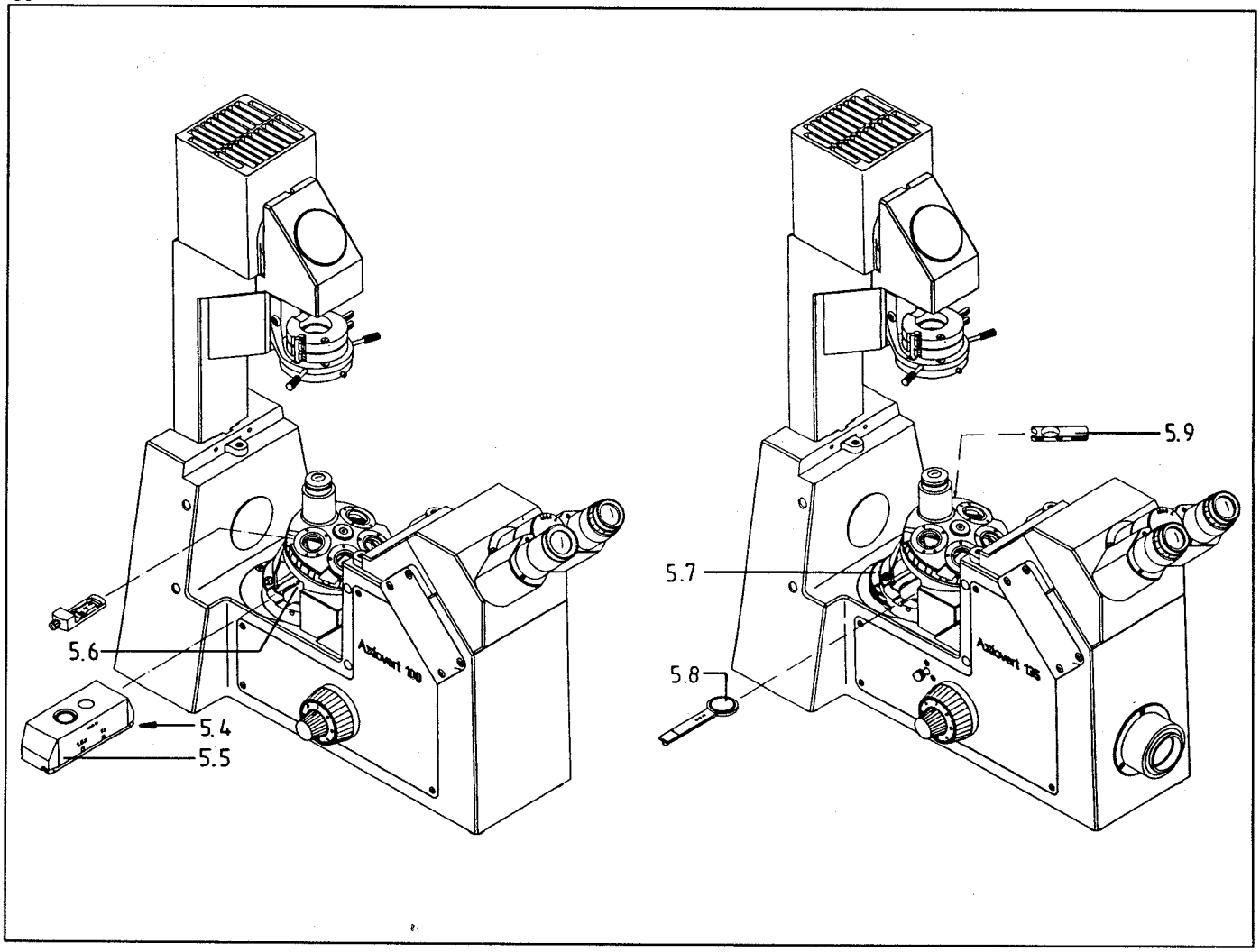

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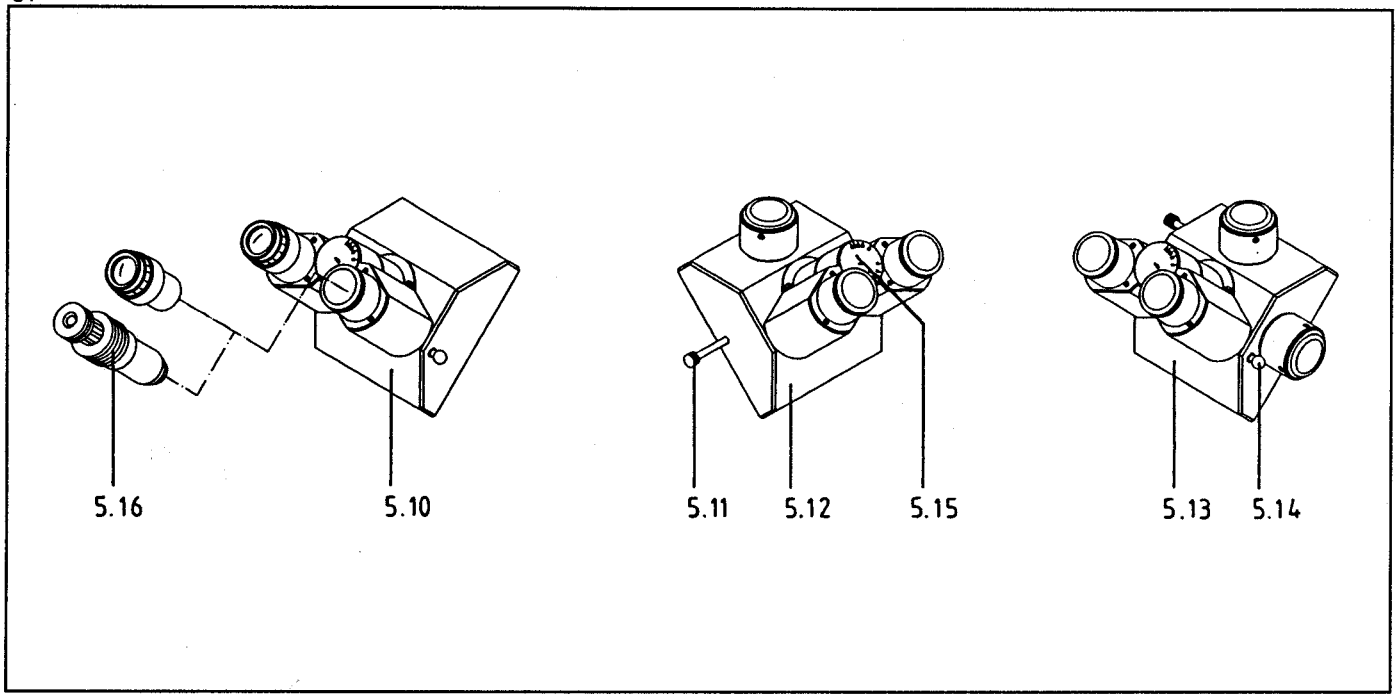


Most frequently used is
5.10 Binocular tube (45 13 20) producing an upright, unreversed image of the specimen, which moves synchronously with the specimen in x and y.

5.12 Binocular phototube 70/30 (45 13 22)
with left-hand
5.11 pushrod which when
pushed in: 100% of the light for observation, or
pulled out: 30% of the light for observation and 70% to the camera, used only
- for photography with Axiovert 100 or
for attachment of a TV camera,
- for the use of a large-format camera or 2 cameras on Axiovert 135 or 135 M,
- for top mounting of a TV camera on Axiovert 135 and 135 M.

Binocular phototube 100/100 (45 13 21)
similar to tube **5.12**. A left-hand pushrod with 2 positions provides for 100% of the light either for observation or for photography/TV.

Binocular phototube P (45 13 74) for microscope photometry which centers asnd reflects the photometric measuring diaphragm into the system.

5.13 Binocular phototube with 2 ports (45 13 25)
Phototube with left-hand pushrod with 2 beam splitting positions:
pushed in: 70% of the light to the upper port and 30% to the binocular tube,
pulled out: 70% of the light laterally reflected out and 30% to the binocular tube.
A microscope camera may be mounted on the top port, a TV camera on the lateral port.

All tubes have
- 45° viewing angles
- PDs from 55 . . . 75mm are adjusted by turning the tubes in or out.
- 2 different viewing heights, 2 PD scales **5.15**
- Eyepiece shutter
Right-hand shutter **5.14** pulled out:
Shutter remains open for observation.
Pushrod pushed in: the shutter on the eyepiece side is closed, no observation through the tube, no entrance of straylight.

Convenient observation of the objective pupil, especially for phase-contrast centration by swung-in Bertrand optics or
5.16 Centering telescope (44 48 30) fitted in either of the tubes instead of the eyepiece.

6.0 Photographic equipment

Either of the above-mentioned binocular phototubes may be used on all Axiovert microscopes for mounting of MC 80 or MC 100 microscope camera for 35mm or large-format photography.
The following items are required to mount microscope cameras on **6.2** binocular phototube:

- For MC 80 and MC 100 microscope camera:

6.1 adapter (45 60 06)
for MC 80 with plug-in projection lens P 2.5x (45 60 21) for 35mm film cassette
or projection lens P 10x (45 60 23) for the large-format;
for MC 100 with plug-in photo eyepiece S-PL 10x or S-PL 12.5x.

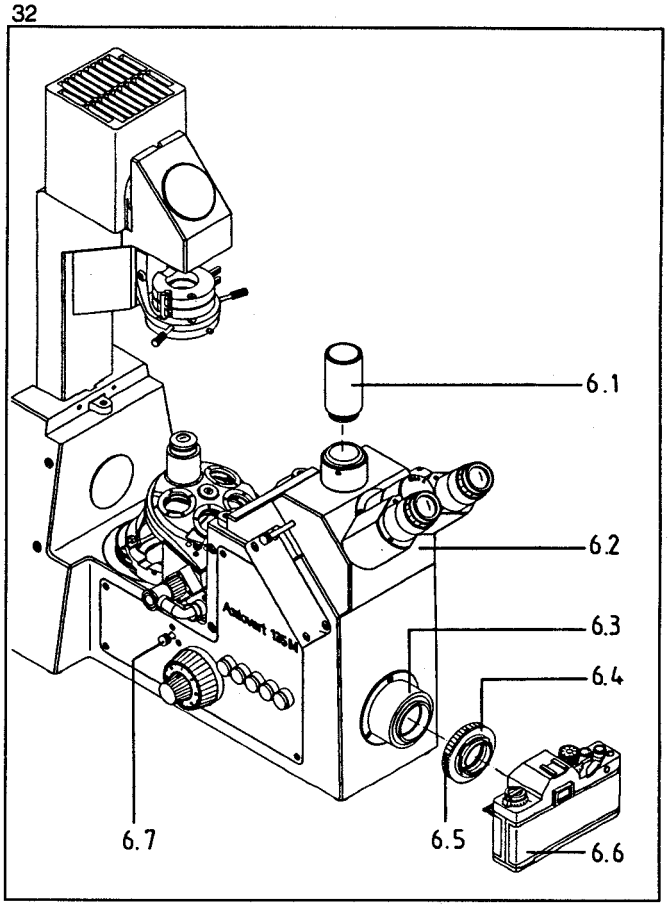
SLR cameras **6.6** can be connected only to the integrated camera light path of the Axiovert microscopes 135 or 135 M.

The use of the photography systems is described in the relevant Operating Instructions:
G 42-401 Microscope Camera MC 100
G 42-407 Microscope Camera MC 80

Axiovert microscope 135 or 135 M

6.7 Pushrod for beam splitting prism
pushed in: 100% of the light for observation
pulled out: 30% of the light for observation, 70% to the camera.
The photo reticle integrated in the microscope is also used for correct focusing through either of the two focusing eyepieces.
Switching the beam splitting prism brings it automatically into the light path; it displays the 24x36mm photographic format.
6.4 Special T2 adapter (41 60 10) to mount e.g.
6.6 SLR camera housing with automatic exposure control. Bring the camera in horizontal position: loosen the 3 screws **6.5** on T2 adapter, turn the camera into horizontal position and tighten the 3 screws. To prevent blurring use only cable release and do not use exposure times below 1/60s.
Relevant instructions by the camera manufacturer should also be observed.
The image scale on the film is the product of objective magnification and camera factor 2.5.

32



7.0 Mounting of TV equipment

using a phototube
is possible on all Axiovert microscopes. The image is upright and unreversed and free access for micromanipulations guaranteed.

Required for top port of **7.3** phototube
- For TV cameras with standard C mount:

7.1 Adapter TV 1x (45 61 05)

- For TV cameras 3C-CTV with ENG bayonet mount:

7.2 Adapter for 3C-CTV 1x ENG bayonet (45 61 15), or
Adapter for 3T-CTV 0.8x ENG bayonet mount (45 61 17).

The adapter is used without eyepiece.

By means of the binocular phototube with two ports (45 13 25) it is possible to mount simultaneously a TV camera and a microscope camera or two TV cameras.

7.5 TV port (45 13 75) to mount
TV cameras weighing max. 1kg on the side of the stand. The image becomes upright and unreversed by turning the TV camera through 135° (stand mount pointing diagonally upwards).
The mounting should be made by our maintenance service.
Two switch positions at **7.6** (marked by arrows) for:
100% for observation;
30% for observation, 70% reflected out at the side (hinged mirror), provided pushrod **7.4** for the photographic beam path is pushed in on Axiovert 135 or 135 M microscope.
It is possible to connect TV cameras using the following items (without eyepiece):
- TV cameras with standard C mount:

Adapter TV 1x (45 61 05) **7.1** or
- TV cameras 3C-CTV with ENG bayonet mount:

Adapter for 3C-CTV 1x ENG bayonet (45 61 15) **7.2**, or
Adapter for 3T-CTV 0.8x ENG bayonet mount (45 61 17).

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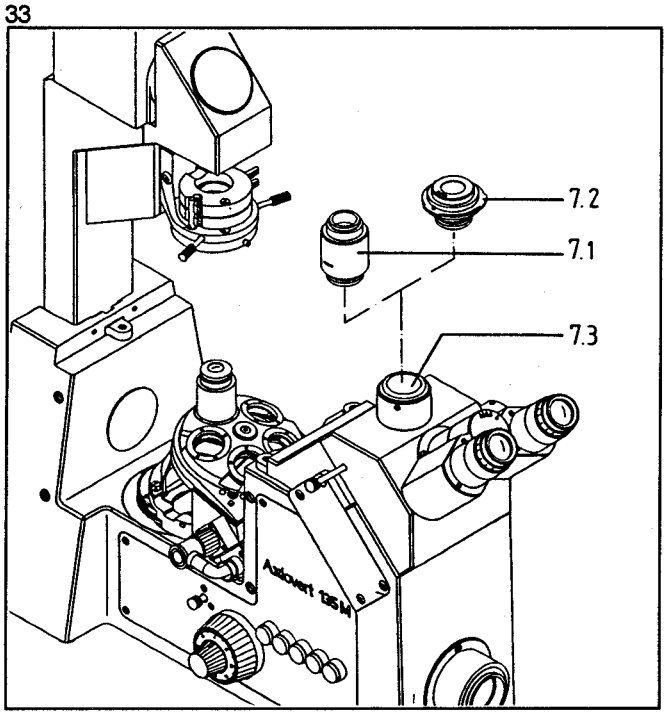

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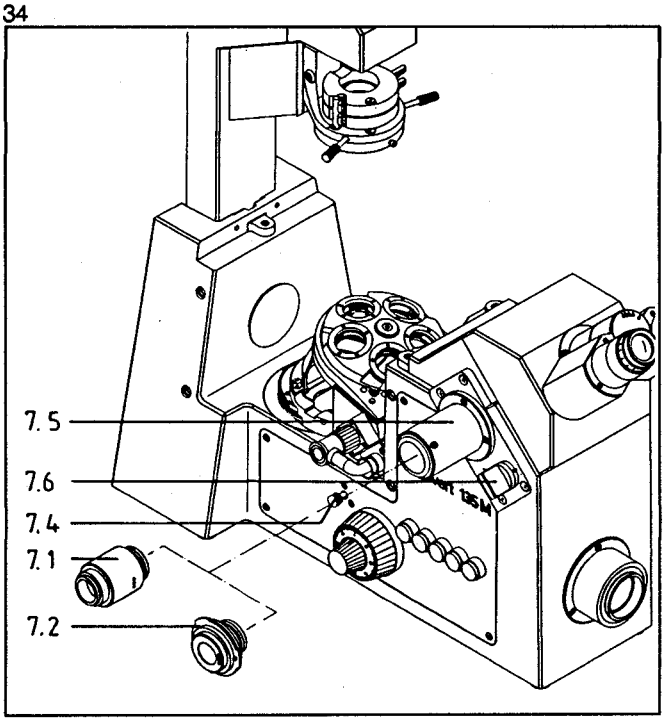

The examination of specimens in chambers or microtest plates requires free space between specimen plane and illumination system, which is provided by LD condensers.

In brightfield

Condenser LD 0.3 H, Ph 1, 2 (1) which may be supplemented by front lens attachment 0.55 (3) for higher illuminating apertures, or condenser LD 0.55 H, Ph 1, Ph 2, DIC or
LD 0.55 H/Ph 2, 3/DIC (8).

See page 5 for microscope adjustment for brightfield.
The following refers only to the use of the LD condensers.
Set turret of LD condenser 0.3 H, Ph 1, Ph 2 to position H.
The iris diaphragm (2) acts as contrast (aperture) diaphragm so that adjustment of the luminous field diaphragm is not necessary.

Set the turret (6) of LD condenser 0.55 H/Ph/DIC (8) to either of the two DIC apertures, with inserted DIC prism, remove polarizer (7) from the light path or set the analyzer slider to free light path.
Luminous field diaphragm is provided at (5),
aperture diaphragm at (9).

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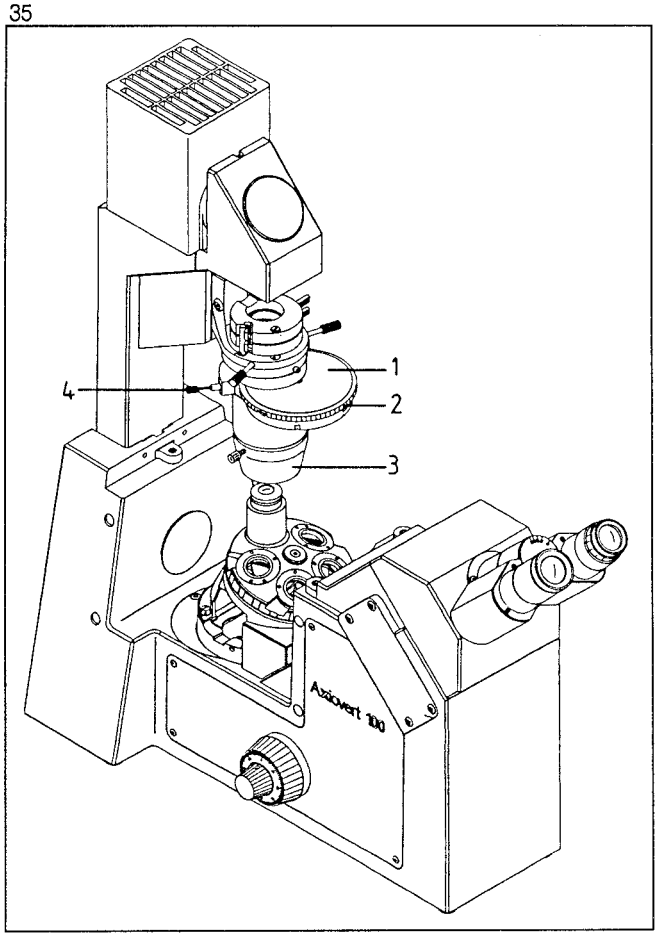

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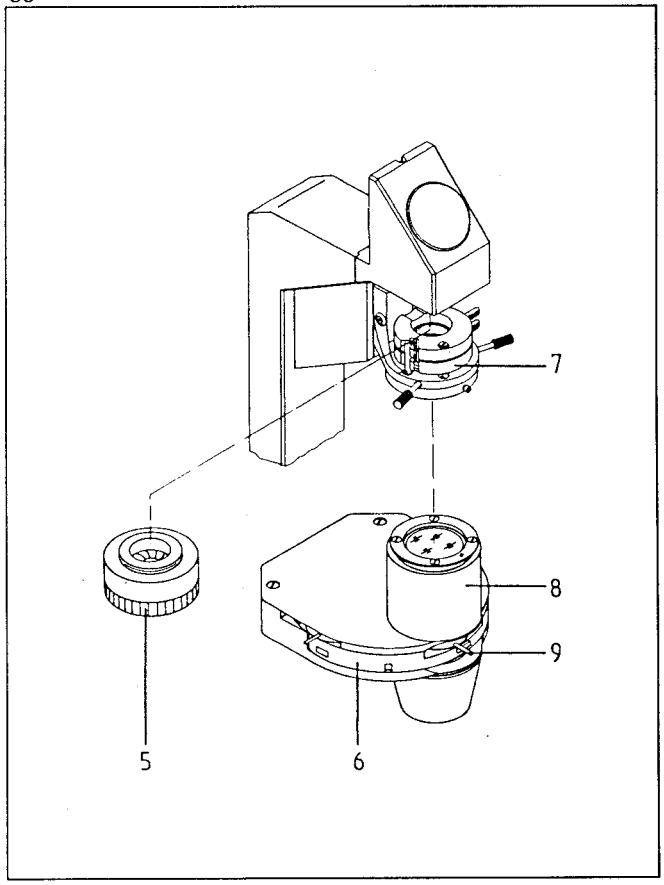

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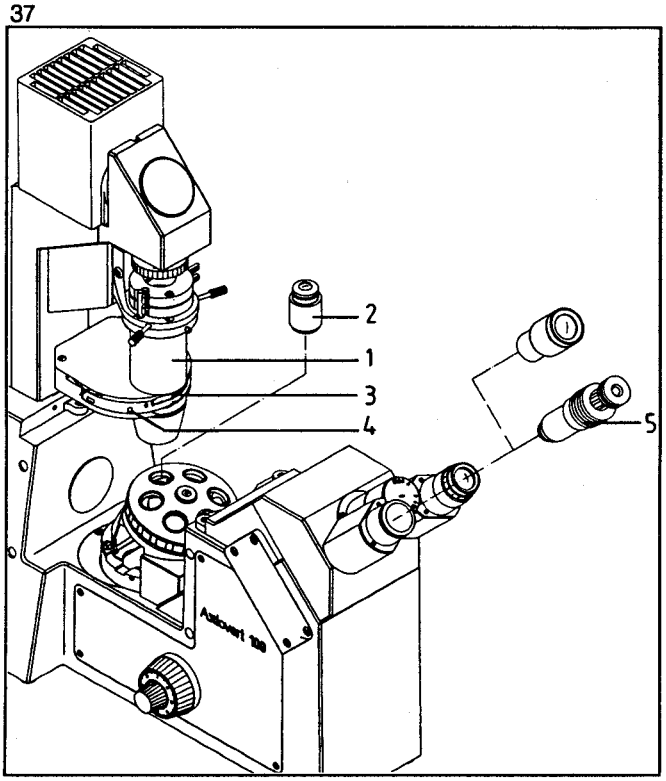

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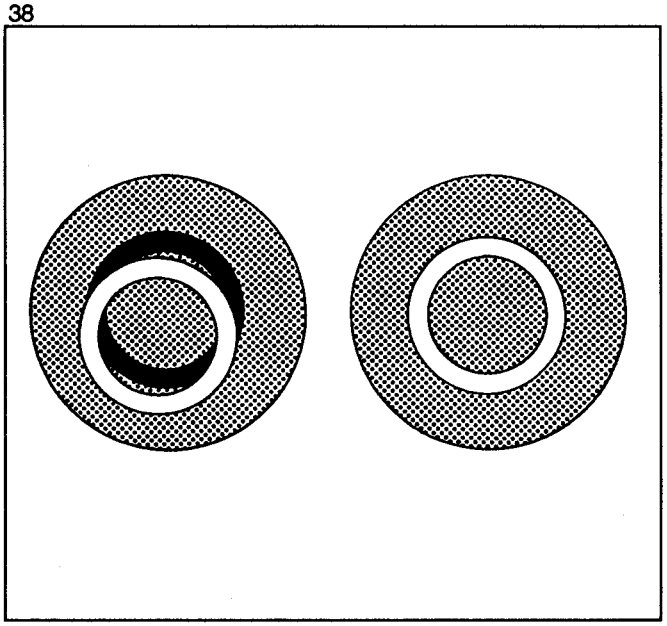

In phase contrast

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

Required phase contrast equipment
Objectives (2) designated Ph and equally well suited for brightfield.
- For microscopy with long illumination distance: LD condenser 0.3 H, (35.1 means fig. 35, part 1) Ph 1, 2 (front lens attachment 0.55 may not be used in phase contrast)
or LD condenser 0.55 H/Ph/DIC. (1)
Both condensers feature turrets (3) which contain the phase stops.

Further adjustments for phase-contrast microscopy with 12V 100W transmitted-light illuminator:
The annular phase stops in the objectives are of different size and displayed on the objective (2) by Ph 1, Ph 2 and Ph 3. The suitable ring size 1, 2 or 3 for a turned-in objective is selected using turret.

Perfect phase contrast is obtained only if dark ring in the objective and bright ring in the condenser exactly coincide (see Fig. 38). After insertion of the centering telescope this may be controlled using the Bertrand optics and focusing by moving the eyelens of the centering telescope (5) which is held on its knurled ring. The Bertrand optics may be used instead of the centering telesope. Without either of the attachments the control is similar to the condenser diaphragm control with the eyepiece removed (described on page 5).
The phase stops of the LD condenser 0.3 H, Ph 1, Ph 2 are centered with both integrated wrenches (35.4) , those of the LD condenser 0.55 H/Ph/DIC with a screwdriver on (4).

For phase contrast adjustment with 6V 20W illuminator see page 15.

Special notes:
Even more than brightfield phase contrast requires clean glass-to-air surfaces of the specimen (no fingerprints!). The diaphragm ring of LD condenser 0.3 H, Ph 1, Ph 2 is functionless because no iris diaphragms are provided in the phase-contrast apertures.

In Differential Interference Contrast (DIC)

DIC is used, for example, 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, interferes with the observation of small features.

Required DIC equipment:

- Plan-Neofluar objectives, for most exacting demands Plan-Neofluar Pol objectives, and LD Achroplan objectives (20, 32 and 40 corr.) for long working distances between specimen and objective.
- A special nosepiece (5) with slots to accommodate
- DIC sliders (1) bearing on the bottom type, magnification and aperture of the objective for which they are intended. Slide DIC slider - inscription facing down - all the way into slot.
- LD condenser 0.55 H/Ph/DIC (4) with built in DIC-Prisms for microscopy using long illumination distance.
- A polarizer (3) which is swung in above the condenser.
- An analyzer (2) which is slid into a slot beneath the nosepiece.
- The slider with auxiliary object λ (47 37 04) slid into the slot **1.13**, s. page 6, beneath the nosepiece produces color contrast.

Further DIC adjustments:

Condenser turret to positions .3 - .4 or .5 - 1.3. The DIC prisms are provided in these positions, which are required for objectives with the corresponding NAs.
An iris diaphragm is used in DIC, which is opened first (generally the last step of adjustment) and may be moderately closed for further contrast enhancement.

Polarizer (3) and analyzer (2) are crossed. They should be in crossed (extinction) position before starting to work in DIC. Screw the polarizer from above into lower filter holder and re-adjust. Remove objectives, DIC slider, eyepieces and condensers from the light path, swing in illumination system and from below turn polarizer until extinction is obtained.
Bring removed microscope items back into light path.

The contrast may be optimized using a knurled screw on the DIC slider in the nosepiece.

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).

DIC uses polarized light; "optically active" elements between polarizer and analyzer will interfere with the method. Such elements may be mica plates used for cytological sections or culture dishes made entirely of plexiglass (dishes with strainfree glass bottoms are available). The use of such materials may cause a performance loss.

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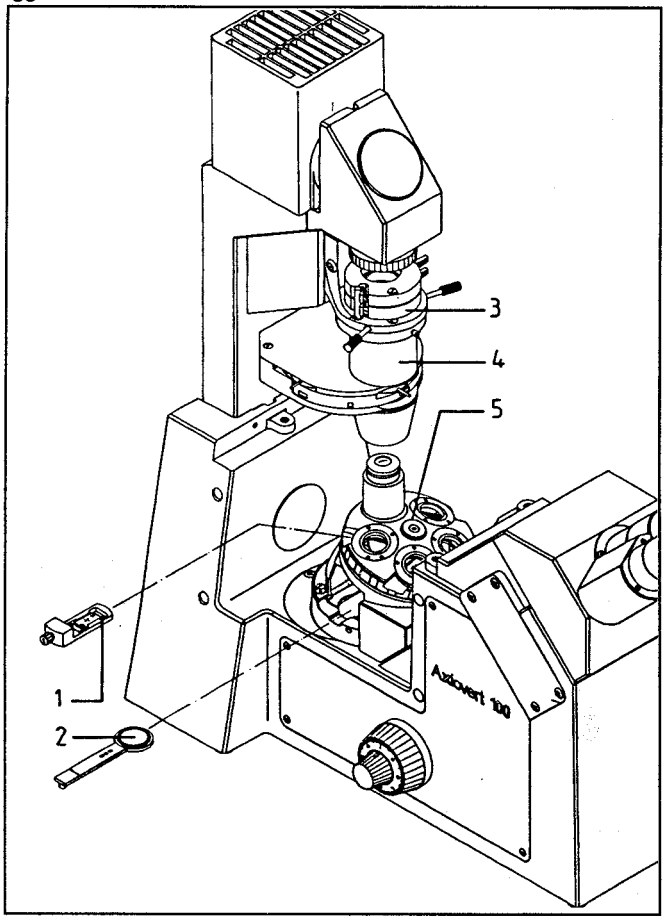

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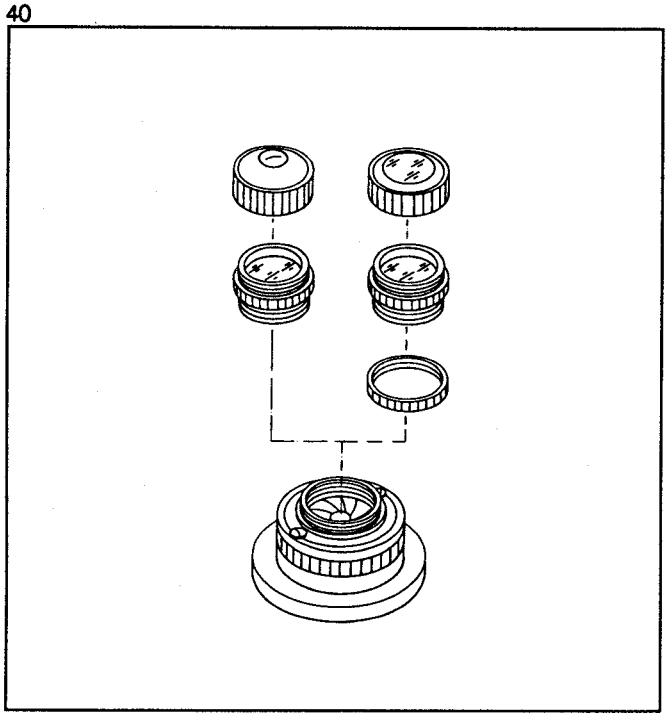

You should use condensers which fully utilize the resolving power of high-aperture objectives. Köhler illumination will provide for a homogeneously illuminated object field, a brilliant image without refletions or glare and optimum protection of the specimen.

In brightfield

The necessary adjustments are described on page 5. Use the achromatic-apalantic condenser system 0.32 Pol*) with front lens 0.63 Pol or 1.4 Pol (Fig. 40), or turret position J for brightfield of condenser II Z *) (see condenser description on page 14).

In phase contrast

For high apertur objectives use the phase contrast condenser II Z 0.9 Ph 1, 2, 3 (45 17 14)*), see page 14.
Working with phase contrast is described on page 26 including objectives Ph, the Bertrand optics or the centering telescope.

In Differential Interference Contrast (DIC)

The necessary adjustments are described on page 27.
Use the achromatic-aplanatic condenser system Pol*) with front lens 0.63 Pol or 1.4 Pol and corresponding DIC prism fitted aligned in the condenser:

DIC prism (43 44 10), NA 0.3 - 0.4/0.63, for front lens 0.63 with 5x, 10x, LD 20x and LD 32x objectives.

DIC prism (44 52 94), NA 0.5 - 1.4/1.4,
for 20x, 40x, LD 40x and 100x objectives.
Mounting of DIC prism in achromatic-aplanatic condenser system see page 31.

*) The condensers are mounted on the condenser mount with iris (see Fig. 17).
For mounting set the lateral notch against the bolt of the clamping screw, put on the entire dovetail ring mount and tighten screw using Allen wrench.

Fluorescence microscopy

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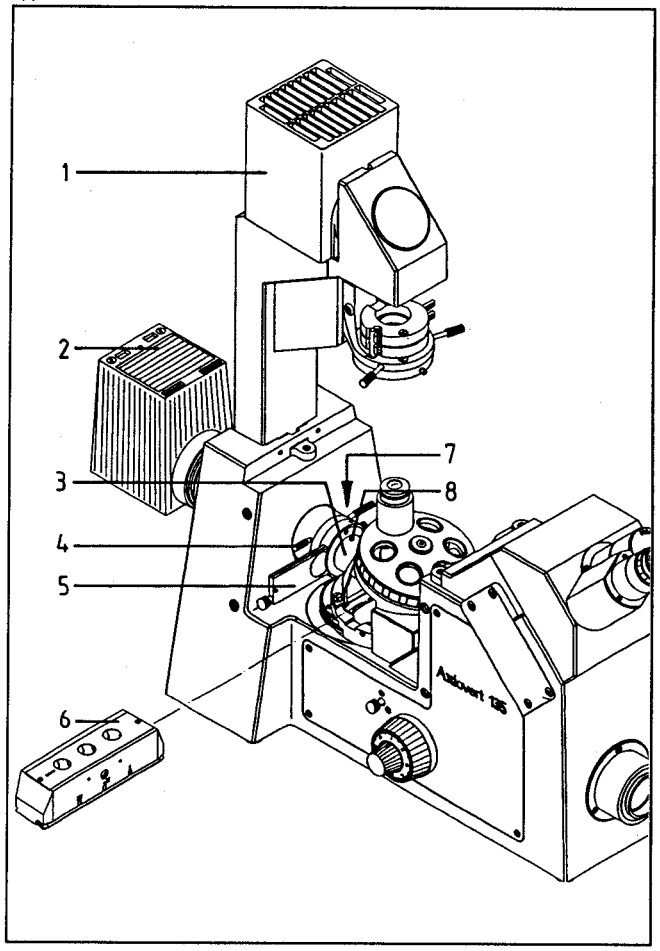

Required equipment
- Plan-Neofluar objectives for UV excitation.
- Special incident -light illuminator, see page 16.

Procedure:
- Set reflector slider (6) to free aperture, which adjusts the selected specimen feature in transmitted-light brightfield or phase contrast. Use the upper illuminator with halogen lamp (1) for transmitted light.

Switch on illuminator with HBO 50W lamp (2), but block its light path with dark slider (5).

- Switch off transmitted-light illumination (or reduce its brightness considerably), select on the reflector slider 3 FL the position with the type of excitation you want, and remove slider (5) from light path.
- In fluorescence an aperture diaphragm in the illumination system would not influence contrast, etc.; only a luminous field diaphragm is, therefore, provided, which is closed with lever (7) so far that it becomes visible in the image. After loosening screw (8) focussing with tube (3) and centering with (4) it is opened so far that it disappears from the field of view.

Special notes:

You should start the initial fluorescence adjustment using a 20x objective and a highly fluorescent specimen. Suitable specimens are available but you may also prepare them yourself. Quite popular are anthracene crystals or crushed preparations of green plant specimens which display chlorophyll well. (You may even use a specimen label to control the illumination.)

Which filter set you choose depends on the problem; the sets are accommodated in the reflector slider. Each set comprises 25mm dia. exciter and barrier filters enclosing a 26x36mm chromatic beam splitter. For details about the filters and their replacement see page 32.

Additional exciter filters may be loosely fitted in the filter holder of the incident-light fluorescence system FL. Required are for 32mm dia. filters holding ring 46 72 52 (e.g. a polarizer), and for 18mm dia. filters an additional adapter ring 18/32mm dia. (46 78 93).

For FITC use the 12V 100W illuminator Hal for fluorescence excitation in incident light.

The microscope may be retrofitted for the dual-wavelength method (e.g. for Ca and pH analysis).

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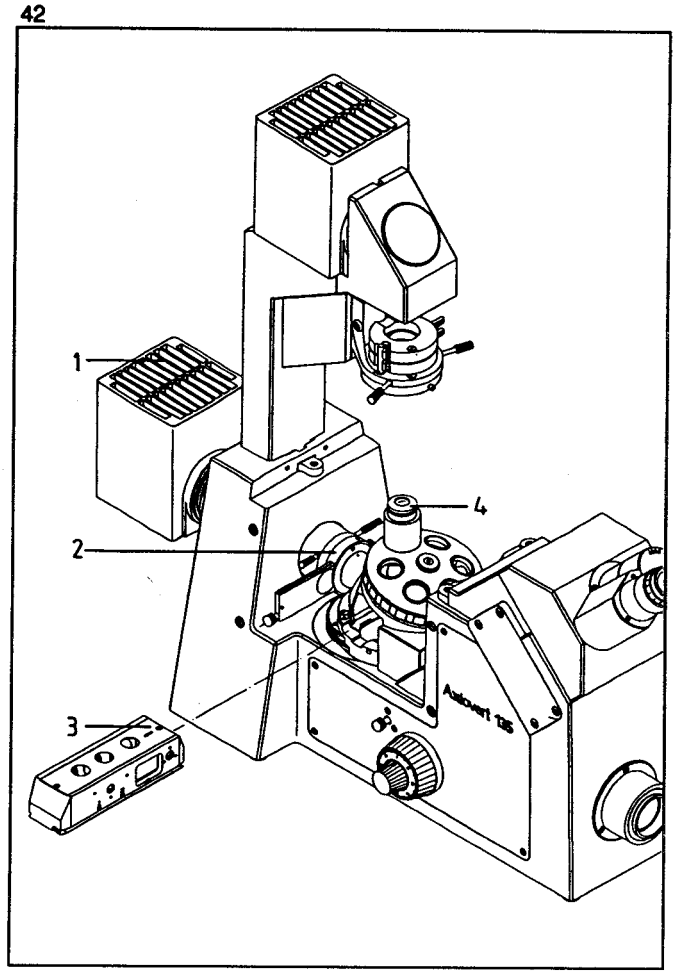

Antiflex method

The Antiflex method enhances the image contrast if weakly reflecting specimens are examined in reflected light. In cell cultures, for example, part of the light is reflected by the cell bottom, part by the top surface of the culture dish bottom. Superposition of these reflections results in characteristic interference colors, which may be applied, for example, to recognize growth structures.

Characteristic interferences may be represented with high contrast only if disturbing reflections by lens surfaces are eliminated, especially in the objective. Objectives with a rotary $\lambda/4$ plate between front lens and specimen assure this.

Required equipment:
- Incident-light fluorescence system (2)
- Reflector slider Antiflex/2 FL (45 13 67) (3) with built-in polarizer and analyzer.
- Antiflex Plan-Neofluar objective 63x/1.25 oil Ph 3 (44 04 69) (4) with rotary $\lambda/4$ plate.
- Microcope illuminator 100 Hal (1).

Reflection contrast is produced by turning the objective front part with λ /4 plate until max. brightness is obtained in the field of view.

For incident-light fluorescence and Antiflex used in combination, you need deflecting mirror for 2 illuminators (44 72 30) with attached illuminator Hal with 12V 100W halogen lamp and HBO/XBO illuminator with HBO 50W mercury short-arc lamp, see page 18.

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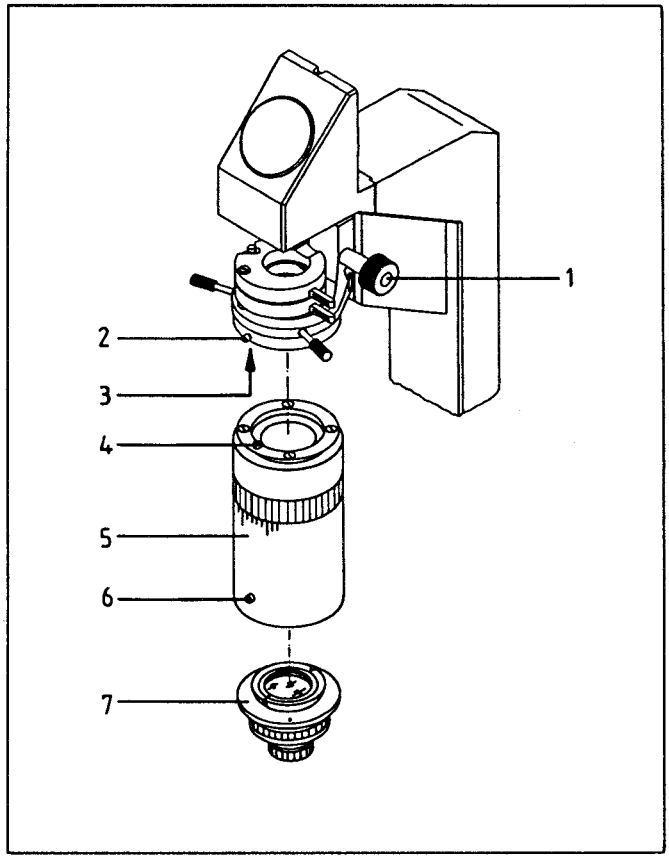


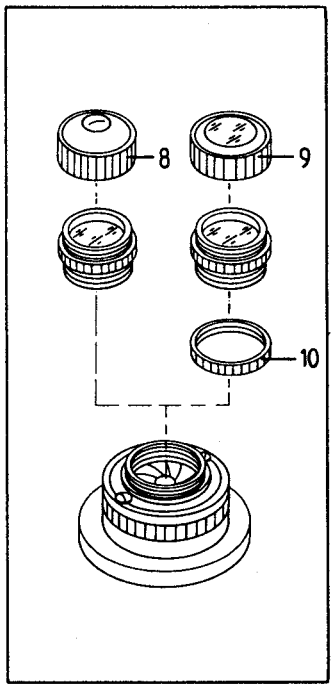
Should minor changes be necessary the following information may be helpful.

Condenser exchange
To replace an LD condenser by a condenser system, lift condenser as far as possible using (1), swing back the carrier, hold the condenser, loosen screw at (2) and take condenser out of its holder.

Be careful when mounting the condenser!
Plug alignment pin on the condenser dovetail or condenser holder with iris (4) into alignment notch at (3) of the condenser holder, fit condenser dovetail mount completely in holder and secure with screw at (2) or (6). The condenser is aligned.

The condenser holder with iris (45 13 55) (5) is required for high-aperture condensers, such as condenser 0.9 Z (44 52 30), achromatic-aplanatic condenser system (7) or phase-contrast condenser II Z 0.9 Ph 1,2,3 (45 17 54). First fit the condenser holder with iris aligned on the carrier and then mount the condenser.

44



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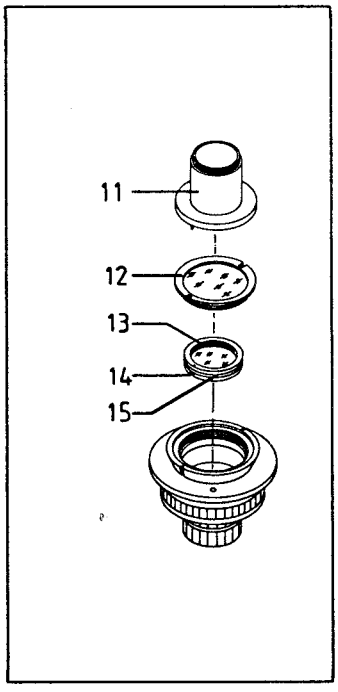

Replacement of condenser front lens
Achromatic-aplanatic condenser system 0.32 may be equipped with front lens 1.4 Pol (8) and 0.63 Pol (9) (for long back focal distance: 7mm in air, 11mm in glass). Both front lenses are screwed on to condenser 0.32. An extra supplied spacer ring 0.63 (10) is required between condenser body and condenser part 0.63 for front lens 0.63 Pol.

Fitting a DIC prism in achromatic-aplanatic condenser system (44 52 45)
Remove auxiliary lens (12) using the supplied wrench (11). With built-in DIC prism screw external thread of wrench into interal thread of prism mount (13). Pull up prism (15) and lift it out.
(A wire ring in the holder engages ring notch (14) of the prism mount.)
Correct fitting is assured if notch (14) marked by an engraved dot engages the corresponding pin of the holder. Check the assembly for correct seating to prevent mechanical and optical disturbances. Screw in auxiliary lens (12).

Fitting DIC prisms in LD condenser 0.55 H/Ph/DIC

Turn condenser turret with DIC prism you want to replace through 2 positions into working position. The prism is under the cover plate which you raise. Remove retaining ring.
To assure correct fitting the pin must engage the notch of the mount. Check for correct seating to prevent mechanical and optical disturbances.

Fluorescence reflector (Fig. 46)

Exchange of filters (4) and chromatic beam splitters (2). Exchange of built-in filter sets after unscrewing retaining rings (5). Please refer to the supplied notes on the mounting of the filters for replacement of the barrier filters. After removal of plate (1) the plate carrying the chromatic beam splitters (2) is accessible. The beam splitting coating must face the exciter filter. The plate rests on an elastic sheet-metal mask and should not be touched. Jigs (3) need usually not be removed for replacement of the beam splitter on the mask; it suffices if they are slackened.

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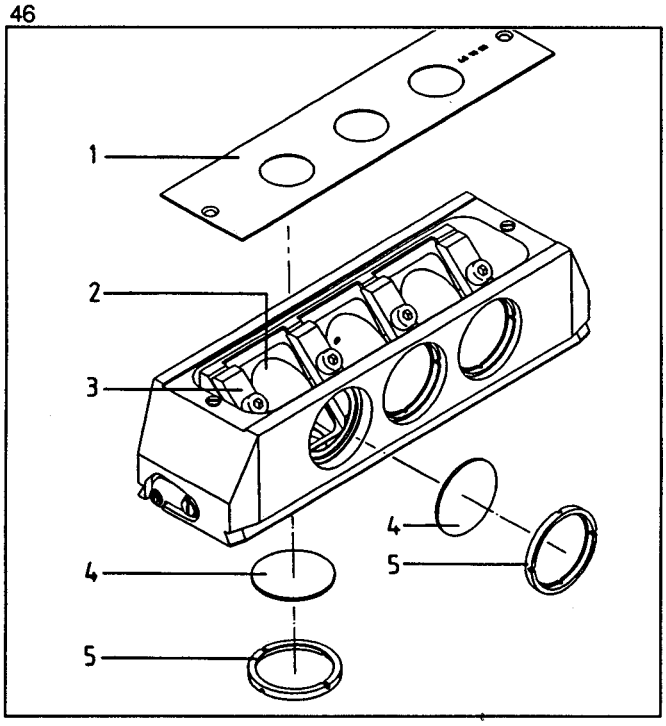

Filter sets for incident-light fluorescence

Excitation filter	Set	Exciter filter	chrom. beam spl.	Barrier filter
UV-G 365	48 79 02 48 80 02	G 365 G 365	FT 395 FT 395	LP 420 LP 420
Blue-violet G 436	48 79 07	G 436	FT 510	LP 520
UV-H 365	48 79 01 48 80 01	BP 365/12 BP 365/12	FT 395 FT 395	LP 397 LP 397
Blue-violet H 436	48 79 06	BP 436/10	FT 460	LP 470
Blue H 485	48 79 16 48 80 16	BP 485/20 BP 485/20	FT 510 FT 510	LP 520 LP 520
Blue H 485 SB	48 79 17	BP 485/20	FT 510	BP 515-565
Green H 546	48 79 15 48 80 15	BP 546/12 BP 546/12	FT 580 FT 580	LP 590 LP 590
UV-violet 390-420	48 79 18 48 80 18	BP 390-420 BP 390-420	FT 425 FT 425	LP 540 LP 540
Blue-violet 395-440	48 79 05 48 80 05	BP 395-440 BP 395-440	FT 460 FT 460	LP 470 LP 470
Blue 450-490	48 79 09 48 80 09	BP 450-490 BP 450-490	FT 510 FT 510	LP 520 LP 520
Blue 450-490 SB	48 79 10 48 80 10	BP 450-490 BP 450-490	FT 510 FT 510	BP 515-565 BP 515-565
Green 510-560	48 79 14	LP 510- KP 560	FT 580	LP 590
Green 530-585	48 79 00 48 80 00	BP 530-585 BP 530-585	FT 600 FT 600	LP 615 LP 615
Green H 546	48 79 20	BP 546/12	FT 560	BP 575-640
FURA-2 UV 340+380	48 79 21	BP 340/10 BP 380/10	FT 425	BP 500-530
Dual excitation 485 + 546	48 79 23	DBP 485/20 and 546/12	FT 505/ FT 585	DBP 515-530 585-635

Some filter sets come in two versions. The sets supplied under Cat. No. **48 80** .. feature wedge-free barrier filters to prevent image displacement when changing between filter sets.

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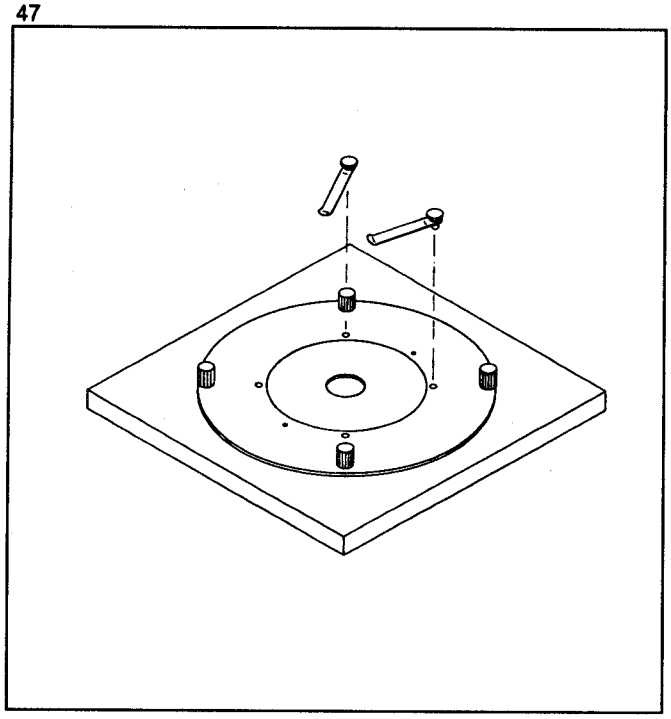

Specimen stage

Hinge back carrier with transmitted-light illuminator.
Unscrew 3 Allen screws and take off specimen stage. Mount new stage on the microscope and secure it with the 3 screws.
Mounting attachable mechanical stage (45 13 36) on specimen stage (45 13 35) and fitting mounting frames see page 8.
Special note: attachable mechanical stage and mechanical stage may be mounted with the coaxial drive either to the left or right.

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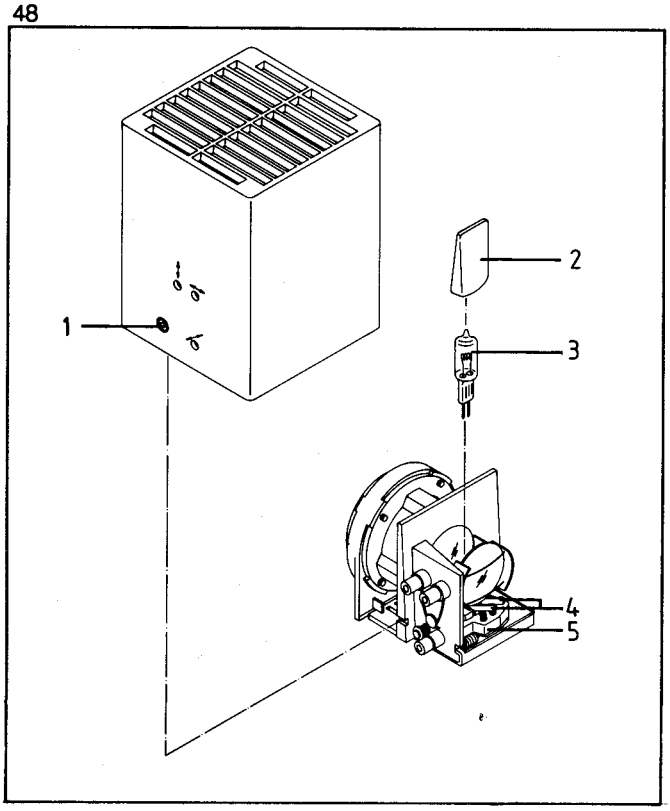

Replacement of 12V 100W halogen lamp (38 00 79-9540)

- Pull plug on back of stand.
- Detach illuminator from microscope, loosen screw (1) and lift out lamp housing.
- Press down spring clips (4) and take defective lamp out of socket (5).
- Insert new lamp (3) with protective cover (2):
- Pull lamp out of protective cover until the contact pins project completely.
- Hold bulb with protective cover on upper end and let it snap into socket by pressing down spring clips (4). Pressing spring clips down again will automatically align the halogen lamp in the socket.
- Stick supplied label of halogen lamp into frame on the back of the lamp housing Hal.
- Attach lamp housing and secure with screw (1). Connect illuminator cable.
- Adjust lamp (see page 11).

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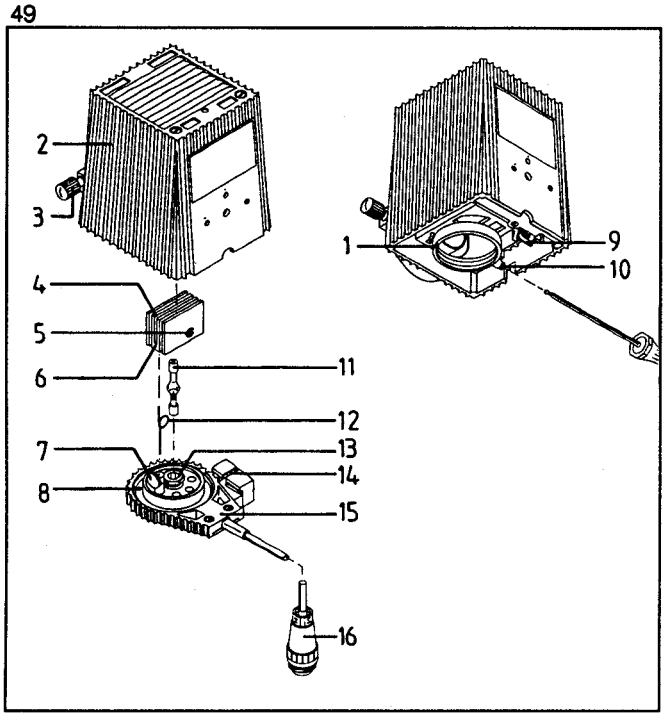

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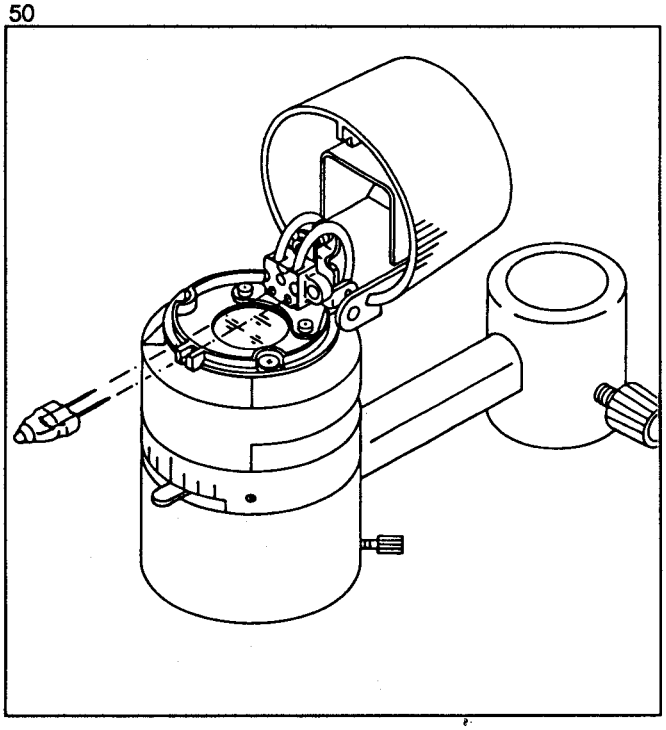

Replacement of HBO 50W lamp

Pull plug (16) of lamp socket on power supply and loosen screw. Detach lamp housing HBO/XBO (2) from microscope. With (3) bring collector to foremost position and loosen clamping screw (10). You may now take lamp socket (15) out of lamp housing. Loosen (7) and (13) and pull out lamp (11) and wire loop (12); loosen (5) and (6) and remove dissipator (4).
Hold new HBO 50 on bottom of metal socket and insert it in dissipator so that the mirror surface is in the lower part of the lamp; secure lamp using supplied SW 3 screwdriver on screw (5). The melt tip should be aligned parallel to the dissipator. Remove fingerprints on the bulb to prevent them from burning in. Plug wire loop in dissipator, align parallel and secure.
Guide lamp with dissipator and supply line in the corresponding openings of the lamp socket and secure with hexagon nut (13) and knurled screw (7); securely fit supplied fork wrench to prevent it from slipping (danger of smashing the bulb!). Tighten knurled screw (7). The longer side of the dissipator, the supply line contact, loop and fuse tip should be in one line.

Carefully tighten all clamping screws to prevent contact failure if strong heat develops during operation.

Insert socket (15) with new lamp in lamp housing so that alignment pin (1) engages borehole (8) of the lamp socket. Contact plate (9) on the lamp housing engages the notch of contact switch (14) on the lamp socket. Tighten screw (10).
Adjusted voltage and frequency are displayed in the window (back of the instrument). They must comply with the local line data.
Set the switch on the back of the power supply to the lamp type used (L1 or L2), plug in lamp plug and connect instrument to the line. The power switch for the lamp is on the front panel of the power supply unit.
The lamp should be replaced after approx. 100 operating hours. (see running time meter).

Replacement of 6V 20W halogen lamp

- Pull plug of illuminator 20 on power supply.
- Hinge back black lid of illuminator 20.
- Pull out lamp and insert new one.

	Ordering number
Fuses for built-in DC 12V 100W power supply and for 6V 20W	
primary for 230V: 2x SB 2A	127.024
for 115V: 2x SB 4A	144.060
Fuse for power plug 230V (45 84 05) SB 125 mA	38 01 27-0110
Fuse for power plug 115V (45 84 06) SB 250 mA	38 01 42-2830

32mm dia. filters for photomicrography	
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 3200-5500K	46 78 47
Blue filter CB 6	46 78 51
Blue filter CB 3	46 78 52
Green interference filter	46 78 03

12V 100W halogen lamp	**38 00 79-9540**
Voltage	12V
Output	100W
Color temperature at 11.5V:	3200 K
Mean life at 12V:	50hrs

6V 20W halogen lamp	**38 01 43-1350**
Voltage	6V
Output	20W
Color temperature at 6V	3200 K
Mean life	100hrs

HBO 50W mercury short arc lamp	**38 16 19**
Voltage	L1: 39...45V / L2: 34...39V
Current	L1: 1.30A / L2: 1.45A
Output	50W
Line spectrum	
Mean light flux	2 000lm
Mean life	100hrs
Lumnious surface	0.3x1 mm^2

L1, L2: The HBO 50W/Ac lamp is produced in 2 classes; to which a lamp belongs is indicated on the label. The power supply should be adjusted to L1 or L2.

General

- Specifications subject to change.
- Comprehensive knowledge of the instrument is absolutely essential for safe operation. Please read these instructions carefully before putting the instrument into operation. You may obtain further information from our maintenance service or authorized representatives.
- Special packing should be stored for later use.

Notes on installation

- The tube openings should be closed by the eyepieces and other ports or apertures by covers or plugs to protect them from dust and humidity.
The microscope should be covered by the dust cover if not in use.
- The microscope should not be set up in damp rooms.
- Use only power cables and plugs which are in perfect condition.
- After assembly of microscope and attachments check all connecting pieces (screws, nuts) for correct fitting, which serve the instrument safety or have supporting functions. Tighten any loose or slackened connections.

Notes on instrument use

- The microscope may be used only by specifically trained and instructed persons.
- The orderly condition of instrument and equipment should be checked every time the instrument is put into operation.
- Always use the main switch to switch off the instrument.

Waste disposal

- Used HBO mercury short arc lamps are subject to special waste disposal in compliance with legal provisions.

Regulations

- The user is liable to observe the legal provisions for the prevention of accidents.
- Manufacture, inspection, assembly, maintenance and repair are made in compliance with German and international instructions (Good Manufacturing Practice).
- The instrument is radio-screened, short-circuit-proof and complies with the applicable VDE, IEC, CSA and UL provisions; it is categorized as a protection class I, type B instrument.

Maintenance

- It is recommended to conclude a service contract with the local Zeiss representatives in order to assure correct functioning of the microscope.
- Changes and/or repairs of the instrument should be carried out only by the manufacturer or persons expressly authorized to do so.
- Always pull power plug before lamp replacement, voltage change (here, fuses should also be replaced), etc.!
- Repair and maintenance of damaged instruments or instrument parts should only be made by our maintenance service.
- The manufacturer of the instrument shall not be liable for any damage caused by accident, negligence or misuse of the instrument, especially the removal of components or the use of accessories of other make. Such damage forfeits the right to any warranty claims.
- We shall not be liable for any damage caused by the use of accessories of other make. Such damage forfeits the right to any warranty claims.
- Cleaning polished surfaces of the stand:
Clean soiled surfaces using a cloth soaked in a mixture of water and detergent and dry with a clean cloth.
Do not use solvents.
- Cleaning glass surfaces:
Breathe over smears (e.g. fingerprints) on glass surfaces and wipe off with clean optical cleaning tissue. Wipe off heavy contamination, e.g. mascara using optical cleaning tissue soaked in a mixture of distilled water and detergent, breathe over the surface and wipe with a clean dry cloth. Wipe off lints or dust with a clean soft brush.

Additional notes on instrument safety

■ The Axiovert 100, S 100, 135 and 135 M microscopes were designed, produced and tested in compliance with DIN EN 61010-1 (IEC 1010-1), Safety requirements for electrical measuring, control and laboratory instruments. This annex to the operating manual contains information and warnings which must be observed by the user.

■ The Axiovert 100, S 100, 135 and 135 M light microscopes have been designed in compliance with the latest scientific and technical development for the visual photomicrographic and videotechnological examination of specimens. The instruments may only be used for their intended application and have not been designed for unattended constant operation!

■ The microscopes are not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive or otherwise hazardous to health. The admissible specimen weight (≤ 5 kg) must not be exceeded.

■ Check whether the line voltage complies with the value indicated at the rear of the instrument. The power plug must be inserted in a socket featuring a grounding (earth) contact. The grounding effect must not be nullified by an extension cable which does not have a protective ground wire. If a transformer is used to adapt the line voltage, it must not nullify the effect of the ground wire.

■ When the microscopes have been connected to the line, connecting clamps inside the instrument can contain dangerous voltage, and the opening of covers or the removal of components (if not required for a function) may expose components containing dangerous voltage. Therefore, the instruments must be disconnected from the line before they are opened for adjustment, change of components, maintenance or repair. If adjustment, maintenance or repair of the live instrument cannot be avoided, this must be performed by specialized personnel who is aware of the danger involved.

■ The effect of existing ventilation slats on the lamp housing must not be nullified by covers. This also applies to ventilation slats on the instrument rear. Tools, objects of any kind and liquids must not enter the instrument via the ventilation slats or other instrument openings. Always disconnect the instrument from the line before changing the lamp and allow the lamp to cool down to room temperature (cooling time approx. 15 min).
The lamp housing heats up during operation!

■ Be careful when using gas discharge lamps:
In unfavorable circumstances and with improper use, gas discharge lamps (e.g. Xenon lamps or the HBO 50 or HBO 103 mercury pressure short-arc lamp) can explode, flinging splinters of glass through the air and causing injury. Therefore, be sure to follow any special hints and safety instructions provided by the lamp manufacturer. Gas discharge lamps emit ultraviolet radiation which can cause burns on the eyes and skin. Never look directly into the light of these lamps and avoid direct, unprotected incidence of their light on your skin.

■ When changing the instrument fuses, make sure to use only those of the rated power required and the type indicated. The use of makeshift fuses and the short-circuiting of the fuse holders are not permitted.

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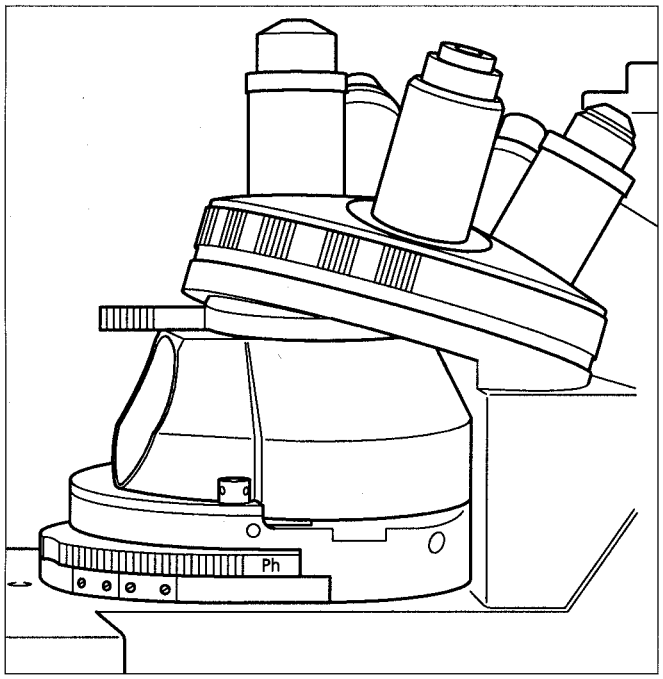

The S 100 stand features an integrated **Optovar turret** for precise phase contrast setting and for additional magnifications without any need to change the objective.

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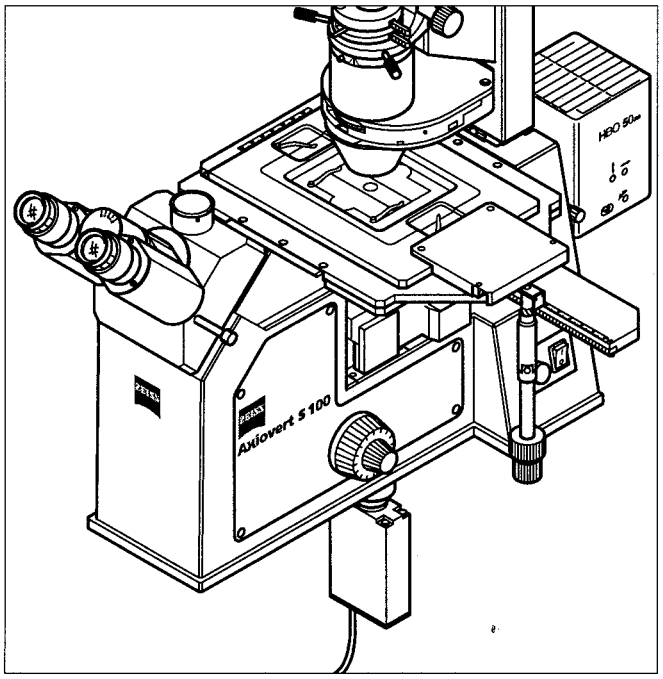


The special models S 100 TV and 135 TV allow the additional attachment of a TV camera to the base of the stand. Therefore, all of the light from the specimen directly reaches the receiver – without any beam deflection.

Lateral TV adapter

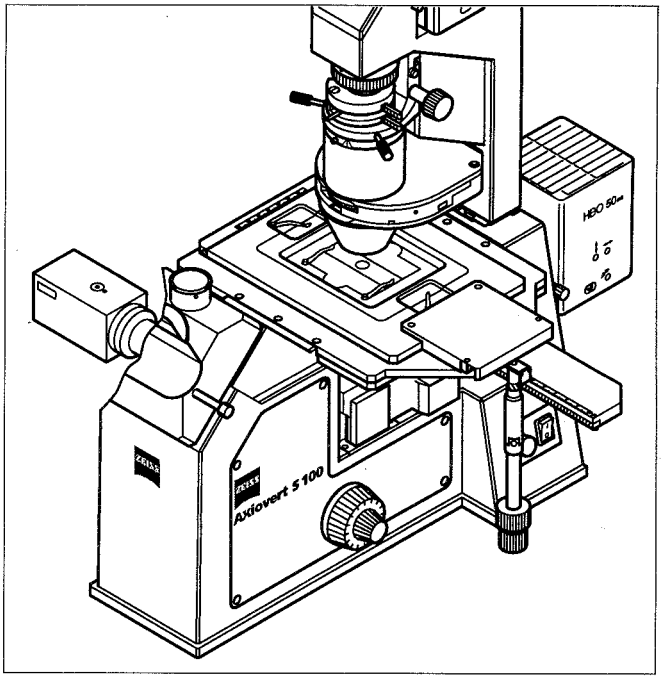
The **lateral** attachment of commercially available TV cameras to the Axiovert is possible via standard TV adapters. The two models below permit the light distribution to be set via a knob.

- Lateral TV adapter with beam splitting and change between 100% observation and 30% observation/ 70% TV (Cat. No. 451375-9901).

- Lateral TV adapter with change between 100% TV and 100% observation (Cat. No. 451377-9901).

The integrated mirror can be easily changed for a dichroic beam splitter by the users themselves.

53



Proceed as follows:

- Remove the four Phillips screws (54/5).

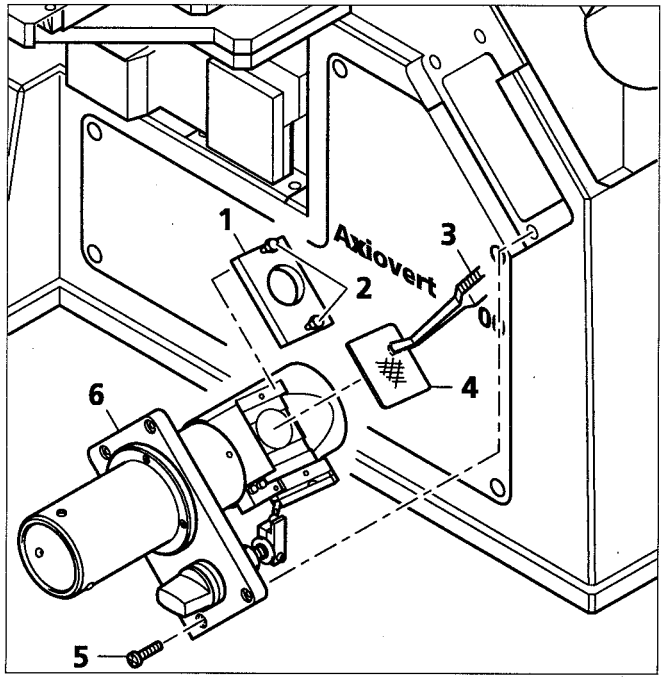
- Pull out lateral TV adapter (54/6) from the stand.

- Loosen the two captive hexagonal screws (54/2) and remove the spring plate (54/1).

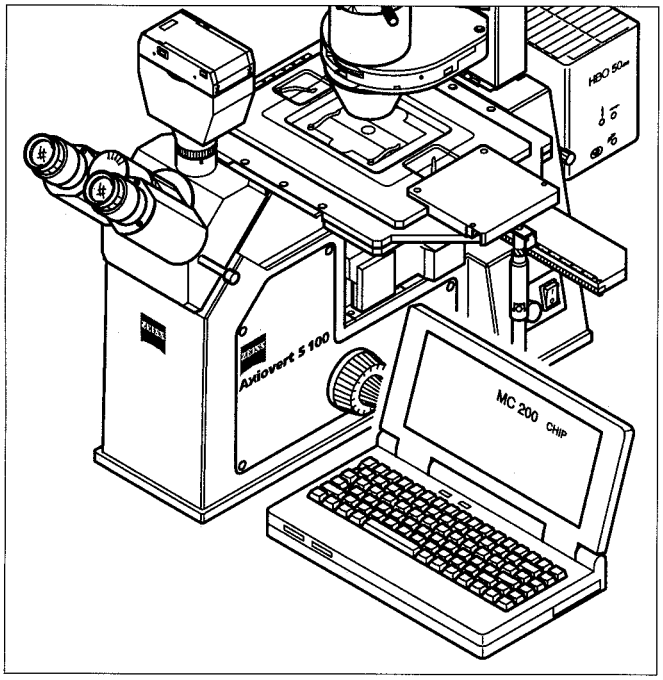
- Remove mirror or dichroic beam splitter (54/4) using flat tweezers (54/3).

- Exchange the mirror/beam splitter as required and reassemble stand in reverse order.

54



55



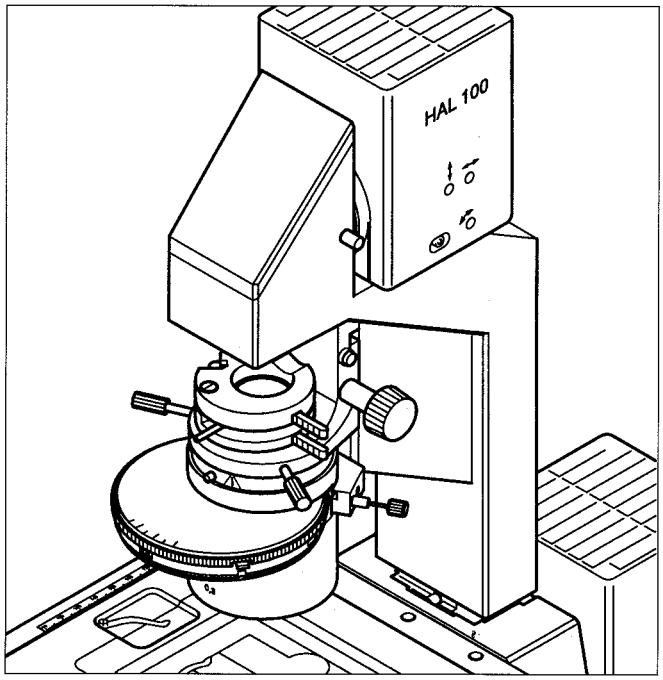
MC 200 CHIP microscope camera on standardized camera port

The MC 200 CHIP microscope camera can also be attached to the Axiovert stand via the standardized camera port of the binocular phototubes.

All the camera functions are controlled via a special photomicrography software.

The software, which is pre-installed on a notebook, can also be installed under WINDOWS from version 3.1 or WINDOWS 95 on any customer PC meeting the system requirements.

56



Condenser system

The four changeable condensers of the condenser system of the Axiovert permit five different transmitted light contrasting techniques: brightfield, darkfield, phase contrast, DIC and VAREL. The following condensers are new:

LD condenser 0.3 (451756)

Working distance: 70 mm

Brightfield H via aperture diaphragm

Phase contrast Ph 0, Ph 1 and Ph 2 for low and medium magnifications (e.g. objective 5x Ph 0)

LD condenser 0.55 (DIC) (451359)

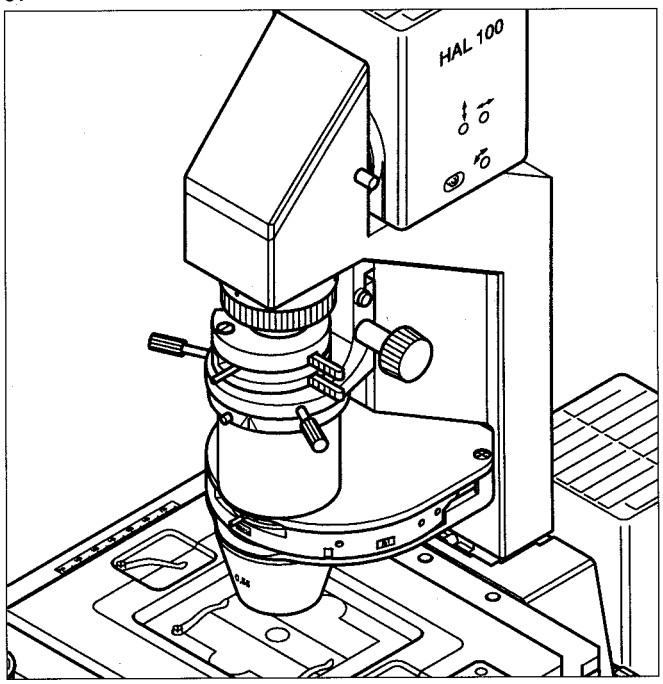
Working distance: 22 mm

Brightfield H via aperture diaphragm

Phase contrast Ph 1, Ph 2 and Ph 3

DIC 0.3–0.4 and DIC 0.5–1.4

57



LD condenser 0.55 (VAREL) (451358)

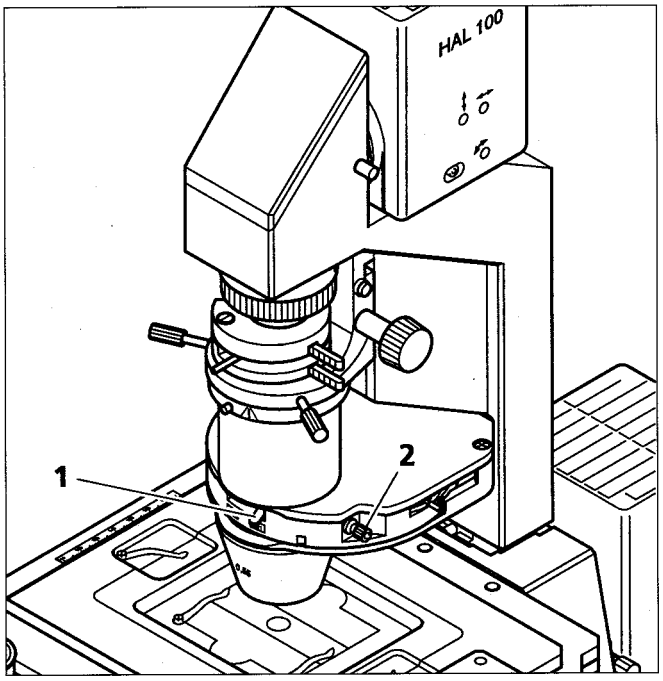
Working distance: 22 mm

Brightfield H via aperture diaphragm

Phase contrast Ph 1 and Ph 2

Relief contrast Var 1/Var 2

58

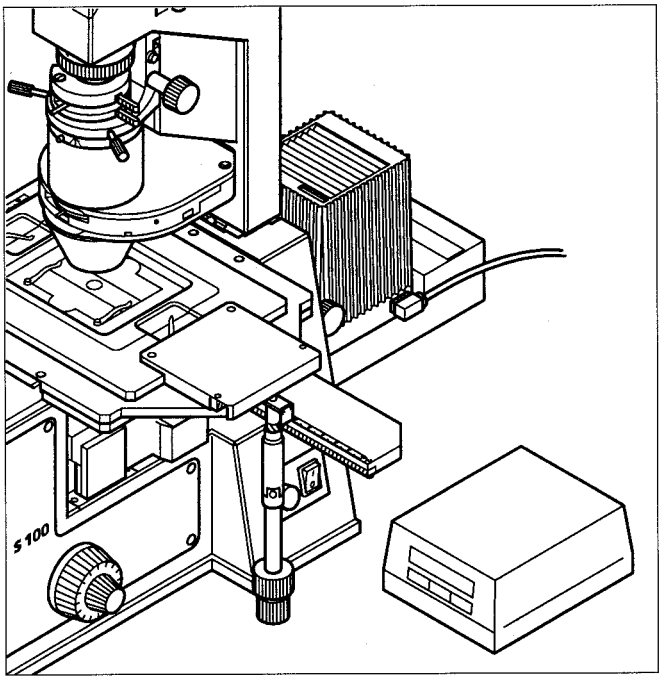


VAREL contrast is obtained as follows:

- Select the VAREL stop Var 1 or Var 2 suitable for the objective via the lever (58/1).

- Move the VAREL aperture using the setting screw (58/2) until optimum VAREL contrast has been obtained (also see Fig. 62).

59

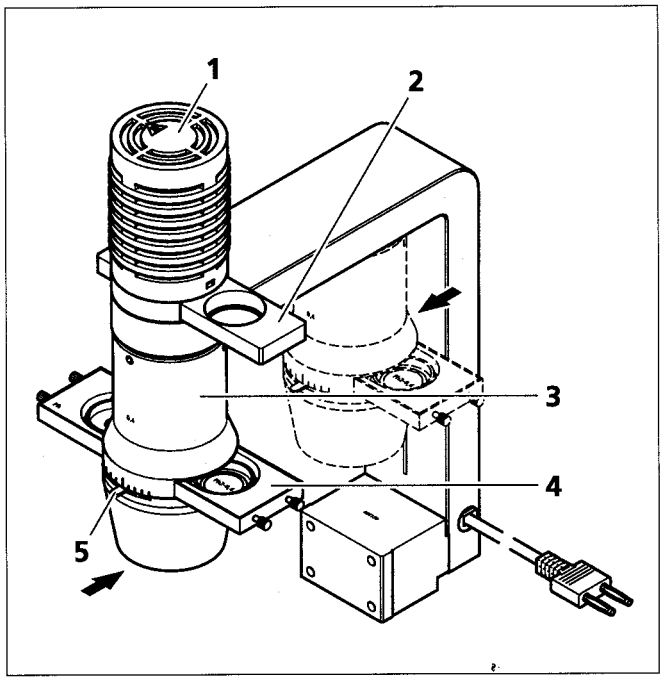


AttoArc™ (419175-9042)

In combination with AttoArc™, the 100 W HBO lamp, used instead of the HBO 50 illuminator, permits continuous light control between 15% and 100%. This avoids the fast bleaching of fluorescence dyes.

Simultaneous observation in fluorescence and DIC is also possible.

60



25 W transmitted light illuminator on the Axiovert S 100

On the customer's request, the carrier for the HAL 100 transmitted light illumination can be replaced with the 6 V 25W carrier for transmitted light illumination. *)

- In that case, the power supply integrated in the Axiovert S 100 is factory-aligned to 6 V 25 W and this is shown on the label on the stand. **)

- The 6 V 25 W illuminator (60/1) features an integrated filter slider (60/2); the suitable filters (green filter, attenuation filter) are easy to insert by the customers themselves.

- The condenser 0.4 (60/3) with aperture diaphragm (60/5) can be pushed out of its standard position. This enables object fields to be entirely illuminated even when low-power objectives are used.

This condenser position is also required for particularly high culture vessels in order to increase the free working distance.

*) This allows the benefits of the Axiovert 25, i.e. low-power illumination without condenser change and free specimen area for high vessels, to be used.

**) In case of retrofitting by the service, a separate 6 V transformer can also be used.

The three different sliders (60/4) pushed in the condenser feature 3 positions:

Ph/HF/Ph or Ph/HF/VAREL or VAREL/HF/VAREL

- For phase contrast examinations, the phase ring of the objective and the ring diaphragm (61/1) must be matched and congruent to each other. This is obtained using two centering screws (61/3).

- The center position of the phase slider permits bright-field illumination, where the brightness is matched to that of the phase contrast image via an attenuation filter (gray filter) (61/2).

NOTE

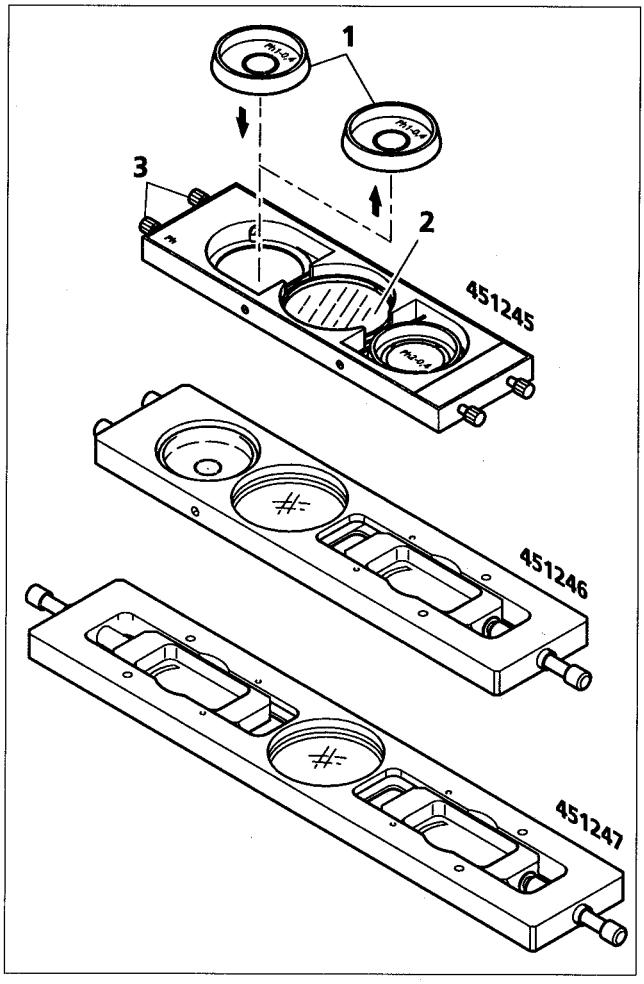
- Shifting the VAREL illumination to outside the pupil corresponds to unilateral darkfield illumination.

- Shifting the VAREL illumination between the Ph and VAREL rings of the objective corresponds to oblique brightfield illumination.

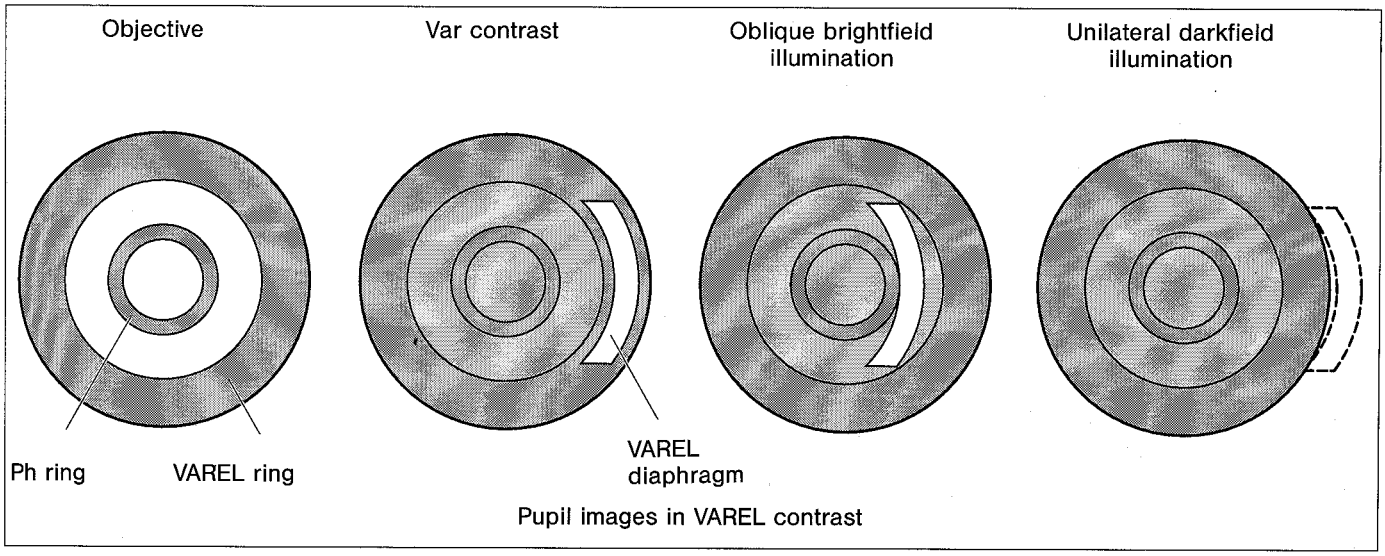
- The center stop position of the Ph/HF/VAREL slider permits brightfield illumination.

- The left stop position of the Ph/HF/VAREL slider permits the fast change from VAREL to Ph contrast.

61



62



Pupil images in VAREL contrast

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