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ZEISS

Operating Instructions

Axiolab® Microscope

for transmitted light
and incident-light fluorescence

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- The 6- or 10-digit numbers, e.g. 45 74 65, are ordering numbers of instruments or components.
- 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.

Installation

- The tube openings should be closed by the eyepieces and other ports or apertures by covers or plugs/sliders 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 (maximum relative humidity 80%).
- Permissible room temperature between 5°C and 40°C.
- Contamination degree 1 not to be exceeded.

- Use only power cables and plugs which are in perfect condition.

- The instrument may be connected to an orderly installed earth-contact socket only.

- 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.

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.

- For notes on the use of an HBO 50 mercury short-arc lamp, please see page 14.

- Always pull power plug before lamp replacement, voltage change (here, fuses should also be replaced), etc.!

- 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 on safety

- 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 complies with the TÜV-GS regulations according to IEC 1010-1.
It is categorized as a protection class I instrument.
The instrument is radio-screened in accordance with the EN 55014 regulations (DIN-VDE-Norm resp. BMPT-AmtsblBfg. 242/1991).

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.

- 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.

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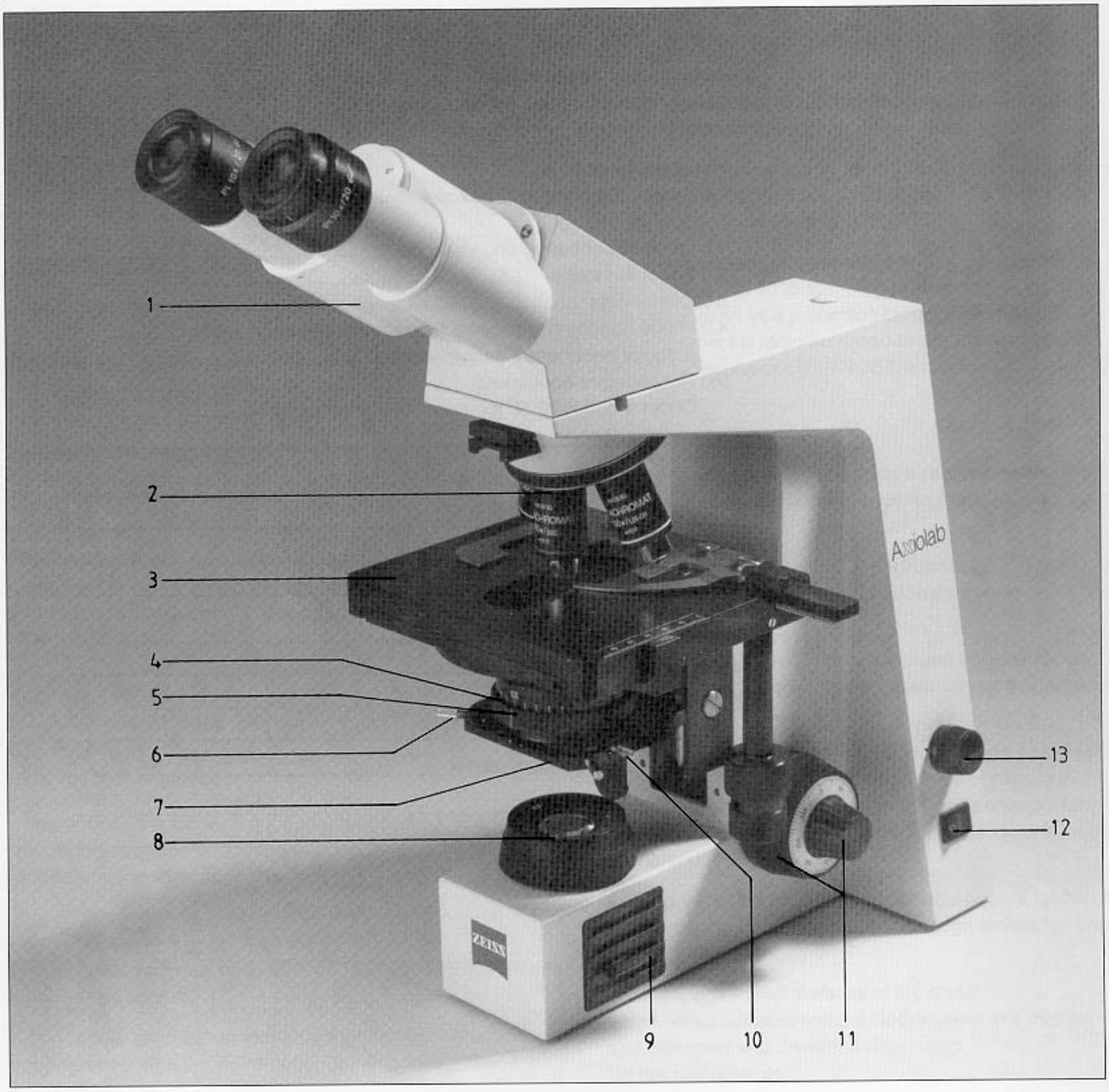


Fig. 1: **Axiolab** binocular microscope for brightfield

1 Binocular tube with eyepieces
2 Nosepiece 5x
3 Mechanical stage
4 Abbe condenser 0.9/1.25
5 Condenser (aperture) diaphragm
6 Condenser centering screws
7 Condenser carrier
8 Luminous field diaphragm
9 Ventilating grid in front of 6V 25W halogen lamp
10 Condenser clamping screw
11 Coaxial coarse/fine focusing control
12 ON/OFF switch with power signal lamp
13 Potentiometer for integral illuminator

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Special note: Numbers such as **1.1** refer to the full description of the instrument starting on page 6.

- Check voltage on power rating plate on instrument back, connect microscope to the line by power cable, switch on with (12), and adjust 3V with (13). The lamp in the switch is on.
- Load a high-contrast specimen on specimen stage (3) with the smaller, thinner cover glass face up.
- Turn in 10x objective (yellow ring) on nosepiece (2)
- Control 0-position on eyepiece scale.

With knob at the condenser carrier (7) move Abbe condenser (4) to topmost position (see also **3.2** on page 8).

- Close aperture diaphragm (5) about half.

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

For the adjustment of KÖHLER illumination proceed as follows:

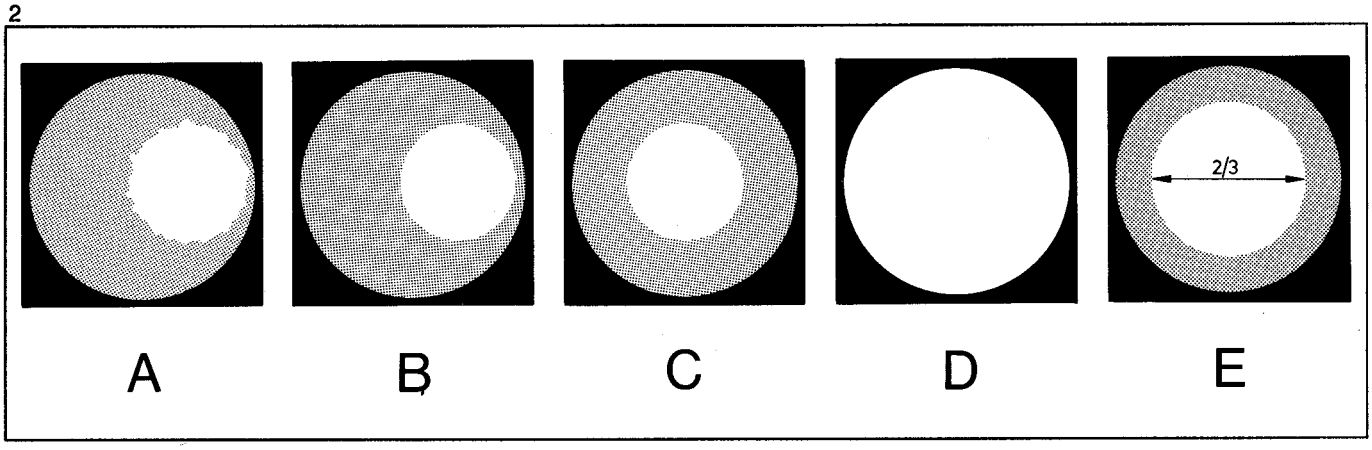
- Focus the specimen with coarse/fine focusing control (11). (If your eyes have different powers, and for microscopy without spectacles, see **4.3** and **4.4** on page 10.)
- Close luminous field diaphragm (8) which becomes visible in the image (A).
- Focus diaphragm image by slightly lowering the Abbe condenser (7).
- With screws (6) move the diaphragm image to the center of the field of view (C), and
- open luminous field diaphragm (8) until it just disappears from the field of view (D).

Depending on the specimen the contrast is now adjusted with the condenser diaphragm (5). - If you are not certain how far to stop down, approx. 2/3 of the rear element of the objective should be illuminated if the specimen is of moderate contrast (E). The rear element is visible at the bottom of the tube when the eyepieces are removed or the centering telescope is inserted.

- Plug eyepieces into binocular tube.

Field of view and objective aperture change, of course, with each objective exchange, so that the last-mentioned manipulations must be repeated in each case.
Use the condenser with low-power system for 2.5x objectives.
For more details see the description of the condensers on pages 8 and 9.

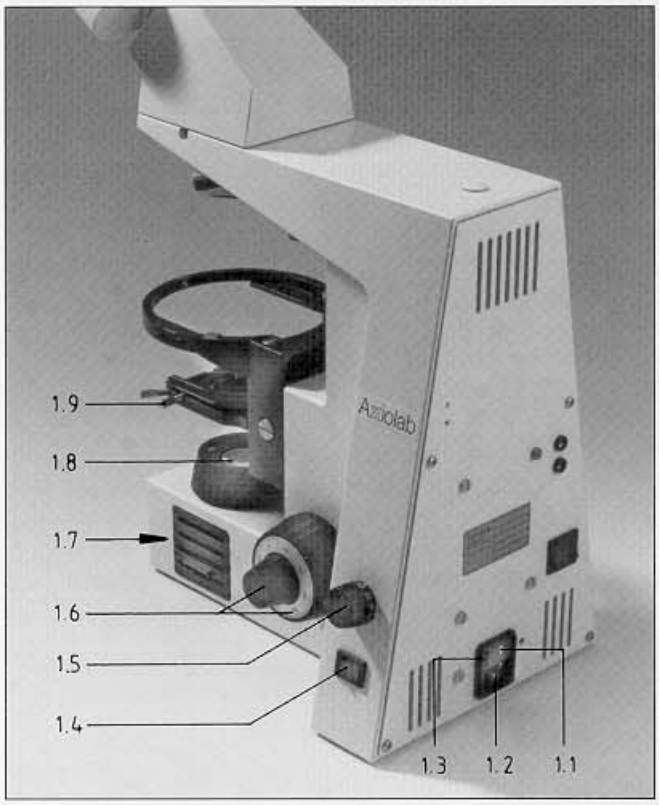
2



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Special notes
The tool supplied with the equipment is an SW 3 hexagon key for the tube clamping screws on the stand and the fluorescence illuminator and the key for clamping the stage.

3



1.0 Lamp power supply and stand base

The 6V 25W lamp power supply is integrated in the stand.
1.1 power rating plate, displays the adjusted input voltage of the instrument:
230V or 115V line voltage.
The instrument voltage is adjustable.
The output voltage is stabilized and variable from 1.5 . . . 6V.
The power consumption is max. 60VA.

1.2 Instrument power plug with integrated fuses.
Change of instrument voltage:
- Switch off instrument and pull power plug.
- Pull out rack with fuse holder at **1.3** using screwdriver.
- Plug suitable fuse for existing line voltage (see page 19) into fuse holder.
- Insert fuse holder in rack so that the adjustable voltage is visible from the outside. Insert rack in slot.

1.4 Power switch with power signal lamp
1.5 Potentiometer supplying 6V when turned fully clockwise. The adjusted voltage is displayed on the scale. The color temperature of 3200 K for color photography is displayed by the symbol ■ (potentiometer turned fully clockwise).

1.6 Coaxial coarse/fine focusing control acting on the stage carrier vertically adjusts the specimen stage with the Abbe condenser. Knob turned towards the user lowers the stage.
Total travelling range (including fine focusing control): 21mm.
One revolution of the coarse focusing control corresponds to 4mm travel.
Gear ratio of the 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. 4μm.

1.7 Integral illuminator containing a lamp socket with 6V 25W halogen lamp (41 70 30-9001) firmly adjusted by the manufacturer.
A diffusing screen, a collector and a lens working also as dust cover glass above the lamp serve to correctly illuminate the field and the pupil.
For lamp exchange see page 18.
1.8 Luminous field diaphragm adjusted with knurled ring.
A 32mm dia. filter can be accommodated on the dust cover glass on top of it.
Frequently used 32mm dia. filters:
Green interference filter (46 78 03) for contrast enhancement in B/W photography of stained sections and in phase contrast.
Conversion filter CB 12 (46 78 47) for daylight color film.

1.9 Condenser clamping screw.

1.10 Clamping screw for the rotatable mechanical stage.

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2.0 Specimen stages

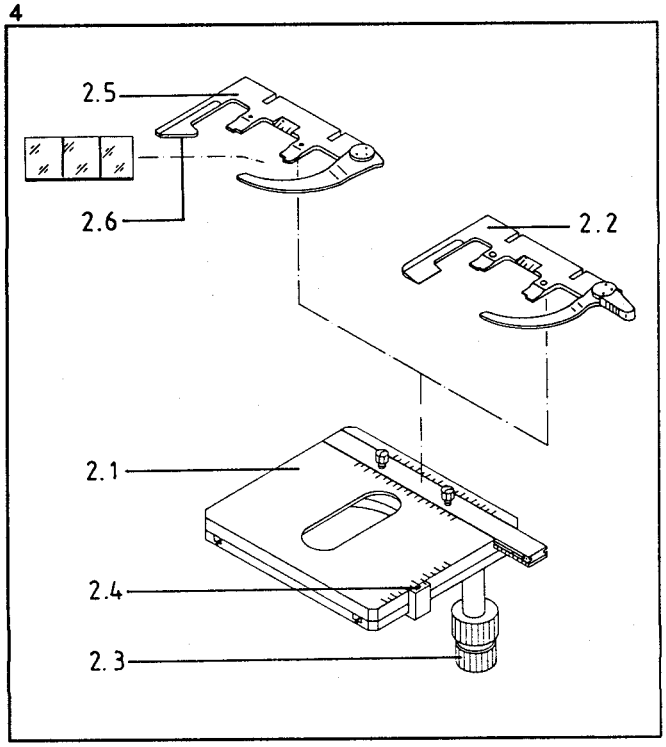
There are two different ways of mounting the microscope stage on the Axiolab microscope:
- The stands (45 09 07/08) are equipped with the rigidly mounted mechanical stage 75x30 mm.

- The stands (45 09 05/06) feature a stage carrier with mount for the rotary mechanical stage with dovetail (see below).

2.1 Mechanical stage 75 x 30mm or 75x50 mm
with controls to the right or left.
Mounted on the stage
2.2 Specimen holder with spring clip to the right (47 34 48) for standard 26x76mm specimen slides and special 26x45mm specimen slides; or

2.5 Specimen holder (45 35 48) for single-hand use which eases slide-in of the specimen.
Place the specimen on the specimen stage, press it lightly down with two fingers, and slide it into the specimen holder along the
2.6 guide edge.
Travelling range of
2.3 coaxial drives in x and y is 75x30mm or 75x50mm.
2.4 Graduations and verniers to relocate specific specimen features.

4



Rotary mechanical stages with dovetail
These mechanical stages can be rotated through approx. 210° in their center position, e.g. to adjust an object in the photo or TV format.

Rotatable mechanical stage 75x30mm R/210° with extended, low-mounted stage drive to the right	45 35 10
Rotatable mechanical stage 75x30mm L/210° with extended, low-mounted stage drive to the left	45 35 11
Rotatable mechanical stage 75x50mm R/210° with drive to the right	45 35 12
Rotatable mechanical stage 75x50mm L/210° with drive to the left	45 35 13

Mounting a rotatable specimen stage
in the dovetail ring mount of the stage carrier:
- Lower stage carrier, turn clamping screw **1.10** all the way back with the SW 3 hexagon key supplied, hold dovetail ring mount of mechanical stage at an angle, fit it at rear in stage holder and put it on uniformly. Secure stage by clamping screw with the supplied key.
It is not possible to center the mechanical stage.
- Before turning the stage loosen stage clamping screw lightly to prevent play when turning.

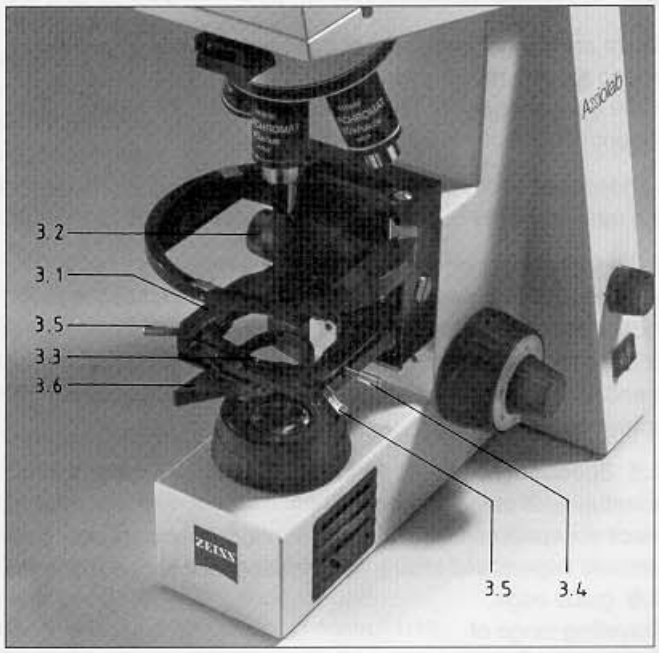
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3.0 Condensers

3.1 Condenser carrier (Fig. 5)
with the following operating controls:
3.2 Knob for vertical condenser adjustment; the stiffness is factory-adjusted.
The knurled screw on the right under the condenser holder serves to define the upper stop. This prevents a specimen from being pressed out by mistake.
Loosen the knurled screw, move condenser to the desired height and tighten the stop screw.
3.3 Orientation slot to mount the condenser with turret.
3.4 Condenser clamping screw.
3.5 Two condenser centering screws to center the luminous-field-diaphragm image for illumination adjustment (see page 5).
3.6 Low-power system, swivel-mounted (44 53 12) to illuminate the field of 2.5x objectives; it can be mounted at the bottom of the condenser carrier with 2 screws.
When using the low-power system, open the luminous-field diaphragm completely.

For polarisation contrast the polarizer (45 36 17) can be mounted in the same way.

5



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3.7 Abbe condenser 0.9/1.25 (44 53 02) (Fig. 6)
can be used for 4x[1] to 100x objectives without supplementary optics.
3.8 Slide ring with scale with aperture diaphragm. This scale is pertinent if no immersion oil is used on the condenser.
With immersion oil the maximum numerical aperture is 1.25.
Plug-in items for this condenser:
3.9 Darkfield stops for 10x and 40x objectives
3.10 Phase stops Ph 1, Ph 2, Ph 3 in centering mounts for phase contrast.
3.11 Low-power system (44 53 04) for 2.5x objective
3.12 Polarizer D (45 36 16).

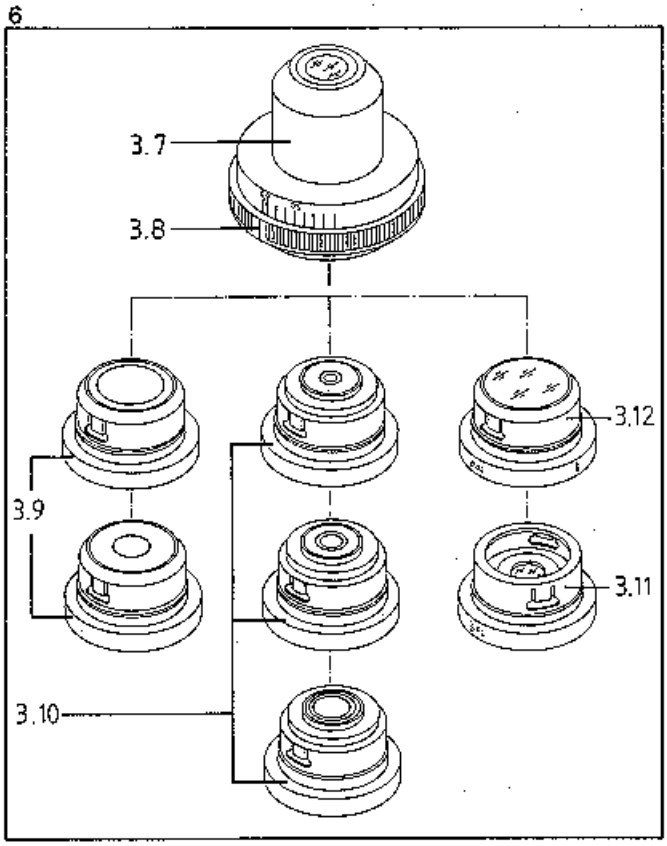
3.13 Abbe condenser 0.9/1.25 with turret 5x H, D 0.65/0.9 and Ph 1, Ph 2, Ph 3 (44 53 03) (Fig. 7)
3.14 Turret with click stops equipped for the following illumination methods:
- Brightfield (H)
- Phase contrast Ph 1, Ph 2, Ph 3
- Darkfield for objectives up to 40x mangification and ≤ 0.65 numerical aperture.

3.15 Centering screws for Ph phase stops and darkfield diaphragms.
The left centering screw **3.15** is equipped with a locking screw which must be loosened for centering and tightened again afterwards if switchover is made between the selected diaphragm and the brightfield position.
The size of the aperture diaphragm can be adjusted at **3.16.**

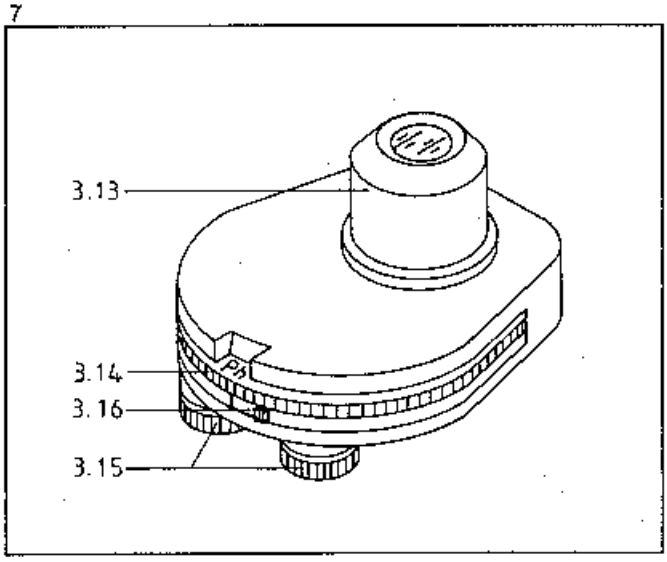
Inserting the condensers in the carrier **3.1:**
- Move condenser carrier all the way down.
- Turn condenser so that the aperture scale is easily visible from the front and slide it into the slot. Tighten clamping screw **3.4** (page 8).

[1] Use eyepieces with the max. field of view number 18.

6



7



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4.0 Image forming components

4.1 The ICS objectives are the most important elements of the microscope, and their front lens surfaces in particular should always be meticulously clean.
The objective designation may be:
CP-Achromat 40/0.65 ∞ /0.17, where:
40 is the (individual) magnification;
0.65 the numerical aperture;
∞ infinite image distance
0.17 signifies that the objective is calculated for a cover glass thickness of 0.17mm.

The (individual) magnification multiplied by the eyepiece magnification (generally 10x) results in the microscope magnification, e.g. 400x.

The numerical aperture multiplied by 1000, that is 650 in this example, is the highest useful magnification; no more details will be revealed above this value.
The aperture is important in darkfield illumination for the selection of the darkfield diaphragm.

The symbol " ∞ " is to remind the user that these objectives cannot be used on microscopes with objectives engraved "160".

The cover glass thickness of 0.17mm must be the more adhered to the higher the numerical aperture of the objective. Certain objectives (with correction mounts) are adjustable to different cover glass thicknesses: use a high-contrast specimen feature and find out in which position of the correction mount the focus is optimized (re-focusing will always be necessary).
Only immersion objectives are insensitive to differences in the cover glass thickness. Because of their short working distances, 25x objectives and those with higher powers have spring mounts for specimen protection. To prevent immersion objectives from contaminating the specimen with oil when turning the nosepiece, it is possible to lock in the spring mount of some objectives in topmost position by a clockwise turn.
Do not forget to disengage them from lock-in position!
The air between an immersion objective and the cover glass is replaced by a liquid, generally immersion oil. We supply with the instrument a plastic oiler containing 50ccm immersion oil.Some experience is needed to obtain a bubble-free layer.
Some microscopists turn the objective from the side into the oil drop on the cover glass, whereas others lower it from lock-in position of the spring mount.
A check of the exit pupil (look through the empty tube, see page 5) should always be made, because this reveals instantly any bubbles. If turning in the objective repeatedly does not eliminate the bubbles, clean the specimen and repeat the procedure.

4.2 Nosepiece 5x, rigidly connected with the stand

4.3 and **4.4** eyepieces PL 10x/18 Br. with 10x magnification, field-of-view number 18 and angular field 39°. They are equally well suited for spectacle wearers (Br) and provided with an exchangeable rubber ring to protect the spectacles (folding eyecups are available under ordering number 44 48 01).
The binocular tube is provided with two fixed tubes; one of the eyepieces must, therefore, be a focusing eyepiece (foc). If your eyes have different powers or you work without spectacles, proceed as follows:
- Look through the fixed eyepiece with the less ametropic eye and focus the specimen.

Leave the microscope adjustment unchanged, and adjust the focus for the more ametropic eye with the eyelens of the focusing eyepiece until the focus is the same for both eyes.
Adhesive rings in both tubes make the eyepieces torsion-proof, which is important for the use of reticles.

Spectacle wearers who work without spectacles will experience unsatisfactory objective parfocalization. If you have a "cylinder" in your spectacles you should always wear spectacles for microscopy.

Reticles are provided in the eyepiece diaphragm plane and are used mainly for measurement and format display if a microscope camera is used. Reticles can only be accommodated in focusing eyepieces; the resulting slight image displacement is considered on the diopter scale by the red dot which displays the zero position instead of the white one.
Because of the high demands on cleanliness and exact alignment reticles should be exchanged only by specialists. (The bottom part of the eyepiece may be unscrewed; the scale-bearing surface of the reticle must face down.)
Two "foc" eyepieces are required if a reticle is used. Proceed as follows to focus on the specimen:
- Look through the eyepiece with reticle with the less ametropic eye.
- Turn the adjusting ring of the eyepiece with reticle until the reticle is in focus.
- Now bring the specimen in focus in the eyepiece with reticle using the coarse and fine control.
- Leave the microscope adjustment unchanged, and adjust the focus for the more ametropic eye using the adjusting ring of the other eyepiece until the focus is the same for both eyes.

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The binocular tubes are adjustable to PDs from 55 ... 75mm by turning the tube halves in or out. The tube factor is 1x. The tube is mounted on the stand after loosening
4.5 Allen screw, and secured by aligning it to the front.

Binocular tube 35°/20 (45 29 08), viewing angle 35°, see fig. 1, positon 1.

4.6 Binocular phototube 35°/20 (45 29 09),
viewing angle 35°,
for eyepieces with the field of view number ≤ 20.
4.7 Pushrod pushed in: 100% of the light for observation
Pushrod pulled out: 100% of the light to the camera
Align the eyepiece with photo reticle in the viewing tube parallel to the camera.
An adhesive ring is provided in each eyepiece tube. It secures the orientation of the photo reticle after turning the eyepiece. The orientation must be corrected after each change of the PD.

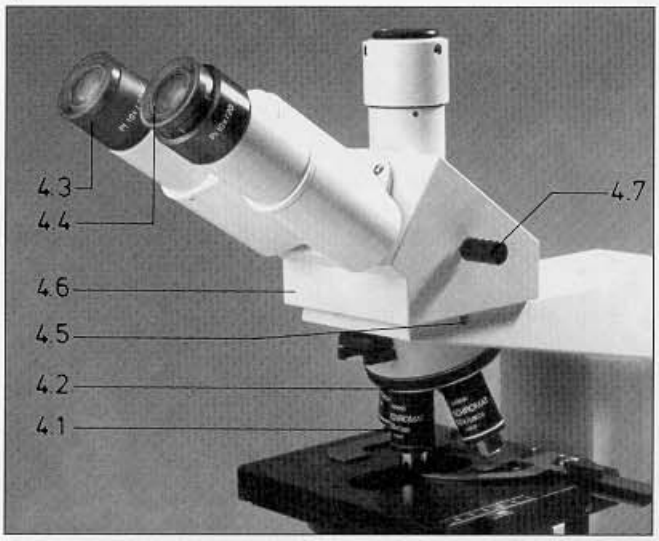
Cameras which can be mounted on the upper tube port:
MC 80 Microscope Camera by means of
4.8 Adapter (30mm dia.) (45 60 06)
with plug-in projection lens P 2.5x (45 60 21) for 35mm format or plug-in projection lens P 10x (45 60 23) for large format.

MC 100 Microscope Camera by means of
4.8 Adapter (30mm dia.) (45 60 06)
with plug-in photo eyepiece S-PL 10x/20 (44 40 39) or plug-in photo eyepiece S-PL 12.5x/16 (44 40 49).

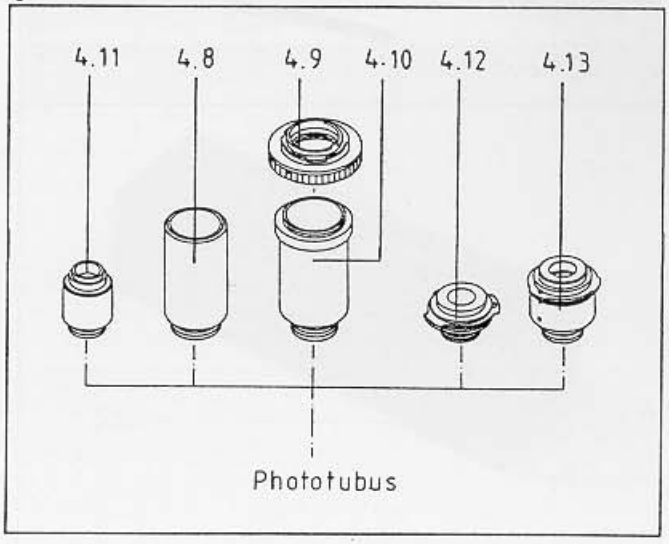
SLR camera housing with
4.9 T2 adapter and
4.10 Adapter for SLR cameras 2.5x for T2 (45 60 05)
without eyepiece

For further details about the microscope cameras see the following instructions:
G 42-401 MC 100 Microscope Camera
G 42-407 MC 80 Microscope Camera
G 42-406 SLR cameras on microscopes

8



9



TV cameras with standard C mount by means of:
4.11 1" TV camera adapter 1x (45 61 05),
2/3" TV camera adapter 0.63x (45 61 07) or
1/2" TV camera adapter 0.5x (45 61 06).

TV cameras with 3C-CTV ENG bayonet mount by means of:
4.12 2/3" TV camera adapter 1x 3 C-CTV (45 61 15) or
4.13 2/3" TV camera adapter 0.8x 3 C-CTV (45 61 17).

TV cameras are always mounted without eyepiece.

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4.13 Drawing eyepiece P 8x/18 (44 41 26) (in preparation)

The drawing eyepiece is an attachment for drawing with the microscope. It contains a beam splitting prism for simultaneous observation of microscopic image and drawing surface.

Adjustment

- Microscope adjustment see page 5.
- Take out one eyepiece, plug drawing eyepiece into tube and rotate it that the projection is symmetric to the sheet of paper in front of the stand.
- Secure the drawing eyepiece.
- Illuminate drawing surface until the drawing surface and the microscopic image are of equal quality. If necessary, reduce the brightness of the microscopic image by means of the potentiometer.

A special pencil facilitates drawing.

Coobservation bridge (in preparation)

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5.0 Fluorescence equipment

The fluorescence tube converts any Axiolab microscope into an incident-light fluorescence microscope.

5.1 Binocular fluorescence tube (45 09 60), factor 1x, is provided with photo/TV port, luminous field diaphragm, several slots for filters and reflector sliders.
5.2 Fluorescence illuminator, equipped with aspheric collector and HBO 50 mercury vapor short-arc lamp, supplied from its own power supply.
5.3 Hexagonal socket-head screw for attachment of the fluorescence illuminator.

5.4 Shutter and filter slider with 2 apertures for 25mm dia. filters

5.5 Luminous field diaphragm with scale division, centrable.

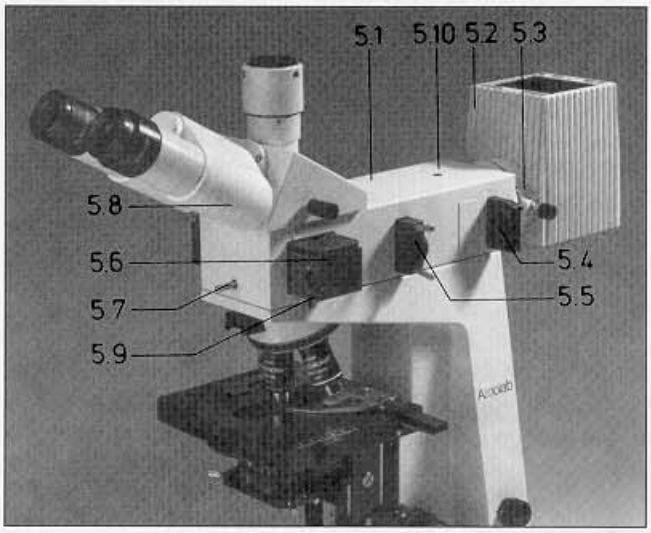
5.6 Reflector slider 3 FLwith 3 apertures -
Central aperture: generally unoccupied for brightfield or phase contrast observation.
Outer apertures: contain sets of exciter filter, dichroitic beam splitter and barrier filter (selection optional). The base equipment includes filter set 09 for blue excitation 450-490.
5.7 Stop screw. For insertion of the reflector slider it must be turned out until the slider can be slid in. Turn the screw all the way in in central postion of the slider.

5.8 Binocular viewing tube with photo tube 30°/20.
The beam splitting ratio is either 100% of the light to the viewing tube (pushrod slid in) or
100% of the light to the phototube (pushrod pulled out).
For attachment of a microscope camera or TV camera,
see page 11.

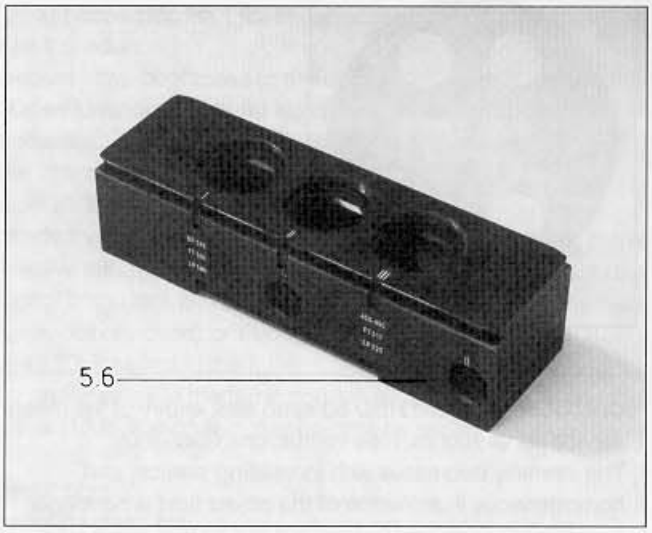
Mounting the fluorescence tube FL:

- Remove cover of borehole **5.10** in stand for the locking screw.
- Insert fluorescence tube in mount port on the stand, align it flush with the stand edges and lightly tighten clamping screw **5.9**. Then tighten locking screw at **5.10** with screwdriver SW 3 and secure intermediate tube with screw **5.9**.

11

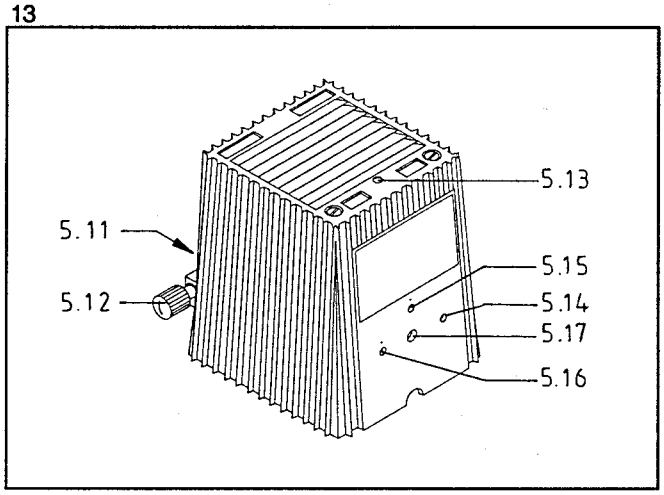


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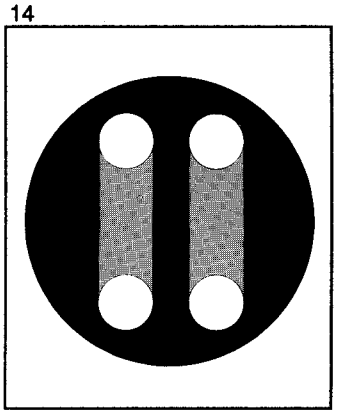


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14



CAUTION: Replace HBO 50 lamp after expiry of the mean service life of 100hrs. (see instructions G 42-160).
The intensity decreases with increasing service, and homogeneous illumination of the object field is no longer guaranteed. Furthermore, there is the **risk of explosion.**
The life of the lamp is displayed on the time meter of the power supply.

For further details and relevant safety precautions see operating instructions:
G 42-160 HBO 50 Fluorescence Microscope Illuminator.

Features of HBO 50 microscope illumination (Fig. 13):
5.11 Light exit port.
Dovetail ring mount to mount the illuminator on the fluorescence tube FL:
- Unscrew clamping screw **5.3** (page 13) on the dovetail ring mount port sufficiently.
- Insert ring mount of lamp housing inclined in opening opposite clamping screw, then tilt illuminator on to the seating surface and tighten screw.

5.12 Knob for collector adjustment
5.13 Vertical lamp adjustment
5.14 Horizontal lamp adjustment
5.15 Vertical adjustment of reflector image (red dot)
5.16 Horizontal adjustment of reflector image (red dot)
5.17 Focusing of reflector image

Centering the HBO 50 lamp:
Caution: never look directly into ignited lamp
to prevent injury to your eyes.
- Put on protective eyewear, e.g. sunglasses to protect your eyes when aligning the ignited lamp.
- Carefully remove switched-on illuminator from the microscope after loosening screw **5.3** with screwdriver SW 3.
- Do not look into the light spot when manipulating the ignited lamp, to prevent damage to your eyes by UV radiation.

With knurled knob **5.12** for collector adjustment image the brighter of the two light arc images focused on a white wall approx. 3m away.
- With screwdriver SW 3 on adjusting screws **5.13** and **5.14** move the light arc image to the center.
- With adjusting screw **5.17** focus the unsharp light spot; lamp image and reflector image should be of equal size.

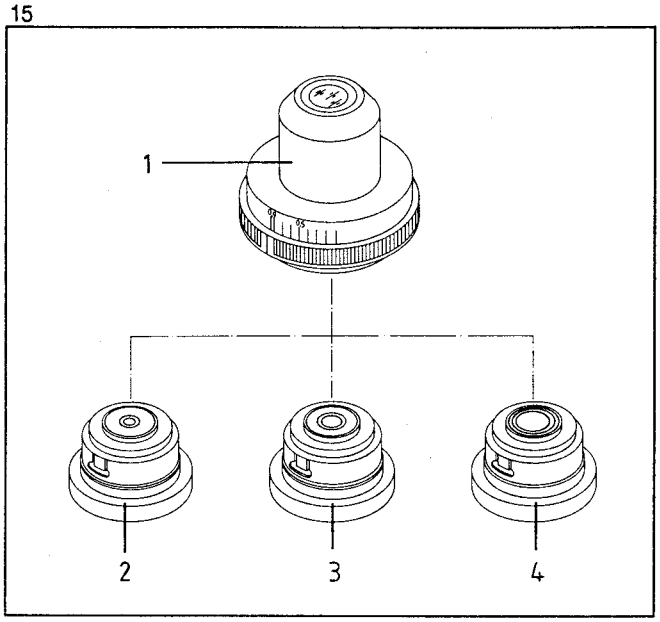
With the red adjusting screws **5.15** and **5.16** move the focused reflector image next to the lamp image, see Fig. 14.
The two images must not overlap.

- Attach illuminator to microscope and secure with clamping screw **5.3.**
- Unscrew objective and observe the light source image on a paper in the object plane (on the specimen stage).
- Make corrections with collector adjustment at **5.12** and adjusting screws **5.13** to **5.17** .
- Screw in objective.
- Set reflector slider **5.6** to blue excitation,

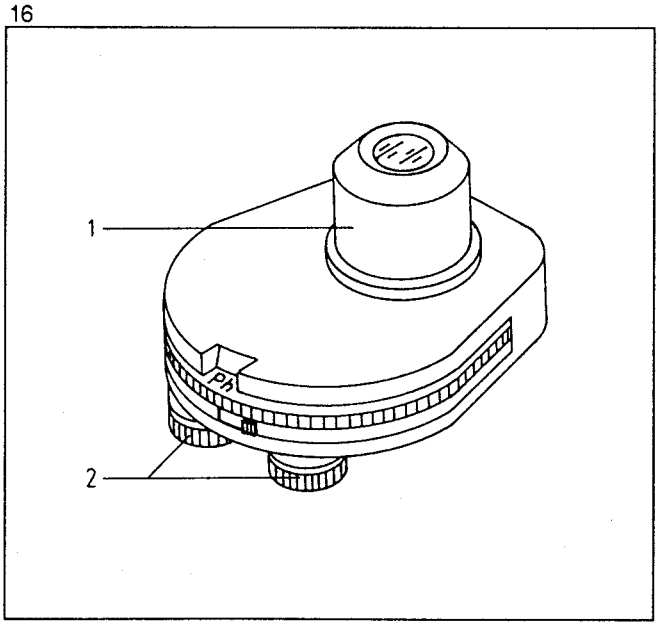
e.g. with filter set 09 for FITC fluorescence.
- Set slider at **5.4** to free light path.

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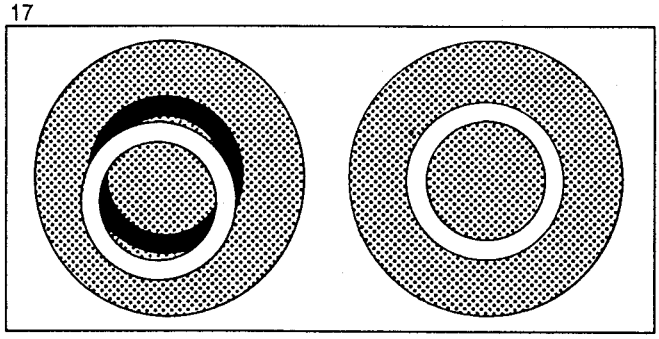
15



16



17



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

Required equipment

either (A)
- Abbe condenser 0.9/1.25 (44 53 02) (15.1)*) with plugged-in
- phase stop Ph 1 (44 53 05) (15.2)
  phase stop Ph 2 (44 53 06) (15.3)
  phase stop Ph 3 (44 53 07) (15.4)
- Objectives designated "Ph".

or (B)
- Abbe condenser 0.9/1.25 features a turret with 5 positions: H (brightfield), D 0.65/0.9, Ph 1, Ph 2 and Ph 3 (44 53 03) (16.1)
- Objectives designated "Ph", which are equally well suited for brightfield microscopy.

The necessary adjustments using equipment (A)
- Adjust object in brightfield, see page 5.
- Turn in 10x objective Ph 1.
- Plug phase stop Ph 1 for Ph objective into condenser or let it snap in.
- Adjust lamp brightness to the object.
- Open luminous field and aperture diaphragms in the condenser.

(The aperture diaphragm of the turret condenser is open automatically in the Ph-positions).
- Perfect phase contrast is produced only if the dark ring in the objective and the bright phase stop exactly coincide (Fig. 17). Control both rings by looking through the empty binocular tube (eyepieces removed) or through the centering telescope (44 48 30) inserted in the tube.

The phase stops in the turret condenser are centered using the screws (16.2), the plug-in diaphragms by sliding the plug mount.

Special note

Especially clean glass-to-air surfaces on the specimen are required in phase contrast even more than in brightfield (no fingerprints).

*) That means figure 15, item 1.

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It is applied

- to study minute objects or object features such as treponemas, spirochaetae, flagellates, bacteria etc., or emulsions, if phase contrast does not supply sufficient contrast.
- if the specific colors or natural (unstained) objects are well visible (living organisms in water like algae, unicellular organisms, lower animals).

Required equipment

- Always a condenser with central stop and a numerical aperture which is higher than that of the objective used.

- For Abbe condenser 0.9/1.25 (44 53 02) (18.1) the plug-in darkfield diaphragms
  0.25/0.9 (44 53 08) for 10x objective (18.2)
  0.65/0.9 (44 53 09) for 40x objective,
  perture ≤ 0.65 (18.3)
- For turret condenser 0.9/1.25 (44 53 03) position D for objectives up to 40x, aperture ≤ 0.65.

- Axioplan darkfield condensers are suitable for higher apertures.

Necessary adjustments:

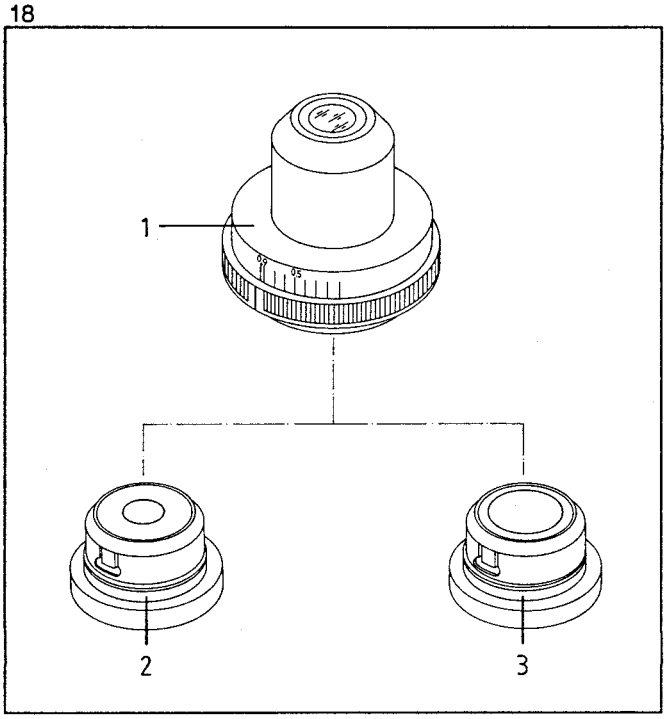
- Illumination adjustment as in brightfield. Imaging and centration of the luminous field diaphragm are also necessary. Adjust the condenser vertically so that the narrowed luminous field diaphragm visible in the center of the field of view is in focus.

- Open the aperture diaphragm of the Abbe condenser 0.9/1.25 completely. (In position D of the turret condenser, this diaphragm is open automatically).
- The darkfield diaphragm can be centered on the turret condenser via the screws (16.2, page 15).

The centering condition can be checked in the back lens of the objective when the eyepieces are removed or the centering telescope is inserted.

The apertures of objectives with iris diaphragms are too high for darkfield; using the iris they must be stopped down as far as the limit aperture. Very important is the absolutely black background of the eyepiece image, which may be influenced by the position of the luminous field diaphragm, especially at the edge of the field of view.

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Special notes:

The cleanliness of darkfield specimens is still more important than that of specimens for other methods; grease films (fingerprints) are particularly disturbing because they light up the background.

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Required equipment

- Polarizer (45 36 16) (19.2) in plug mount for Abbe condenser 0.9 (44 53 02) (19.1) is plugged in from below and turned.

or Polarizer (45 36 17) in swing-out holder (20.1) , which can be attached below the condenser carrier.

- Slider with analyzer (45 36 85) (19.3).
The oscillation direction is oriented NORTH-SOUTH.
It can be turned through ± 5° by its lever.

- Slider with λ plate first order red (45 37 05) (19.4).
It can be turned through ± 5° by its lever to optimize contrast ajustment.

Necessary adjustments

- Adjust illumination as for brightness (see page 5), but without polarizer.

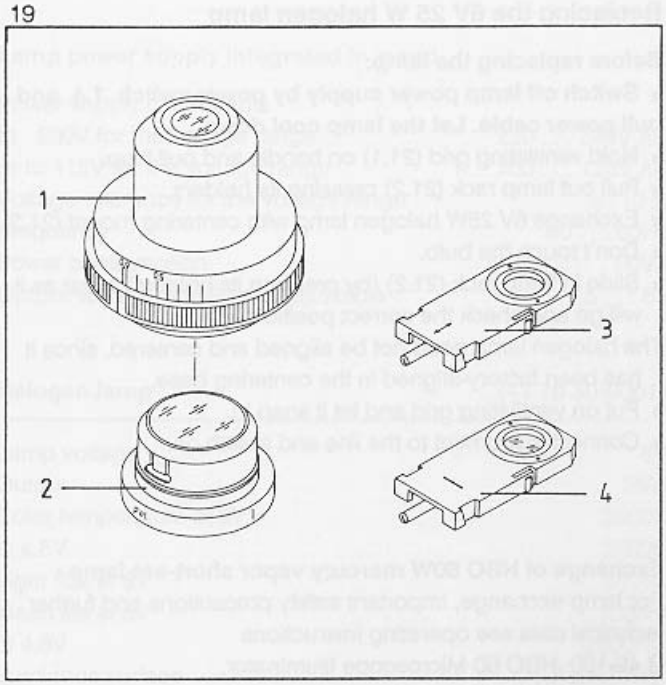
- Slide analyzer slider (19.3) all the way into upper carrier slot (20.2). Set its lever in middle position.

- Plug polarizer (19.2) into Abbe, condenser (19.1) from below and turn it until the "Pol" marking points to the front and the field of view displays maximum darkness, or swing in polarizer (20.1) until stop and reset analyzer to distinction position.

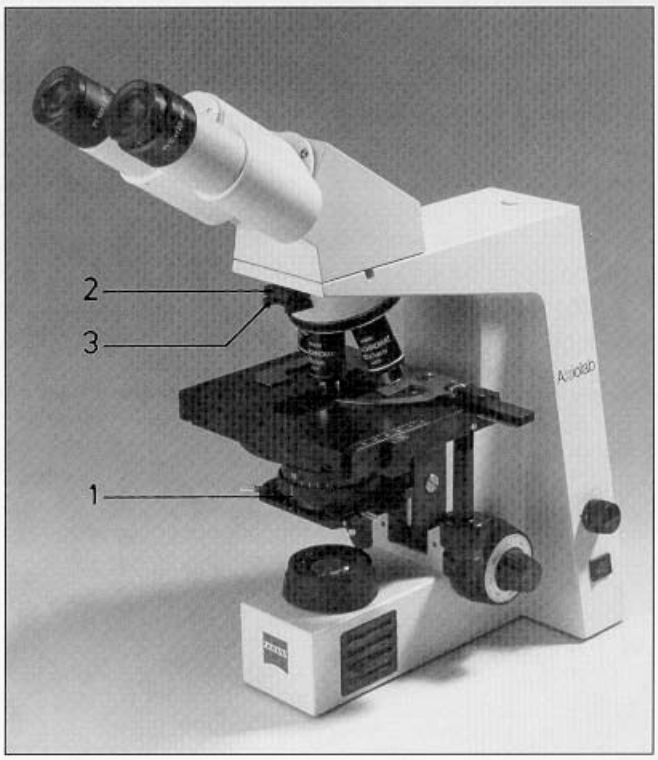
- Increase the lamp brightness and set the aperture diaphragm to approx. 0.2 to enhance the contrast when objectives with a high aperture are used.

- For color polarizing contrast slide slider with λ plate first order red (19.4) all the way into lower carrier slot (20.3).
The color intensity is variable by the lever.

19



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Replacing the 6V 25 W halogen lamp

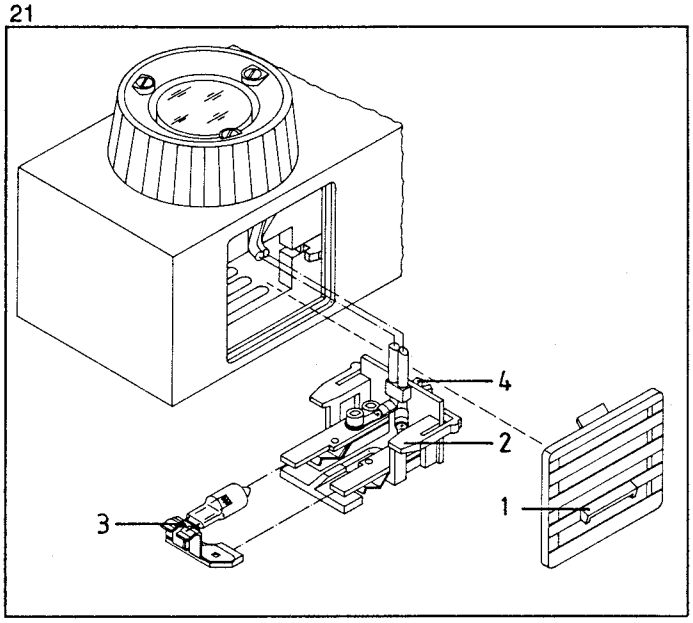
Before replacing the lamp:

- **Switch off lamp power supply by power switch 1.4 and pull power cable. Let the lamp cool down.**
- Hold ventilating grid (21.1) on handle and pull it up.
- Pull out lamp rack (21.2) pressing its holders.
- Exchange 6V 25W halogen lamp with centering mount (21.3).
- Don't touch the bulb.
- Slide in lamp rack (21.2) (by pressing its holders) as far as it will go and check the correct position.

The halogen lamp need not be aligned and centered, since it has been factory-aligned in the centering base.

- Put on ventilating grid and let it snap in.
- Connect instrument to the line and switch on.

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Exchange of HBO 50W mercury vapor short-arc lamp

For lamp exchange, important safety precautions and further technical data see operating instructions
G 42-160 HBO 50 Microscope Illuminator.
Centering of the HBO 50 lamp, s. page 14.

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Replacement parts are listed here in the order and with the numbers of the instrument description starting on page 6.

1.2 Spare fuses for the lamp power supply

for 230V: 0.4 A/L	(INR 127.016)
for 115V: 0.8 A/L	(INR 142.290)

4.1 Plastic oiler containing 50ccm immersion oil ISO 8036/1 (46 29 58)

Use only 6V 25W halogen lamps (41 70 30-9001), see page 18. Other commercial lamps will cause optical and perhaps also mechanical difficulties.

Lamp power supply integrated in stand

Power supply changeable	
to 230V for the voltage range	220 . . . 240V AC
or to 115V for the voltage range	100 . . . 120V AC
Voltage tolerance for the voltage range	± 10 %
Frequency	50 . . . 60Hz
Power consumption	60VA
Output voltage, stabilized, adjustable	1.5 . . . 6V

Halogen lamp	**(41 70 30-9001)**
Lamp voltage	6V
Output	25W
Color temperature at 6V*	3200K
at 4.8V	2800K
Light flux at 6V	510lm
Mean life at 6V	100hrs
at 4.8V	2000hrs
Luminous surface	1.7 x 1.7mm^2

* For photography using artificial-light color reversal film sensitized for 3200K, adjust the halogen lamp to 6V using knob **1.5** s. page 6.

HBO 50 W/AC mercury vapor short-arc lamp	**(38 16 19)**
Lamp voltage	L1: 39...45V/L2: 34...39V
Lamp current	L1: 1.30A/L2: 1.45A
Output	50W
Line spectrum	
mean luminous flux	2 000lm
mean life approx.	100hrs
Luminous surface (light arc)	0.3x1 mm

L1; L2: The HBO 50W/AC lamps are produced in two different types; the type is indicated on the enclosed ticket. The power supply unit must be set to L1 or L2.

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Further notes on instrument safety

■ The Axiolab microscope was designed, produced and tested in compliance with DIN 61010-1 (IEC 1010-1), Safety requirements for electrical measuring, control and laboratory instruments, and meets the requirements of the relevant CSA and UL directives.
This annex to the operating manual contains information and warnings which must be observed by the user.

■ The Axiolab light microscope has been designed in compliance with the latest scientific and technical development for the visual photomicrographic and video-technological examination of specimens. The instrument may only be used for its intended application. The instrument has not been designed for unattended constant operation!

■ The microscope is not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive or otherwise hazardous to health. The admissible specimen weight (≤ 2 kg) must not be exceeded.

■ To avoid glare, an attenuation filter must always remain in the beam path. It may only be removed from the beam path when the light intensity is too low.

■ 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 may not nullify the effect of the ground wire.

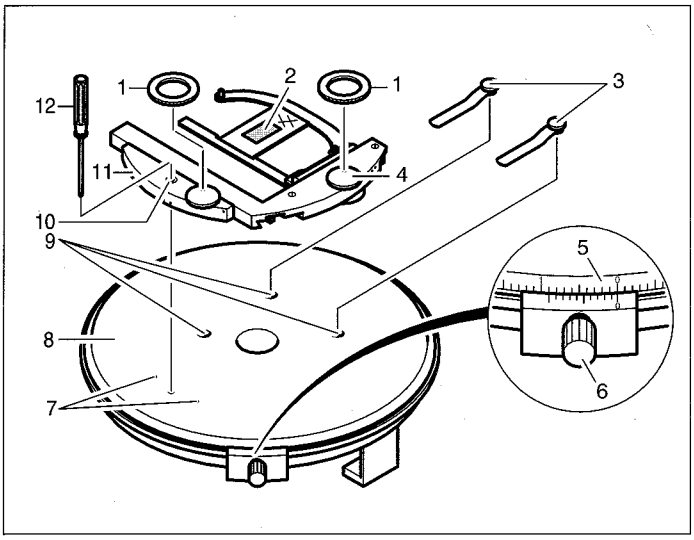
■ When the microscope has been connected to the line, connecting clamps inside the instrument may contain dangerous voltage, and the opening of covers or the removal of components (if not required for a function) may expose components containing dangerous voltage. Therefore, the instrument must be disconnected from the line before it is 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 minutes).
The lamp housing heats up during operation.

■ 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 optional polarization equipment includes the following components:

- Pol rotary stage with stage carrier, condenser carrier and drive, including 2 stage clips 453556-0000-000
- Pol object guide 473325-0000-000
- ABBE condenser 0.9/1.25 445302-0000-000
- Analyzer 6 x 25 453687-0000-000

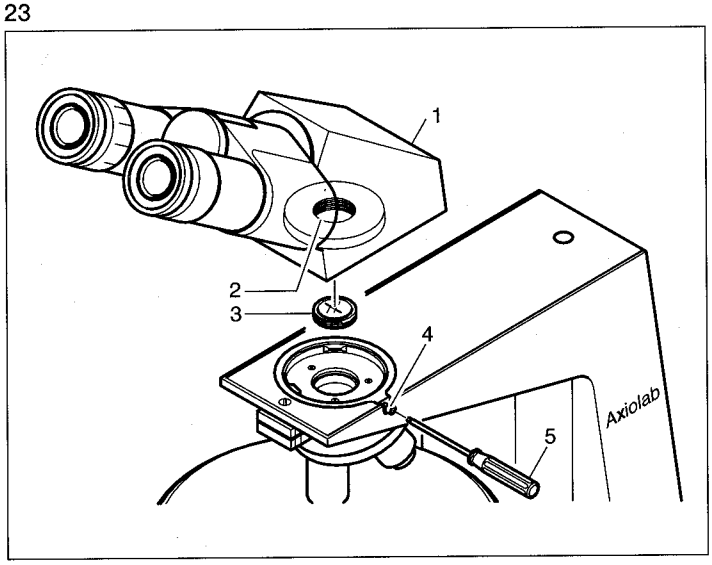
Working with the Pol rotary stage

The stage clips (22/3) supplied fix the specimen on the rotary stage (22/8). They are inserted into two of the three drilled holes (22/9) and used either diagonal, opposite or parallel to each other, depending on the size of the microscope slide or the scanning area.
The Pol object guide (22/11) (accessory 473325) provides a more convenient possibility. It is inserted into the drilled holes (22/7) and fixed using the screw (22/10).
For this purpose, use the ball-headed screwdriver (22/12) supplied. The plastic rings (22/1) supplied are particularly beneficial during screening to increase the knob diameter (22/4) and thus to allow the convenient operation from the side.

Note:
To ensure that the scanning area of the microscope slide is as large as possible, place the object on the stage as shown in Fig. 22/2.

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Insertion of the depolarizer

A depolarizer is absolutely necessary particularly for examinations of dichroic and pleochroic materials. For the Axiolab, the depolarizer 453675-0000-000 is offered as an accessory.

Assembly

■ Loosen screw (23/4) using the ball-headed screwdriver (23/5), remove the tube from the stand and turn it around.

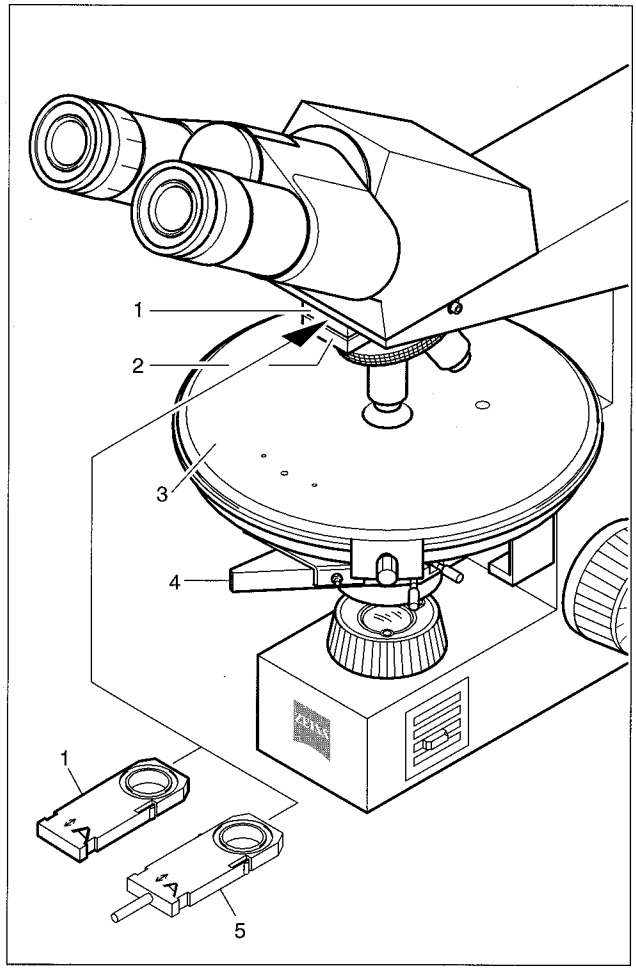
■ Screw depolarizer (23/3) into the opening of the dovetail (23/2).

IMPORTANT:
The number on the mount must be upside down.

■ Attach tube to the stand again, align it and tighten the screw (23/4).

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Polarization in transmitted light –
Detection of birefringence

This technique is used for the examination of transparent, birefringent objects. Birefringence can be recognized, with crossed polarizer and analyzer, by the otherwise dark field of view being brightened 4 times when the specimen stage is rotated about 360°. Irrespective of birefringence, thickness and orientation of the object, the interference colors range from just visible gray (e.g. in biological objects) to white, yellow, red, blue, etc. and high-order white.

- Set the microscope as usual for brightfield examinations.

- Swing in polarizer (24/4).

- Push in analyzer (24/1) until stop so that the field of view is dark.

Note:
When a (±5° rotary analyzer (24/5) is used, the analyzer must be in the center stop position.

- Move the object into the field of view and turn the Pol rotary stage (24/3) containing the object.

Note:
Birefringence is indicated by the either colorless or colored brightening of the object. However, optically anisotropic materials can also remain dark if an isotropic direction, e.g. of optically uniaxial or biaxial crystals, is oriented parallel to the observation direction.
The conoscopic viewing method makes it possible to find out whether the object is isotropic or anisotropic.

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Polarization in transmitted light –
Determination of the vibration direction nγ
and determination of the path difference

Application:
The position of the two directions with the relatively highest (nγ) and the relatively lowest (nα) refractive index, or the absolutely highest (nγ) and the absolutely lowest (nα) refractive index in relation to morphological directions of crystal surfaces, crystal needles or fibers, is an important criterion for recognition. It is used, for example, for the diagnosis of biocrystals (gout, pseudogout).

Settings:
■ Set the microscope as usual for brightfield examinations.
– The fiber lights up in the field of view.

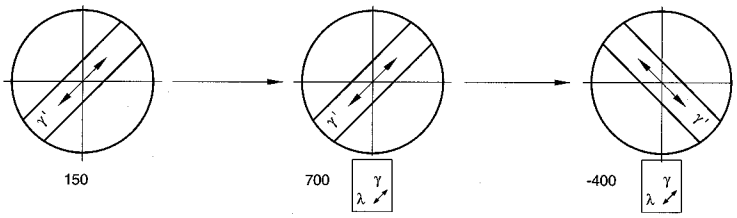
■ Turn stage until the first stop position, loosen stop screw and turn the stage until the object features maximum darkness.

■ Activate stage stop mechanism and turn stage around 45° until the next stop so that the longitudinal axis of the fiber is oriented in NE-SW direction.
– The object now features its maximum brightness (diagonal position), e.g. grayish white.

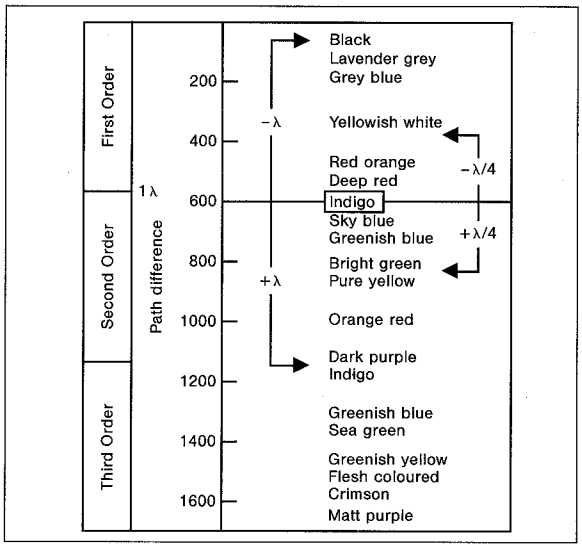
■ Allocate a path difference of approx. 150 nm to this color on the MICHEL LEVY color chart.
– When the λ-compensator (24/2) is pushed in, the color of the fiber changes to yellow-orange (path difference approx. 500 nm).
– When the stage is turned around 90°, the color of the fiber changes to greenish blue (path difference approx. 700 nm).

Conclusions:
The nγ direction of the λ-compensator is NE-SW oriented. The environment of the fiber exhibits a dark, first-order red (path difference is one λ, approx. 550 nm). The fiber itself appears greenish blue (path difference approx. 700 nm). The higher interference color (700 nm) can only have been created by addition of the path differences of the object (approx. 150 nm) and that of the λ-compensator (approx. 550 nm), while the lower one (approx. 400 nm) must have been created by subtraction.
Addition is possible only if nγ of the compensator and nγ of the object are parallel. Therefore, nγ of the object also lies in NE-SW direction in the case of a higher interference color and is oriented parallel to the longitudinal axis of the fiber.

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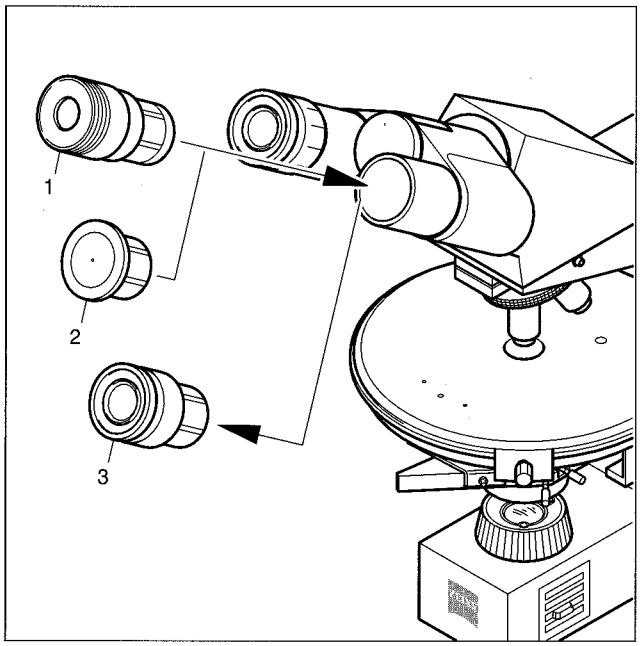


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Summary:
Compare the interference colors (path differences) in the two diagonal positions. The higher path difference results if both nγ directions are parallel, which defines the nγ direction of the object.

Note:
If the thickness of the specimen is known or can be measured, the MICHEL LEVY color chart (also see Fig. 26) can be used to determine the birefringence $\Delta n\gamma$ of minerals, strained glass, biocrystals or erythrocytes depending on the orientation of the object. Starting at the intersection point of the Γ-value (e.g. 551 nm) and of the thickness d (e.g. 30 µm), the inclined line is followed to the outside right up to the edge of the chart. Here, the birefringence (e.g. 0.018) can be read off.

Polarization in transmitted light –
Determination of the optical character of crystals

Application:
The optical character of transparent and weakly absorbing crystals must be determined for the diagnosis of crystals. The determination is made in conoscopic observation. The main field of application is classical petrography. However, it is also possible to identify and characterize synthetic crystals, industry minerals and plastics (e.g. films).

Settings:
■ Set the microscope in the same way as for usual bright-field examinations and for polarization in transmitted light.

■ Swing in low-power objective.
– In conoscopic observation, those crystals (e.g. of a mineral section) are optimally oriented which alter the brightness least during the stage rotation. In that case, the optical axis of uniaxial crystals or one of the optical axes of biaxial crystals is almost parallel to the observation direction.

■ Move one such crystal into the center of the switchable crosslines. Then swing in the objective with the highest dry aperture (40x/0.65) and the condenser front lens.

■ Check whether the condenser stop is fully open and lift the condenser until the image of the luminous-field diaphragm is in focus.

■ Now close the field of view diaphragm until the grain borders of the selected crystal are no longer visible. This avoids the axis image of the examined crystal being overlaid by axis images of neighboring crystals. When the stage is turned, the object must be oriented to the center of the crosslines. Therefore, it remains in the cut-off area durig rotation.

Note:
For conoscopic viewing, the fixed eyepiece (right or left) (27/3) is removed and replaced with the diopter pinhole 444020-0000-000 (27/2) or the auxiliary lens 444830-0000-000 (27/1).

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Uniaxial crystals
If the optical axis of a uniaxial crystal is oriented parallel to the observation direction, a dark cross which can be surrounded by concentric interference rings (depending on birefringence and specimen thickness) becomes visible in the conoscopic observation mode. These interference rings are also called isochromats (from the Greek isos = equal and chroma = color).

The cross is maintained when the stage is turned. Observe the NE quadrant of the cross (1st quadrant; counting is made counterclockwise).

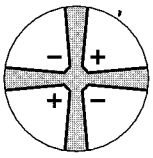
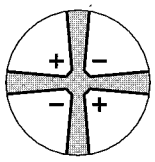
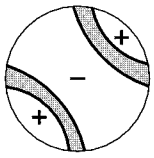
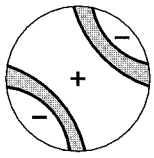
If you use the λ-compensator:
■ After insertion of the λ-compensator, the following appears in the first and third quadrant near the center of the dark cross:
– blue = optically negative
– yellow = optically positive

Biaxial crystals
If biaxial crystals show a cross in conoscopic observation which is resolved into two hyperbola legs when the stage is turned, the acute bisectrix (1st center line) is oriented parallel to the observation direction. Turn the stage until the dark hyperbola legs (isogyres) are in the first and third quadrant.

If you use the λ-compensator:
■ The following appears after insertion of the λ-compensator:
– blue = optically negative
– yellow = optically positive

Note:
If one optical axis of a biaxial crystal is oriented parallel to the observation direction, only one hyperbola leg is visible in conoscopic observation, the vertex of which lies in the center of the field of view. When the stages are turned, the hyperbola leg moves around its vertex. The determination of the optical character is analog.

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	Optically uniaxial		Optically biaxial		
	positive	negative	positive	negative	
λ-plate (white → blue → yellow)					+ = blue – = yellow

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