Operating Manual

Axiovert 40

Inverted Microscope

The manual is not covered by an update service.

Issued by:

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CONTENTS

Page

2 OPERATION

Page

LIST OF ILLUSTRATIONS

Page

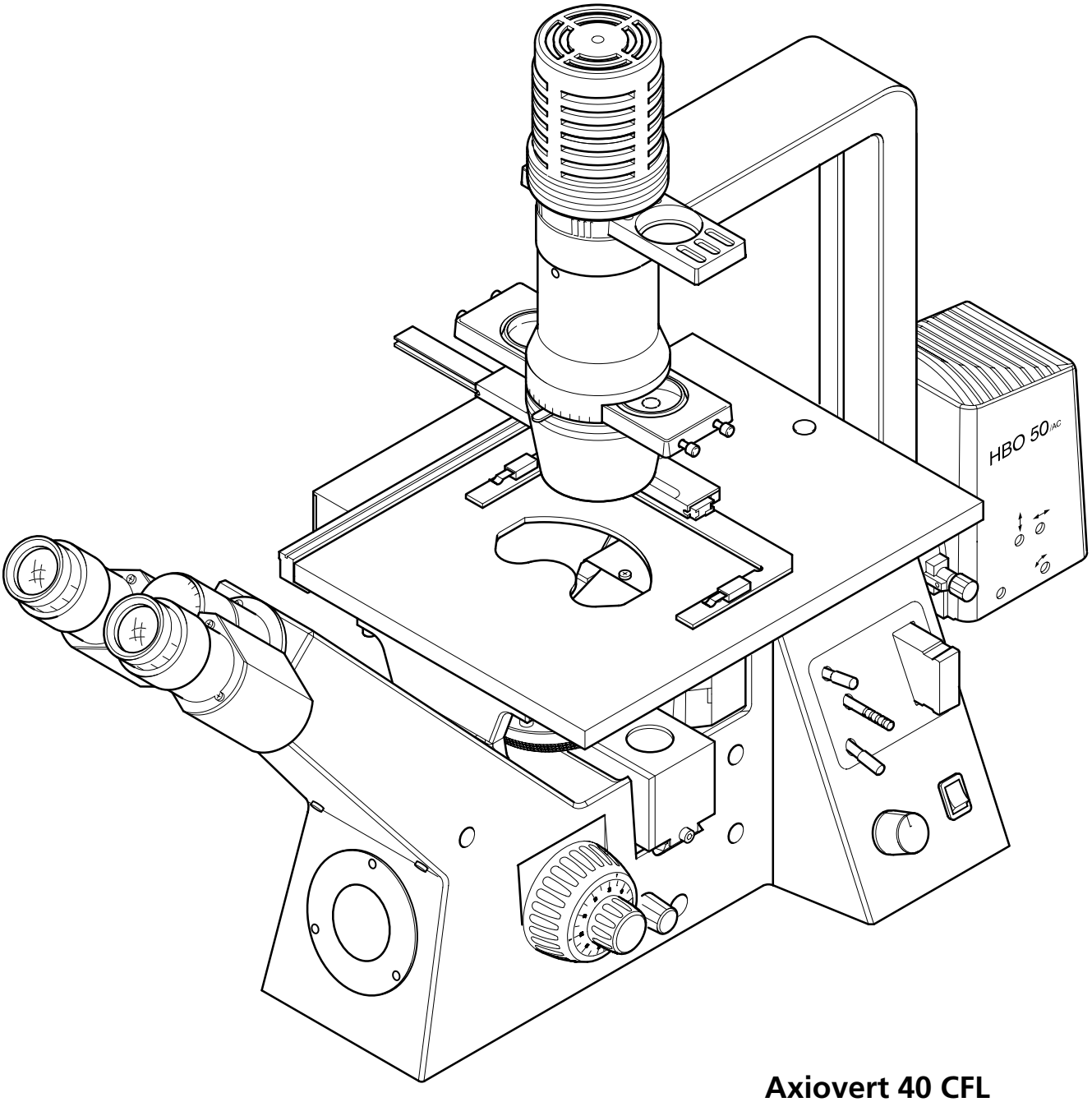
NOTE

- The figures integrated in the text are numbered and captioned.
  "Figure 2-8", for example, refers to figure No. 8. Items referred to in the text are marked by a reference line and an item number. For example, "eyepiece tube (2-8/**4**)" means that the eyepiece tube in figure 8 in section 2 is marked with the item number **4**.

- Abbreviations are explained in the Annex.

- This operating manual refers to the Axiovert 40 configurations (see section 1.4) including accessories. When working with other instrument models, the instructions apply analogously.

GENERAL VIEW



Axiovert 40 CFL

NOTES ON INSTRUMENT SAFETY

The Axiovert 40 microscopes have been designed, produced and tested in compliance with DIN 61010-1 (IEC 1010-1) and DIN EN 61010-2-101 safety requirements for electrical measuring, control and laboratory instruments, and meet the requirements of Appendix I of directive 98/79/EC.

Conformity to the given standards is documented by the CE marking.

This operating manual includes information and warnings that must be observed by the user.

NOTE
This symbol is a warning, which you must observe under all circumstances.

CAUTION
This symbol is a warning, which indicates a hazard to the instrument or instrument system.

CAUTION
This symbol is a warning, which indicates a hazard to the user of the instrument.

CAUTION
Hot surface!

CAUTION
Emission of UV radiation!

CAUTION
Disconnect the instrument from the line before opening it!

The Axiovert 40 microscopes, including original accessories, may only be used for the microscope techniques and applications described in this manual.

Particular attention must be paid to the following warnings:

The manufacturer cannot assume any liability for any other applications, including that of individual modules or single parts. This also applies to all service or repair work that is not carried out by authorized service personnel. All warranty claims shall be forfeited.

Axiovert 40 Inverted Microscopes are classified as Protection Class I instruments.
The power plug must be inserted in an outlet featuring a grounding (earth) contact. The grounding effect must not be made ineffective by an extension cable that does not have a protective ground wire.

If it is determined that protection measures are no longer effective, the instrument must be switched off and safeguarded against inadvertent operation. Please contact a Zeiss service agency or the Carl Zeiss Microscopy Service to repair the instrument.

The Axiovert 40 Inverted Microscopes are equipped with a new, wide input range power supply allowing line voltages to be used in the range between 100 and 240 V AC ±10 %, 50 / 60 Hz, without the voltage setting on the instrument having to be changed.

Before switching on the HBO 50 power supply, check whether it is suitable for the available line voltage. Always disconnect the instrument from the power outlet before opening the instrument and before changing the fuses.
Take care to ensure you only use fuses of the rated power required. Use of makeshift fuses and short-circuiting of the fuse holders are not permitted.

The Axiovert 40 Inverted Microscopes are not equipped with any special devices for protection from substances that are corrosive, potentially infectious, toxic, radioactive, or other substances that could be hazardous to health. Make sure to observe all legal regulations, particularly the relevant national accident prevention regulations when handling such substances.

Be sure to read the safety notes provided with Immersol 518 N® immersion oil.

Immersol 518 N® immersion oil irritates the skin. Avoid any contact with skin, eyes and clothing.

After skin contact, wash the oil off with plenty of water and soap.
After eye contact, immediately rinse the eye with plenty of water for at least five minutes.
If the irritation persists, consult a specialist.

Proper disposal.
Take care to ensure that immersion oil does not enter surface water or the sewage system.

Avoid touching the hot lamp housing. Always pull the power plug before changing the lamps and allow the instrument to cool down for some 15 minutes.

When fluorescence filters are used, the heat protection filter for heat emitted by the microscope illuminator must not be removed, since fluorescence filters are sensitive to heat, and their performance could be impaired.

Dust and dirt may impair the instrument's performance. Therefore, the instrument must be protected from these influences as far as possible and covered with the dust cover when not in use. Always check whether the instrument is switched off before you cover it.

Placing objects against or covering ventilation slats may lead to heat build-up that will damage the instrument and, in extreme cases, cause a fire. Always keep the ventilation slats clear and ensure that no objects enter the instrument through the ventilation slats.

The instruments may only be operated by trained personnel who must be aware of the possible dangers involved with microscopy and the particular application concerned. The Axiovert 40 Inverted Microscopes are high-precision instruments that can be impaired in their performance or destroyed when handled improperly.

Gas-discharge lamps, e.g. XBO 75, HBO 50 or HBO 103, emit ultraviolet radiation, which can cause burns to the eyes and skin. Therefore, never look directly into the light of these lamps and avoid direct, unprotected incidence of their light on your skin. When using the microscope, always use the protective devices belonging to the instrument (e.g. special attenuation filters). When they are hot, gas-discharge lamps are under high internal pressure. Therefore, change them only when they have cooled down, and make sure to wear protective gloves and goggles (for detailed information please see Operating Manual B 40-065 e).

Defective microscopes must not be disposed of with household waste. Dispose of the microscope in compliance with the relevant legal requirements.

1 Description

1.1 Name and intended use

Manufacturer's designation: Axiovert 40 Inverted Transmitted-Light Microscope

In the product family of inverted transmitted-light microscopes, the Axiovert 40 microscopes rank as follows:

Laboratory microscopes	**Research microscopes**
- Axiovert 40 C	- Axiovert 200
- Axiovert 40 CFL	- Axiovert 200 M

The Axiovert 40 microscopes are universally applicable modular light microscopes of inverted design (inverted microscopes). They are used in biology and medicine for the examination of plant and animal cells and/or tissue specimens, but also of specimens of the human body. Observation and cultivation vessels include, for instance, culture bottles, Petri dishes and microtiter plates.

The following microscopy techniques are possible:

Transmitted light	- Brightfield
	- Phase contrast
	- VAREL
	• Oblique brightfield illumination
	• Unilateral darkfield illumination
	- PlasDIC
Incident light	- Fluorescence

1.2 Instrument description

The Axiovert 40 microscopes are available as high-performance desktop units with two different microscope stand versions:

- **Axiovert 40 C**	Inverted transmitted-light microscope with HAL 12 V 35 W illuminator and documentation
- **Axiovert 40 CFL**	Inverted transmitted-light/incident-light fluorescence microscope with HAL 12 V 35 W and HBO 50 illuminators and documentation

Major instrument features include:

- Modular design for optimum adaptation to applications
- Microscope stand of compact design; ergonomic operation provided by a stage height of only 185 mm on Axiovert 40 C and low-positioned controls
- Integrated 12 V 35 W power supply
- Continuously adjustable illumination intensity
- Upright, non-reversed images
- Fixed Köhler illumination, changeable condenser, numerical apertures of 0.55, 0.4 or 0.2 and working distances of ≥ 31 mm, ≥ 53 mm or ≥ 90 mm; brightfield illumination for large object fields for vessel heights of up to 190 mm
- User-friendly phase contrast by means of slider with two annular diaphragms
- Brightness compensation by attenuation filters providing dazzle-free switching between phase contrast and brightfield illumination
- VAREL (variable relief) with continuous transition from unilateral darkfield to oblique brightfield, contrasting of the wells of microtiter plates up to the edge without special preparations
- PlasDIC for relief-like imaging especially of thick objects with variable contrasting, contrasting of the wells of microtiter plates up to the edge
- Ph and PlasDIC: Simple changeover to phase contrast or PlasDIC
- Incident-light fluorescence contrast usable alternatively to or simultaneously with brightfield, phase contrast and VAREL
- Possibility of adapting incident-light fluorescence to the respective application by replacing the fluorescence filter combination
- Variable stage use by mounting the attachable object traverser M
- Use of different mounting frames including marker strips for various culture vessels
- Specimen stage can be replaced by specimen stage glass for optimum visibility of the nosepiece, a heatable stage or a temperable microscope stage for demanding experiments
- Standardized mounts for objectives, eyepieces and lamps
- Photo/video port for single-reflex cameras, compact digital cameras, digital cameras, e.g. AxioCam, and video cameras

1.2.1 Mechanical design

Axiovert 40 C stand

- Integrated wide input range power supply powering a 12 V 35 W halogen lamp and accepting line voltages of 100 to 240 V AC without the need for changing the voltage setting
- NA 0.55, 0.4 and 0.2 condensers; replaceable and movable
- Coaxial coarse and fine drive with focusing acting on the nosepiece
- Binocular tube adjustable to two heights with one fixed and one focusing eyepiece
- Quintuple nosepiece
- Specimen stage prepared for fitting the attachable object traverser M with the possibility of using different mounting frames
- Optional diaphragm sliders for VAREL and phase contrast
- Photo/video port

Axiovert 40 CFL stand

- Same as Axiovert 40 C
- Optional diaphragm sliders for VAREL, phase contrast and PlasDIC
- Upgradeable for fluorescence by HBO 50, FL incident-light fluorescence equipment, 3-position reflector slider P&C, reflector modules FL P&C with fluorescence filter sets

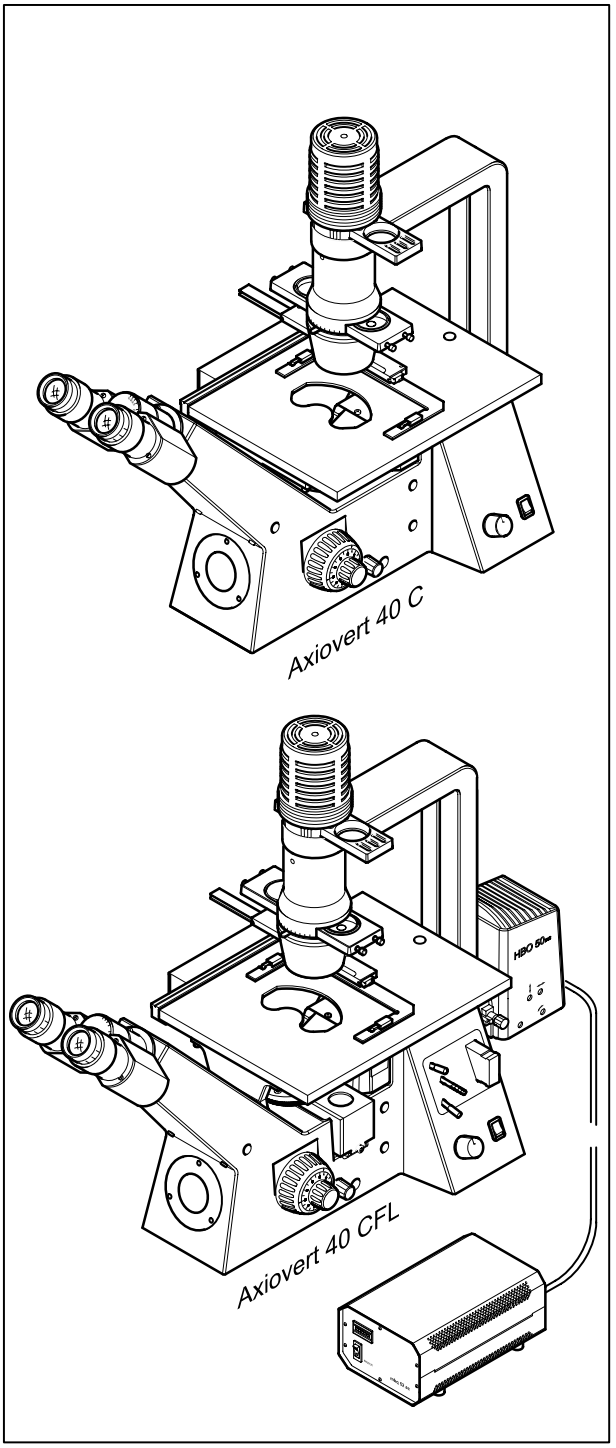


Fig. 1-1 Instrument models

1.2.2 Optical design

(shown using the example of the Axiovert 40 CFL)

The tried and tested ICS optics (Infinity Color-corrected System) guarantees high optical performance for all methods (up to field-of-view number 20, tube factor 1x). Different combinations of objectives and eyepieces provide optimum customization to the intended application.

Depending on the configuration, the Axiovert 40 microscopes are equipped with a 12 V 35 W halogen lamp and a HBO 50 illuminator.

An universal port is available for documentation. Type-specific adapter modules are available for photomicrography and video microscopy.

The correct color temperature for color photography is obtained automatically at full lamp voltage.

A light path selector directs 100 % of light either to the viewing or the photo/video port.

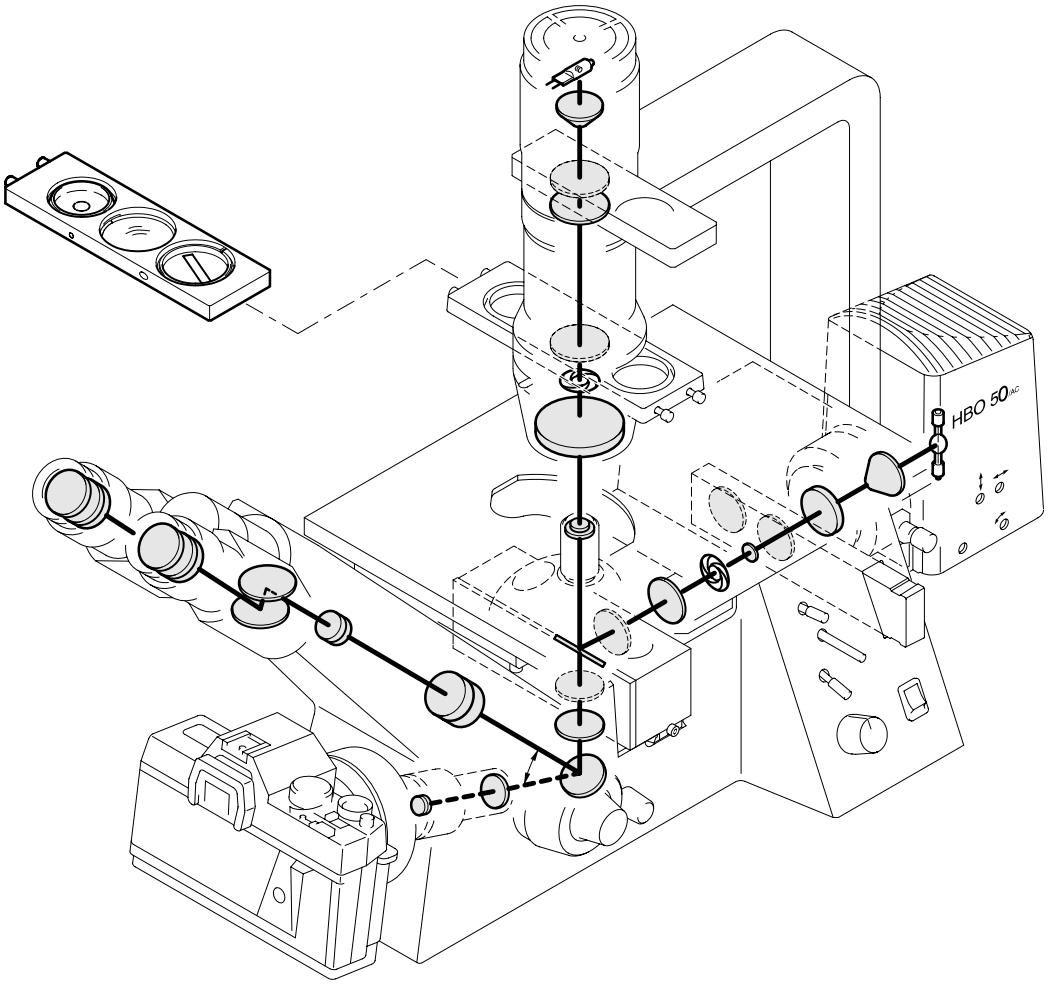


Fig. 1-2 Optical diagram of Axiovert 40

1.3 Technical data

(1) Dimensions and weight

Dimensions (width x depth x height)

Axiovert 40 C	245 x 556 x 505 mm
Axiovert 40 CFL	245 x 666 x 530 mm

Foot print

Axiovert 40 C	245 x 362 mm
Axiovert 40 CFL	245 x 362 mm
Power supply for HBO 50	150 x 200 mm

Weight

Axiovert 40 C	Approx. 14 kg
Axiovert 40 CFL	Approx. 16 kg

(2) Ambient conditions

Transport (in packaging)

Permissible ambient temperature	-40 to +70 °C

Storage (in packaging)

Permissible ambient temperature	+10 to +40 °C
Permissible relative humidity	≤ 75 %

Operation

Permissible ambient temperature	+10 to +35 °C
Permissible relative humidity	≤ 75 %
Altitude of installation site	2000 m
Atmospheric pressure	800 hPa to 1060 hPa
Pollution degree	2

(3) Operating data

Operating environment	closed room
Protection Class	I
Protection Type	IP 20
Electrical safety	in compliance with DIN EN 61010-1 (IEC 1010-1) including CSA and UL directives
Overvoltage category	II

Radio interference suppression	in accordance with EN 55011, Class B
Noise immunity	in accordance with DIN EN 61326 / A 1
Line voltage	100 to 240 V AC (±10 %)
Line frequency	50 to 60 Hz
Power consumption of internal power supply	max. 100 VA

mbq 52 ac-z transformer for HBO 50

Operating environment	closed room
Protection Class	I
Protection Type	IP 20
Line voltage, switchable between	100, 110, 120, 127, 230, 240 V AC
Line frequency, switchable between	50 and 60 Hz
Power consumption if operated with HBO 50	max. 350 VA

Fuses acc. to IEC 127

Microscope stand Axiovert 40 C	T 1 A/H, 5x20 mm
Mikroskopstativ Axiovert 40 CFL	T 1 A/H, 5x20 mm
mbq 52 ac-z transformer for HBO 50	100 V to 127 V: 2x T 4 A 220 to 240 V: 2x T 2.5 A
HBO 100W power supply	T 2.0 A/H, 5x20 mm

(4) Light sources

Halogen bulb	12 V 35 W
Adjustment of light source	continuous, ≤ 1.5 to 12 V
Mercury-vapor lamp for fluorescence	HBO 50
Power consumption of HBO 50 power supply	350 VA

(5) Optical/mechanical data

Stand with objective focusing	with coarse drive (7.5 mm/rev) and fine drive (0.75 mm/rev) Total lift > 8 mm (focus position ranging from 1 mm below to > 7 mm above the stage surface)
Binocular tube	interpupillary distance adjustable from 55 to 75 mm with constant tube length

Viewing port	tube factor 1x
Variable viewing height (2 positions)	for interpupillary distance 56 mm → 350 or 390 mm 65 mm → 355 or 385 mm
Viewing angle	45°
Specimen stage (width x depth)	210 x 290 mm working height on Axiovert 40 C 185 mm working height on Axiovert 40 CFL 210 mm
Attachable object traverser M, attachable on left or right side	allowing the use of mounting frames M search range 130 x 85 mm
Working distance for specimen vessels (between stage and condenser)	≥ 31 mm for condenser 0.55 ≥ 53 mm for condenser 0.4 ≥ 90 mm for condenser 0.2 extendable to 190 mm through slide-out condenser
Condenser 0.2, 0.4 or 0.55	adjustable aperture diaphragm with scale, max. aperture 0.2, 0.4 or 0.55 mount for diaphragm sliders
Objectives	ICS objectives with W 0.8" x 1/36 thread
Objective change	manual via quintuple nosepiece
Eyepieces	30 mm tube diameter use of eyepieces up to field-of view number 20 standard: E-PL 10x/20 Br. and E-PL 10x/20 Br. foc
Photo/video port	for connection of: video adapter V25 C 1/2" (3 CCD) 0.5x for video cameras; video adapter V25 ENG 1/2" (3 CCD) 0.5x for video cameras; video zoom V25 C 1/3" (3 CCD) 0.32x-0.8x for video cameras; video adapter V25 C 1" (3 CCD) 1.0x for Axiocam digital camera; camera adapter V25 T2 2.5x for SLR cameras; adapter V25 1.0x, d=30 for microscope camera with digital camera adapter D 40 M37/52x0.75 for compact digital cameras
3-position reflector slider P&C	for accommodation of 3 optionally selectable reflector modules P&C

1.4 System overview

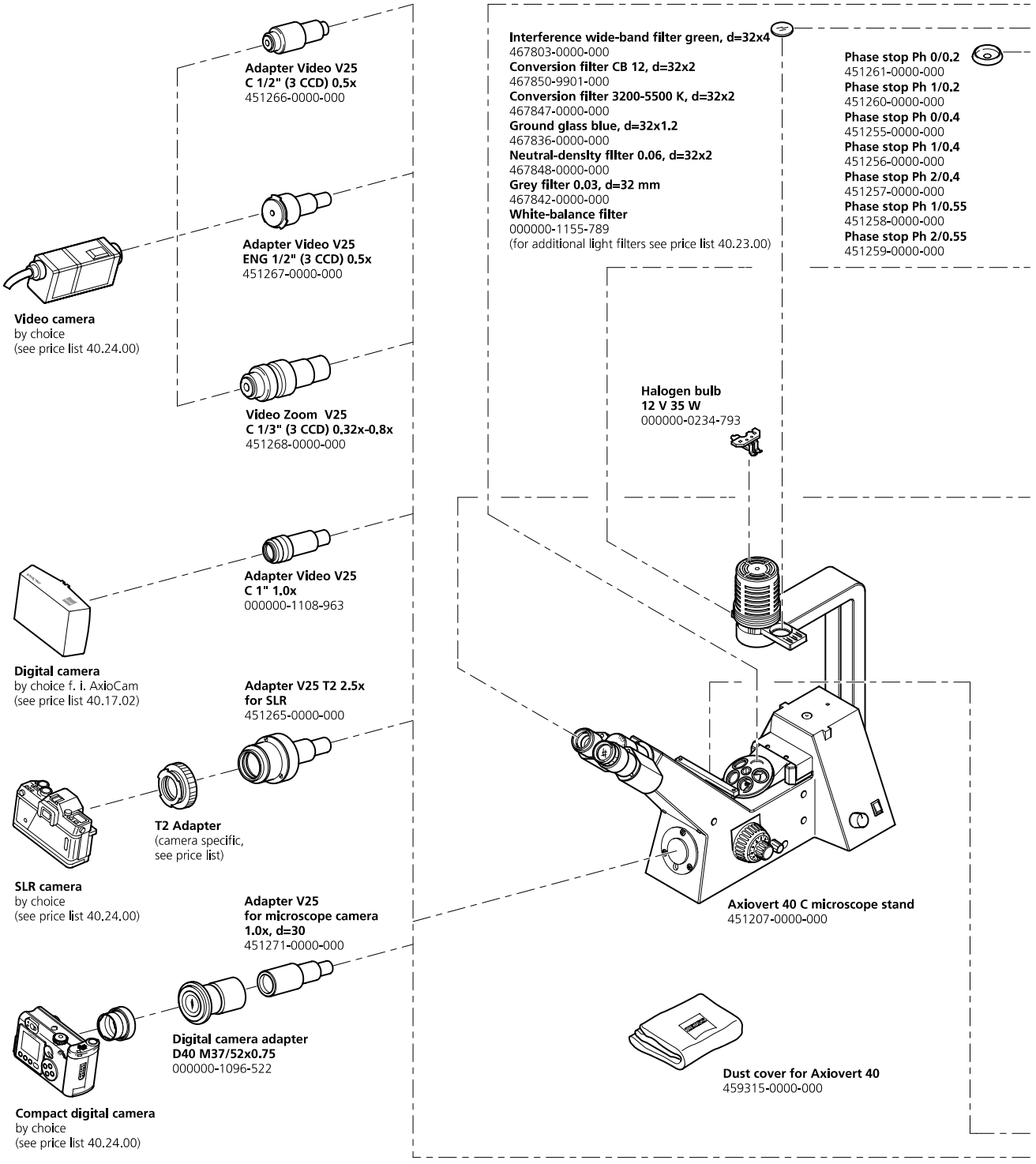


Fig. 1-3/1 Axiovert 40 system overview

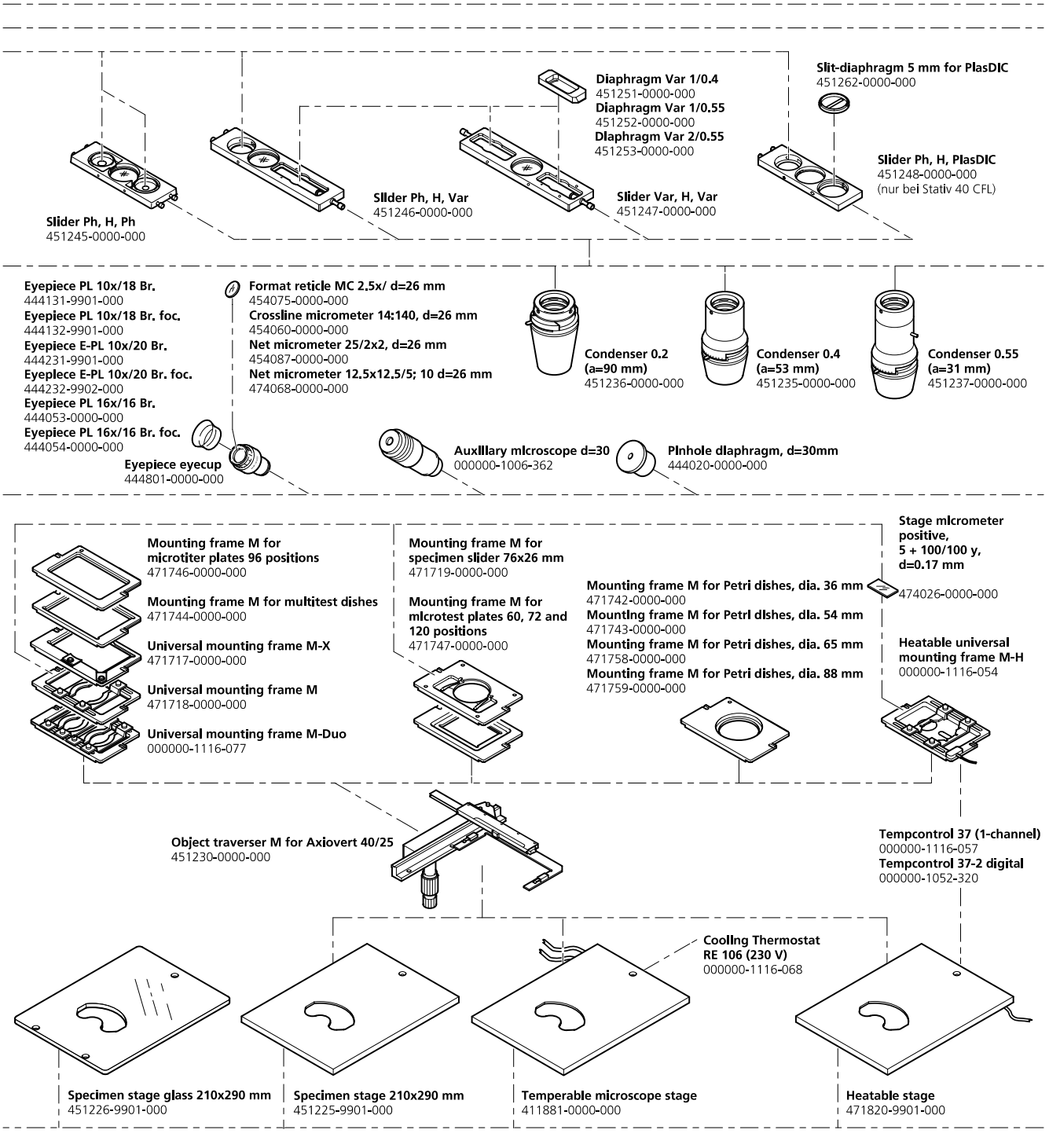


Fig. 1-3/2 Axiovert 40 system overview

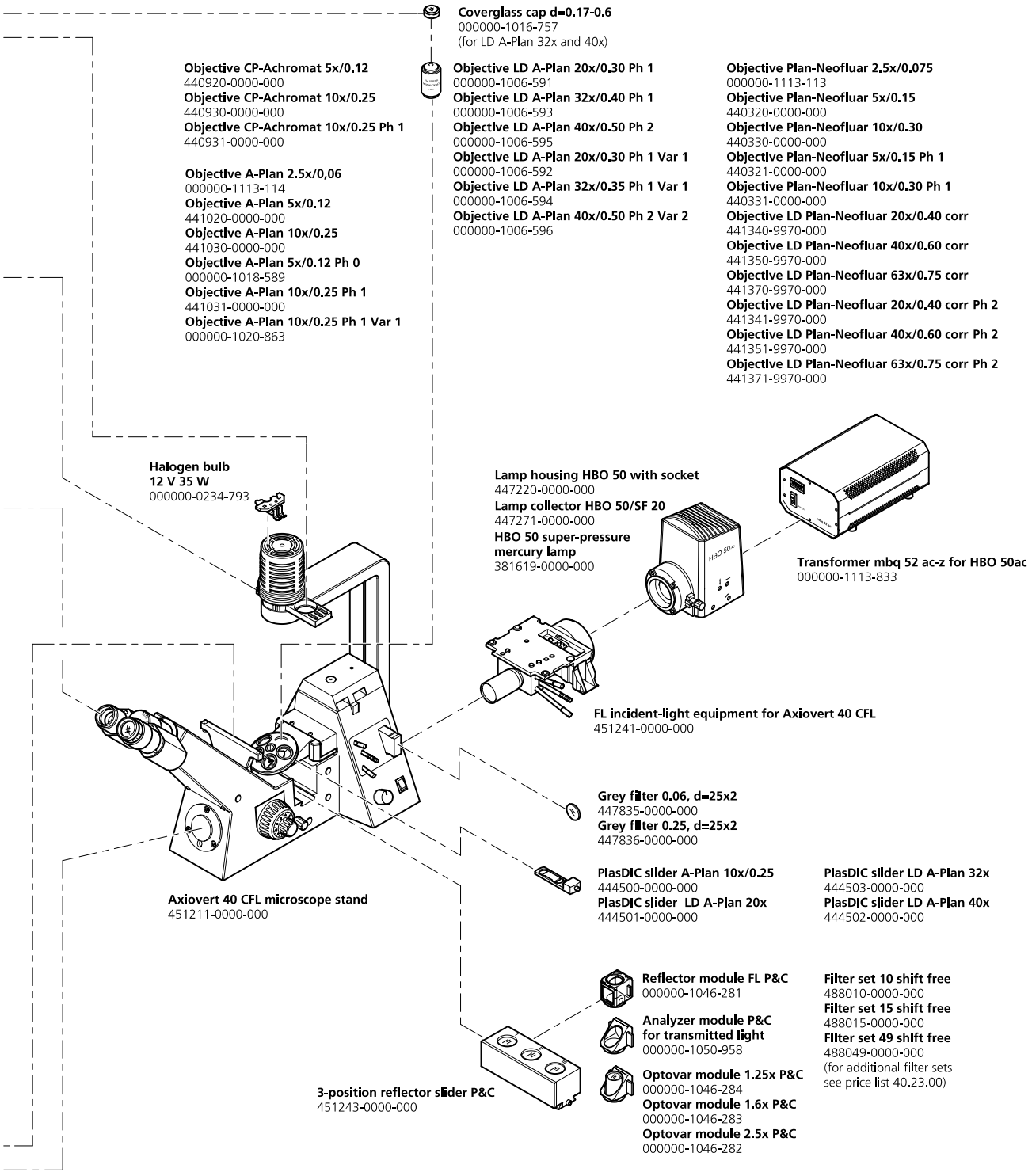


Fig. 1-3/3 Axiovert 40 system overview

2 Operation

2.1 Installation of the microscope

2.1.1 Unpacking

The Axiovert 40 models including their accessories are supplied in customary packaging. It is advisable to retain the transport containers in case the instrument must be stored for a longer period of time or returned to the manufacturer.

- Open the packaging.
- Remove the cardboard box (2-1/**1**) containing the accessories.
- Holding the polyethylene packing (2-1/**2**) of the microscope stand at the grip openings (2-1/**3**), remove it from the cardboard box and place it on the side.
- Remove the upper part of the packing.
- Remove the microscope stand from the lower half of the packing, but do not hold it at the illumination arm or the binocular tube.
- Make sure all items specified on the packing list are present.
- Store the packing material in the transport box or dispose of it as indicated on the labels.

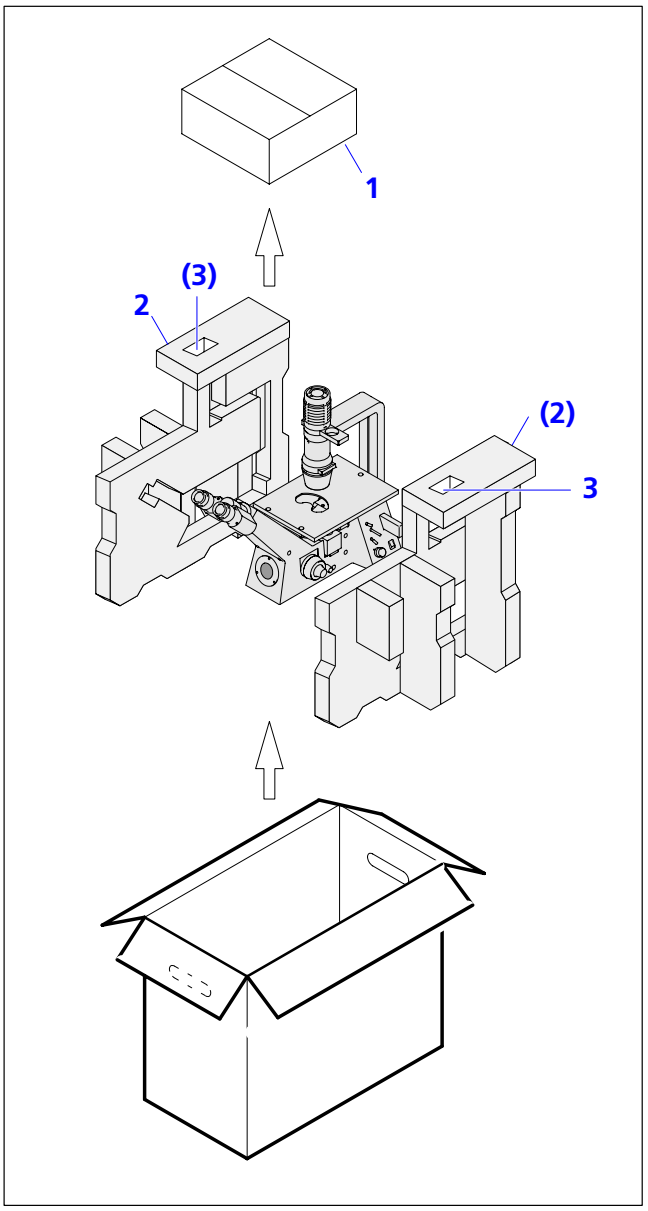


Fig. 2-1 Axiovert 40 packing units

2.1.2 Installation

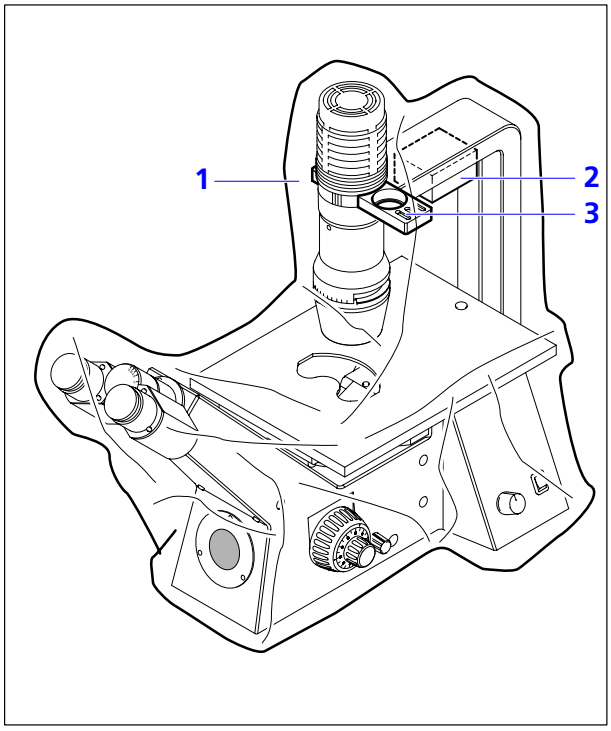


Fig. 2-2 Unpacking and installation

(1) Preparations

- Place the microscope stand on a suitable tabletop.
- Remove the plastic cover (2-2/**1**).
- Remove the foam part (2-2/**2**) that locks the condenser slider.
- Remove the foam part (2-3/**1**) located above the nosepiece.

NOTE Filter slider (2-2/**3**) is firmly mounted; the appropriate filters (green filter, attenuation filter) are not inserted during transport.

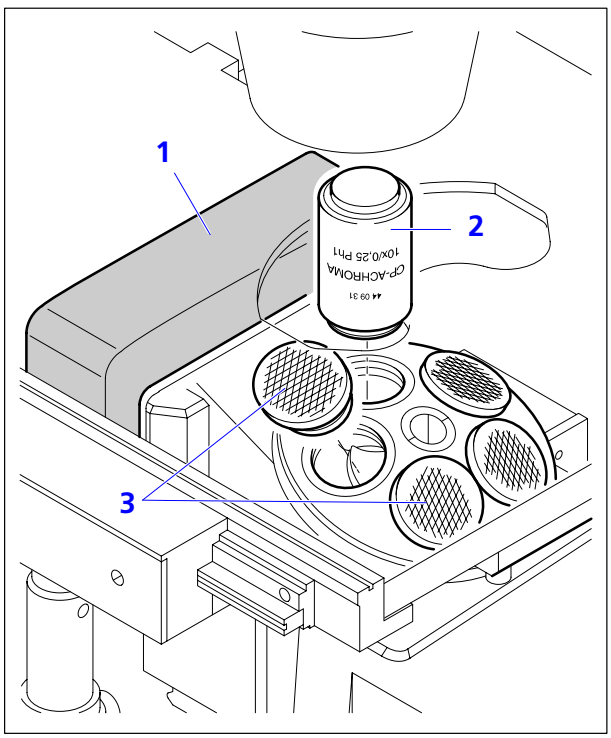


Fig. 2-3 Screwing in objectives

(2) Screwing in of objectives

- Remove the dust covers (2-3/**3**) from the objective mounts you want to use. Screw the objectives (2-3/**2**) into the mounts in ascending order of magnification factors.

(3) Inserting diaphragm sliders

- Remove dust protection slider (2-4/**1**) from the slider mount and replace it by the
  - Slider Ph, H, Ph (2-4/**2**) or the
  - Slider Ph, H, Var (2-4/**3**) or the
  - Slider Var, H, Var (not illustrated) or the
  - Slider Ph, H, PlasDIC (2-4/**4**).

  Push it into the middle position (brightfield).

NOTE The label on the slider must be legible upright and the inserted diaphragms must correspond to the condenser/ objective combination used (refer to the table on page 2-14).

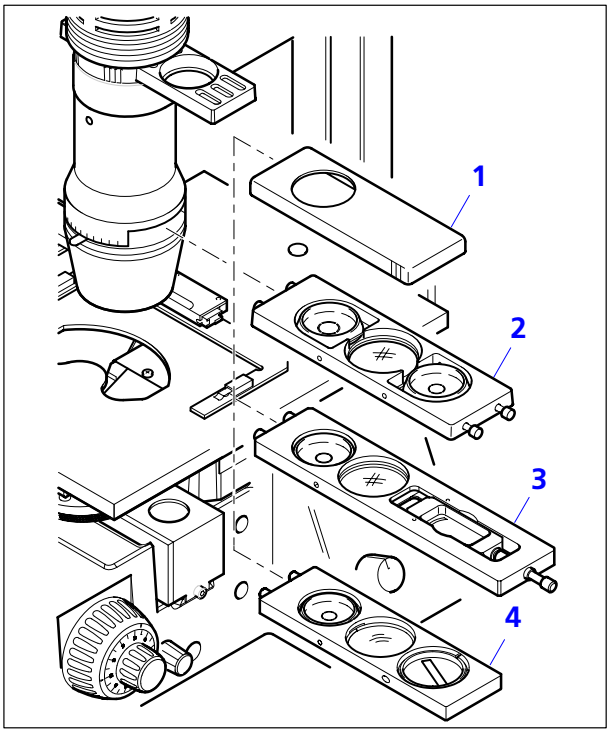


Fig. 2-4 Inserting diaphragm sliders

(4) Mounting the HBO 50 illuminator

- Attach the HBO 50 illuminator (2-5/**3**) equipped with mercury vapor short-arc lamp to the corresponding dovetail mount of the Axiovert 40 CFL and tighten the fastening screw by means of the SW 3 ball-headed screwdriver.
  For information on lamp replacement, refer to Section 3.2 (3).

- Establish the connection between the illuminator and the HBO 50 power supply (2-5/**1**). Connect the power cable (2-5/**2**) to the power outlet.

- Switch on the power supply.

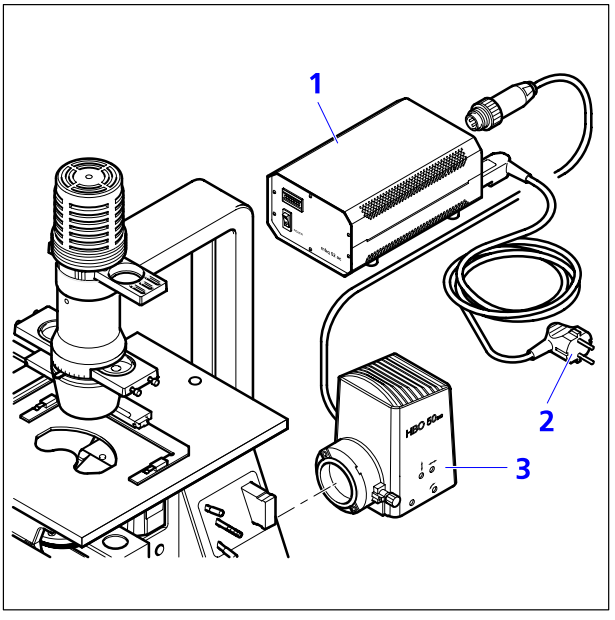


Fig. 2-5 Mounting the HBO 50 illuminator

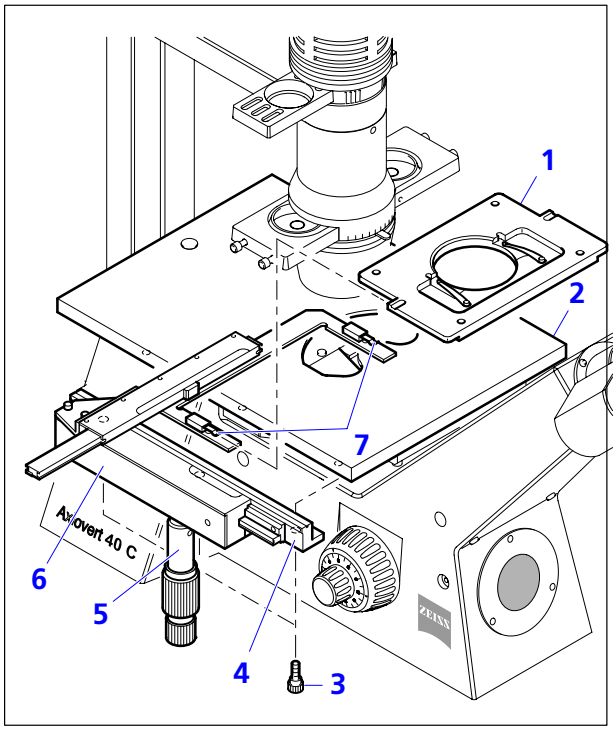


Fig. 2-6 **Attaching the attachable object traverser M**

(5) Mounting the attachable object traverser M

- Attach the attachable object traverser M for Axiovert 40 (2-6/**6**) to the left or right of the specimen stage (2-6/**2**) and secure it from below with three knurled screws (2-6/**3**).

NOTE The L rail (2-6/**4**) of the attachable object traverser M must be flush with the specimen stage.
The attachable object traverser M is moved by means of a coaxial drive (2-6/**5**) (bottom drive knob for X movement and top drive knob for Y movement).

- Select the mounting frame (2-6/**1**) appropriate for the specimen vessel to be used and fit the corresponding scales in the recesses on the attachable object traverser M and adhere them.

NOTE When using the mounting frames, make sure that the mounting frames are inserted correctly in the attachable object traverser M, i.e. the mounting frames must engage in the two holding clips (2-6/**7**).

- Section 2.5 (”Working with attachable object traverser M and mounting frames”) gives a selection of available mounting frames and the appropriate specimen vessels. However, the vessels are not included in the standard equipment and must be provided by the user.

(6) Connection to the power outlet

CAUTION Verify the operating voltage indicated on the rear panel of the instrument agrees with the available line voltage.

- Plug power cable (2-7/**4**) into the instrument socket and connect to the power outlet.
- Switch on the instrument at the power switch (2-7/**3**) on the right of the instrument.

NOTE When the instrument is switched off, the "0" marking is visible on the power switch.

- The instrument is ready for operation when the microscope lamp is on.
- In the event of a defect, check the two fuses (2-7/**2**):
  for 100…240 V AC: T 1 A/H, 5 x 20 mm (acc. to IEC 127)

For this purpose, remove the fuse holder (2-7/**1**) from the housing by pressing the two springs simultaneously in the direction indicated by the arrows.

CAUTION Only use the correct fuses.

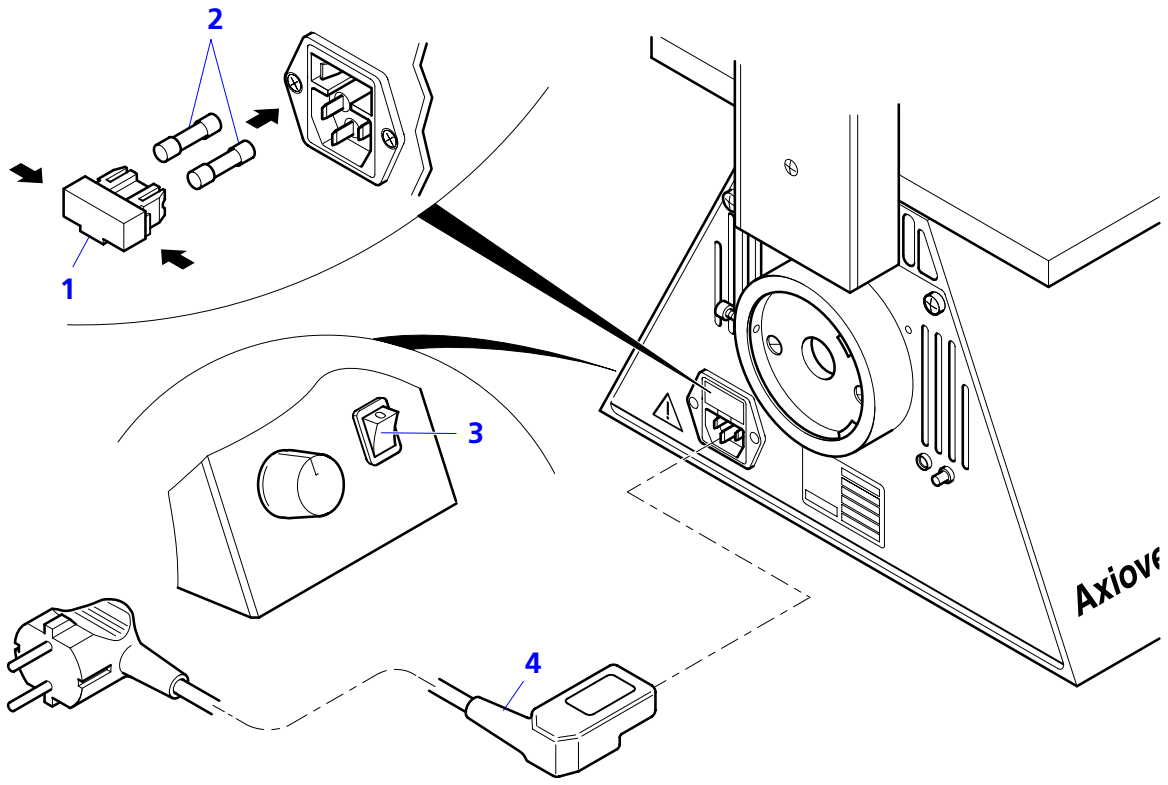


Fig. 2-7 Connection to the power outlet

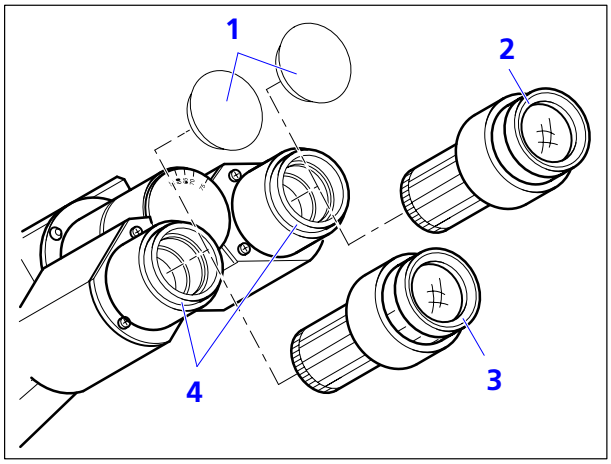


Fig. 2-8 **Inserting the eyepieces**

(7) Inserting the eyepieces

- Remove the dust covers (2-8/**1**) and insert the fixed eyepiece (2-8/**2**, **3**) and the focusing eyepiece in the eyepiece tubes (2-8/**4**).

NOTE Depending on the configuration, one or two eyepieces may permit focusing to compensate for varied degrees of defective vision of the two eyes.

(8) Compensation for defective vision

1) With one fixed and one focusing eyepiece

- Looking through the fixed eyepiece (2-8/**2**), turn the focusing drive to focus on the specimen. Then, looking through the focusing eyepiece (2-8/**3**), turn its eyelens until the specimen is in focus for the other eye, too.

2) With two focusing eyepieces

- Turn the eyelens of the first focusing eyepiece (2-8/**3**) to focus on the eyepiece reticle or, if there is no eyepiece reticle, on the edge of the field of view.
- Looking through the eyepiece with the reticle, turn the focusing drive until the image of the specimen on the specimen stage is in focus.
- Then, when the image of the specimen and that of the reticle are in focus in the above eyepiece, turn the eyelens of the second focusing eyepiece until the image appears sharply for the other eye, too.

NOTE In the last operation, make sure not to change the position of the focusing drive.

(9) Using the binocular tube

- Adjust the eyepiece distance to your interpupillary distance by swinging the eyepiece tubes symmetrically towards or away from one another.
- A higher (2-9/**A**) or lower (2-9/**B**) viewing height is set by swiveling the binocular tube

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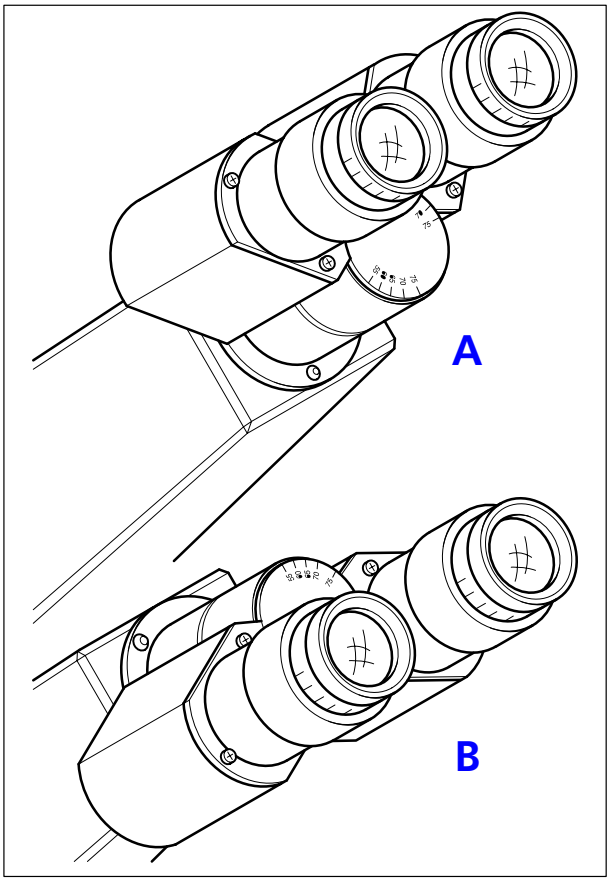


Fig. 2-9 Positions of the binocular tube

(10) Inserting an eyepiece reticle (26 mm dia.)

- The PL 10x/18 Br. foc. and E-PL 10x/20 Br. foc. eyepieces are intended for use with reticles.
- The slight image shift produced by the additional path through the glass is taken into account on the diopter scale by the fact that the zero position is indicated by the red dot (R) instead of the white dot (W).
- The reticles (2-10/**2**) have been cemented into screw-in mounts (2-10/**1**) for easy replacement.
- To replace a reticle, unscrew the entire mount and replace it by one containing the required reticle.
- Finally, check the image focus and readjust it, if necessary (see Section 2.1.2 (8), page 2-6).

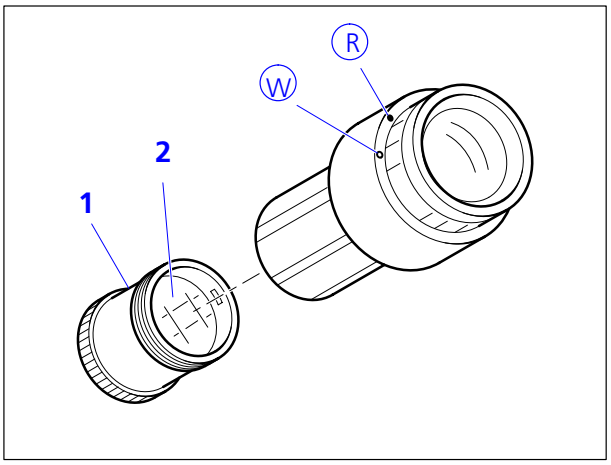


Fig. 2-10 Inserting an eyepiece reticle

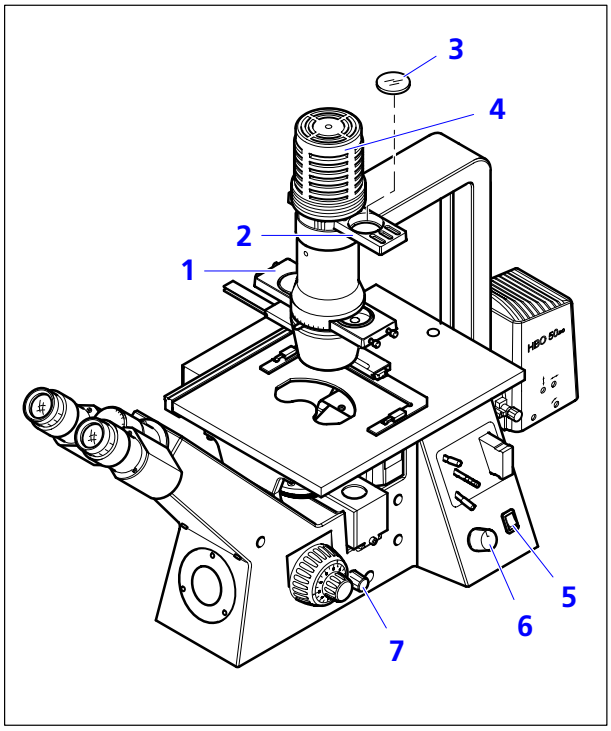


Fig. 2-11 **Switching on/setting the instrument**

(11) Switching on/setting the instrument

- Unless already done, switch on the instrument at the power switch (2-11/**5**).
- After switching on, the lamp in the illuminator (2-11/**4**) must light up.
- Adjust the required brightness by turning the brightness control (2-11/**6**).
- If required, insert the appropriate attenuation filter, green filter, conversion filter or white balance filter (2-11/**3**) in the filter slider (2-11/**2**) and push the slider into the light path.
- Move slider Ph, H, Ph (2-11/**1**, slider Ph, H, Var (2-4/**3**), slider Var, H, Var or slider Ph, H, PlasDIC (2-4/**4**) to its mid-position.

NOTE If no light appears in the viewing path of the Axiovert 40 C or Axiovert 40 CFL, actuate the "observation/camera" path selector switch (2-11/**7**).

2.2 Start-up

When starting up the Axiovert 40 microscope for the first time, the instrument has to be unpacked in accordance with Section 2.1.1, installed in accordance with Section 2.1.2, connected to the power outlet and made ready for operation.

The Axiovert 40 microscope is supplied with factory-centered halogen bulb, which need not be recentered even after you replaced the halogen bulb.
After any change of the lamp of the HBO 50 illuminator, however, you have to center this lamp (for that, refer to Section 3.2 (3)).

Minor adjustment procedures, such as the adjustment of an annular diaphragm, are explained in the sections covering the respective microscopic techniques.

Otherwise, settings are restricted to changing the objectives (swinging them into the light path) and the handling of LD objectives.

2.3 Use of LD objectives

The bottom thickness of the vessels typically used for examination on inverted microscopes considerably differs from the standard coverglass thickness of 0.17 mm.

Normally, such distances are easily bridged by the free working distance (FWD) of low-power objectives:
- CP Achromat 5x/0.12: FWD (in air) 10.9 mm or
- CP Achromat 10x/0.25: FWD (in air) 5.3 mm.

Already in the medium magnification range, however, these working distances are reduced to values around or below 1 mm. Such objectives can then no longer be used for thicker bottoms.

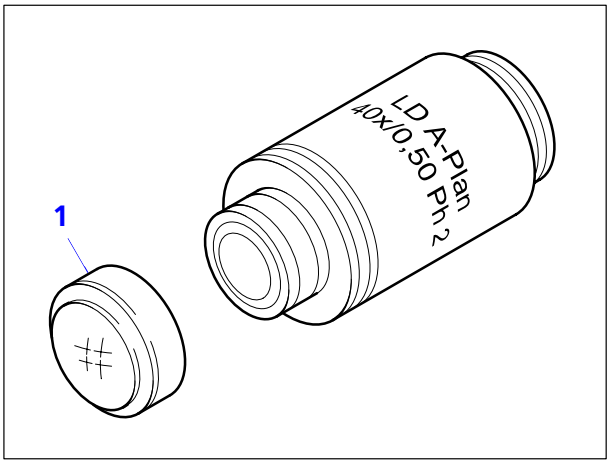


Fig. 2-12 Working with LD objectives

This shortcoming is remedied by special LD (Long Distance) objectives. These objectives feature a relatively long working distance, but also the standard 45 mm parfocalization length of all other objectives.

For adaptation to the bottom thickness in use, plug the corresponding coverglass cap (2-12/**1**) onto the objective (see table below for assignments).

Table of free working distances (FWD) on Axiovert 40

Objective Name	Catalogue No.	FWD without coverglass cap for bottom thickness			FWD with coverglass cap (000000-1016-757) for bottom thickness 0.17 - 0.6
		D=0,17 [mm]	D=1 [mm]	D=1,2 - 1,8 [mm]	
CP-Achromat 5x/0.12	440920-0000-000	10.9	10.4	10.2 ... 9.8	
CP-Achromat 10x/0.25	440930-0000-000	5.3	4.8	4.6 ... 4.2	
A-Plan 2.5x/0.06	000000-1113-114	9.4	8.9	8.7 ... 8.3	
A-Plan 5x/0.12	441020-0000-000	9.8	9.2	9.1 ... 8.7	
A-Plan 10x/0.25	441030-0000-000	4.4	3.9	3.7 ... 3.3	
LD A-Plan 20x/0.30 Ph 1	000000-1006-591	4.8	4.3	4.2 ... 3.8	
LD A-Plan 32x/0.40 Ph 1	000000-1006-593		3.2	3.1 ... 2.7	3.1 ... 2.8
LD A-Plan 40x/0.50 Ph 2	000000-1006-595		2.0	1.9 ... 1.5	1.9 ... 1.6
Plan Neofluar 2.5x/0.075	000000-1113-113	9.55	9.0	8.9 ... 8.5	
Plan Neofluar 5x/0.15	440320-0000-000	13.6	13.1	12.9 ... 12.5	
Plan Neofluar 10x/0.30	440330-0000-000	5.58	5.0	4.9 ... 4.5	
		Corr objectives D=0.17 - 1.5			
LD Plan Neofluar 20x/0.40 corr Ph 2	441341-9970-000	8.34 ... 7.46			
LD Plan Neofluar 40x/0.60 corr Ph 2	441351-9970-000	3.27 ... 2.57			
LD Plan Neofluar 63x/0.75 corr Ph 2	441371-9970-000	2.11 ... 1.24			

The working distances of PhVar objectives correspond to those of the Ph objectives.

Use of objectives with correction mount (corr objectives)

Exact observance of the coverglass thickness is important for high-quality imaging.
For this reason, special objectives are available with a correction mount allowing them to be set to different coverglass thicknesses. To this end, search a specific specimen area, and find out the position of the correction ring where optimum focus and image contrast are obtained (refocusing is invariably required).

CAUTION

1. Because of their large diameter, the installation options of LD corr objectives depend on the nosepiece type used: On the quintuple nosepiece H of the Axiovert 40 C microscope, it is not possible to use adjacent objective mounts. On the quintuple nosepiece (2x H, 3x PlasDIC) of the Axiovert 40 CFL microscope, screw the LD corr objectives in the PlasDIC positions. That way, two LD corr objectives can be accommodated in two adjacent mounts without any restrictions to an adjacent standard objective mount.
2. The specimen plane must not be higher than 2.5 mm above the stage surface, to avoid that the LD corr objective collides with the underside of the specimen stage. Objects on a 1 mm thick vessel bottom can be focused, if the vessel is moved via attachable object traverser M and mounting frame.
   If these conditions are met, all objectives can be swiveled through under the specimen stage or specimen stage glass without colliding with the specimen stage. However, when using the heatable or temperable stage, the nosepiece must be lowered completely, before swiveling in or out the LD corr objectives.

Use of immersion objectives

When immersion objectives are used, the air between the coverglass and the objective is replaced by a liquid, the so-called immersion oil. For this purpose, apply a little, bubble-free drop of Immersol™ immersion oil to the front lens of the objective and put the cultivation vessel or the specimen with the coverglass onto the specimen stage or into the mounting frame so that it is above the objective. Then, carefully lift the objective and focus on the specimen. After every examination, make sure to remove the immersion oil with a soft cloth (possibly moistened with petroleum ether) from the objective. On inverted microscopes, too large amounts of immersion oil may get into the mechanical system and impair its function.

CAUTION Observe the safety notes regarding the use of immersion oil given on page X.

2.4 Illumination and contrast techniques

The description/application of illumination/contrast techniques is based on the following microscope settings:

- The Axiovert 40 microscope has been switched on.
- The brightness control (2-14/**5**) has been turned to mid-position.
- The observation/camera path selector switch (2-11/**7**) has been set to observation.

2.4.1 Use of condensers

Condenser	0.2	0.4	0.55
Working distance between condenser and stage surface	≥ 90 mm	≥ 53 mm	≥ 31 mm plus 6 mm vertical travel
Objective magnification	5x, 10x, 20x	5x, 10x, 20x, 32x, 40x	5x, 10x, 20x, 32x, 40x
Microscopic technique	Brightfield phase contrast	Brightfield phase contrast VAREL (10x up to 32x) PlasDIC	Brightfield phase contrast VAREL PlasDIC

- To fully illuminate object fields even when low-power objectives (< 5x) are used, push condenser (2-14/**3**) out of its work position (dashed line in Fig. 2-14).
- This position of the condenser is also required when particularly high culture vessels are used in order to increase the free working distance to approx. 190 mm.

NOTE Only simple survey illumination is obtained, if no condenser is used!

- When using the condenser again for illumination, move it exactly up to the stop position.

NOTE Loosening the three screws (2-14/**7**) of the condenser changer permits the condenser 0.4 to be replaced by the condenser 0.2 or 0.55. In doing so, make sure to hold the condenser.

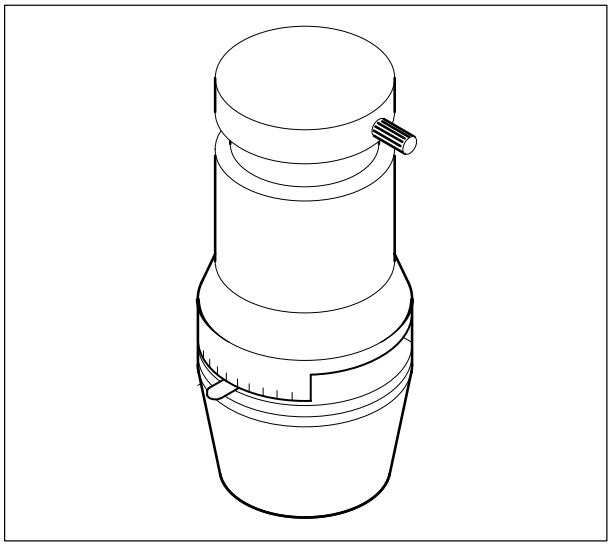


Fig. 2-13 Condenser 0.55

Height adjustment of the condenser 0.55

The height of the condenser 0.55 can be adjusted in order to obtain homogeneous illumination of the object field and optimum intensities in all the focusing positions of the objective.
When the fixation screw has been loosened, the bottom part of the condenser can be moved relative to the upper part and clamped in any position.
In phase contrast and VAREL, the phase rings and stops remain superimposed after the adjustment.

- If the object plane is 0...2.5 mm above the stage surface, keep the condenser in the lower position.
- If the object plane is more than 2.5 mm above the stage surface, move the bottom part of the condenser upwards and clamp it when the maximum illumination intensity in the object field has been achieved.

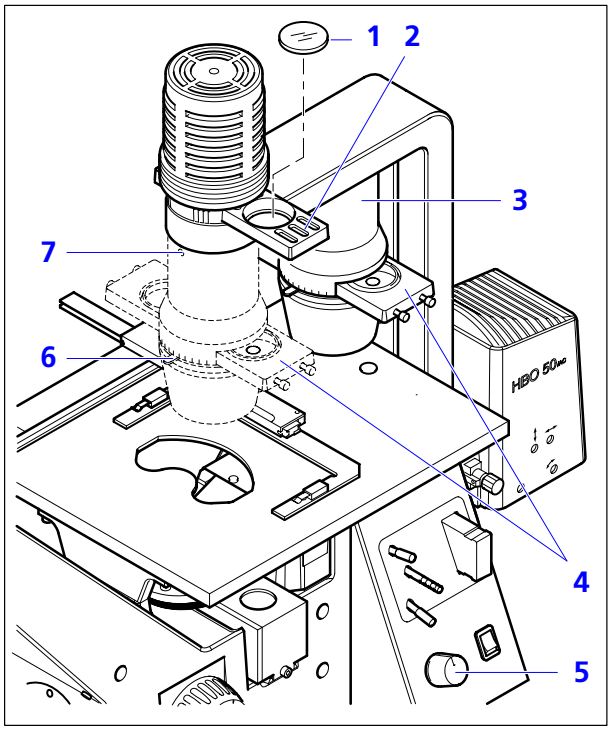


Fig. 2-14 Various modes of brightfield illumination

2.4.2 Brightfield illumination

- Prepare the microscope as described in Sections 2.4. and 2.4.1.
- Set slider Ph, H, Ph (2-14/**4**) to mid-position.
- Place the specimen on the specimen stage and, using a low-power objective, e.g. the CP Achromat 10x, focus on it by turning the focusing drive.
- Swing lever (2-14/**6**) to close the aperture diaphragm until the image contrast is optimal.

NOTE Do not use the aperture diaphragm for controlling the image brightness (loss in image quality)!

- If necessary, turn control (2-14/**5**) to change the lamp voltage and thus brightness or insert an attenuation filter (2-14/**1**) in the filter slider (2-14/**2**) and push it into the light path.

2.4.3 Phase contrast

- Prepare the microscope as described in Sections 2.4. and 2.4.1.

NOTE Phase-contrast objectives are labeled in green.

- Swing phase-contrast objective (2-15/**3**) in the light path.
- Open the aperture diaphragm (2-15/**2**) fully.
- Insert the eyepiece telescope (2-15/**4**) in one of the tubes in place of the eyepiece. Focus its eyelens to make the phase rings of the objective visible.
- The phase ring of the phase-contrast objective appears as a gray ring in the bright pupil (2-16/**A**).

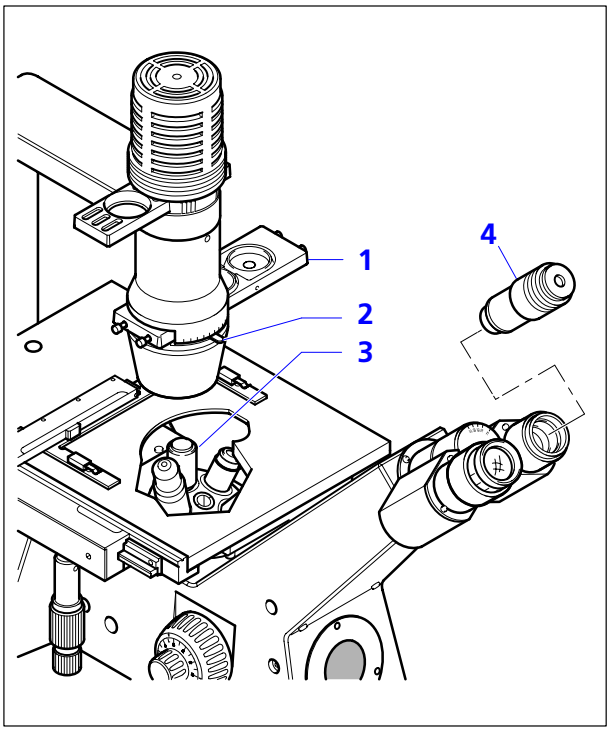


Fig. 2-15 Observation in phase contrast

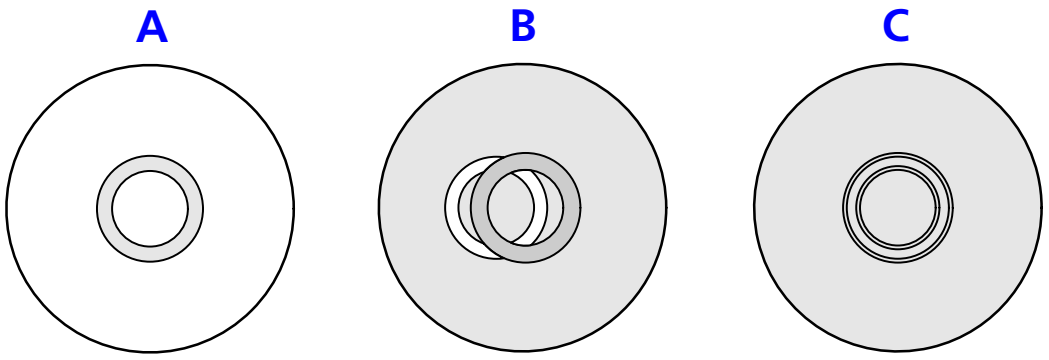


Fig. 2-16 Illustration of phase-contrast adjustment

- Slide the annular diaphragm (2-17/**1**) of a slider with phase diaphragm positions (2-15/**1**) matching the objective and the inserted condenser in the light path.
  The phase ring of the objective appears centrally in the field of view, while the bright annular stop may be off-center (2-16/**B**).
- Turn the centering screws (2-17/**3**) on the slider until phase ring and annular diaphragm are superimposed. The adjustment is correct, if the gray phase ring of the objective completely covers the bright ring stop (2-16/**C**).

NOTE Should the setting range of the centering screws not be sufficient, please check whether the condenser is at the front stop and the slider has engaged. Exact imaging of the annular diaphragm requires a plane-parallel object.

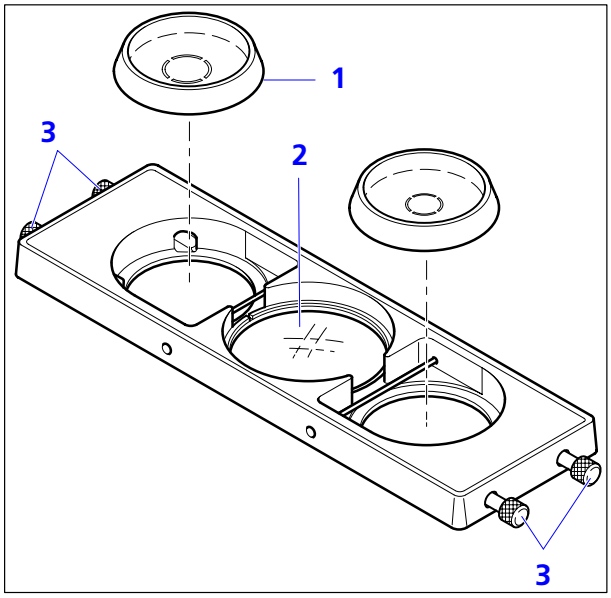


Fig. 2-17 Inserting the annular diaphragms

- Replace the eyepiece telescope by the eyepiece again.
- The center position of the phase-contrast sliders permits brightfield illumination, the brightness of which is matched to that of the phase-contrast image by means of an attenuation filter (gray filter) (2-17/**2**).
- When using different Ph objectives with Ph 0, Ph 1 and Ph 2, pay attention to the correct assignment of the annular diaphragms (see table below).

Objective	Annular diaphragms for					
	condenser 0.55		condenser 0.4		condenser 0.2	
A-Plan 5x/0.12 Ph 0	-	-	Ph 0/0.4	451255	Ph 0/0.2	451261
CP-Achromat 10x/0.25 Ph 1	Ph 1/0.55	451258	Ph 1/0.4	451256	Ph 1/0.2	451260
A-Plan 10x/0.25 Ph 1	Ph 1/0.55	451258	Ph 1/0.4	451256	Ph 1/0.2	451260
LD A-Plan 20x/0.30 Ph 1	Ph 1/0.55	451258	Ph 1/0.4	451256	Ph 1/0.2	451260
LD A-Plan 32x/0.40 Ph 1	Ph 1/0.55	451258	Ph 1/0.4	451256	Ph 1/0.2	451260
Plan Neofluar 5x/0.15 Ph 1	Ph 1/0.55	451258	Ph 1/0.4	451256	Ph 1/0.2	451260
Plan Neofluar 10x/0.30 Ph 1	Ph 1/0.55	451258	Ph 1/0.4	451256	Ph 1/0.2	451260
LD A-Plan 40x/0.50 Ph 2	Ph 2/0.55	451259	Ph 2/0.4	451257	-	-
LD Plan Neofluar 20x/0.40 corr Ph 2	Ph 2/0.55	451259	Ph 2/0.4	451257	-	-
LD Plan Neofluar 40x/0.60 corr Ph 2	Ph 2/0.55	451259	Ph 2/0.4	451257	-	-
LD Plan Neofluar 63x/0.75 corr Ph 2	Ph 2/0.55	451259	Ph 2/0.4	451257	-	-

- Loosen the centering screws to allow the inserted annular diaphragm to be exchanged for another one.

2.4.4 VAREL

VAREL (variable relief contrast) provides a relief-like image of specimens and can be used as an alternative to phase contrast. VAREL can also be used on curved surfaces, e.g. 96-well microtiter plates, which cannot be contrasted with phase contrast as the phase rings cannot be superimposed exactly!

- Prepare the microscope as described in Sections 2.4. and 2.4.1.
- Fully open the aperture diaphragm (2-18/**1**) on condenser (2-18/**2**).
- Insert the specimen with the appropriate mounting frame in the holding clips (2-18/**4**) of the attachable object traverser M.
- From the right, push the slider Ph, H, Var (2-18/**3**) into the slider mount on the 0.4 or 0.55 condenser up to the VAREL position.
- Swivel in the required VAREL objective.
- Use the adjusting screw (2-18/**5**) to move the left or right VAREL diaphragm aperture (after selecting the engaging position) until optimum contrast is achieved (relief-like impression).

NOTE Microtiter plates:
Select the opposite VAREL ring of the diaphragm for illumination at the edge of the well; in the middle of the well, you can use the left or right VAREL ring.

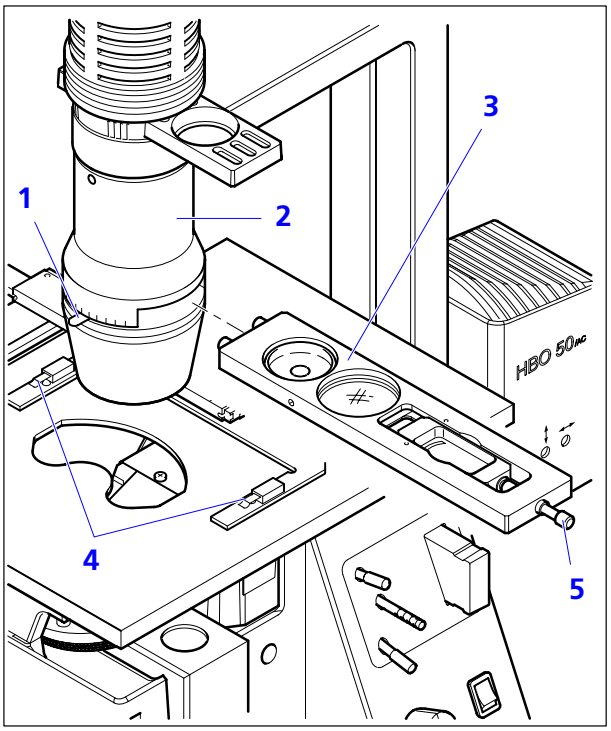


Fig. 2-18 Observation with VAREL

Specimen field with corresponding pupil image of VAREL diaphragm (diaphragm appears rotated by 180°):

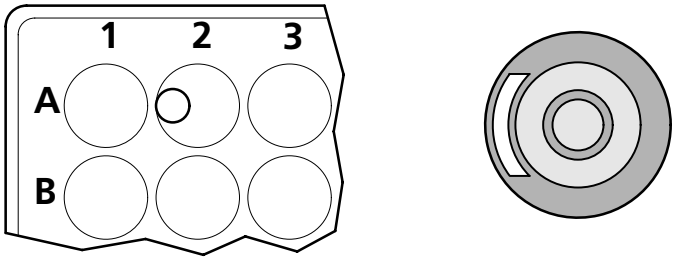


Fig. 2-19 VAREL with microtiter plates

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 mid-position of the slider Ph, H, Var and the slider Var, H, Var allows brightfield illumination.
- The left click-stop position of the slider Ph, H, Var allows fast change from VAREL to phase contrast.

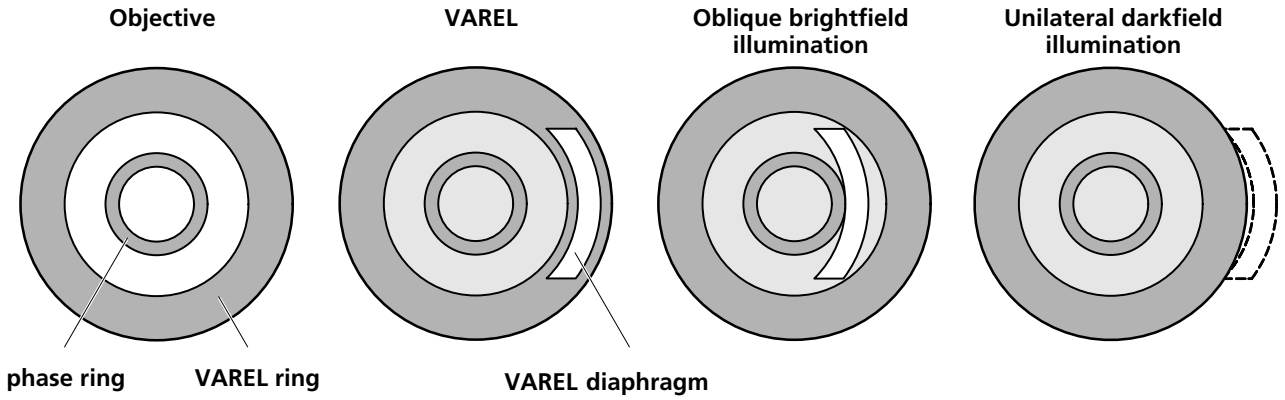


Fig. 2-20 Pupil images with VAREL

Preparing the slider Var, H, Var for phase contrast

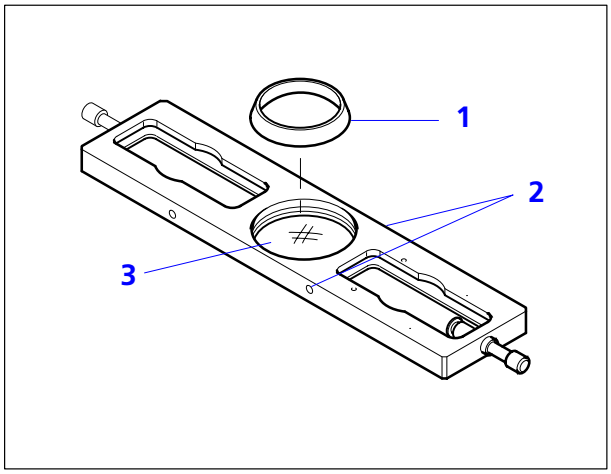


Fig. 2-21 Slider Var, H, Var

- Remove the attenuation filter (2-21/**3**) from the central mount of the slider (remove spring ring and filter).
- Insert the ring diaphragm mount (2-21/**1**) (supplied with the slider). If necessary, loosen centering screws (2-21/**2**) by means of the key.
- Insert ring diaphragm in ring diaphragm mount and center it to the phase ring of the objective using the centering screws (one centering screw at each side of the slider).

Objective	VAREL diaphragms for				
	condenser 0.55		condenser 0.4		condenser 0.2
A-Plan 10x/0.25 Ph 1 Var 1	Var 1/0.55	451252	Var 1/0.4	451251	
LD A-Plan 20x/0.30 Ph 1 Var 1	Var 1/0.55	451252	Var 1/0.4	451251	
LD A-Plan 32x/0.35 Ph 1 Var 1	Var 1/0.55	451252	Var 1/0.4	451251	
LD A-Plan 40x/0.50 Ph 2 Var 2	Var 2/0.55	451253			

2.4.5 PlasDIC

PlasDIC is an innovative interference contrast technique that can be used on the Axiovert 40 CFL in combination with the following components: Quintuple nosepiece with slot for PlasDIC slider, slider Ph, H, PlasDIC, slit-diaphragm 5 mm for PlasDIC, 3-position reflector slider P&C with analyzer module D P&C.

PlasDIC provides a relief-like image and can be used especially with thick specimens. The contrast is variably adjustable. The wells of microtiter plates can be contrasted up to the edge. It is not necessary to use cultivation vessels with glass bottom.

- Prepare the microscope as described in Sections 2.4. and 2.4.1.
- Equip the 3-position reflector slider P&C with the analyzer module D P&C (for that, refer to Section 3.2 (4)).
- Equip the slider Ph, H, PlasDIC with the slit-diaphragm 5 mm for PlasDIC (see next page).
- Remove the factory-fitted protective slider from the slot on the nosepiece and push the PlasDIC slider (2-22/**4**) appropriate for the used objective (A-Plan 10x, LD A-Plan 20x, 32x or 40x) into the slot.
- Fully open the aperture diaphragm (2-22/**1**) on the condenser (2-22/**2**).
- Insert the specimen with the appropriate mounting frame in the holding clips (2-22/**7**) of the attachable object traverser M.

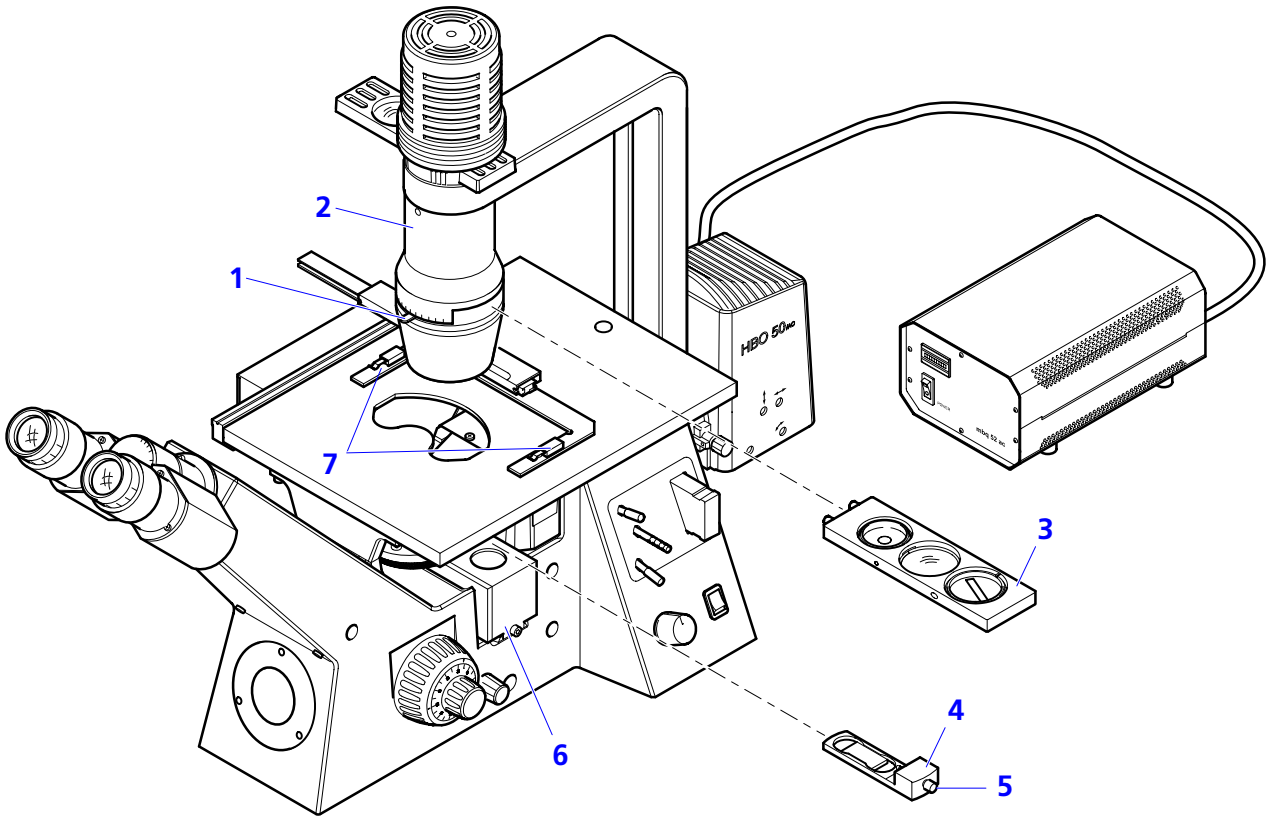


Fig. 2-22 Observation with PlasDIC

- From the right, push the slider Ph, H, PlasDIC (2-22/**3**) into the respective mount on the condenser 0.4 or 0.55 up to the PlasDIC position. When switching from brightfield to PlasDIC, you have to increase the brightness.
- Push the 3-position reflector slider P&C (2-22/**6**) with mounted analyzer module D P&C into the slot on the microscope stand until it is in the light path.
- Swivel in the required objective. You can use the following objectives for PlasDIC:
  A-Plan 10x, LD A-Plan 20x, 32x and 40x.
- Turn adjusting screw (2-22/**5**) of the PlasDIC slider to adjust the contrast. The specimen structures can be imaged in relief or pseudo darkfield presentation. The relief image provides the best contrast.

NOTE If no longer used, you can plug the PlasDIC slider (2-22/**4**) for storage into one of the two mounts on the microscope stand below the specimen stage. Before doing so, slip the supplied black protective cover over the PlasDIC slider.

Preparing the slider Ph, H, PlasDIC

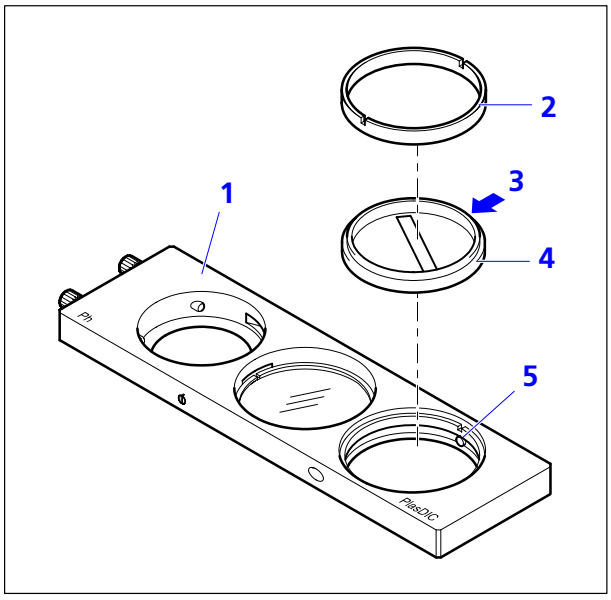


Fig. 2-23 Slider Ph, H, PlasDIC

- Unscrew the retaining ring (2-23/**2**) from the Plas-DIC mount of the slider Ph, H, PlasDIC (2-23/**1**).
- Insert the slit-diaphragm 5 mm for PlasDIC (2-23/**4**) in the PlasDIC mount. In doing so, take care that it is correctly seated: Orientation of the slit-diaphragm is by its groove (2-23/**3**) and the pin (2-23/**5**) of the diaphragm mount.
- Screw down the retaining ring (2-23/**2**) again.

NOTE Normally the Ph diaphragm mount is equipped with an annular diaphragm (see Section 2.4.3). This allows fast changeover from PlasDIC to phase contrast.

2.4.6 Incident-light fluorescence

Incident-light fluorescence requires the use of the Axiovert 40 CFL microscope with the following components:
FL incident-light equipment, HBO 50 illuminator with mbq ac-z transformer, 3-position reflector slider P&C and reflector module FL P&C (equipped with set of filters for fluorescence excitation).

- Select the specimen area to be examined in transmitted light brightfield or phase contrast. To do this, push reflector slider (2-24/**10**) to the blank position, switch on halogen bulb (2-24/**2**) and move diaphragm slider (2-24/**3**) to brightfield or phase contrast position.
- Switch on the HBO 50 lamp (2-24/**5**) at the power supply (2-24/**4**), but block the light path using the additional filter slider (2-24/**6**), which in its mid-position does not allow the light to pass.
- After you have selected the specimen area of interest, switch off the halogen bulb.
- With the 3-position reflector slider P&C (2-24/**10**) move the required filter combination (2-24/**9**) into the light path and release the light path by removing the additional filter slider (2-24/**6**).
- Move the push-pull rod (2-24/**7**) to close the luminous-field diaphragm until it is visible in the field of view. Center it by means of centering screws (2-24/**8**) and open it up to the edge of the field of view.
- In the additional filter slider (2-24/**6**) of the FL incident-light equipment, you can insert additional 25-mm excitation filters, which must be held by means of O-rings.

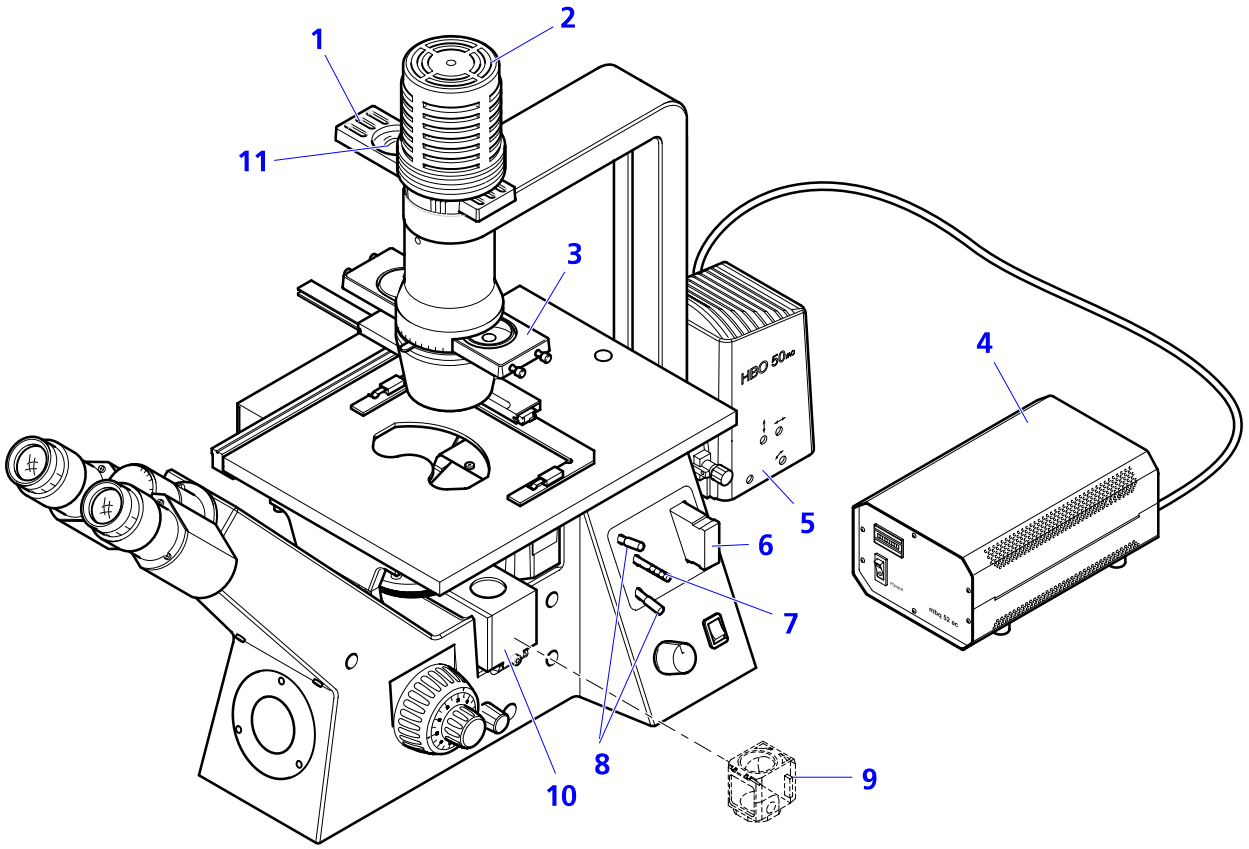


Fig. 2-24 Observation in incident-light fluorescence

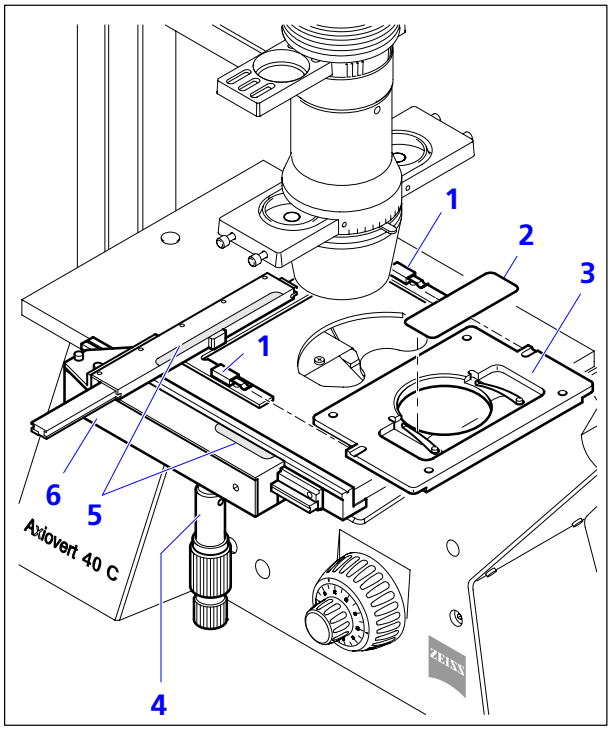


Fig. 2-25 **Working with attachable object traverser M**

2.5 Working with attachable object traverser M and mounting frames

- Prepare the microscope as described in Section 2.4. and 2.4.1.
- Mount attachable object traverser M as described in Section 2.1.2 (5).
- In combination with various mounting frames (2-25/**3**), e.g. for 76 x 26 mm specimen slides (2-25/**2**), attachable object traverser M (2-25/**6**) provides sensitive movement by means of a coaxial drive (2-25/**4**).
  Adhesive scales (2-25/**5**), which are supplied with the chosen mounting frame and which must be attached to recesses on the attachable object traverser M provide defined position display of the specimen vessel. Mounting frames are secured by means of holding clips (2-25/**1**).

The following mounting frames are available (other mounting frames for COSTAR, CORNING and tissue culture flasks, Hamax plates, Coates plates as well as plankton chambers on request):

Mounting frame M for 76 x 26 mm specimen slides

Mounting frame M for Petri dishes; ∅ 36, 54, 65, 88 mm

Mounting frame M for 96-well microtiter plate

Mounting frame M for microtest plates with 60, 72, 120 pos.

Mounting frame M for multidishes (133.5 x 88.5 mm)

Universal mounting frame M for the use of:

- Petri dishes in the range from 35 to 60 mm
- Petriperm dishes
- Terasaki plates
- Hamax plates
- Cell cultivation chambers, such as
  - TSCS-1, -2 and -3
  - POC-R chamber
  - DVORAK-STOTLER chamber and others
- Specimen slides and all vessels based on specimen slides

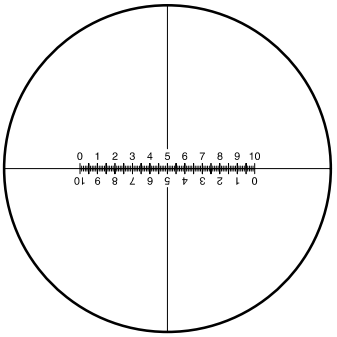
Universal mounting frame MX (max.133 x 88 mm, min.124 x 83 mm) for:

- Multidishes with 6, 12, 24, 48 or 96 wells
- Petri dishes with 87 to 92 mm diameter

Heatable universal mounting frame M-H

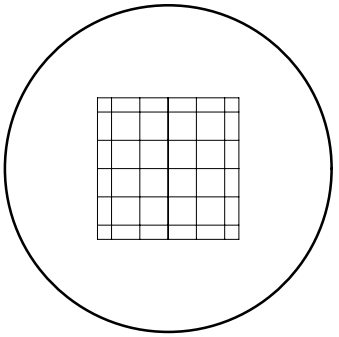
2.6 Counting, measuring and framing reticles

Microscopic counting and measuring, and the imaging of frame sizes requires the use of special reticles. A small selection is given below:



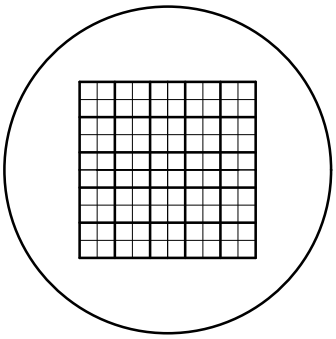
Crossline micrometer 14:140, d=26

Graduation length = 14 mm
Increments = 0.1 mm
Graduation tolerance ≤ 0.001 mm
Catalogue No. 4540060-0000-000



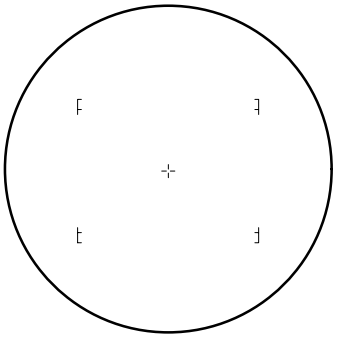
Net micrometer 25/2x2, d = 26 mm

Catalogue No. 454087-0000-000



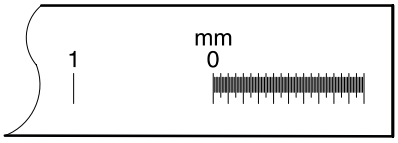
Net micrometer 12.5x12.5/5;10 / d = 26 mm

Catalogue No. 474068-0000-000



Framing reticle MC 2.5x / d = 26 mm

Catalogue No. 454075-0000-000



Stage micrometer, positive, 5+100/100y
d = 0.17 mm

Graduation to one side: 5 mm in 5 intervals,
Graduation to other side: 1 mm in 100/100 mm = 10 µm. Accuracy ± 1 µm.
Catalogue No. 474026-0000-000

Fig. 2-26 Selected eyepiece reticles and stage micrometers

NOTE

- Handling of eyepiece reticles is described in Section 2.1.2 (10).
- Other eyepiece reticles of d = 26 mm may also be used (refer to Price List).

2.7 Working with micromanipulators

The Axiovert 40 stands are prepared as follows for the attachment of micromanipulators:

- Three M4 threaded holes (2-27/**1**) on the left and right underside of the specimen stage.
- Three M5 threaded holes (2-27/**2**) on the left and right sides of the stand.
- Four M5 threaded holes (2-27/**3**) on the underside of the stand for securing the stand.

Refer to the instructions of the manufacturer of the micromanipulator for further details of how to attach the micromanipulator, if necessary using special adapters.

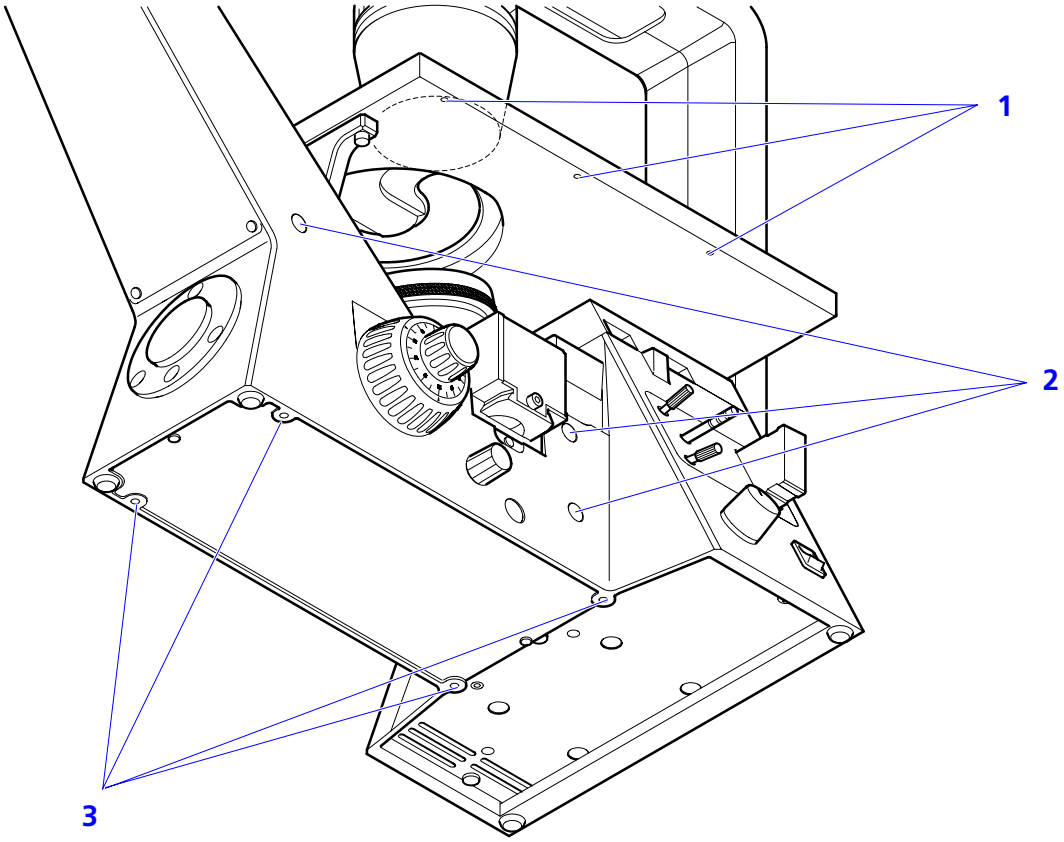


Fig. 2-27 Mounting facilities for micromanipulators

2.8 Replacing the specimen stage

- Remove the screw cover (2-28/**1**) from the stage surface.
- Using the SW 3 ball-headed screwdriver, remove three fastening screws (2-28/**2**) and the stage (2-28/**3**).
- Fit and fasten the selected specimen stage (specimen stage glass (Section 2.8.1) heatable stage or temperable microscope stage (Section 2.8.2)).

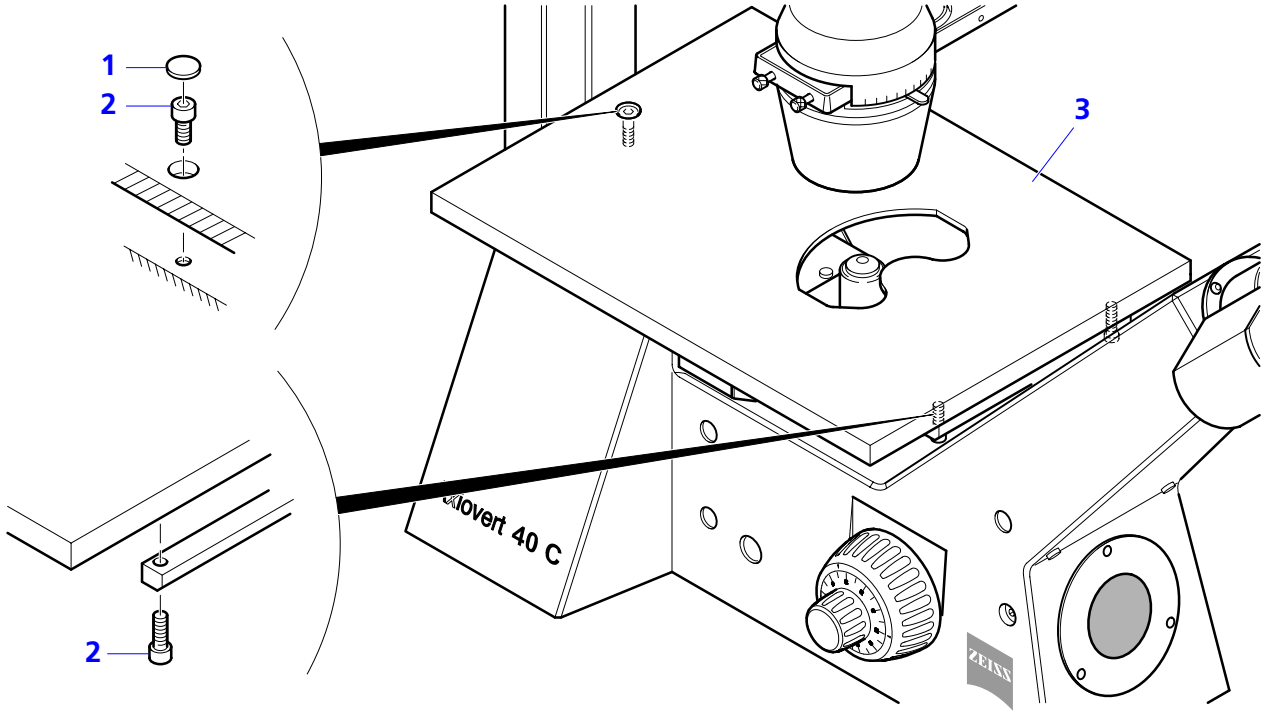


Fig. 2-28 Replacing the specimen stage

2.8.1 Specimen stage glass

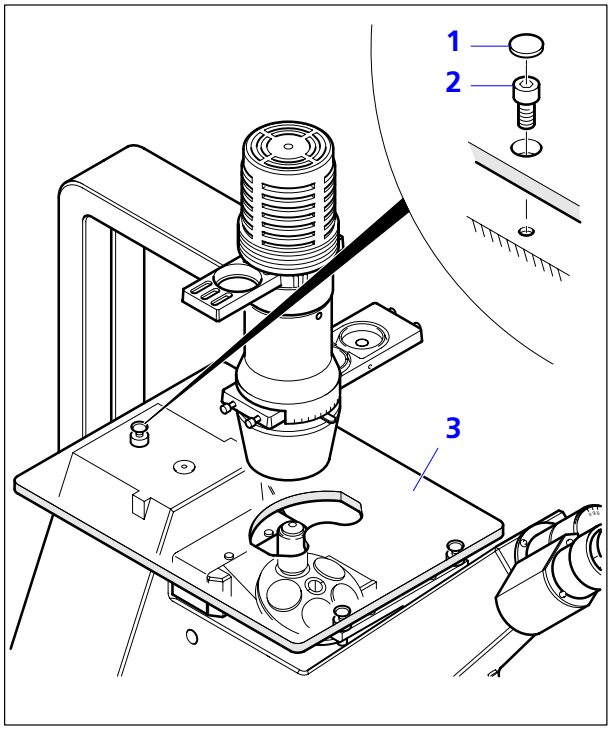


Fig. 2-29 Mounting the specimen stage glass

The specimen stage glass (2-29/**3**) enables convenient working with simultaneous observation of the objectives.

Installation

- Put the specimen stage glass onto the stage mounting point using the spacers.
- Using fastening screws (2-29/**2**), fasten the specimen stage glass to the stand.
- Cover the rear hole on the surface of specimen stage glass with cap (2-29/**1**).

2.8.2 Heatable stage and temperable microscope stage

A heatable stage and a temperable microscope stage are available for the Axiovert 40 microscope.

- Heatable stage (471820-9901-000) including three spacers
- Temperable microscope stage (411881-0000-000) including three spacers

The use of the heatable stage requires:

- Control unit Tempcontrol 37 (1-Kanal) (000000-1116-057)

If additionally you need a pre-heating plate, it is recommended to order the Tempcontrol 37-2 control unit (000000-1052-320).

The use of the temperable microscope stage requires:

- Cooling thermostat RE 106 (230 V) (000000-1116-068). This device is also available for 105 V lines.

2.9 Documentation

On the Axiovert 40 C / 40 CFL microscopes, you can switch between observation and photomicrography by means of a path selector switch. Since this is a 100%/100% selector, simultaneous observation and photography is not possible. The digital adapter D 40 M37/52x0.75 and the V25 adapter for microscope cameras 1.0x, d=30 allow compact digital cameras to be mounted to the microscope. Additionally, other camera types can be connected via special adapters (refer to the system overview in Section 1.4).

Mounting compact digital cameras

- Remove the dust-protective cap from the camera body (2-30/**1**, e.g. A 70 Canon).
- Fix the camera-specific filter adapter (2-30/**2**) to the camera body. The filter adapter is included in the camera equipment, when ordered from Carl Zeiss.
- Screw the digital adapter D 40 M37/52x0.75 (2-30/**3**) onto the adapter of the camera.
- Plug the V25 adapter for microscope cameras 1.0x, d=30 (2-30/**4**) into the digital camera adapter D 40 M37/52x0.75 (2-30/**3**) and tighten grub screw (2-31/**4**, see next page).
- Remove dust-protective cap (2-30/**6**) from camera port and attach the pre-installed camera system to this port. Align it horizontally and fasten it by means of the clamping screw (2-30/**7**) using the SW 3 ball-headed screw driver (2-30/**5**).

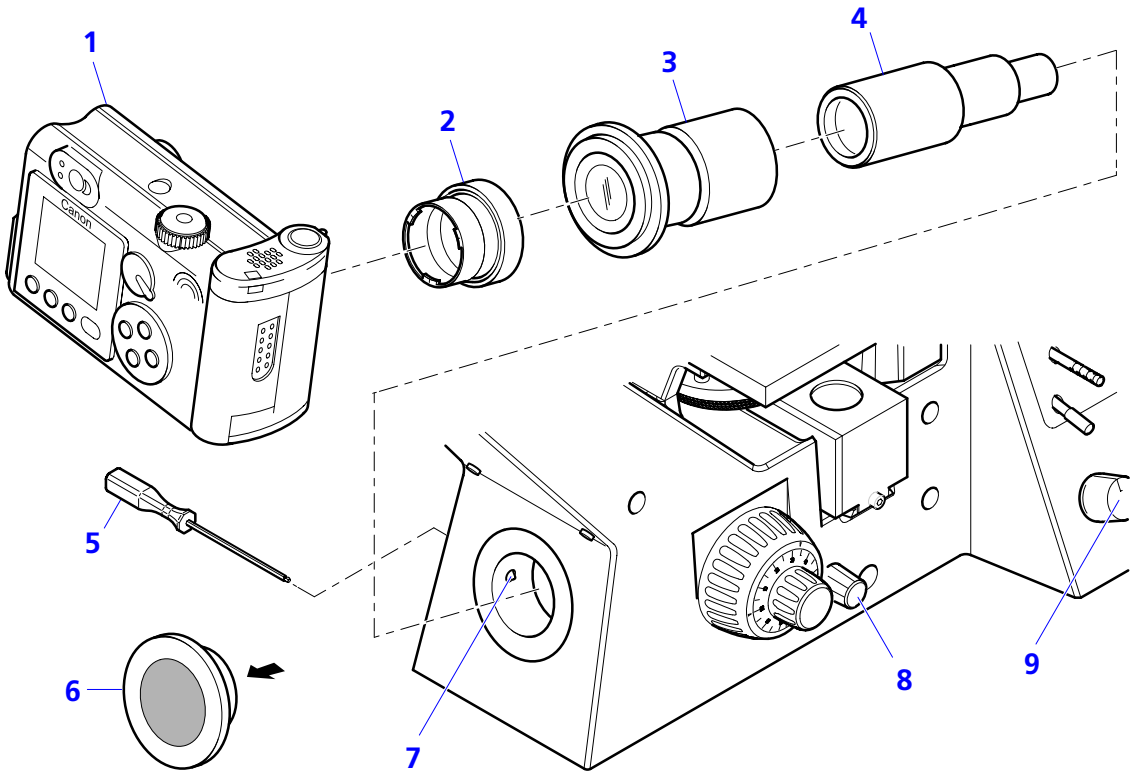


Fig. 2-30 Camera system

- Switch on the microscope, turn the brightness control (2-30/**9**) to maximum intensity (at right stop: 3200 K). Use an attenuation filter, if necessary.
- Select the specimen detail to be photographed via the binocular tube.
- Switch the path selector (2-30/**8**) from observation to photomicrography to release the camera light path thus directing 100 % of the light to the camera.

Notes on the adjustment of the digital camera adapter D 40 M37/52x0.75 and the camera settings

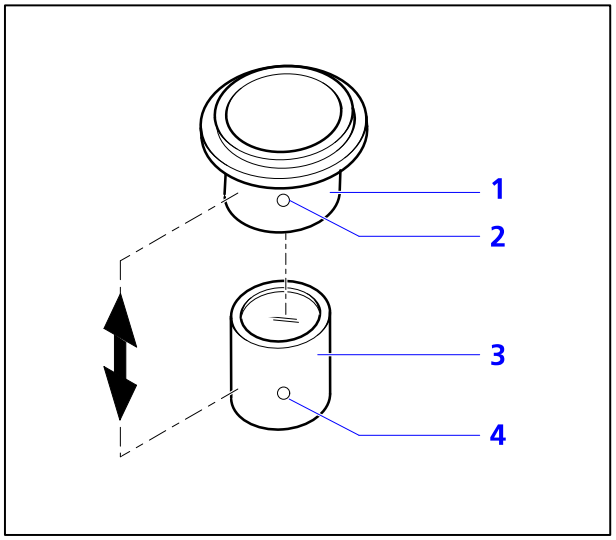


Fig. 2-31 Adjusting the digital camera adapter

Depending on the microscope equipment and the camera used, it may be necessary to optimize the distance between camera lens and lens mount (2-31/**3**) (see double-headed arrow). This is the case, in particular, if it is impossible to obtain an unmasked image in any zoom position of the camera lens.

Set the camera as follows:

- Switch off the autofocus.
- Set the distance to infinity (∞).
- Set exposure control to aperture-priority auto exposure.
- Choose an aperture as wide as possible (i.e. a small f-stop number!).
- Not all cameras provide these options. Please consult the operating instructions of the camera.
- Loosen grub screw (2-31/**2**).
- Vary the distance between camera lens and lens mount in steps, i.e. move the sliding mount (2-31/**1**) with the camera on the lens mount (2-31/**3**) by defined steps.
- Zoom the camera lens from the wide-angle (W) to the telephoto position (T).
- Perform the test until the image fills the frame without masking or vignetting.
- Tighten grub screw (2-31/**2**) again.

If you use a camera/adapter combination not expressly recommended by Carl Zeiss, it is quite possible that you will be unable to obtain an unmasked image.

3 Maintenance and troubleshooting

3.1 Maintenance

Maintenance of the Axiovert 40 microscope is limited to the following operations.

- Cover the instrument with the dust cover after every use.
- Clean uncovered optical components whenever required.
- Carefully remove condensation or precipitated aggressive vapor using a dry cloth.
- Protect the instrument from temperatures above 50 °C, frost, humidity, chemically aggressive vapor and substances.
- Remove dust from optical surfaces using a rubber blower or a natural hair brush which can be degreased in alcohol and dried afterwards. Remove stubborn dirt and fingerprints using a dustfree cloth or leather; breathe on the dirty surface, if required.
  Clean the front lenses of the objectives using petroleum ether, but do not use alcohol.
- Use commercially available optics cleaning cloths to remove stubborn dirt; if necessary, lightly moisten the cloths with petroleum ether.

When using the Axiovert 40 microscope in humid climatic zones, proceed as follows:

- Store the instrument in bright, dry and well-ventilated rooms with a humidity of less than 75 %; optical components and accessories that are particularly susceptible to fungus growth, e.g. objectives and eye-pieces, should be stored in a drying cabinet.
- When storing the equipment in closed cases for a longer period of time, the growth of fungus can be avoided by putting in cloths soaked in fungicide.

NOTE The risk of fungus growth on opto-mechanical instruments invariably exists in the following conditions:

- Relative humidity in excess of 75 % and temperatures between +15 °C and +35 °C for more than three days.
- Installation in dark rooms without air ventilation.
- Dust deposits and fingerprints on optical surfaces.

3.2 Troubleshooting and service

Troubleshooting on the Axiovert 40 microscope is limited to a few actions:

- Checking the line voltage
- Checking the illuminating equipment
  - Fuse replacement according to paragraph (1)
  - Replacement of halogen bulb according to paragraph (2)
  - Replacement of HBO 50 according to paragraph (3)
  - Replacement of reflector module according to paragraph (4)

(1) Checking the line voltage

- Check power cable (3-1/**3**) and replace it, if necessary.
- Remove the fuse holder (3-1/**1**) by simultaneously pressing it in the direction indicated by the arrows. Check the fuses (3-1/**2**) and replace the defective fuse(s).

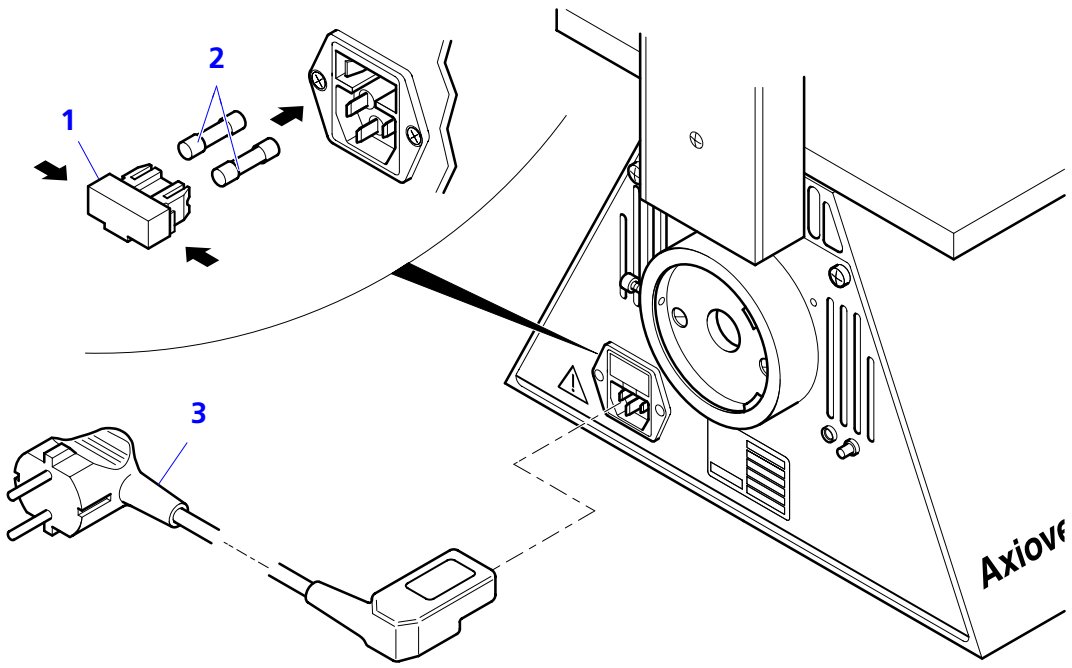


Fig. 3-1 Checking the line voltage

(2) Replacing the halogen bulb

CAUTION
Hot surfaces on bulb housing (3-2/**1**), heat sink (3-2/**2** and halogen bulb:
Make sure to let them cool down sufficiently!

Replace the halogen bulb following this procedure:

- Disconnect the power cable and let the halogen bulb cool down for about 15 minutes.
- Unlock the bulb housing (3-2/**1**) by slightly turning it anticlockwise.
- Withdraw the heat sink (3-2/**2**) upward.
- Remove the halogen bulb with alignment base (3-2/**3**).
- Remove new 12 V 35 W halogen bulb with adjustment base from the packing box. Insert it in the mount taking care that the tip of the mount engages in the centering notch of the carrier plate.

CAUTION Do not touch the bulb with your bare hands; if required, clean the bulb using pure alcohol **before** switching the lamp on for the first time in order to prevent contaminations from burning in.

- After you have replaced the halogen bulb, fit on the heat sink, attach the bulb housing and lock it.

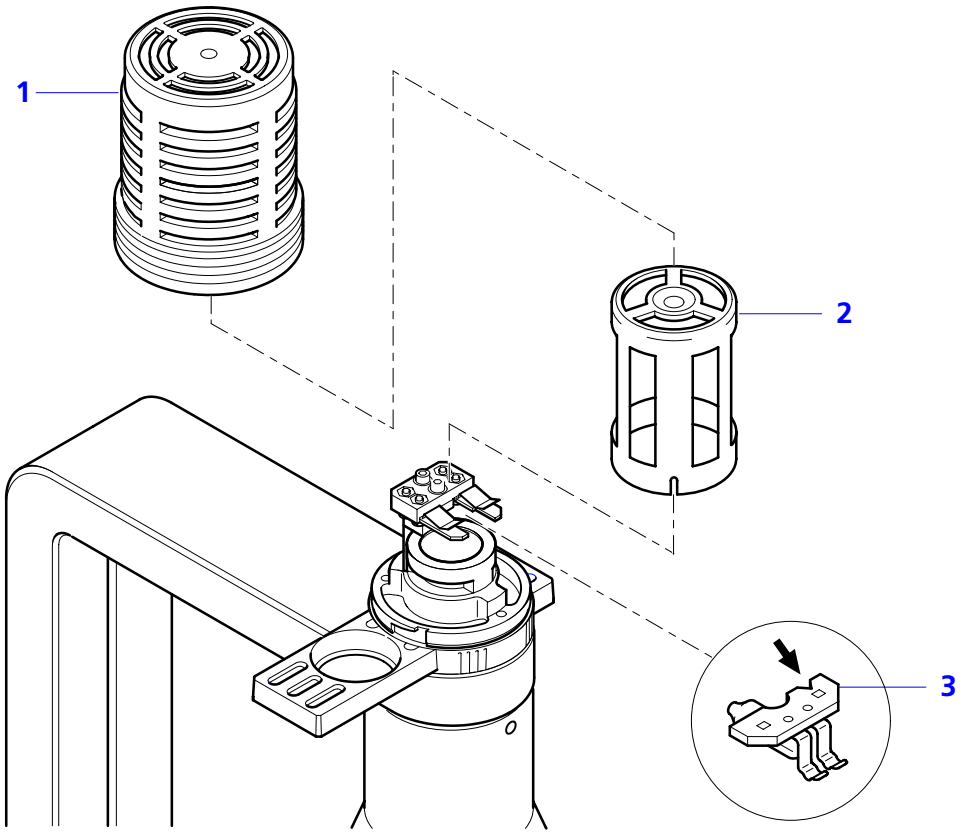


Fig. 3-2 Replacing the halogen bulb

(3) Replacing the HBO 50

The HBO 50 microscope illuminator is used for incident-light fluorescence excitation. It comprises the following items:

- HBO 50 illuminator 447220-0000-000
- HBO 50/SF 20 collector 447271-0000-000
- HBO 50 mercury vapor short-arc lamp 381619-0000-000
- Transformer mbq52ac-z for HBO 50ac 000000-1113-833

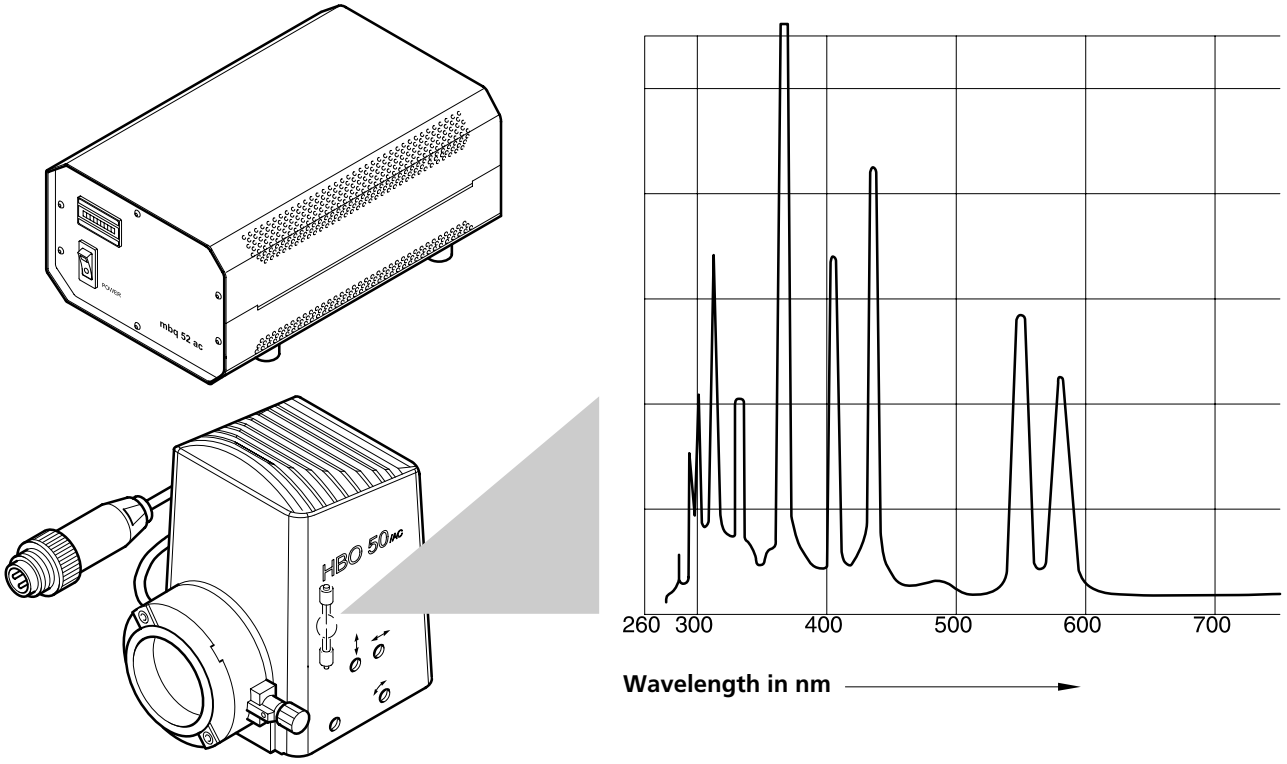


Fig. 3-3 HBO 50 illuminator with line spectrum

Safety notes for the use of the HBO 50

CAUTION While in operation, the HBO 50 is under high pressure. It may therefore only be operated in a closed microscope illuminator.

- The cooling process of the lamp housing must not be obstructed by covers.
- Before you can change the lamp, the HBO 50 must cool down for approx. 15 minutes.
- While in operation, the lamp emits UV radiation. Avoid direct exposure of eyes and skin. It is advisable to wear protective eyewear when handling the microscope illuminator.

CAUTION The HBO 50 must be replaced after expiry of its average life of 100 h. The risk of explosion increases when the average life is exceeded.
The operating time of the HBO 50 can be read from the hour meter.

This warning label on the rear of the illuminator means:
Caution: Hot surface!
Allow the lamp to cool down before touching it.

Replacing the HBO 50 lamp

CAUTION Switch off power supply and disconnect the power plug of the lamp from the socket of the power supply. Allow lamp and lamp housing to cool down (for at least 15 min.).

- Loosen the clamping screw on the microscope stand to remove the HBO 50 illuminator from the stand.
- Place the illuminator on a flat work surface.
- Using the provided SW 3 ball-headed screwdriver, loosen screw (3-4/**1**) and remove the lamp housing (3-4/**2**).
- Depress spring lever (3-4/**5**) and, holding lamp (3-4/**4**) on heat sink (3-4/**3**), withdraw the lamp from its holder.
- Place the heat sink with lamp on the work surface so as to make the clamping screw on the heat sink accessible.

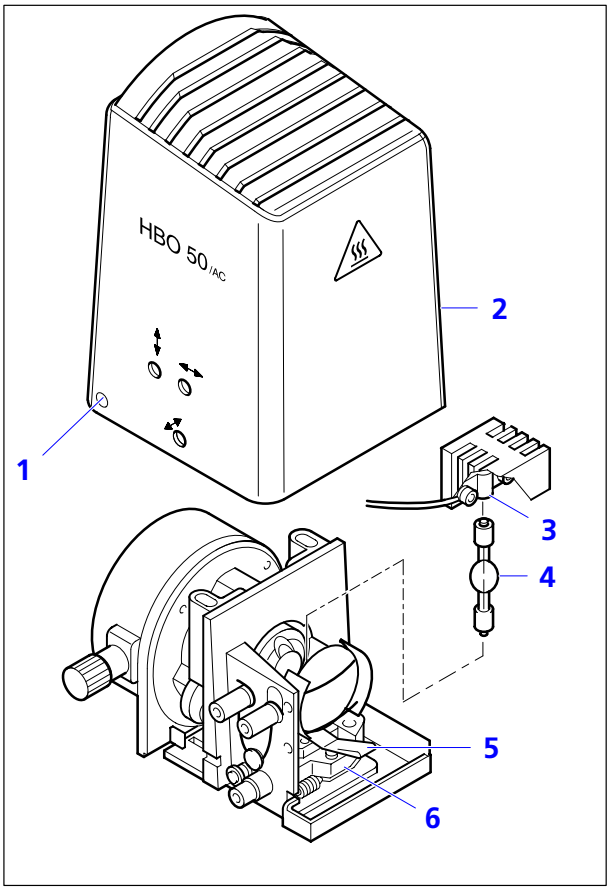


Fig. 3-4 **Replacing the lamp of the HBO 50**

- Use the SW 3 ball-headed screwdriver to loosen the clamping screw on the heat sink and remove the used bulb.

CAUTION You must not remove the cable from the heat sink.

- Hold the new bulb on the labeled metal base and insert it in the heat sink in such a way that its silvered end is at the bottom when inserted in the holder. The silvered melt-off part of the bulb must point to the side (so that it does not affect optical imaging).
- Carefully tighten the clamping screw on the heat sink.
- Avoid touching the glass bulb; remove fingerprints immediately, if necessary.
- Depress spring-loaded lever and, holding the lamp only at the heat sink, insert the assembly of lamp and heat sink into the holder.
- Slowly release the spring-loaded lever while letting the heat sink go.

CAUTION The heat sink must be aligned parallel to the lamp housing. You may correct the position of the heat sink by depressing the spring-loaded lever and turning the heat sink.

- Attach the illuminator cover to the illuminator base and tighten the clamp screw (3-4/**1**).
- Attach the microscope illuminator to the microscope stand.
- Note down the reading of the hour meter of the power supply. The HBO 50 lamp must be replaced when the nominal life of 100 hours has been reached.

Adjustment of the HBO 50

CAUTION Never look directly into the ignited lamp in order to avoid possibly irreparable damage to your eyes.
Use protective eyewear, e.g. sunglasses, to protect your eyes when observing the bright light spot.

- Unscrew an objective and check the image of the light source falling through the blank aperture onto a piece of paper in the object plane (on the specimen stage).
- Turn knurled knob (3-5/**4**) to focus the collector until the brighter light arc is imaged sharply.
- Use adjusting screw (3-5/**1**) to adjust the lamp axially to the reflector until both light arcs are equally sharp, as shown in Figure 3-6.
- Use SW 3 ball-headed screwdriver and the adjusting screws for vertical (3-5/**2**) and lateral (3-5/**3**) adjustment of the arc image beside the arc, as shown in Fig. 3-6.
- Screw the objective in the nosepiece again.
- Set the reflector slider to blue excitation, e.g. by using filter set 09.
- Set the additional filter slider to the blank aperture position.

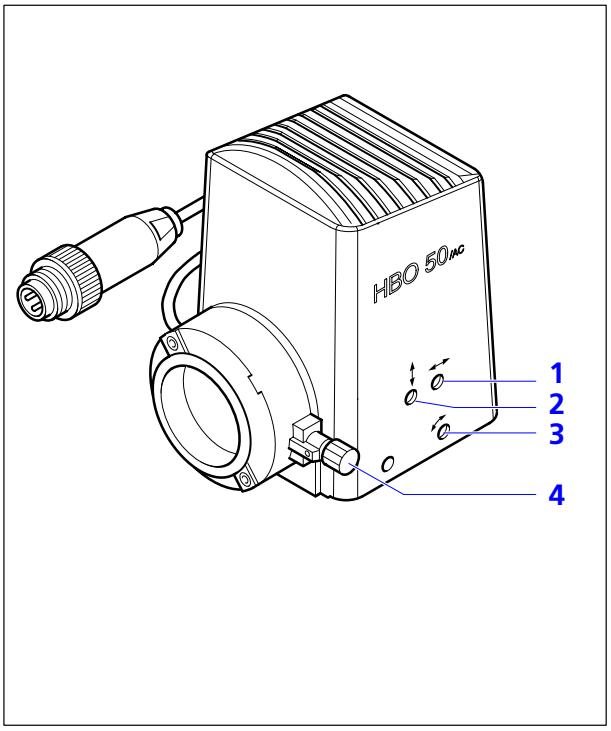


Fig. 3-5 **Adjustment of HBO 50**

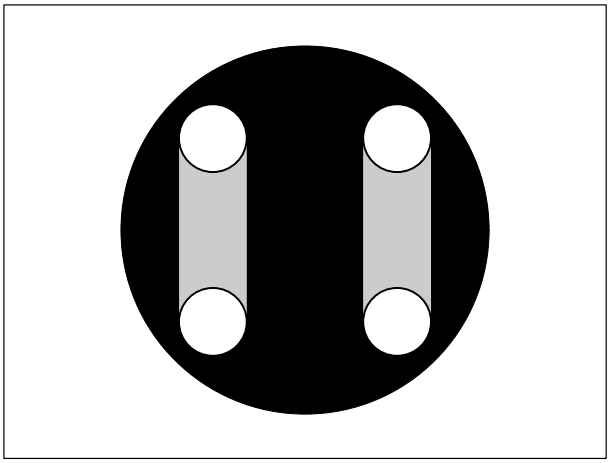


Fig. 3-6 **Lamp image**

(4) Replacing reflector modules FL P&C on 3-position reflector slider P&C

The reflector modules FL P&C are equipped in the factory with the appropriate filter sets consisting of excitation filter, dichroic beam splitter and barrier filter.
However, the reflector modules may be equipped with any FL filter set.

Two examples of available filter sets:

Filter set 09 shift-free (blue excitation) (4888009-0000-000)	consisting of	EX BP 450-490 BS FT 510 EM LP 515
Filter set 15 shift-free (green excitation) (488015-0000-000)	consisting of	EX BP 546/12 BS FT 580 EM LP 590

For further filter sets, refer to the Price List.

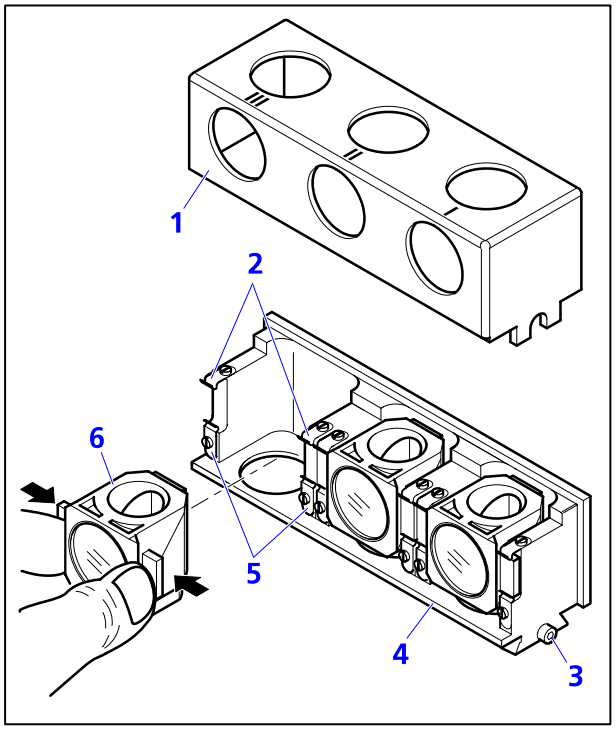


Fig. 3-7 Replacing the reflector modules FL P&C

- Remove 3-position reflector slider P&C from Axiovert 40 CFL microscope.
- Loosen fastening screws (3-7/**3**) on the right and left and remove cover (3-7/**1**) upward.
- To remove a reflector module FL P&C no longer used, withdraw it first from the upper clips (3-7/**2**) and then from the lower ones (3-7/**5**), and then lift it out obliquely.
- Holding it on the holding elements arranged on the right and left (see arrows), insert the reflector module FL P&C (3-7/**6**) obliquely from top in the bottom clips on the bottom part (3-7/**4**) of the reflector mount (in the required place I, II or III). Then, push against the top part of the module until it reliably snaps in the upper clips, too.
- When all reflector modules FL P&C have been inserted, reattach cover (3-7/**1**) and tighten both fastening screws.

(5) Changing the filter set

CAUTION Basically, it is not advisable to convert the factory-fitted reflector modules because of the sensitivity of their components! However, if a specific application requires such a conversion, proceed with extreme care to avoid damage and contamination of the optical components.

The filter sets for the reflector module FL P&C can be individually configured and mounted by the customer.

- Remove the reflector module FL P&C (3-8/**3**) from the 3-position reflector slider P&C and put it down on a soft support.
- Using mounting plate (3-8/**6**) included in the tool kit, unscrew retaining rings (3-8/**1**).
- Turn reflector module upside down so that the filter (3-8/**2** or **5**) falls out on the soft support.
- Insert the barrier filter (emission filter) in (3-8/**2**), the excitation filter in (3-8/**5**) and lock them in place by means of the retaining rings (3-8/**1**).

Barrier filters and excitation filters may be marked on their periphery with their names and an arrow. The arrow indicates the mounting direction of the respective filter in the reflector module FL P&C. It must always point to the inside (see arrows in Fig. 3-8).

To minimize the image displacement with multiple fluorescence photographs, the barrier filter may carry an additional mark indicating the position of the wedge angle.

This mark has to be aligned to the orientation groove (3-8/**4**) when mounting the respective barrier filter to the reflector module used. This is to ensure that on the used reflector module the wedge angle of the barrier filter has the same, defined position thus compensating or minimizing the anyway already small module-to module image displacement with the used Zeiss filter sets.

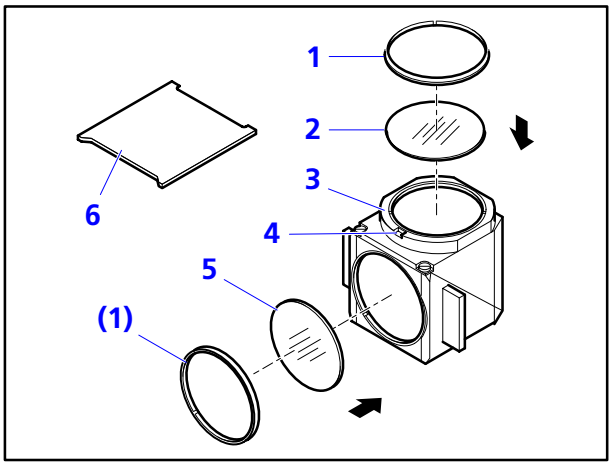


Fig. 3-8 **Changing the fluorescence filter set**

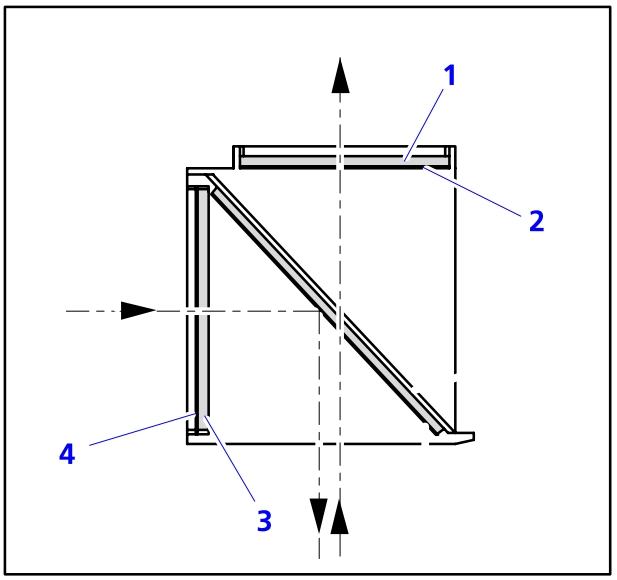


Fig. 3-9 **Inserting filters and dichroic beam splitter**

If it is required to mount a filter that does not carry an orientation mark (arrow), we recommend you follow this procedure:
Insert the filter having reflecting, dielectric coatings in such a way, that the

- reflective coating of the excitation filter (3-9/**3**) points to the outside (3-9/**4**), referred to the reflector module)

and the

- reflective coating of the emission filter (3-9/**1**) points to the inside (3-9/**2**).

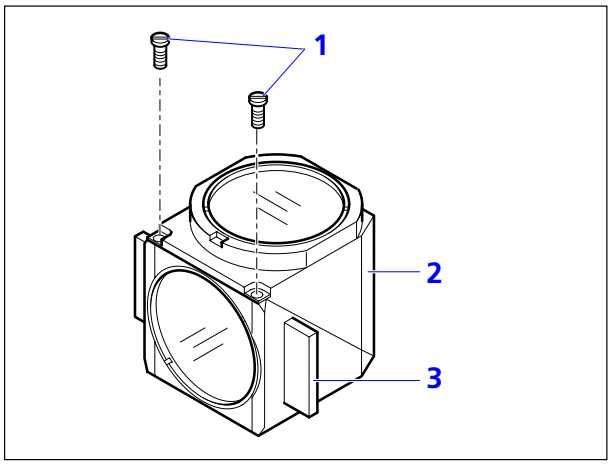


Fig. 3-10 Changing the dichroic beam splitter

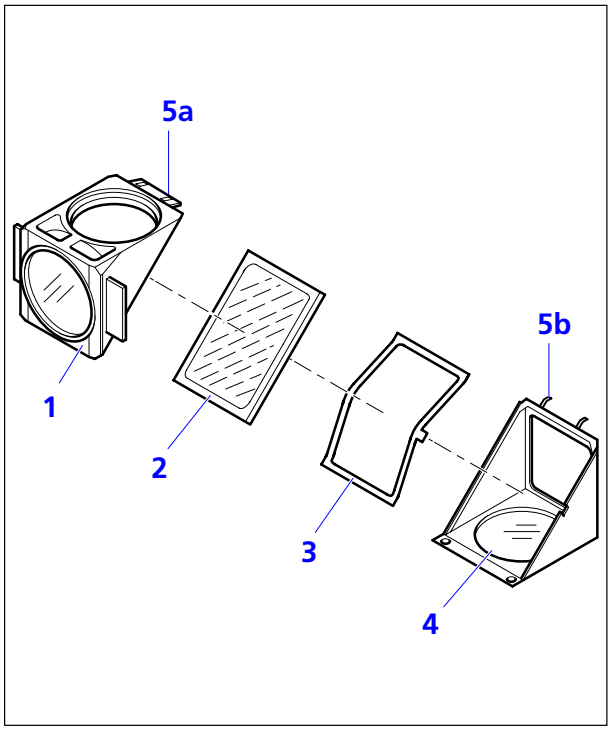


Fig. 3-11 Mounting the dichroic beam splitter

(6) Changing the dichroic beam splitter

CAUTION Basically, it is not advisable to convert the factory-fitted reflector modules because of the sensitivity of their components! However, if a specific application requires such a conversion, proceed with extreme care to avoid damage and contamination of the optical components.

To replace a dichroic beam splitter, follow this procedure:

- Use the screwdriver to loosen the two slotted screws (3-10/**1**).
- Hold both halves of the module (**emission** half 3-10/**2** and **excitation** half 3-10/**3**) of the reflector module FL P&C together, turn it round and put it aside.
- Tilt upward the **excitation** half of the module (3-11/**1**) now being on top and lift it out of the holding elements (3-11/**5b**) of the bottom **emission** half (3-11/**4**).
- Take the dichroic beam splitter (3-11/**2**) and the spring-loaded frame (3-11/**3**) out of the bottom half of the module.
- Remove the old dichroic beam splitter and carefully put the new one with the reflective side on top onto the spring-loaded frame (3-11/**3**). Insert both parts together in the bottom half of the module. Take care that the lateral tongue of the spring-loaded frame engages with the corresponding recess of the bottom half of the module.

NOTE The reflective (coated) side (3-12/**3**) of the dichroic beam splitter is provided with a beveled edge (3-12/**1**) or corner (3-12/**2**).

- Put the **excitation** half (3-11/**1**) onto the **emission** half (3-11/**4**) of the module (holding elements 3-11/**5b** and eyes 3-11/**5a** interlock). Holding both halves together, turn them over in mounting position again.
- Reinsert the slotted screws and tighten them.
- Finally affix the adhesive label with the designation of the filter combination to the side of the module.
- Insert the reflector module FL P&C into the 3-position reflector slider P&C as described above.

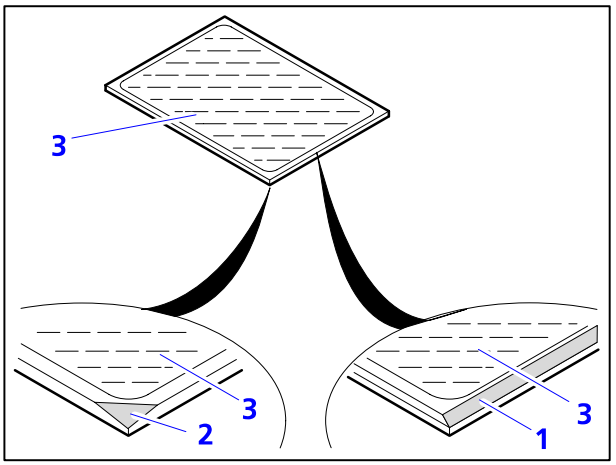


Fig. 3-12 Marking of dichroic beam splitter

(7) Service

Upgrading of the Axiovert 40 CFL stand with the FL incident-light fluorescence equipment is solely performed by service personnel.

All repairs of mechanical, optical or electronic components inside the instrument and of the electrical system of the Axiovert 40 may only be performed by Carl Zeiss service staff or specially **authorized** personnel.

To ensure optimum setting and trouble-free function of your microscope over a longer period of time, we recommend that you enter into a service/maintenance agreement with Carl Zeiss.

For subsequent orders or when service is required, please get in touch with your local Carl Zeiss representative.

For additional information, contact us at

micro.service@zeiss.de

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ANNEX

- Index
- List of abbreviations

Index

Page

F

H

I

L

Page

M

O

P

R

Page

S

T

U

V

W

List of abbreviations

BP	Band pass
Br.	Suitable for eyeglass wearers
C	Camera
CB	Conversion Blue (filter name)
CP	Clinical Plan (objective)
D	Coverglass thickness
DIN	Deutsches Institut für Normung (German Standards Institute)
DF	Darkfield
EG	European Community (EC)
EN	European standard
ENG	Electronic News Gathering
FL	Fluorescence
foc.	focusing
fot.	Photographic
FT	Dichroic beam splitter
FWD	Free working distance
HAL	Halogen lamp
HBO	Mercury vapor short-arc lamp
HF, (H)	Brightfield
ICS	Inifinity Colour-corrected System
IEC	International Electrotechnical Commission
IP	International Protection (protection by instrument casing)
ISO	International Organization for Standardization
Korr. / Corr.	Correction mount or correction ring
LD	Long Distance
LP	Long pass
Ph	Phase
Pl	Plane, flat field
SF	Field of view
SFZ	Field-of-view number, field number
SLR	Single Lens Reflex
SK	Protection Class
Var	VAREL
vis	Visual