

Operating Manual
Axiovert 200 / Axiovert 200 M
Inverted Microscopes

Knowledge of this manual is required for the operation of the instrument. Would you please therefore make yourself familiar with the contents of this manual and pay special attention to hints concerning the safe operation of the instrument.

The specifications are subject to change; the manual is not covered by an update service.

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CONTENTS

	page
INTRODUCTION	0-2
Copyright	0-2
Contents.....	0-3
Notes on instrument safety.....	0-7
Notes on warranty	0-10
Overall view of the Axiovert 200.....	0-11
Overall view of the Axiovert 200 M.....	0-11
Microscopy in transmitted-light brightfield in a few steps.....	0-12
CHAPTER 1 INSTRUMENT DESCRIPTION	1-3
1.1 Name and intended application	1-3
1.2 Instrument description and main features	1-5
1.3 Microscope configurations and modules	1-6
1.4 Objectives	1-10
1.5 Eyepieces	1-12
1.6 Condensers.....	1-12
1.7 Specimen stages and mounting frames.....	1-13
1.8 Binocular tubes	1-13
1.9 Technical Data	1-14

	page
CHAPTER 2 START-UP.....	2-5
2.1 Unpacking and installation of the microscope.....	2-5
2.2 Attachment of binocular (photo) tube	2-6
2.2.1 Inserting the eyepieces and the centering telescope	2-6
2.2.2 Inserting the eyepiece reticle	2-7
2.3 Attachment of transmitted-light illumination	2-8
2.3.1 Attachment of carrier for transmitted-light illumination (100 W)	2-8
2.3.2 Attachment of carrier for transmitted-light illumination (30 W)	2-8
2.4 Screw in objectives	2-9
2.5 Attachment of microscope stages	2-10
2.5.1 Attachment of mechanical stage 130x85 R/L and mounting frame for mechanical stage (K).....	2-10
2.5.2 Attachment of scanning stage.....	2-10
2.5.3 Attachment of specimen stage 250x230, object guide and mounting frame for object guide (M).....	2-11
2.5.4 Attachment of heating stage	2-12
2.5.5 Attachment of gliding stage Z.....	2-12
2.6 Attachment of condensers	2-13
2.6.1 Condensers for the Axiovert 200.....	2-13
2.6.2 Condensers from the Axioplan 2 imaging / Axioskop 2 product line	2-13
2.6.3 Changing the DIC prism in the condenser turret	2-14
2.7 Reflector turret	2-15
2.7.1 Attachment of reflector turret.....	2-15
2.7.2 Equipment of reflector turret	2-15
2.7.3 Changing the filter set in the FL reflector module	2-16
2.7.4 Changing the beam splitter in the FL reflector module.....	2-16
2.8 Connection to the line	2-18
2.9 Interfaces of the Axiovert 200 M	2-19
2.10 Switch microscope and ebq 100 dc power supply on and off.....	2-19
2.11 Equipotential bonding terminals	2-19
2.12 HAL 100 halogen illuminator.....	2-20
2.12.1 Change / attachment of the HAL 100 halogen lamp	2-20
2.12.2 Coarse alignment of halogen illuminator	2-21
2.12.3 Attachment of halogen illuminator.....	2-21
2.12.4 Fine alignment of halogen illuminator	2-22

	page
2.13	Illuminator N HBO 103 2-23
2.13.1	Attachment / change of the HBO 103 W/2 mercury pressure short-arc lamp..... 2-23
2.13.2	Coarse alignment of illuminator N HBO 103..... 2-25
2.13.3	Attachment of illuminator N HBO 103 2-26
2.13.4	Fine adjustment of illuminator N HBO 103 using the adjusting aid 2-27
2.14	Attachment of Aqua Stop 2-28
CHAPTER 3	OPERATION..... 3-3
3.1	Axiovert 200 (manual)..... 3-4
3.1.1	Operation and function controls on the Axiovert 200 (manual)..... 3-4
3.1.2	Switching on and basic settings on the Axiovert 200 (manual)..... 3-14
3.2	Axiovert 200 M (motorized)..... 3-16
3.2.1	Operation and function controls on the Axiovert 200 (motorized)..... 3-16
3.2.2	Switching on and basic settings on the Axiovert 200 M (motorized) 3-23
3.3	Illumination and contrasting techniques..... 3-28
3.3.1	Setting of transmitted-light brightfield for KÖHLER illumination..... 3-28
3.3.2	Setting of transmitted-light phase contrast 3-33
3.3.3	Setting of differential interference contrast (DIC) in transmitted light..... 3-35
3.3.4	Setting of VAREL contrast in transmitted light..... 3-38
3.3.5	Setting of fluorescence contrast in reflected light..... 3-40
3.4	Documentation 3-43
3.4.1	Image orientation of camera ports..... 3-43
3.4.2	Photomicrography with SLR camera..... 3-45
3.4.3	Photomicrography using a digital camera and videomicroscopy 3-46

	page
CHAPTER 4 CARE, MAINTENANCE, TROUBLESHOOTING AND SERVICE.....	4-3
4.1 Care	4-3
4.2 Maintenance	4-4
4.2.1 Performing checks	4-4
4.2.2 Changing the fuses on the microscope.....	4-4
4.2.3 Changing the fuses on the ebq 100 dc power supply	4-5
4.3 Service.....	4-6
 ANNEX	 A-1
List of abbreviations.....	A-3
List of key words.....	A-5
Certification in accordance with DIN ISO 9001 / EN 46001	
EC Declaration of Conformity	

Notes on instrument safety

The Axiovert 200 / Axiovert 200 M microscopes were designed, produced and tested in compliance with DIN 61010-1 (IEC 1010-1), Safety requirements for electrical measuring, control and laboratory instruments, and meet the requirements of appendix I of directive 73/23/EC and the relevant CSA and UL directives. The instruments meet the requirements of the EC directive 89/336/EC and the EMC legislation of November 9th 1992. This operation manual includes information and warnings which must be observed by the user.

The following warning and information symbols are used in this manual:

**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**

UV radiation is emitted!

**CAUTION**

Disconnect the instrument from the line before opening it!

The Axiovert 200 microscopes, including original accessories, may only be used for the microscope techniques described in this manual.

Particular attention must be paid to the following notes:

 The manufacturer cannot assume any liability for any other applications, possibly also involving individual modules or single parts. This also applies to all service or repair work which is not carried out by authorized service personnel. Furthermore, this forfeits all the claims against warranty

 The power plug must be inserted in a socket featuring a grounding (earth) contact. The grounding effect must not be made ineffective by an extension cable which does not have a protective ground wire.

 If it is established that the protection measures are no longer effective, the instrument must be switched off and safeguarded against inadvertent operation. For repair of the instrument, contact the Carl Zeiss microscope service in Germany (see page 4-6) or your local Carl Zeiss agency.


 The Axiovert 200 microscopes are not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive or otherwise hazardous to health. All the legal regulations for accident prevention, particularly those in the respective countries, must be observed when handling such substances.

 Before switching on the power unit for the HBO 50/100, check whether it is suitable for the line voltage present.

 Always disconnect the instrument from the line before opening the instrument and before changing the fuses.

 Make sure to use only fuses of the rated power required. The use of makeshift fuses and the short-circuiting of the fuse holders are not permitted.



Gas discharge lamps, e.g. HBO 100, emit ultraviolet radiation which can cause burns on 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 hot, gas discharge lamps are under high internal pressure and must therefore be changed when cooled down by using protective gloves and goggles (for detailed information, please see the operating instructions B 40-065 e).



When fluorescence filters are used, the filter protecting from heat emitted by the microscope illuminator must not be removed, since fluorescence filters are sensitive to heat and their function might therefore be impaired.



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



Avoid touching the hot lamp housings. Always pull the power plug before changing the lamps and allow the instrument to cool down for approx. 15 mins.



Dust and dirt can impair the performance of the instrument. Therefore, the instrument must be protected against these influences as far as possible, and covered with the dust cover if it is not used for longer periods of time. Always check whether the instrument is switched off before you cover it.



The instruments may only be operated by trained personnel who must be aware of the possible danger involved with microscopy and the relevant application.



The Axiovert 200 microscopes are opto-mechanical precision instruments which can be impaired in their performance or damaged when handled improperly.

Notes on warranty

The manufacturer guarantees that the instrument has no material and production defects when delivered. You must inform us of any defects immediately and we must do anything to minimize the damage. If the manufacturer is informed of such a defect, he is obliged to remove it; it is his decision whether he does this by repairing the instrument or by delivering an instrument free of any defect. No guarantee is provided for defects caused by natural wear (wearing parts in particular) and improper use.

The instrument manufacturer is not liable for damage caused by faulty operation, negligence or any other meddling with the instrument, particularly the removal or replacement of instrument components, or the use of accessories from other manufacturers. This forfeits all the claims against warranty.

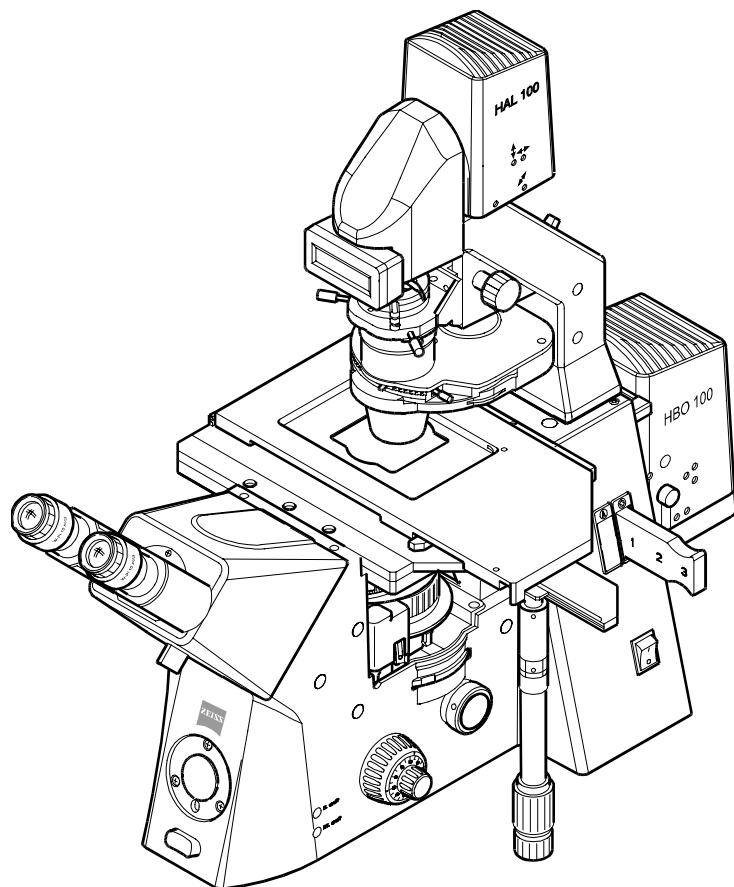
With the exception of the work specified in this manual, no maintenance or repair of the microscopes may be performed. Repairs may only be performed by Carl Zeiss service staff or specially authorized personnel. Should any defect occur with the instrument, please get in touch with the Carl Zeiss microscopy service in Germany (see page 4-6) or your local Zeiss agency.

INTRODUCTION

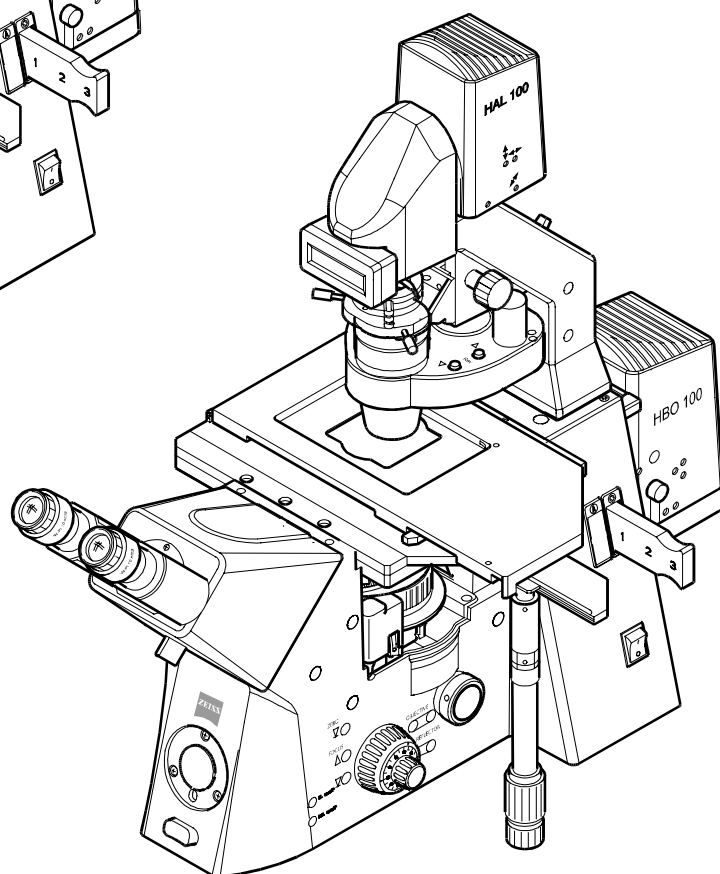
Axiovert 200

Overall view of the Axiovert 200 and Axiovert 200 M

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**Overall view of the
Axiovert 200 (manual)**



**Overall view of the
Axiovert 200 M (motorized)**

Microscopy in transmitted-light brightfield in a few steps

 Before starting to use the Axiovert 200, make sure to read the notes on instrument safety and the chapters entitled "**Instrument Description**" (Chapter 1) and "**Start-up**" (Chapter 2).

- Make the microscope ready for operation as described in chapter 2 and switch it on via the On/Off switch (0-2/1).
- Select the objective with the lowest magnification (e.g. 10x) on the nosepiece (0-2/2). Set factor 1x on the setting wheel (0-2/4) of the Optovar turret.
- Open the luminous-field diaphragm or the aperture diaphragm completely by pulling lever (0-2/16) to the front until stop or by turning the setting wheel (0-2/20) to the front until stop.
- Turn the setting ring (0-2/19) to move the condenser turret in position **H** for brightfield (or **DIC**).
- Move reflector turret (0-2/5, if available) into the position without filter combination via the setting ring.
- If required, remove analyzer slider (0-2/3) or switch to free light path.
- Turn setting wheel for Sideport right / left / vis (0-2/22) to position 100 % vis (visual ☉).
- Turn setting knob for Frontport / Baseport / vis (0-2/23) to position 100 % vis ().
- Set beam splitting ratio to 100 % vis (0-2/10) on the tube. Switch off the Bertrand lens (if available). Move combined rotary / slider knob (0-2/9) to position 100 % vis (.
- Place a high-contrast specimen on the microscope stage (0-2/21). Adjust the binocular component.
- Use the coarse / fine focusing drive (0-2/6) to focus on the selected detail of the specimen. Should no light be visible in the eyepieces, switch on the halogen illuminator via the HAL on / off switch (0-2/7).

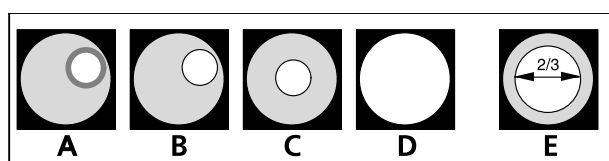
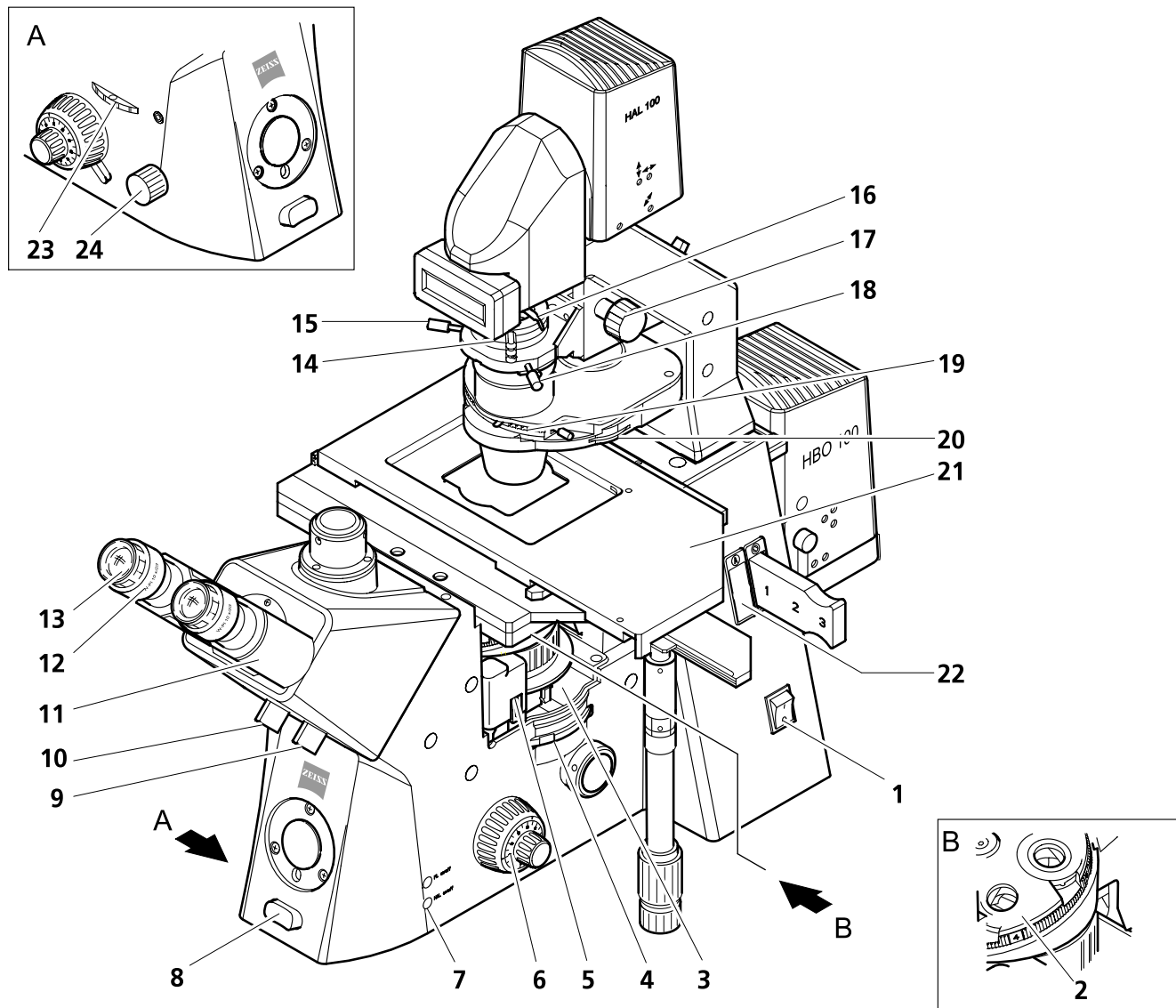


Fig. 0-1 Diaphragm settings in transmitted-light brightfield according to KÖHLER

- Use the toggle switch (0-2/8) to set the light intensity to comfortable brightness.
- Close luminous-field diaphragm (0-2/16) until it is visible in the field of view, even if not in focus (0-1/A).
- Focus on the edge of the luminous-field diaphragm (0-1/B) by moving the condenser (0-2/17) vertically.
- Center (0-1/C) luminous-field diaphragm via the centering screws (0-2/15 and 18) and open it until the edge of the diaphragm just disappears from the field of view (0-1/D).
- Remove one eyepiece from the eyepiece tube (or swing in Bertrand lens) and set aperture diaphragm (0-2/20) to approx. 2/3 of the diameter of the objective exit pupil (0-1/E). Optimum contrast setting is dependent on the respective specimen.
- Insert the eyepiece again (or swing out Bertrand lens) and refocus, if required, via the fine drive.
- After the microscope has been set to transmitted-light brightfield in this way, changing to this special contrasting technique is now possible (see chapter 3 of this manual).



- | | | |
|--|---|--|
| 1 On / Off switch | 10 Turning or sliding knob for Bertrand lens and manual shutter | 18 Centering screw for condenser |
| 2 Objective nosepiece | 11 Binocular tube component | 19 Turret disk of condenser |
| 3 Compartment for slider Analyzer | 12 Setting ring of the eyepiece | 20 Setting wheel for aperture diaphragm on the condenser |
| 4 Setting wheel for Optovar turret | 13 Eyepiece | 21 Microscope stage |
| 5 Reflector turret | 14 Polarizer D with 2-position filter changer | 22 Compartment for aperture diaphragm slider |
| 6 Focusing drive coarse / fine | 15 Centering screw for condenser | 23 Setting wheel for Sideport |
| 7 HAL on / off switch | 16 Setting lever for luminous-field diaphragm | 24 Setting knob for Frontport / Baseport |
| 8 Toggle switch for illumination intensity | 17 Setting knob for vertical adjustment of the condenser | |
| 9 Turning or sliding knob for vis / doc beam splitting | | |

Fig. 0-2 Axiovert 200

INTRODUCTION

Carl Zeiss

Microscopy in transmitted-light brightfield in a few steps

Axiovert 200

INSTRUMENT DESCRIPTION

Contents

1	INSTRUMENT DESCRIPTION	1-3
1.1	Name and intended application.....	1-3
1.2	Instrument description and main features.....	1-5
1.3	Microscope configurations and modules.....	1-6
1.4	Objectives.....	1-10
1.5	Eyepieces.....	1-12
1.6	Condensers	1-12
1.7	Specimen stages and mounting frames	1-13
1.8	Binocular tubes.....	1-13
1.9	Technical Data.....	1-14

List of illustrations

Fig. 1-1	Microscope configurations and modules (sheet 1)	1-6
Fig. 1-2	Microscope configurations and modules (sheet 2)	1-7
Fig. 1-3	Microscope configurations and modules (sheet 3)	1-8
Fig. 1-4	Microscope configurations and modules (sheet 4)	1-9

1 INSTRUMENT DESCRIPTION

1.1 Name and intended application

Manufacturer's name: Inverted microscope for transmitted light and epifluorescence

Brief name: Axiovert 200 (manual version)
Axiovert 200 M (motorized version)

The Axiovert 200 microscopes fit in the product family of inverted transmitted-light microscopes as follows:

Laboratory microscopes

- Axiovert 25
- Axiovert 25 C
- Axiovert 25 CFL

Research microscopes

- **Axiovert 200**
- **Axiovert 200 M**

The Axiovert 200 microscopes are universally applicable inverted microscopes and are mainly used for the examination of cell and tissue cultures and of sediments in culture flasks, Petri dishes, microtiter plates, etc. in transmitted and reflected light.

The Axiovert 200 microscopes permit the performance of the transmitted-light techniques brightfield, phase contrast, differential interference contrast and VAREL contrast, and the epi-fluorescence technique.

The microscopes Axiovert 200 and Axiovert 200 M are the basis for scientific microscopic work on living cells.

- The sturdy stand provides attachment possibilities for various tools (micromanipulation), different light sources, temperature control devices, etc.
- Ample space is available for the specimens and the relevant handling systems on account of the inverted design, the LD illumination system of the microscope and the use of fixed stages. This permits the performance of experiments which would not be possible with upright microscopes.
- The design allows the easy attachment of cameras, lasers, specific stages, etc.

Typical fields of application:

Observation of intracellular processes in living cell cultures, cell/cell interactions, motility, growth, measurements of potential, drug detection, microinjection, IVF (in-vitro fertilization), toxicity examinations, patch-clamp technique, ion measurements, digital recording, long-time / time lapse examinations in combination with the automation of processes, z-sectioning, deconvolution, visualization of molecular structures, Fura (Ca measurement), GFP, optical tweezers and scissors, single molecule detection, TIRF

Accessories for temperature control and incubation are described in the separate manual B 40-610 d/e, "Incubation systems on the Axiovert".

1.2 Instrument description and main features

The Axiovert 200 / Axiovert 200 M is available either as manual or as motorized version. The accessory components are part of a modular system.

For documentation purposes, the Axiovert 200 / Axiovert 200 M can be equipped with the maximum of five camera / TV ports in accordance with the customer's requests.

Adaptation possibilities are provided for heating stages, incubators and micromanipulators.

Major instrument features:

- ICS optics for image creation
- high thermal and mechanical stability
- high flexibility in documentation
- improved ergonomic design
- LCD display of instrument parameters
- 23 mm field of view
- Light Manager
- modular design for optimum adaptation to the relevant application
- 6-position nosepiece, coded
- 5-position reflector turret, manual: can be changed on both sides; motorized: can be changed from the right
- 5-position or 6-position condenser turret
- 3-position Optovar turret
- changeable aperture diaphragm and luminous-field diaphragm sliders in reflected light
- fluorescence shutter
- illuminators: HAL 100 W, HBO 50, N HBO 103, N XBO 75
- Axiovert 200 M, all the major microscope functions are motorized.

1.3 Microscope configurations and modules

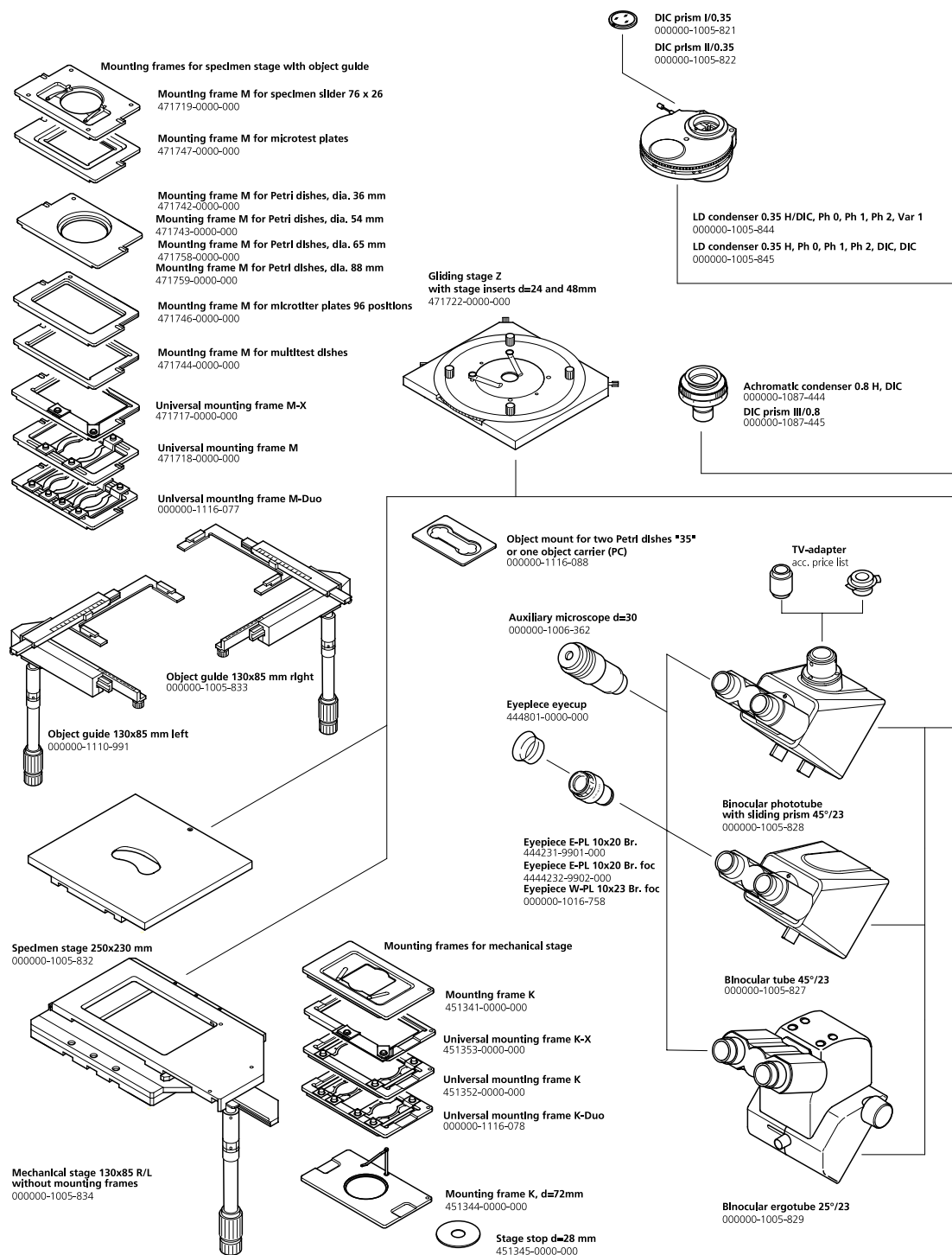


Fig. 1-1 Microscope configurations and modules (sheet 1)

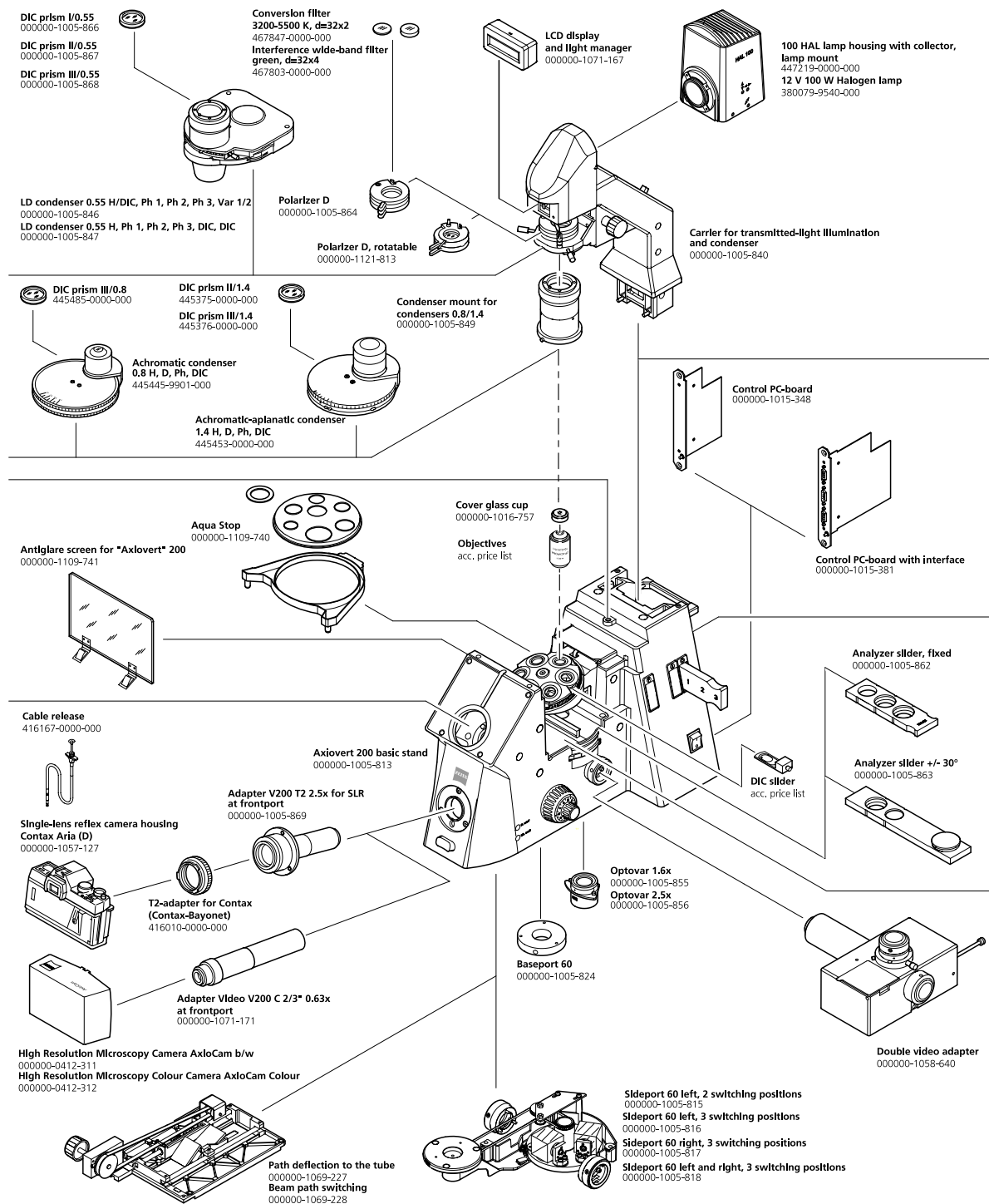


Fig. 1-2 Microscope configurations and modules (sheet 2)

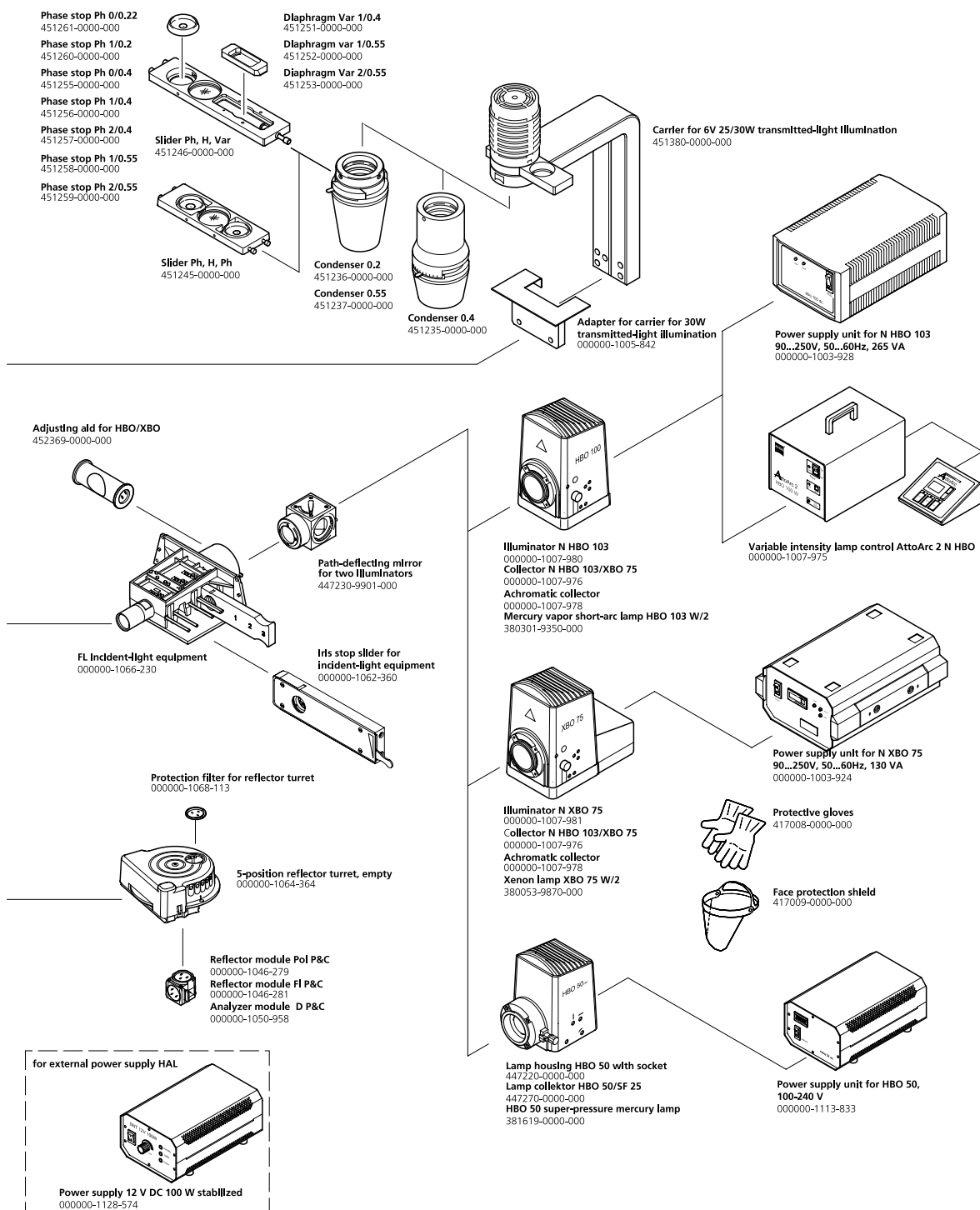
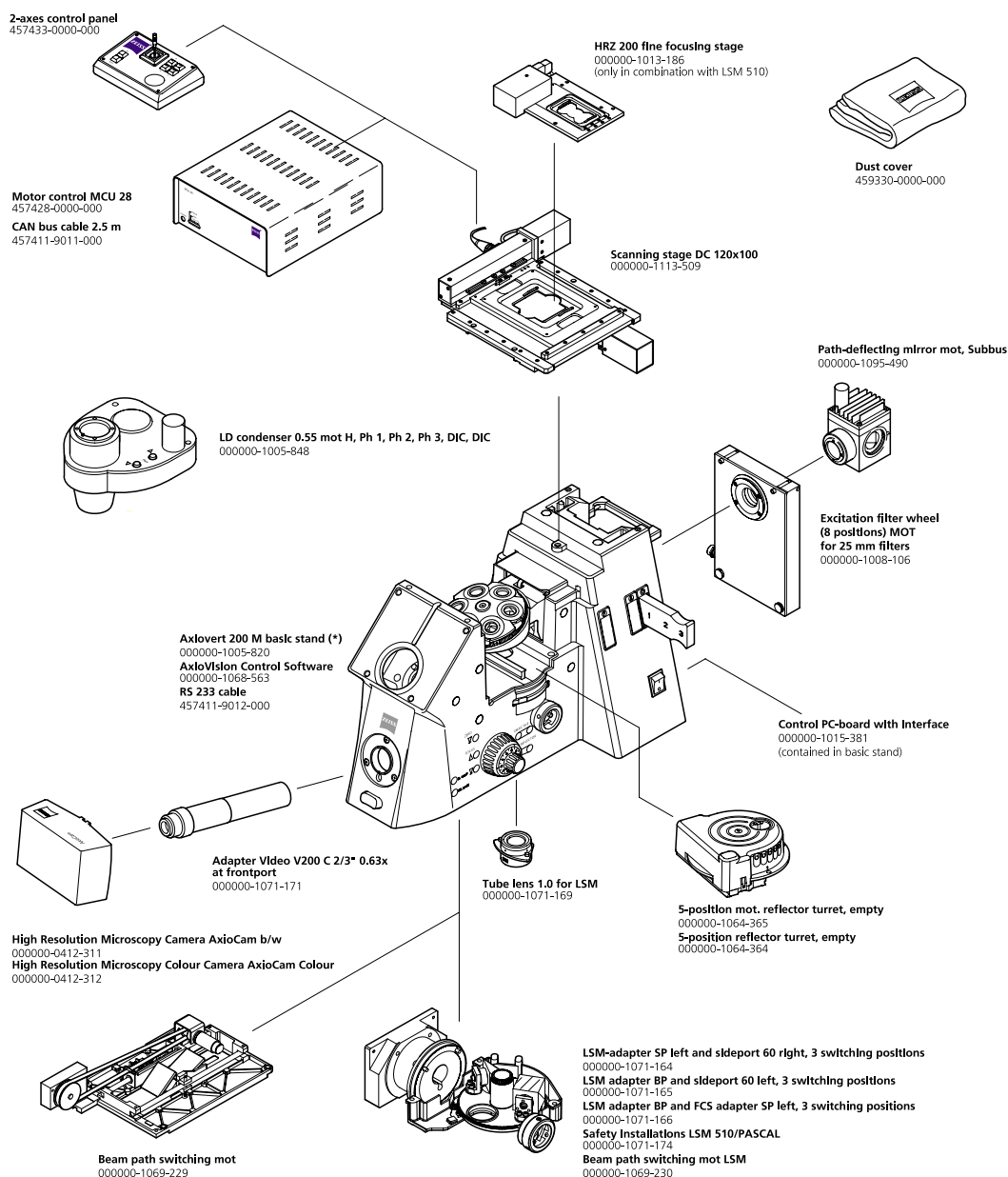


Fig. 1-3 Microscope configurations and modules (sheet 3)



(*) If there are no special motorized components, all components of the manual stand can also be used at the motorized stand.

Fig. 1-4 Microscope configurations and modules (sheet 4)

1.4 Objectives

The objectives are the optical centerpiece of the microscope. The following is an example of how objectives can be labelled:

A-Plan 10×/0.20 HD ∞/-

Key:

10× objective magnification, with a color ring on the objective being allocated to each magnification step (Zeiss color code)

0.20 numerical aperture

HD reflected-light brightfield and darkfield objective

∞ infinite tube length

- can be used without cover slip ($D = 0$ mm) or with cover slip thickness $D = 0.17$ mm

or

0 can only be used without cover slip ($D = 0$ mm)

0.17 can only be used with cover slip thickness $D = 0.17$ mm

and

Oil oil immersion objective

Ph 2 phase contrast objective with a green color ring and phase stop Ph 2

Color ring code for objective magnification:

Color ring on objective	black	brown	red	orange	yellow	green	light blue	dark blue	white
Magnification factor	1.25×	2.5×	4×; 5×	6.3×	10×	16×; 20×; 25×; 32×	40×; 50×	63×	100×; 150×

The objective magnification (e.g. 10×) multiplied with the eyepiece magnification (e.g. 10×) and the Optovar magnification (e.g. 1.6×) results in the visual overall magnification;
example: $10 \times 10 \times 1.6 = 160\times$.

The numerical aperture $\times 1000$, e.g. $0.20 \times 1000 = 200\times$, is the highest useful magnification, i.e. no further details are resolved above that limit.

In transmitted-light applications, the exact observance of the cover slip thickness of 0.17 mm is all the more necessary the higher the numeric aperture of the objective. Therefore, so-called "corr" objectives can be set for different cover slip thicknesses via a correction ring. For this, a specimen area is searched, and the position of the correction ring where optimum focus and image contrast are obtained is determined (refocusing is always required).

Immersion objectives are always insensitive to differences in cover slip thickness.

When immersion objectives are used, the air between the cover slip and the objective is replaced with a liquid, which is immersion oil in most cases.

The following objectives are available for the Axiovert 200 / Axiovert 200 M microscope:

Objective type	Magnification/ numeric aperture	Cover slip thickness D in mm	Free working distance A in mm	Contrasting	Cat. No.
A-Plan					
A-Plan	5x/0.12		a = 9.1	ph0	000000-1018-589
A-Plan	10x/0.20		a = 4.4	ph1 var1	000000-1020-863
LD A-Plan	20x/0.30	D = 0.6 - 1.4	a = 4.3	ph1	000000-1006-591
LD A-Plan	20x/0.30	D = 0.6 - 1.4	a = 4.2	ph1 var1	000000-1006-592
LD A-Plan	32x/0.40	D = 0.6 - 1.4	a = 3.1	ph1	000000-1006-593
LD A-Plan	32x/0.35	D = 0.6 - 1.4	a = 3.1	ph1 var1	000000-1006-594
LD A-Plan	40x/0.50	D = 0.7 - 1.3	a = 2.3	ph2	000000-1006-595
LD A-Plan	40x/0.50	D = 0.7 - 1.3	a = 2.3	ph2 var2	000000-1006-596
Cover slip cap		D = 0.17 - 0.6			000000-1016-757
LD Achroplan *					
Achroplan	4x/0.10	-	a = 11.1		440020-0000-000
Achroplan	10x/0.25	-	a = 4.8	ph1	440031-0000-000
LD Achroplan	20x/0.40	corr D = 0 - 1.5	a = 10.2		440844-0000-000
LD Achroplan	20x/0.40	corr D = 0 - 1.5	a = 10.2	ph2	440845-0000-000
LD Achroplan	40x/0.60	corr D = 0 - 2	a = 1.8		440864-0000-000
LD Achroplan	40x/0.60	corr D = 0 - 2	a = 1.8	ph2	440865-0000-000
LD Achroplan	63x/0.75	corr D = 0 - 1.5	a = 1.57	ph2	440861-0000-000
Plan-Neofluar					
Plan-Neofluar	5x/0.15	-	a = 13.6		440320-0000-000
Plan-Neofluar	5x/0.15	-	a = 13.6	ph1	440321-0000-000
Plan-Neofluar	10x/0.30	D = 0.17	a = 5.6		440330-0000-000
Plan-Neofluar	10x/0.30	D = 0.17	a = 5.6	ph1	440331-0000-000
Plan-Neofluar	16x/0.50 Imm	D = 0.17	a = 0.22		440530-0000-000
Plan-Neofluar	16x/0.50 Imm	D = 0.17	a = 0.22	Ph1	440531-0000-000
Plan-Neofluar	20x/0.50	D = 0.17	a = 2.0		000000-1004-072
Plan-Neofluar	20x/0.50	D = 0.17	a = 2.0	ph2	000000-1004-989
Plan-Neofluar	40x/0.75	D = 0.17	a = 0.5		440350-9902-000
Plan-Neofluar	40x/0.75	D = 0.17	a = 0.5	ph2	440351-9902-000
Plan-Neofluar	40x/1.30 Oil	D = 0.17	a = 0.2		000000-1022-818
Plan-Neofluar	40x/1.30 Oil	D = 0.17	a = 0.2	ph3	000000-1022-819
Plan-Neofluar	63x/0.95 Korr.	D = 0.17	a = 0.12		440364-0000-000
Plan-Neofluar	100x/1.30 Oil	D = 0.17	a = 0.12		000000-1018-595
Plan-Neofluar	100x/1.30 Oil	D = 0.17	a = 0.12	ph3	000000-1031-171
Plan-Apochromat					
Plan-Apochromat	63x/1.40 Oil	D = 0.17			440760-0000-000

* a refers to d = 1

1.5 Eyepieces

The following eyepieces are available for the Axiovert 200 and Axiovert 200 M:

Eyepiece type	Image angle	Cat. No.
Eyepiece W-PL 10×/23 Br. foc.	24.7°	455043-0000-000
Eyepiece W-PL 10×/23 Br. foc.	24.7°	000000-1016-758
Centering telescope d = 30		000000-1006-362

1.6 Condensers

The following condensers are available for combination with the stage carrier D and the transmitted-light equipment on the Axiovert 200 / Axiovert 200 M:

Condenser type	Cat. No.	Comments
LD condenser 0.35, 5 positions: H/DIC, Ph0, Ph1, Ph2, Var1/2	000000-1005-844	from objective 2.5x
LD condenser 0.55, 6 positions: H, Ph1, Ph2, Ph3, DIC, DIC mot.	000000-1005-848	from objective 4x
Condenser 0.35, 6 positions: H/DIC, Ph0, Ph1, Ph2, DIC, DIC	000000-1005-845	
Condenser 0.55, 5 positions: H, Ph1, Ph2, Ph3, Var1/2	000000-1005-846	
Condenser 0.55, 6 positions: H, Ph1, Ph2, Ph3, DIC, DIC	000000-1005-847	
Condenser adapter for condensers 0.8 and 1.4	000000-1005-849	
Condenser 0.8 H, D 0.66 / 0.8 Ph DIC, plus factory-aligned: DIC prism III/0.8	445445-9901-000 445485-0000-000	
Condenser 0.8 H, DIC, plus factory- aligned: DIC prism III/0.8	000000-1087-444 000000-1087-445	
Condenser 1.4, 6 positions: H, D, Ph 2x, DIC 2x	445453-0000-000	

1.7 Specimen stages and mounting frames

The Axiovert 200 microscopes can be equipped with the following specimen stages and mounting frames:

Description	Cat. No.
Specimen stage 250x230 mm with ceramic surface and 3-point support	000000-1005-832
Object guide 130x85 right, can be attached on both sides, accepts various mounting frames	000000-1005-833
Object guide 130x85 left, can be attached on both sides, accepts various mounting frames	000000-1110-991
Mechanical stage 130x85 R/L	000000-1005-834
Mounting frame for object guide (M)	see price list
Mounting frame for mechanical stage (K)	see price list
Gliding stage Z	471722-0000-000
Scanning stage	000000-1113-509

1.8 Binocular tubes

The following tubes can be used with the Axiovert 200 microscopes:

Description	Cat. No.	VIEWING ANGLE / FIELD NUMBER	Light distribution in %
Binocular tube 45°/23	000000-1005-827	45° / 23	100 %
Binocular phototube 45°/23, with Bertrand lens and shutter	000000-1005-828	45° / 23	100-0, 0-100, 50-50
Binocular ergotube 25°/23 vertically variable, with Bertrand lens and shutter	000000-1005-829	25° / 23	100 %

1.9 Technical Data

Dimensions (width x depth x height)

Stand Axiovert 200 approx. 295 × 805 × 707 mm

Weight

Axiovert 200 without fluorescence equipment approx. 26 kg

Axiovert 200 M with fluorescence equipment approx. 40 kg

Ambient conditions

Storage and transport (in packaging):

Permissible ambient temperature..... -40 to +50 °C

Operation:

Permissible ambient temperature..... +10 to +35 °C

Permissible relative humidity (without condensation).....max. 80 %

Altitude of use max. 2000 m

Atmospheric pressure..... 800 hPa to 1060 hPa

Pollution degree..... 2

Operating data

Category of use..... closed rooms

Protection class.....I

Protection type IP 20

Electrical safetyin compliance with DIN EN 61010 (IEC 1010-1)
including CSA and UL directives

Excess voltage category II

Radio interference suppression..... in accordance with EN 55011, Class B

Voltage ranges:

Integrated 12 V, 100 W power unit, stabilized 100 to 240 VAC (± 10 %)

Line frequency	50 to 60 Hz
Power consumption with internal power units	
Axiovert 200	235 VA
Axiovert 200 M	235 VA
and external power units	
Power supply N XBO 75, 100 ... 240 V AC	130 VA
Power supply N HBO 100, 90 ... 240 V AC	265 VA

Fuses in accordance with IEC 127

Axiovert 200

100 ... 240 V AC	T 4.0 A/H; 250 V; 5 × 20 mm
------------------------	-----------------------------

Axiovert 200 M

100 ... 240 V AC	T 4.0 A/H; 250 V; 5 × 20 mm
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Light sources

HBO 50 W/AC mercury pressure short-arc lamp

Lamp voltage for lamp type L1 and L2	L1: 39 ... 45 V/L2: 34 ... 39 V
Power	50 W
Average life	100 h

HBO 103 mercury vapor short-arc lamp	100 W
--	-------

Opto-mechanical data

Stand with stage focusing	with coarse drive (5 mm/rot) and fine drive (0.1 mm /rot) Fine scaling 1 µm/scale part Overall lift approx. 10 mm
Objective change	via 6-position nosepiece
Objectives	with W 0.8" x 1/36" thread
Eyepieces	with plug-in diameter 30 mm and
	field number 23

START-UP

Contents

2	START-UP.....	2-5
2.1	Unpacking and installation of the microscope.....	2-5
2.2	Attachment of binocular (photo) tube.....	2-6
2.2.1	Inserting the eyepieces and the centering telescope.....	2-6
2.2.2	Inserting the eyepiece reticle.....	2-7
2.3	Attachment of transmitted-light illumination.....	2-8
2.3.1	Attachment of carrier for transmitted-light illumination (100 W).....	2-8
2.3.2	Attachment of carrier for transmitted-light illumination (30 W).....	2-8
2.4	Screw in objectives.....	2-9
2.5	Attachment of microscope stages.....	2-10
2.5.1	Attachment of mechanical stage 130x85 R/L and mounting frame for mechanical stage (K).....	2-10
2.5.2	Attachment of scanning stage.....	2-10
2.5.3	Attachment of specimen stage 250x230, object guide and mounting frame for object guide (M).....	2-11
2.5.4	Attachment of heating stage.....	2-12
2.5.5	Attachment of gliding stage Z.....	2-12
2.6	Attachment of condensers.....	2-13
2.6.1	Condensers for the Axiovert 200.....	2-13
2.6.2	Condensers from the Axioplan 2 imaging / Axioskop 2 product line.....	2-13
2.6.3	Changing the DIC prism in the condenser turret.....	2-14
2.7	Reflector turret.....	2-15
2.7.1	Attachment of reflector turret.....	2-15
2.7.2	Equipment of reflector turret.....	2-15
2.7.3	Changing the filter set in the FL reflector module.....	2-16
2.7.4	Changing the beam splitter in the FL reflector module.....	2-16

2.8	Connection to the line.....	2-18
2.9	Interfaces of the Axiovert 200 M	2-19
2.10	Switch microscope and ebq 100 dc power supply on and off	2-19
2.11	Equipotential bonding terminals	2-19
2.12	HAL 100 halogen illuminator	2-20
2.12.1	Change / attachment of the HAL 100 halogen lamp.....	2-20
2.12.2	Coarse alignment of halogen illuminator.....	2-21
2.12.3	Attachment of halogen illuminator	2-21
2.12.4	Fine alignment of halogen illuminator	2-22
2.13	Illuminator N HBO 103	2-23
2.13.1	Attachment / change of the HBO 103 W/2 mercury pressure short-arc lamp.....	2-23
2.13.2	Coarse alignment of illuminator N HBO 103.....	2-25
2.13.3	Attachment of illuminator N HBO 103	2-26
2.13.4	Fine adjustment of illuminator N HBO 103 using the adjusting aid	2-27
2.14	Attachment of Aqua Stop.....	2-28

List of illustrations

Fig. 2-1	Installation of microscope	2-5
Fig. 2-2	Attachment of binocular tube	2-6
Fig. 2-3	Inserting the eyepieces.....	2-6
Fig. 2-4	Inserting the eyepiece reticle	2-7
Fig. 2-5	Attachment of carrier for transmitted-light illumination (100 W)	2-8
Fig. 2-6	Attachment of carrier for transmitted-light illumination (30 W)	2-8
Fig. 2-7	Screw in objectives	2-9
Fig. 2-8	Attachment of mechanical stage 130x85	2-10
Fig. 2-9	Insertion of mounting frame (K)	2-10
Fig. 2-10	Attachment of specimen stage 250x230	2-11
Fig. 2-11	Attachment of object guide and mounting frame.....	2-11
Fig. 2-12	Attachment of heating stage	2-12
Fig. 2-13	Attachment of condenser	2-13
Fig. 2-14	Attachment of condenser adapter.....	2-13
Fig. 2-15	Changing the DIC prism.....	2-14
Fig. 2-16	Insertion of reflector turret.....	2-15
Fig. 2-17	Insertion of reflector modules	2-15
Fig. 2-18	Changing the filter set in the FL reflector module	2-16
Fig. 2-19	Changing the beam splitter.....	2-16
Fig. 2-20	Changing the beam splitter.....	2-17
Fig. 2-21	Axiovert 200 M (rear).....	2-18
Fig. 2-22	Power supply ebq 100 dc (rear).....	2-18
Fig. 2-23	Axiovert 200 M (rear).....	2-19
Fig. 2-24	Power supply ebq 100 dc (front)	2-19
Fig. 2-25	Changing the halogen lamp.....	2-20
Fig. 2-26	Alignment of halogen illuminator.....	2-21
Fig. 2-27	Attachment of halogen illuminator.....	2-21
Fig. 2-28	Changing the diffusion disk	2-22
Fig. 2-29	Power supply ebq 100 dc.....	2-23
Fig. 2-30	Removal of N HBO 103 housing	2-24
Fig. 2-31	Changing the HBO 103 W/2 mercury pressure short-arc lamp.....	2-24
Fig. 2-32	Coarse alignment of mercury vapor short-arc lamp.....	2-25
Fig. 2-33	Focal spots of N HBO 103 before coarse adjustment.....	2-25
Fig. 2-34	Focal spots of N HBO 103 after coarse adjustment	2-25
Fig. 2-35	Attachment of illuminator N HBO 103.....	2-26
Fig. 2-36	Power supply ebq 100 dc.....	2-26
Fig. 2-37	Adjusting aid and illuminator N HBO 103	2-27
Fig. 2-38	Attachment of Aqua Stop	2-28

2 START-UP

On account of the complexity of the equipment and to ensure proper functioning, installation and initial start-up of the Axiovert 200 or Axiovert 200 M at your site will be performed entirely by the responsible Carl Zeiss agency.

The following major services will be performed:

- Installation of the microscope, assembly and alignment of all components (if these are not already factory-aligned).
- Connection of cables and line connection.
- Instrument training

Should you want to install the instrument yourself or move it to another position, proceed as described below.

2.1 Unpacking and installation of the microscope

The basic instrument is supplied in a commercially available polyethylene case in cardboard packaging.

This package contains the following: stand, binocular tube, objectives, eyepieces, condenser, halogen illuminator, fluorescence illuminator and various small components such as filter and diaphragm slider, DIC slider, dust cover, tools, etc.

Further, optional accessories are supplied in a separate case.

- Remove all components from the packaging and use the delivery note to check for completeness.
- Place stand (2-1/1) on a low-vibration, flat worktable.
- Properly dispose of original packaging, or keep it for storage or return of the instrument to the manufacturer.
- Unscrew handle (2-1/2) using the SW 4 Allen key.

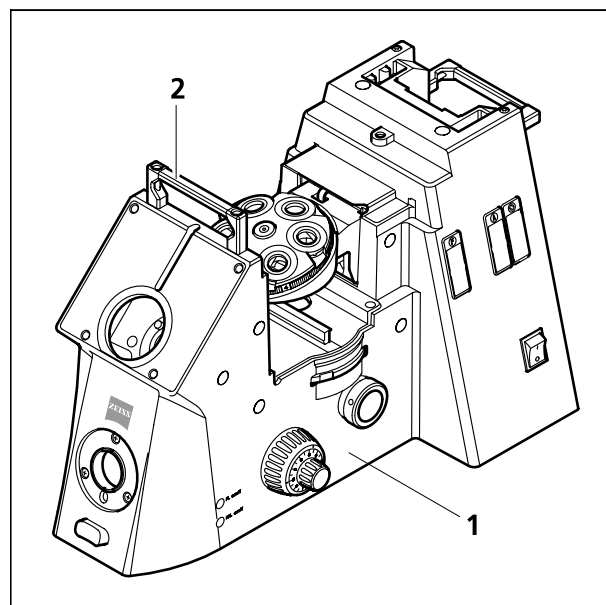


Fig. 2-1 Installation of microscope

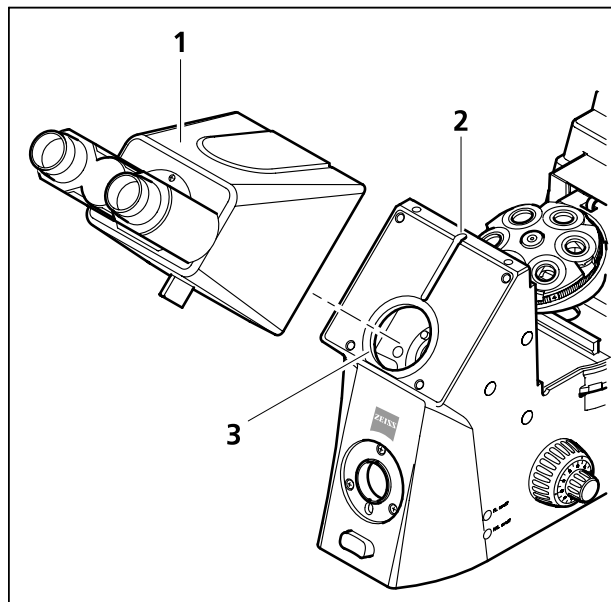


Fig. 2-2 Attachment of binocular tube

2.2 Attachment of binocular (photo) tube

All the binocular tubes listed in the microscope configuration can be attached to the Axiovert 200 and Axiovert 200 M as described below.

Proceed as follows to attach a tube for the first time, or to replace a tube:

- Loosen hexagonal screw (2-2/2) using the SW3 ball-headed screwdriver. If a tube is to be replaced, hold it and remove it in forward direction.
- Remove the dust cap from the tube lens of the tube to be attached.
- Insert dovetail of the binocular tube (2-2/1) into the stand mount (2-2/3), align it with the stand and tighten hexagonal screw (2-2/2) using the ball-headed screwdriver.

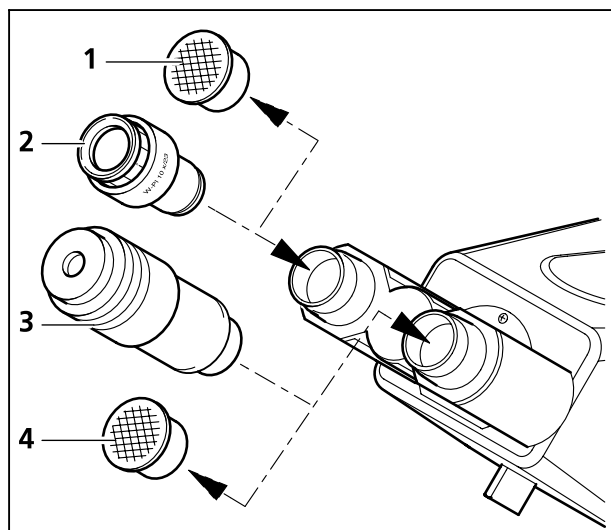


Fig. 2-3 Inserting the eyepieces

2.2.1 Inserting the eyepieces and the centering telescope

- Remove both dust protection caps (2-3/1 and 2-3/4) from the binocular tube.
- Remove both eyepieces (2-3/2) from the cases and insert them in the binocular tube until stop.
- The centering telescope (2-3/3), which is used to view the aperture and phase stops and to center the phase stops, can be inserted in one of the tubes instead of an eyepiece. The variable eyelens permits focusing on these stops.



The eyepieces W-PL 10x/23 Br. foc. can be attached to all tubes.

2.2.2 Inserting the eyepiece reticle

The eyepieces W-PL 10x/23 Br. foc. are intended for use with eyepiece reticles.

The slight image shift caused by the additional path through the glass is taken into account on the diopter scale by the fact that the zero point position is indicated not by the white dot (2-4/**W**) but by the red dot (2-4/**R**).

The eyepiece reticles (2-4/**1**) have been adhered to screw-in mounts (2-4/**2**) by the manufacturer to allow easy replacement.

To replace an eyepiece reticle, remove screw-in mount (2-4/**2**) with eyepiece reticle (2-4/**1**) and replace it with a screw-in mount containing the required eyepiece reticle.

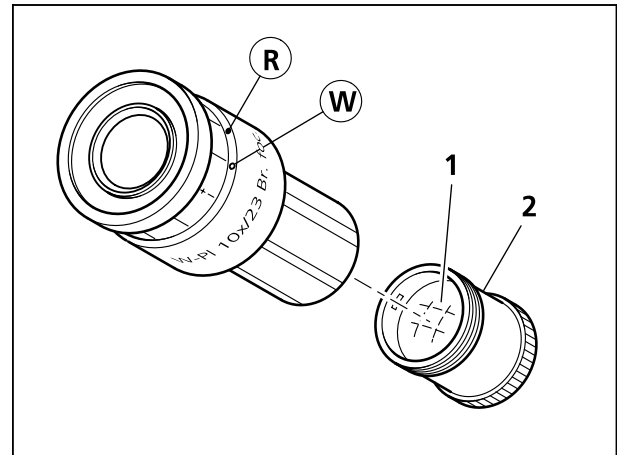


Fig. 2-4 Inserting the eyepiece reticle



If eyepiece reticles are inserted into the unscrewed mount by the customer, attention must be paid to the labelling being visible the right way up after insertion.

Compensation of ametropia when eyepiece reticles are used

For the correct use of an eyepiece reticle, two focusing eyepieces are required to allow the user to compensate for differences in the visual performance of his two eyes.

- Use the focusing eyepiece to focus on the eyepiece reticle; focus on the edge of the field of view if no eyepiece reticle is used.
- Use the focusing drive to focus on the microscope image of a specimen through the eyepiece set as described above.
- Then use the second focusing eyepiece to focus the microscope image for the second eye. The position of the focusing drive on the microscope stand must not be changed.

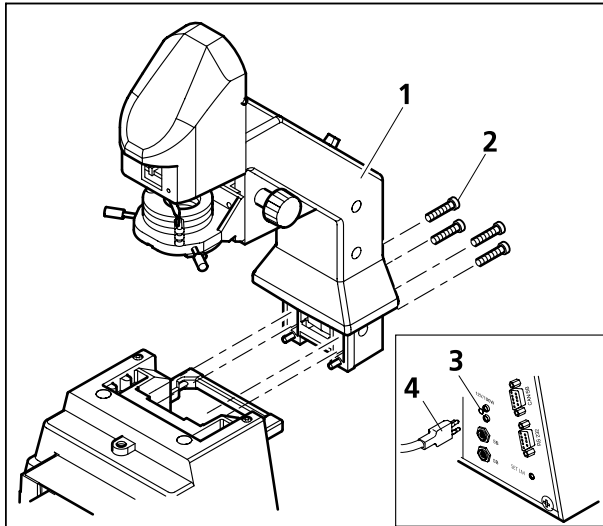


Fig. 2-5 Attachment of carrier for transmitted-light illumination (100 W)

2.3 Attachment of transmitted-light illumination

2.3.1 Attachment of carrier for transmitted-light illumination (100 W)

- Attach carrier (2-5/1) to the relevant contact surface on the rear of the stand and use the SW 4 Allen key to tighten the four enclosed hexagonal screws (2-5/2).
- Connect plug (2-5/4) for the LCD display (if available) to the SB socket (2-5/3) at the instrument rear.

Alignment of the carrier (100 W) is not required.

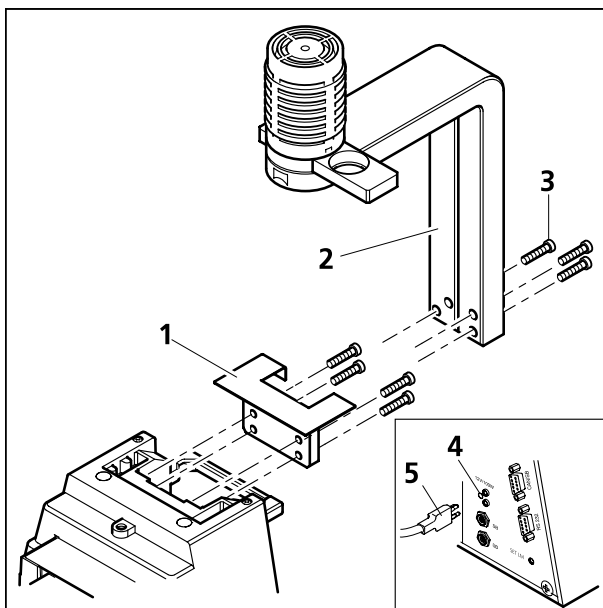


Fig. 2-6 Attachment of carrier for transmitted-light illumination (30 W)

2.3.2 Attachment of carrier for transmitted-light illumination (30 W)



Before attachment of the carrier for transmitted-light illumination 30 W (451380-0000-000) to the stand, the control electronics included in the stand must be changed by Zeiss service staff.

- Remove the HAL 100 and N HBO 103 illuminators from the microscope.
- If required, remove carrier (100 W) by loosening the four hexagonal screws (SW 4) and disconnect plug of the LCD display from the SB socket.
- Screw adapter plate (000000-1005-842, 2-6/1) onto the contact surface on the stand rear using the four hexagonal screws.

- Attach centering pin of the carrier for transmitted-light illumination (30 W) (2-6/2) to the adapter plate and tighten the three SW 4 hexagonal screws (2-6/3).

Alignment of the carrier (30 W) is not required.

- Connect power supply plug of the carrier for transmitted-light illumination 30 W (2-6/5) to the 12 V / 100 W connector (2-6/4) on the rear of the stand.

2.4 Screw in objectives

- Remove dust caps (2-7/1) from the respective openings in the nosepiece.
- Remove objectives (2-7/2) from the case and screw them in the nosepiece (2-7/3), starting with position 1 (see engraved number), in increasing order of magnification factors. Make sure that the objective is correctly and securely locked.



Make sure to use the dust protection caps on those nosepiece eyes which are not required.

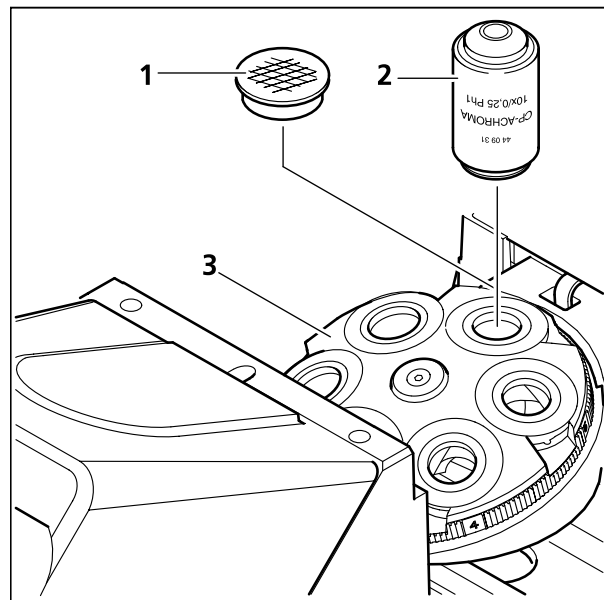


Fig. 2-7 Screw in objectives

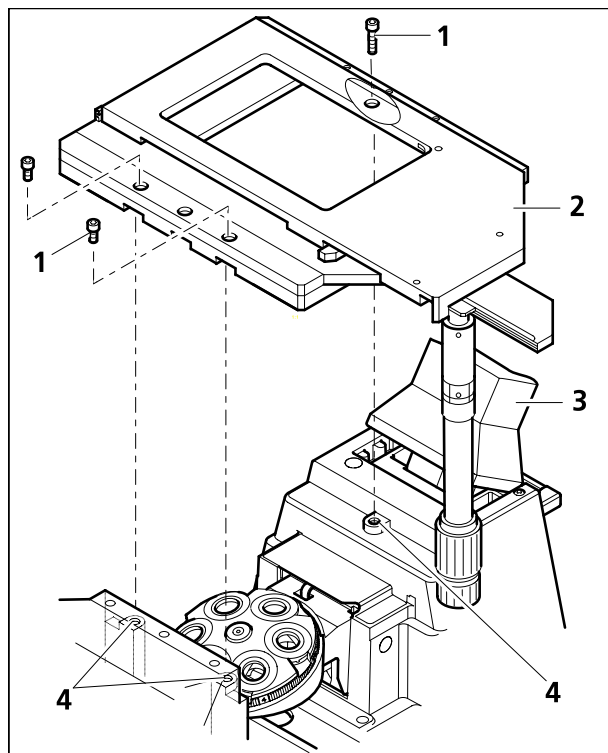


Fig. 2-8 Attachment of mechanical stage 130x85

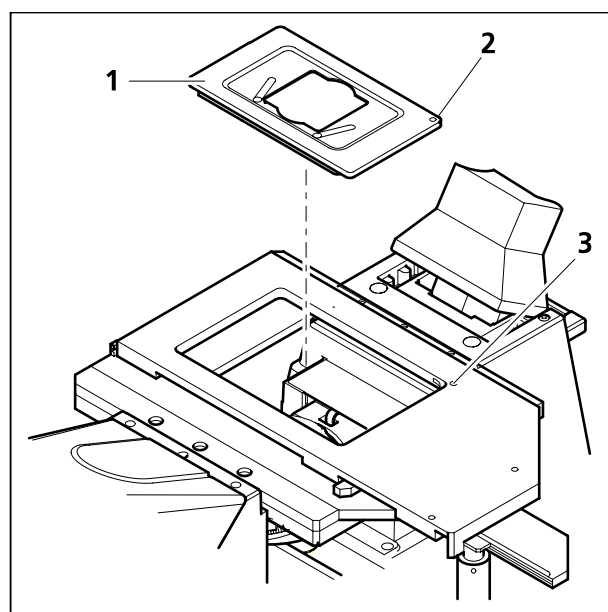


Fig. 2-9 Insertion of mounting frame (K)

2.5 Attachment of microscope stages

2.5.1 Attachment of mechanical stage 130x85 R/L and mounting frame for mechanical stage (K)

The mechanical stage is mounted to the stand directly above three contact points with the relevant drilled holes.

- To improve access during stage assembly, the carrier (2-8/3) for transmitted-light illumination can be tilted backwards.
- Place mechanical stage (2-8/2) on the three contact points (2-8/4) of the stand and fix it in position using three hexagonal screws (2-8/1) (two at the front, one at the rear).

Three countersunk holes each on the front and rear of the mechanical stage 130x85 R/L permit attachment with the drive knobs being positioned on the right or on the left.

- Then insert the mounting frame (K) (2-9/1) into the mechanical stage. For this purpose, position the red dot (2-9/2) of the corner of the mounting frame on the red dot of the mechanical stage (2-9/3) and press the mounting frame diagonally against the springs and downwards into the recess. Make sure that the mounting frame is seated correctly.

2.5.2 Attachment of scanning stage

- The scanning stage is attached in the same way as the mechanical stage. However, the three spacers (4 mm) enclosed with the stand must be inserted before attachment of the scanning stage.
- The cable to the separate motor control unit must then be connected.



Because of the large travel range of the scanning stage, it may happen that the stage frame collides with the objectives at the end of the stage travel.

2.5.3 Attachment of specimen stage 250x230, object guide and mounting frame for object guide (M)

The specimen stage is attached to the contact points of the stand using a spacer bar and a spacer disk.

- Use the two shorter hexagonal screws to screw the spacer bar (2-10/5) to the two front contact points.
- Place spacer disk (2-10/3) on the rear contact point.
- Place specimen stage (2-10/2) on the stand and first fix it to the rear contact point from above using the longer hexagonal screw (2-10/1). Make sure that the screw goes through the drilled hole of the spacer disk.
- Then screw specimen stage to the right and left of the spacer bar from below using two hexagonal screws (2-10/4).
- Also tighten the rear screw (2-10/1).
- Attach object guide (2-11/1) to the specimen stage from the right or left and fix it in position from below using three hexagonal screws (2-11/2).
- Then push mounting frame for object guide (M) (2-11/3) under the two springs of the object guide from below until it clicks into position.

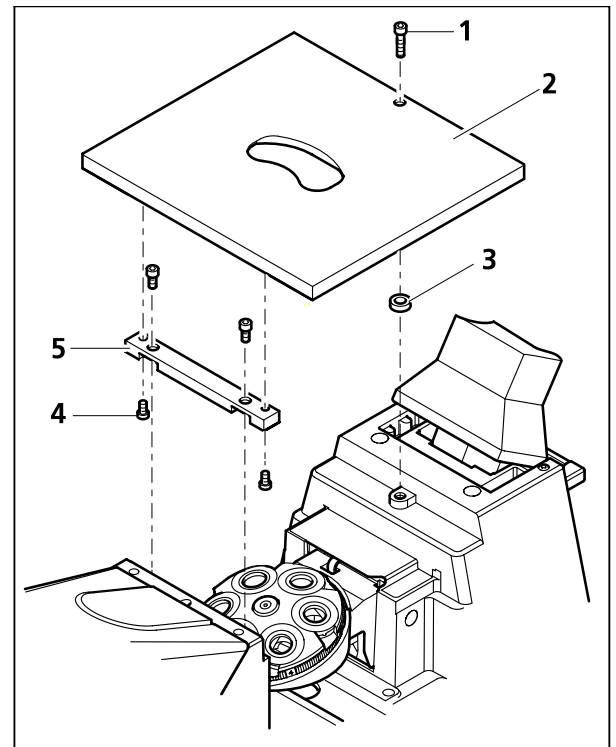


Fig. 2-10 Attachment of specimen stage 250x230

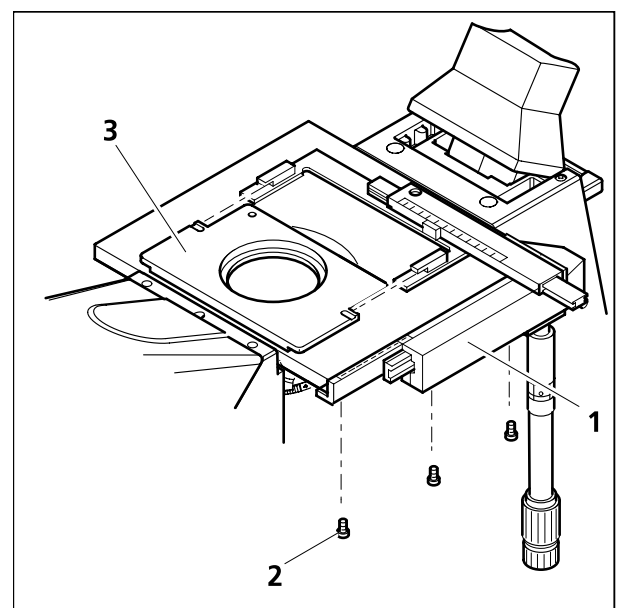


Fig. 2-11 Attachment of object guide and mounting frame

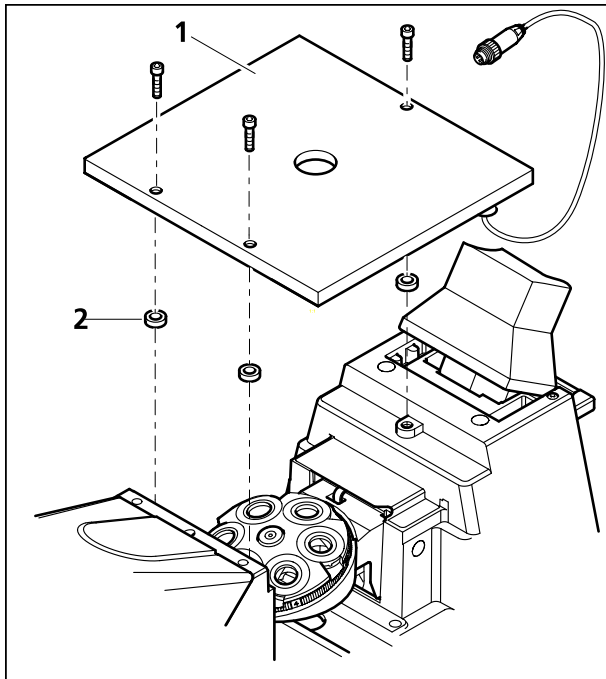


Fig. 2-12 Attachment of heating stage

2.5.4 Attachment of heating stage

The heating stage is attached to the contact points of the stand using three spacer disks.

- Remove any available microscope stage and additional mounting components.
- Place spacer disks (2-12/2) on the three contact points of the stand.
- Place the heating stage (2-12/1) on the contact area and tighten three hexagonal screws from above. Make sure that the screws go through the drilled hole of the relevant spacer disk.
- Then connect the instrument to the power supply as described in the separate operating manual.



When the heating stage is used, the nosepiece must first be moved to the lowest position via the focusing drive before a new objective is swung in, since otherwise collision between the objective and the heating stage might occur.

2.5.5 Attachment of gliding stage Z

The gliding stages are attached in the same way as the heating stage by using three spacer disks.

- Before attaching the gliding stage to the stand, the three support elements on the underside of the gliding stage must be turned out.
- Place spacer disks on the three contact points of the stand.
- Place the heating stage on the contact area and tighten three hexagonal screws from above. Make sure that the screws go through the drilled hole of the relevant spacer disk.



When the heating stage is used, the nosepiece must first be moved to the lowest position via the focusing drive before a new objective is swung in, since otherwise collision between the objective and the heating stage might occur.



After stage assembly, cover the drilled holes visible from above with the caps supplied. Tilt carrier for transmitted-light illumination forward again to move it in its work position.

2.6 Attachment of condensers

2.6.1 Condensers for the Axiovert 200

- Insert condenser (2-13/1) into the condenser carrier on the carrier for transmitted-light illumination with its dovetail pointing upwards. Make sure that the orientation pin of the condenser is positioned at the front and exactly engages into the guiding groove of the condenser carrier.
- Fix condenser in position with clamping screw (2-13/2).
- For the motorized condenser, connect the cable to the SB connector on the rear of the stand.

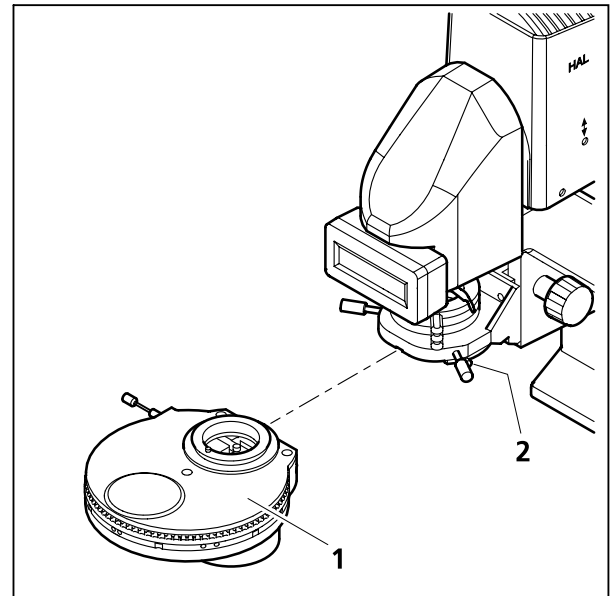


Fig. 2-13 Attachment of condenser

2.6.2 Condensers from the Axioplan 2 imaging / Axioskop 2 product line

The following condensers from the Axioplan 2 imaging / Axioskop 2 line can be used:

- Achromatic condenser 0.8 H, D, Ph DIC, (445445-9901-000)
- Achromatic aplanatic condenser 14 H, D, Ph DIC, (445453-0000-000)



The inverted design of the Axiovert 200 requires reversed integration of the condensers from the Axioplan 2 imaging / Axioskop 2 line, i.e. the turret must point to the back so that the control elements are positioned at the rear and the labeling is upside down.

- Achromatic condenser 0.8 H, DIC (000000-1087-444)

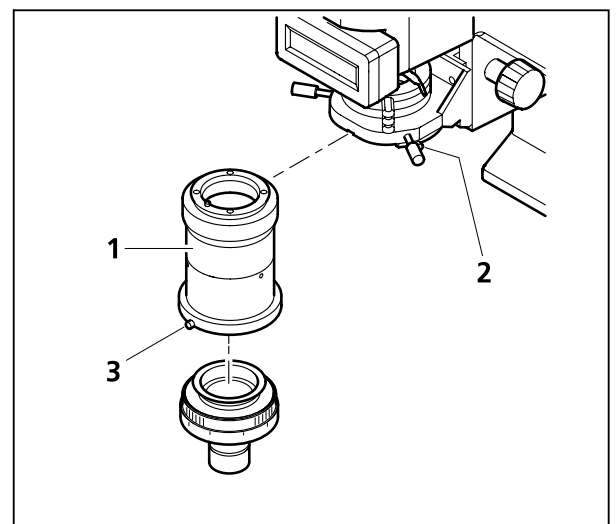


Fig. 2-14 Attachment of condenser adapter

- Insert condenser adapter (2-14/1) into the condenser carrier on the carrier for transmitted-light illumination with its dovetail pointing upwards. Make sure that the orientation pin of the condenser is positioned at the front and exactly engages into the guiding groove of the condenser carrier.
- Fix condenser adapter in position with clamping screw (2-14 /2).
- Then insert the required condenser into the condenser adapter via the dovetail, making sure that the orientation is correct, and fix it into position with clamping screw (2-14/3).

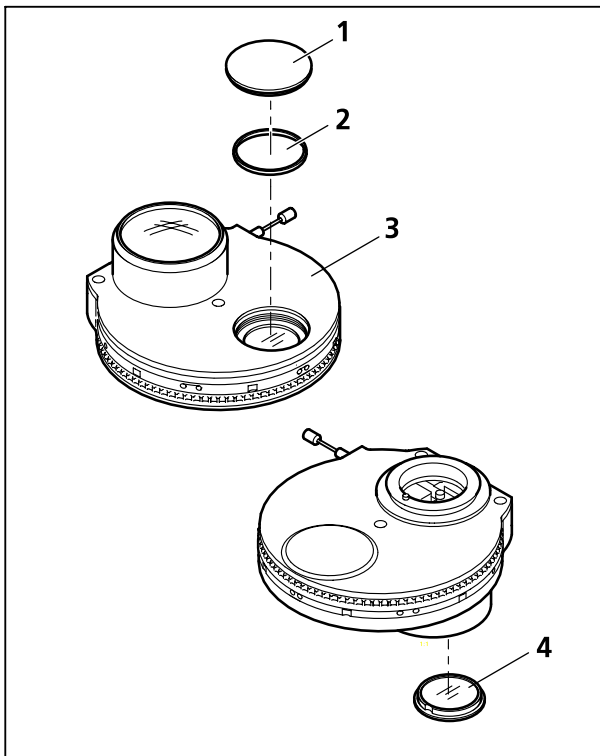


Fig. 2-15 Changing the DIC prism

2.6.3 Changing the DIC prism in the condenser turret

- To change a DIC prism, remove the condenser and place it upside down on a sturdy support to make the underside accessible.
- Remove the plastic cover (2-15/1) from the assembly opening (2-15/3).
- Position the turret disk containing the DIC prism to be exchanged in the assembly opening and hold it on the knurled ring.
- Use mounting device from the tool set to unscrew retainer ring (2-15/2).
- Turn condenser upside down and allow DIC prism (2-15/4) to slide out onto a soft surface.

Installation of the DIC prism is made in reverse order:

- Carefully insert new DIC prism into the assembly opening with the labeling pointing upwards. If required, use tweezers to hold the DIC prism carefully on its outer ring. Take special care of the correct orientation of the DIC prism in the mount (groove of the DIC prism must engage in the pinion of the mount).

- Carefully insert retainer ring again and screw it tight using the mounting device.
- Use plastic cover to close the assembly opening again.
- Make sure that the knurled ring of the turret disk is labelled correctly.
- Turn the condenser around and insert it in the carrier for transmitted-light illumination.

2.7 Reflector turret

2.7.1 Attachment of reflector turret

The manual reflector turret can be pushed into the stand either from the right or from the left, depending on which side is accessible. The motorized reflector turret can only be inserted from the right, with the instrument being switched off.

- Insert equipped reflector turret (2-16/1) in the mount provided below the nosepiece. Ensure the correct stop position.
- Tighten fixation screw (2-16/2) for the reflector turret on the right side.

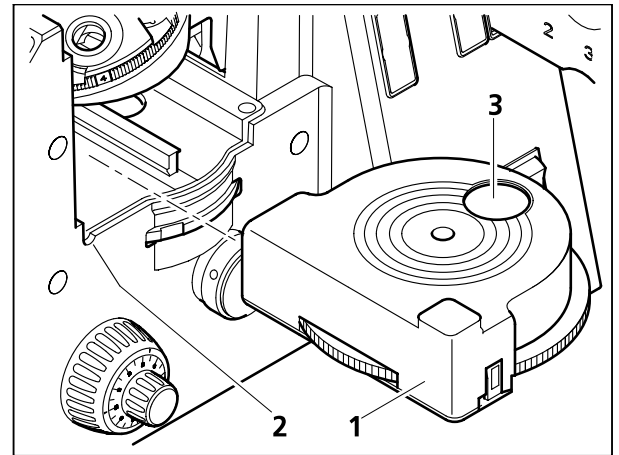


Fig. 2-16 Insertion of reflector turret

👉 When changing the reflector turret, close the FL shutter to avoid stray light.

- The protective lens available as an option can be inserted into the opening of the reflector turret (2-16/3).

2.7.2 Equipment of reflector turret

The reflector turret is usually entirely equipped when delivered from the factory. However, equipment with the filter sets contained in the reflector modules can also be easily performed by the customers themselves.

- Loosen fixation screw for reflector turret (2-16/2) on the right side.
- Pull reflector turret out of the stand and place it on a suitable support (sturdy worktable).
- Carefully press both holding catches (2-17/2) on the right and left of the plastic hood away from the pins in outward direction and remove plastic hood in upward direction.
- Insert reflector modules (2-17/1) in the relevant reflector position according to the filter combination (see engraved number), starting with position 1 (emission filter lies at the bottom). First, insert the reflector module into the two lower spring clamps at an angle from above using the holding elements on the right and left, and then press the module against the upper spring clamps from the front until the module click-stops into position.

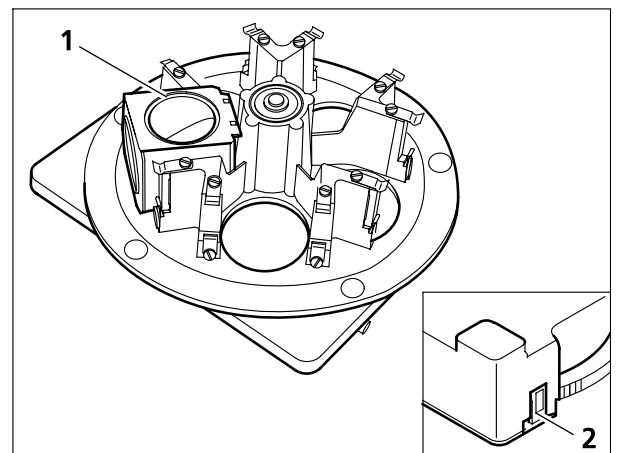


Fig. 2-17 Insertion of reflector modules

- To remove a reflector module no longer required, first pull it out of the upper spring clamps and then from the lower ones.
- When equipment has been finished, attach the plastic hood again and press it downwards until the holding catches on the right and left engage in the lower part of the reflector turret.

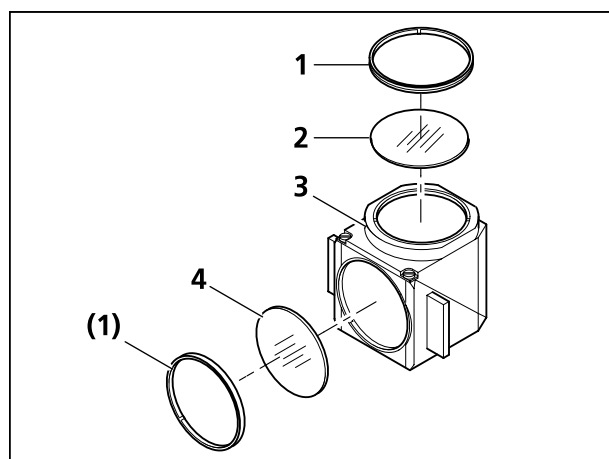


Fig. 2-18 Changing the filter set in the FL reflector module

2.7.3 Changing the filter set in the FL reflector module

The filter sets for the FL reflector module can be compiled and assembled by the customer himself as required. Suitable filter sets or completely assembled FL reflector modules can be ordered from Carl Zeiss.

- Remove FL reflector module (2-18/3) from the reflector turret.
- Use mounting device from the tool set to unscrew retainer ring (2-18/1).
- Turn the reflector module around and allow the filter (2-18/2 or 4) to drop on a soft surface.
- The barrier filter is inserted at (2-18/2), the excitation filter at (2-18/4), and both are secured using the retainer rings (2-18/1).

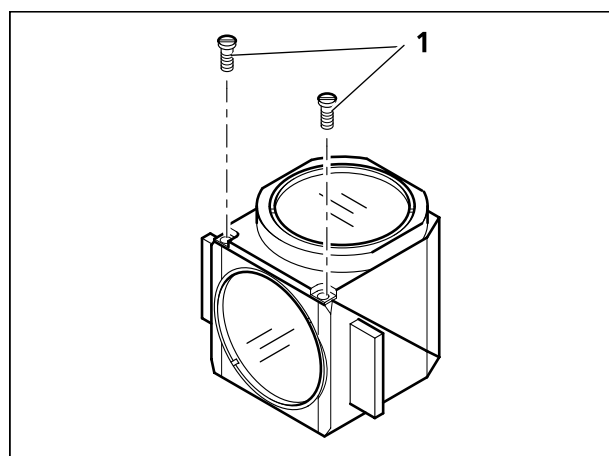


Fig. 2-19 Changing the beam splitter

2.7.4 Changing the beam splitter in the FL reflector module



Assembly of the filter and the beam splitter requires utmost care to prevent damage and contamination of the optical components.

We would recommend you to order completely equipped FL reflector modules, since changing the beam splitter requires much skill.

Otherwise, proceed as follows:

- Remove FL reflector module from the reflector turret.
- Use screwdriver to loosen two slotted screws (2-19/1).

- Hold both halves of the reflector module together, turn them around into the installation position and put them down.
- Now tilt the upper module half (2-20/1) upwards and lift it out of the holding elements (2-20/5b) of the lower module half.
- Remove beam splitter (2-20/2) and spring frame (2-20/3) from the lower module half.
- Remove old beam splitter and carefully place the new one on the spring frame (2-20/4) with the reflecting side pointing downwards, and then insert both parts together in the lower module half. Make sure that the lateral catch of the spring frame is positioned in the relevant recess in the lower module half.

If there is no distance between a wooden pin and its mirror image when such a pin is carefully placed on the surface of the beam splitter, this is the reflecting side of the beam splitter.

- Place upper module half (2-20/1) on the lower one (2-20/4) (holding elements 2-20/5b and lugs 2-20/5a mesh with each other). Hold both halves together and turn them around into the installation position.
- Insert and tighten slotted screws.
- Finally, attach the adhesive label with the name of the filter combination to the side of the module.

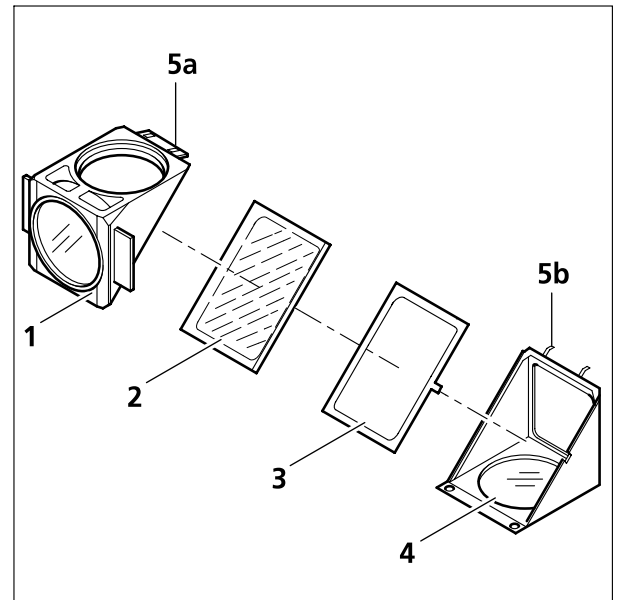


Fig. 2-20 Changing the beam splitter

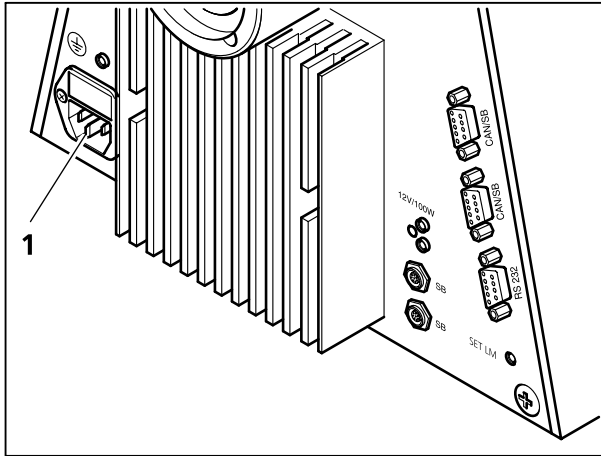


Fig. 2-21 Axiovert 200 M (rear)

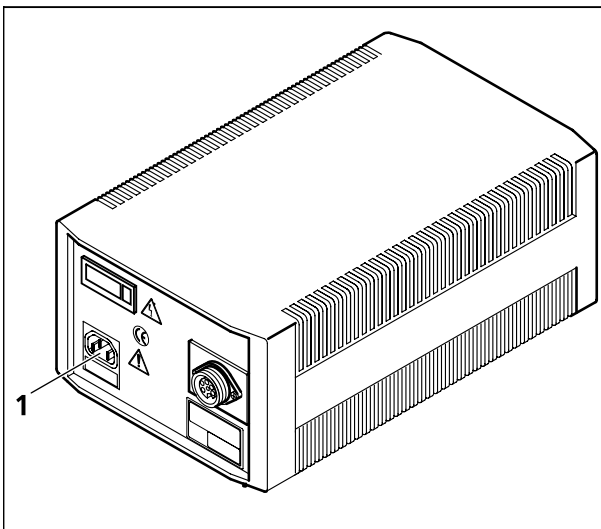


Fig. 2-22 Power supply ebq 100 dc (rear)

2.8 Connection to the line

- Connect microscope socket (2-21/1) to the line socket via a line cable. The microscope can be connected to line voltage between 90 and 264 VAC, 50 - 60 Hz.

The microscope is equipped with a wide-area power unit which automatically adapts to the available line voltage. Voltage change is not required.

The N HBO 103 illuminator (for epi-fluorescence) is supplied via a separate power supply.

- The power supply ebq 100 dc must be connected to the line via the line socket (2-22/1) (also see section 2.13.3, "Attachment of illuminator N HBO 103").

The ebq 100 dc is equipped with a wide-area power unit which automatically adapts to the available line voltage. Voltage change is not required.

2.9 Interfaces of the Axiovert 200 M



Prior to connecting any components, switch off the microscope.

The Axiovert 200 M is connected to a PC via the RS 232 interface (2-23/2).

Motorized components of the Axiovert 200 M (e.g. the motorized condenser) are connected via the SB interfaces (2-23/3).

Further external control components (e.g. the 3-axis motor control MCU 28 of the scanning stage) must be connected to the CAN/SB connectors (2-23/1).

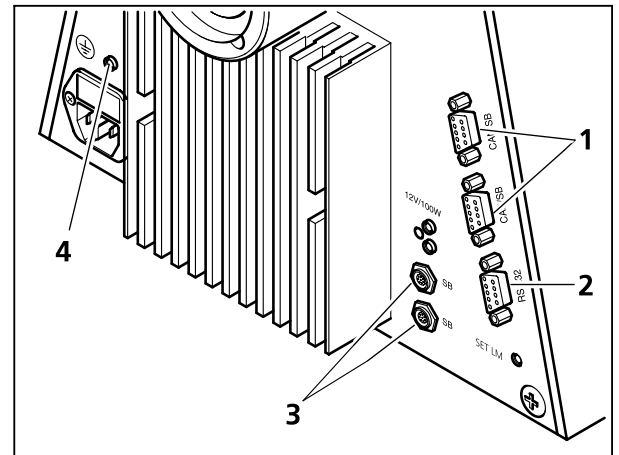


Fig. 2-23 Axiovert 200 M (rear)

2.10 Switch microscope and ebq 100 dc power supply on and off

- Switch the microscope on and off using the line switch (2-24/1).
- If a fluorescence illuminator (e.g. N HBO 103) is connected (see section 2.13.3, "Attachment of illuminator N HBO 103"), switch power supply ebq 100 dc on and off via the line switch (2-24/2).

2.11 Equipotential bonding terminals

Terminals for equipotential bonding for electrophysiological measurements are located on the rear side of the stand (2-23/4) and on the underside of the binocular tubes (000000-1005-827 and 000000-1005-828).

Connection to the stand is by 4mm banana plugs.

The connectors on the tubes are provided with M4 internal thread.

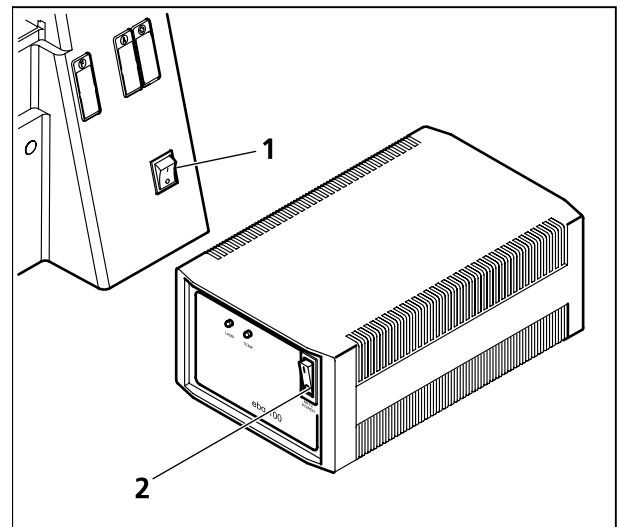


Fig. 2-24 Power supply ebq 100 dc (front)

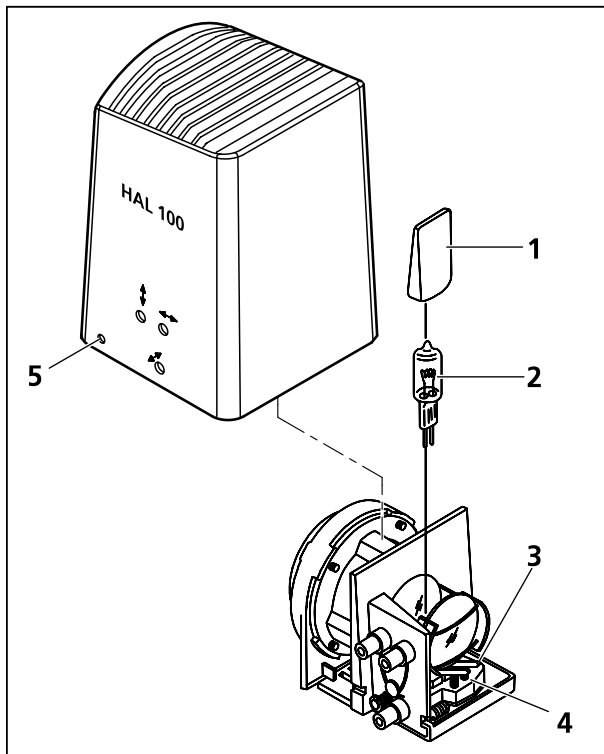


Fig. 2-25 Changing the halogen lamp.

2.12 HAL 100 halogen illuminator

2.12.1 Change / attachment of the HAL 100 halogen lamp

- Switch off the microscope, remove connector (2-27/4) from the 12 V / 100 W socket (2-27/5) and allow the illuminator to cool down for approx. 15 minutes.
- Loosen screw (2-25/5) until the lamp housing can be removed in an upward direction.
- Press both spring levers (2-25/3) downwards and pull out the old halogen lamp in an upward direction.
- Press both spring levers downwards, insert new lamp (2-25/2) with protection cap (2-25/1) in lamp base (2-25/4), let go the spring lever and pull off the protection cap.
- Press spring lever briefly downwards again to center the lamp.
- Attach lamp housing again and tighten clamping screw (2-25/5).



After the lamp change, the halogen illuminator must be realigned.

2.12.2 Coarse alignment of halogen illuminator

- Loosen the clamping screw (2-27/2) and remove the halogen illuminator from the carrier for transmitted-light illumination.
- Switch on the microscope.
- Direct light beam against a projection area (wall) approx. 3 m away.



Make sure not to look into the light exit opening of the illuminator.

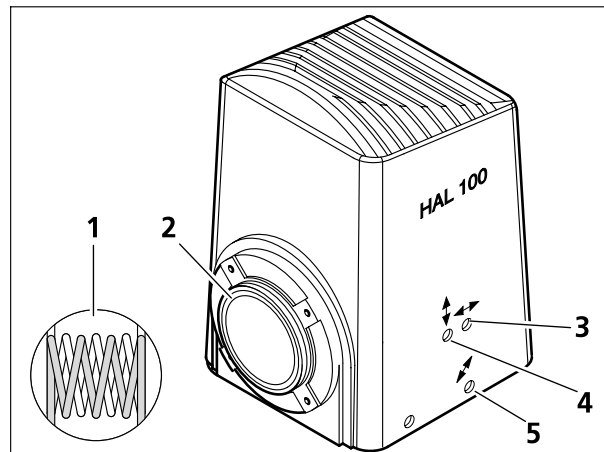


Fig. 2-26 Alignment of halogen illuminator

- Use SW 3 ball-headed screwdriver to set adjusting screw (2-26/3) in such a way that both images of the lamp coil on the projection area are defined as clearly as possible.
- Then set adjusting screws (2-26/4 and 5) in such a way that the lamp coils of one image exactly fill the gaps of the reflector image (2-26/1).

2.12.3 Attachment of halogen illuminator

- If required, remove cover from the lamp mount (2-27/2) in the carrier for transmitted-light illumination.
- Insert dovetail (2-27/2) of lamp housing (2-26/3) in carrier (2-27/2) and use SW 3 ball-headed screwdriver to tighten clamping screw (2-27/1).
- Connect 3-pin lamp plug (2-27/4) to 3-pin 12 V 100 W socket (2-27/5) on the instrument rear.

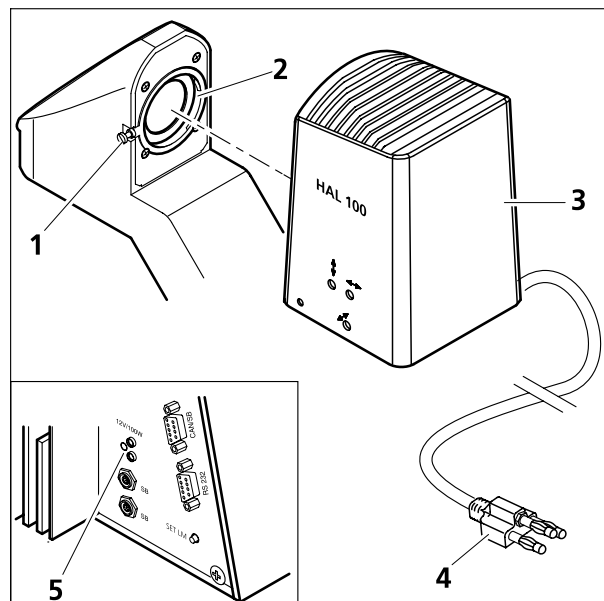


Fig. 2-27 Attachment of halogen illuminator

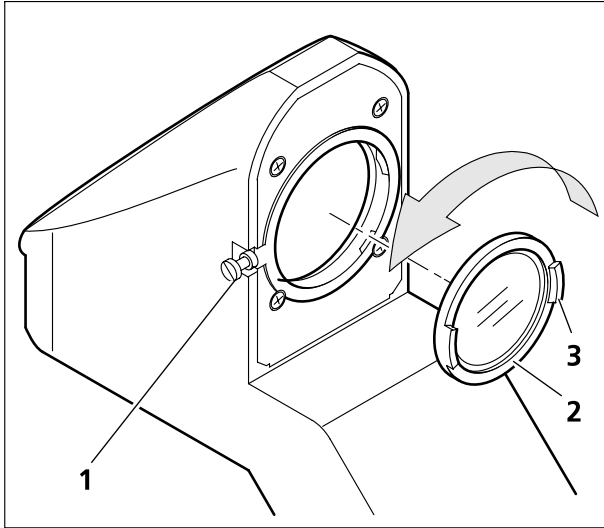


Fig. 2-28 Changing the diffusion disk

2.12.4 Fine alignment of halogen illuminator

Fine alignment requires the diffusion disk to be removed:

- Loosen clamping screw of HAL 100 W (2-28/1) and remove the illuminator from the carrier for transmitted-light illumination.
- Turn diffusion disk (2-28/2) out of the carrier manually (anti-clockwise). Use the projections (2-28/3) on the mount of the diffusion disk to hold the component.
- Attach HAL 100 W and tighten the clamping screw.
- Remove any swung-in filter from the beam path.

- Use objective $\leq 40\times$ to focus on the specimen and search for an empty object spot.
- Remove eyepiece and use adjusting screws (2-26/4 and 5) to center the lamp coil and its reflected image in the pupil image.
- Use adjusting screws (2-26/3) to optimize the homogeneous illumination of the pupil image.
- Remove HAL 100 W after conclusion of the alignment.
- Screw diffusion disk into the carrier again manually.
- Attach HAL 100 W and swing in available filters again.

2.13 Illuminator N HBO 103

2.13.1 Attachment / change of the HBO 103 W/2 mercury pressure short-arc lamp

The illuminator N HBO 103 and the HBO 103 W/2 mercury pressure short-arc lamp are supplied to the customer in separate packaging for safety reasons.

Therefore, insertion of the HBO 103 W/2 into the lamp housing is the first step in the start-up of the illuminator.



Before opening the lamp housing, make sure that no connection to the electrical line is available.



The HBO 203 W/2 lamps may only be changed after they have cooled down. Allow the N HBO 103 microscope illuminator to cool down for approx. 15 minutes to avoid the risk of explosion and burns.

The HBO 103 W/2 lamp may only be removed from the packaging and inserted in the N HBO 103 illuminator if a protection mask and safety gloves are worn.

All electrical clamping connections must be made carefully. Pronounced heat during operation may result in loose contacts.

For start-up or after expiry of the average operation time of 300 h, the HBO 103 W/2 mercury vapor short-arc lamp must be inserted or exchanged.

The operating time of the lamp can be read from the counter (2-29/1) of the ebq 100 dc power supply.

Follow the following steps when inserting / changing the HBO 103 W/2 lamp:

- Pull off or unscrew line cable (near 2-29/2) and N HBO 103 connector (near 2-29/3) from the ebq 100 dc power supply.
- Wear protection mask and safety gloves.

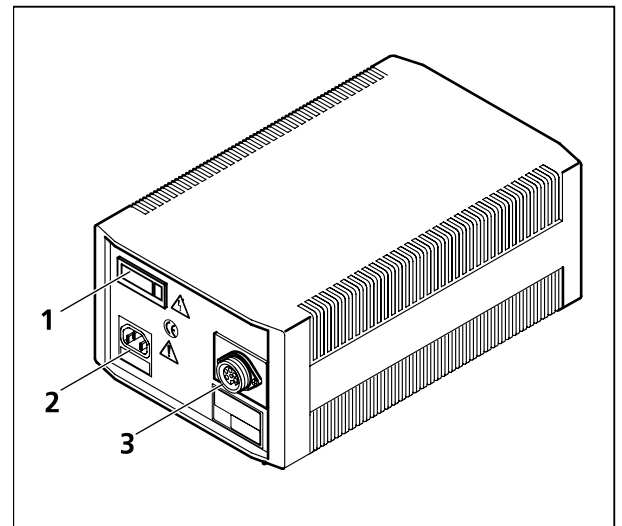


Fig. 2-29 Power supply ebq 100 dc

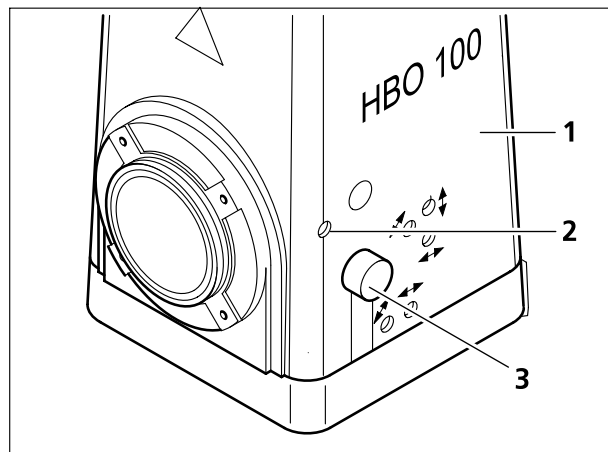


Fig. 2-30 Removal of N HBO 103 housing

- Use focusing knob (2-30/3) to bring collector to the position at the very front (in the direction of the light).
- Use ball-headed screwdriver to loosen clamping screw for lamp housing (2-30/2) and carefully remove lamp housing (2-30/1) from the lamp mount (2-31/3) in an upward direction.
- Press spring lever (2-31/4) downwards and remove defective lamp (or transport lock) and cooling body out of the mount in the lamp base (2-31/2). The wire connection need not be detached.
- Loosen fixation screw (2-31/5) on the cooling body (2-31/6) and remove cooling body. If the cooling body cannot be removed (or attached), unscrew fixation screw entirely, screw it into the adjacent drilled hole and press on the cooling body.

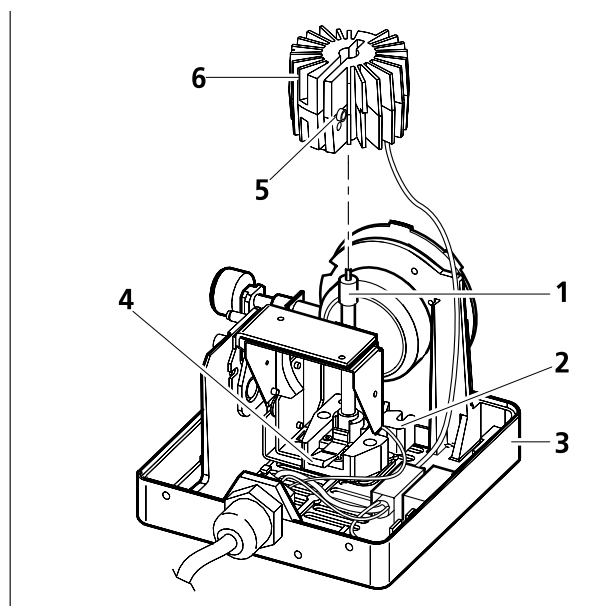


Fig. 2-31 Changing the HBO 103 W/2 mercury pressure short-arc lamp

- Dispose of defective lamp in accordance with the regulations.
- Push new lamp into the cooling body until stop with the smaller diameter.



Ensure the correct hole diameter for insertion and do not exert any force on the lamp.

The mount in the cooling body is marked with **H** (for HBO) or **X** (for XBO).

- Clamp the fixation screw on the cooling body.
- Insert new lamp (2-31/1) with cooling body into the mount in the lamp base (2-31/2) with the spring lever being pressed down (2-31/4). Make sure that the lead to the cooling body remains accessible. Release spring lever. Do not exert any force on the lamp.
- Attach lamp housing to lamp base (2-31/3) and tighten clamping screw (2-30/2).

2.13.2 Coarse alignment of illuminator N HBO 103

The following settings for adjustment of the N HBO 103 illuminator are performed in two steps: the coarse adjustment and the subsequent fine adjustment using the adjustment aid.



During coarse adjustment, UV protection goggles must be worn to protect the eyes from UV radiation.

- Remove illuminator from the microscope stand by loosening the clamping screw (2-35/2).
- Switch on the entirely connected illuminator N HBO 103 (also see page 2-26) via the ebq 100 dc power supply (2-24/2) and allow it to heat to operating temperature. Make sure not to look into the light exit opening of the illuminator.
- Direct light beam against a projection area (wall) approx. 3 m away.
- Use knurled knob (2-32/6) to focus on the brighter focal spot via collector adjustment.
- Use adjusting screw (2-32/3) and SW 3 ball-headed screwdriver to locate and focus on the slightly darker, reflected focal spot.
- Use adjusting screws (2-32/4 and 5) and SW 3 ball-headed screwdriver to set the height and side of the brighter focal spot in accordance with the focal spot image (2-34).

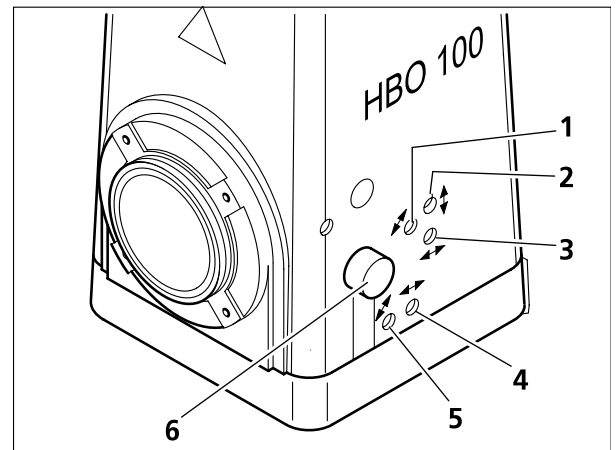


Fig. 2-32 Coarse alignment of mercury vapor short-arc lamp

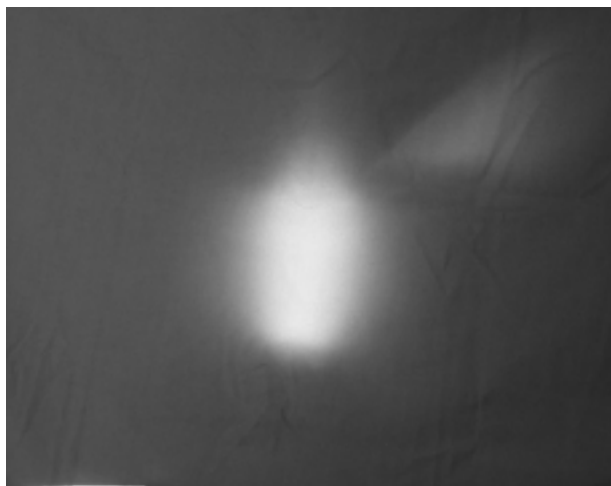


Fig. 2-33 Focal spots of N HBO 103 before coarse adjustment

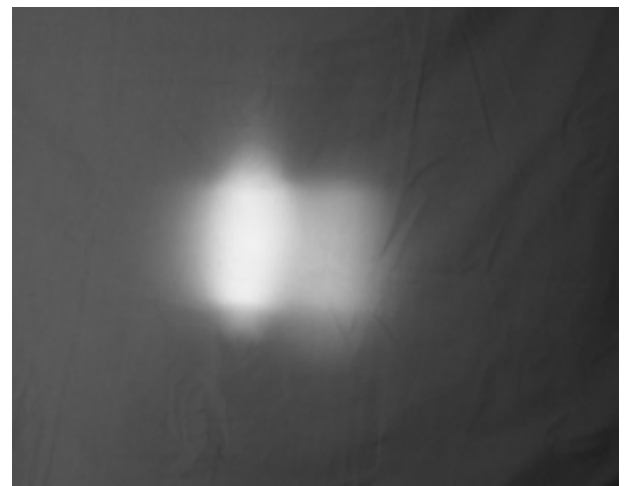


Fig. 2-34 Focal spots of N HBO 103 after coarse adjustment



If required, the above settings for coarse adjustment of the N HBO 103 illuminator can be performed repeatedly. During coarse adjustment of the focal spots, adjusting screws (2-32/1 and 2) marked red must not be changed, since they influence the reflector setting in the HBO 103 illuminator.

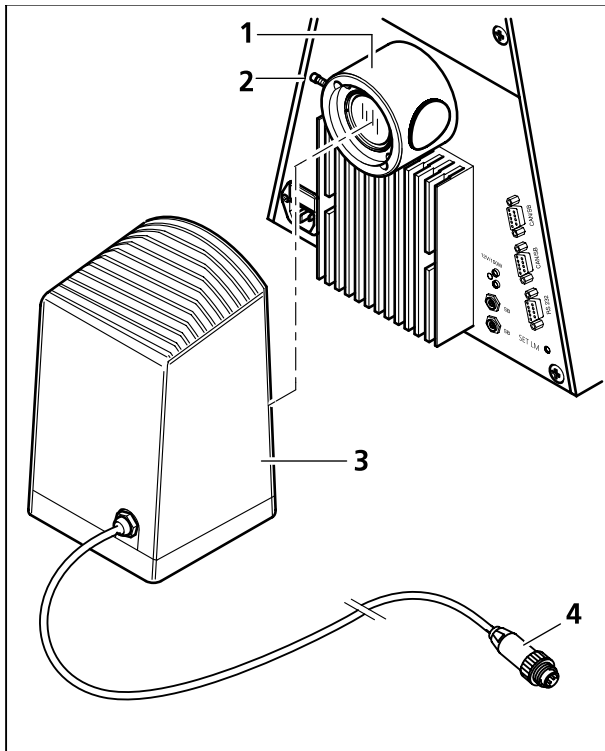


Fig. 2-35 Attachment of illuminator N HBO 103

2.13.3 Attachment of illuminator N HBO 103

- Remove cover from reflected-light tube (2-35/1).
- Insert dovetail of lamp housing in reflected-light tube (2-35/1) on the instrument rear and use SW 3 ball-headed screwdriver to tighten clamping screw (2-35/2).
- Insert multi-pin plug of the N HBO 103 illuminator (2-35/4) into the instrument socket (2-36/2) of the power supply ebq 100 dc and secure it with captive coupling ring (if not already done so after adjustment).
- Connect the line cable to the line socket (2-36/1) of power supply ebq 100 dc first and then to the line (if not already done so after adjustment).

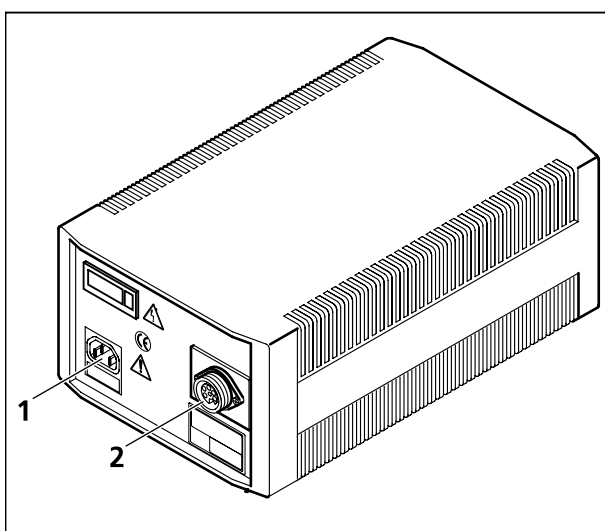


Fig. 2-36 Power supply ebq 100 dc

2.13.4 Fine adjustment of illuminator N HBO 103 using the adjusting aid

Attach illuminator N HBO 103 to the microscope stand, tighten the clamping screw, and switch on the illuminator.

- Pull out adjusting aid (2-37/7) from the incident-light tube. The brighter focal spot of the HBO 103 W/2 lamp and its slightly darker reflector image become visible in the black glass window of the adjusting aid.
- Use knurled knob (2-37/6) to focus on the brighter focal spot via collector adjustment.
- Apply SW 3 ball-headed screwdriver on adjusting screw (2-37/3) to focus on the slightly darker, reflected focal spot of the lamp.
- If required, set knurled knob (2-37/6) and adjusting screw (2-37/3) repeatedly until both focal spots have the same dimension.
- Use adjusting screws (2-37/4 and 5) to adjust the brighter focal spot in the adjusting circle, e.g. on the left side, in accordance with the focal spot image (2-37/8).
- Use adjusting screws (2-37/1 and 2) marked with a dot to adjust the darker focal spot in the adjusting circle, e.g. on the right side, in accordance with the focal spot image.
- Push in adjusting aid again.

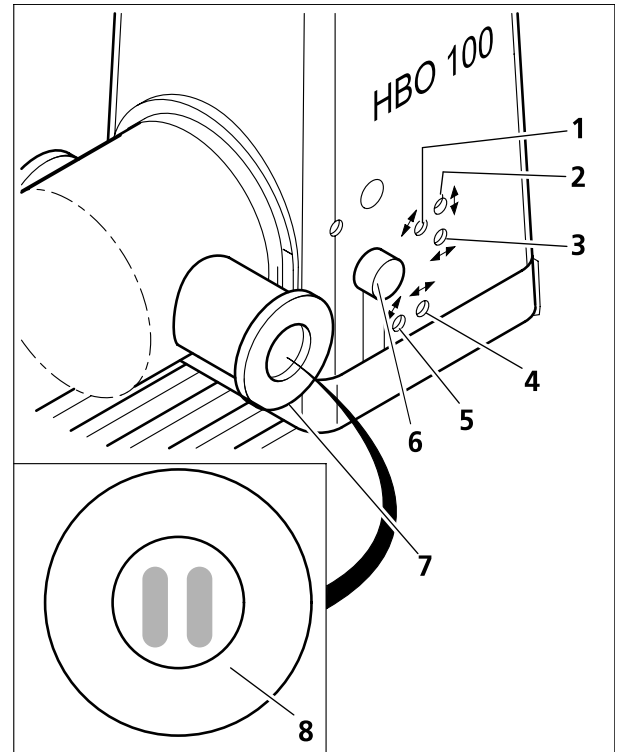


Fig. 2-37 Adjusting aid and illuminator N HBO 103



The two focal spots of the HBO 103 W/2 lamp must **not** superimpose in the adjusting circle of the adjusting aid!

If required, the above settings for fine adjustment of the N HBO 103 illuminator can be performed repeatedly.

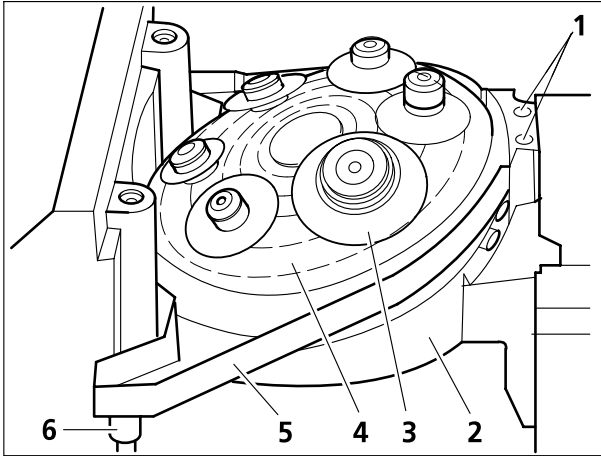


Fig. 2-38 Attachment of Aqua Stop

2.14 Attachment of Aqua Stop

Aqua Stop can be used to protect the objective and the nosepiece from liquids.

- Remove the microscope stage.
- Put collecting trough 2-38/5) onto nosepiece carrier (2-38/2) and screw it on by means of two screws 2-38/1).
- Screw the objectives to be used into the nosepiece.
- Then, use a sharp, pointed scalpel to cut the holes into cover mat (2-38/4).

Four hole sizes are available that are pre-embossed on the underside of the cover mat and labeled from inside to outside with hole size number 1 to 4.

The position ID's of the holes on the mat (1 to 6) correspond to the objective mounts on the nosepiece (1 to 6).

The objective table below specifies the hole size for the most common objectives that must be cut out for the corresponding objective.

- Put cover mat onto nosepiece.

 Press down the cover mat only as far as is possible without noticeably slowing down the motion of the nosepiece.

- Next, put a silicone ring (2-38/3) over every objective.

There are two types of silicone rings available: Size 1 – small, Size 2 – large. The objective table also specifies the size of the silicone ring to be used for the most common objectives.

 On objectives with correction ring, the silicone ring must be above the correction ring. On all other objectives, slide the silicone ring up to about 3 mm before the beginning of the thicker part of the objective to ensure correct function of the objective's specimen protection mechanism.

- Then, slip the cut drain hose over the two drain connectors (2-38/6). Put the other end of the hose into the collecting tank.

 The liquid can only drain if the collecting tank provides venting. This is made possible e.g. by a hole in the lid or by screwing the lid only loosely.

- You can fix the drain hose using the provided clips.



Fix the hose in such a way, that the drain gutter is not bent in focusing.

- Attach the microscope stage again.

After any accident with liquids, you are strongly advised to remove the microscope stage and dab off any liquid drops using a lintless cloth. Particularly, clean the front lenses of the objectives to be able to fully utilize the full performance of the objective.

Objective type	Magnification / num. aperture	Contrasting	Hole size	Silicone ring size	Cat. No.
A-Plan					
A-Plan	5x/0.12	ph0	2	1	000000-1018-589
A-Plan	10x/0.20	ph1 var1	2	1	000000-1020-863
A-Plan	20x	ph2	2	1	441041-0000-000
A-Plan	40x	Ph2	3	1	441051-0000-000
A-Plan	100x	ph3	3	1	441080-0000-000
LD A-Plan	20x/0.30	ph1	2	1	000000-1006-591
LD A-Plan	20x/0.30	ph1 var1	2	1	000000-1006-592
LD A-Plan	32x/0.40	ph1	2	1	000000-1006-593
LD A-Plan	32x/0.35	ph1 var1	2	1	000000-1006-594
LD A-Plan	40x/0.50	ph2	2	1	000000-1006-595
LD A-Plan	40x/0.50	ph2 var2	2	1	000000-1006-596
LD Achroplan					
Achroplan	4x/0.10		2	1	440020-0000-000
Achroplan	10x/0.25	ph1	2	1	440031-0000-000
LD Achroplan	20x/0.40		3	1	440844-0000-000
LD Achroplan	20x/0.40	ph2	3	1	440845-0000-000
LD Achroplan	40x/0.60		4	2	440864-0000-000
LD Achroplan	40x/0.60	ph2	4	2	440865-0000-000
LD Achroplan	63x/0.75	ph2	4	2	440861-0000-000
Fluar					
Fluar	10x		3	1	440135-0000-000
Fluar	20x		3	1	440145-0000-000
Fluar	40x/1.3 Iris		4	1	440257-0000-000

Objective type	Magnification / num. aperture	Contrasting	Hole size	Silicone ring size	Cat. No.
Plan-Neofluar					
Plan-Neofluar	5x/0.15		2	1	440320-0000-000
Plan-Neofluar	5x/0.15	ph1	2	1	440321-0000-000
Plan-Neofluar	10x/0.30		2	1	440330-0000-000
Plan-Neofluar	10x/0.30	ph1	2	1	440331-0000-000
Plan-Neofluar	16x/0.50 Imm		3	1	440530-0000-000
Plan-Neofluar	16x/0.50 Imm	ph1	3	1	440531-0000-000
Plan-Neofluar	20x/0.50		2	1	000000-1004-072
Plan-Neofluar	20x/0.50	ph2	2	1	000000-1004-989
Plan-Neofluar	40x/0.75		2	1	440350-9902-000
Plan-Neofluar	40x/0.75	ph2	2	1	440351-9902-000
Plan-Neofluar	40x/1.30 Oil		3	1	000000-1022-818
Plan-Neofluar	40x/1.30 Oil	ph3	3	1	000000-1022-819
Plan-Neofluar	63x/0.95 Korr.		3	1	440364-0000-000
Plan-Neofluar	63x/1.25 Oil Iris		4	1	440466-0000-000
Plan-Neofluar	100x/1.30 Oil		2	1	000000-1018-595
Plan-Neofluar	100x/1.30 Oil	ph3	2	1	000000-1031-171
Plan-Apochromat					
Plan-Apochromat	63x/1.40 Oil		4	1	440760-0000-000
Plan-Apochromat	10x0.45		3	1	440639-0000-000

OPERATION

Contents

3	OPERATION	3-3
3.1	Axiovert 200 (manual)	3-4
3.1.1	Operation and function controls on the Axiovert 200 (manual)	3-4
3.1.2	Switching on and basic settings on the Axiovert 200 (manual)	3-14
3.2	Axiovert 200 M (motorized)	3-16
3.2.1	Operation and function controls on the Axiovert 200 (motorized)	3-16
3.2.2	Switching on and basic settings on the Axiovert 200 M (motorized)	3-23
3.3	Illumination and contrasting techniques	3-28
3.3.1	Setting of transmitted-light brightfield for KÖHLER illumination	3-28
3.3.2	Setting of transmitted-light phase contrast	3-33
3.3.3	Setting of differential interference contrast (DIC) in transmitted light	3-35
3.3.4	Setting of VAREL contrast in transmitted light	3-38
3.3.5	Setting of fluorescence contrast in reflected light	3-40
3.4	Documentation	3-43
3.4.1	Image orientation of camera ports	3-43
3.4.2	Photomicrography with SLR camera	3-45
3.4.3	Photomicrography using a digital camera and videomicroscopy	3-46

List of illustrations

Fig. 3-1	Operation and function controls on the Axiovert 200 (manual).....	3-4
Fig. 3-2	Nosepiece with mounts for DIC slider	3-7
Fig. 3-3	Binocular tube 45°/23	3-8
Fig. 3-4	Binocular phototube 45°/23	3-8
Fig. 3-5	Setting of eyepiece distance on the binocular tube.....	3-9
Fig. 3-6	LCD display	3-9
Fig. 3-7	LD condenser 0.55, 6-position, H, Ph1, Ph2, Ph3, Var1/2.....	3-10
Fig. 3-8	LD condenser 0.35, 6-position, H, Ph0, Ph1, Ph2, DIC, DIC.....	3-10
Fig. 3-9	5-position reflector turret	3-13
Fig. 3-10	Iris stop slider	3-13
Fig. 3-11	On / Off switch and toggle switch.....	3-14
Fig. 3-12	Key "Set LM"	3-14
Fig. 3-13	Operation and function controls on the Axiovert 200 M (motorized).....	3-16
Fig. 3-14	LCD-Display.....	3-19
Fig. 3-15	Condenser 0.55, 6-position H, Ph1, Ph2, Ph3, DIC, DIC.....	3-20
Fig. 3-16	Axiovert 200	3-30
Fig. 3-17	Diaphragm settings for KÖHLER illumination in transmitted-light brightfield.....	3-31
Fig. 3-18	Centering the phase stop on the condenser	3-34
Fig. 3-19	Centering of phase stop (bright in condenser) with phase ring (dark in objective).....	3-34
Fig. 3-20	Components for the transmitted-light DIC technique on the Axiovert 200	3-37
Fig. 3-21	Setting of VAREL contrast.....	3-38
Fig. 3-22	VAREL contrast with microtiter plates	3-39
Fig. 3-23	Pupil images in VAREL contrast	3-39
Fig. 3-24	Components for epi-fluorescence on the Axiovert 200	3-41
Fig. 3-25	Connection of a SLR camera.....	3-45
Fig. 3-26	Video attachment.....	3-46

3 OPERATION

The Axiovert 200 microscopes are offered in a manual version and in a version with motorized functions and components.

In the "Operation" chapter, the manual setting and operation functions are explained first, and the motorized (coded) functions are then explained separately, where required. However, many operation functions are entirely identical in both instrument versions.

This manual will not deal with the possibilities of operating the motorized Axiovert 200 M together with a connected PC.

Equipment of the stands with an LCD display is possible as an option. The LCD display can also be integrated subsequently, but this must be performed by Zeiss service staff.

The Axiovert 200 microscopes have been designed for use with incubators and micromanipulators. For information about connection possibilities and operation of these units, please see the relevant separate manuals.

3.1 Axiovert 200 (manual)

3.1.1 Operation and function controls on the Axiovert 200 (manual)

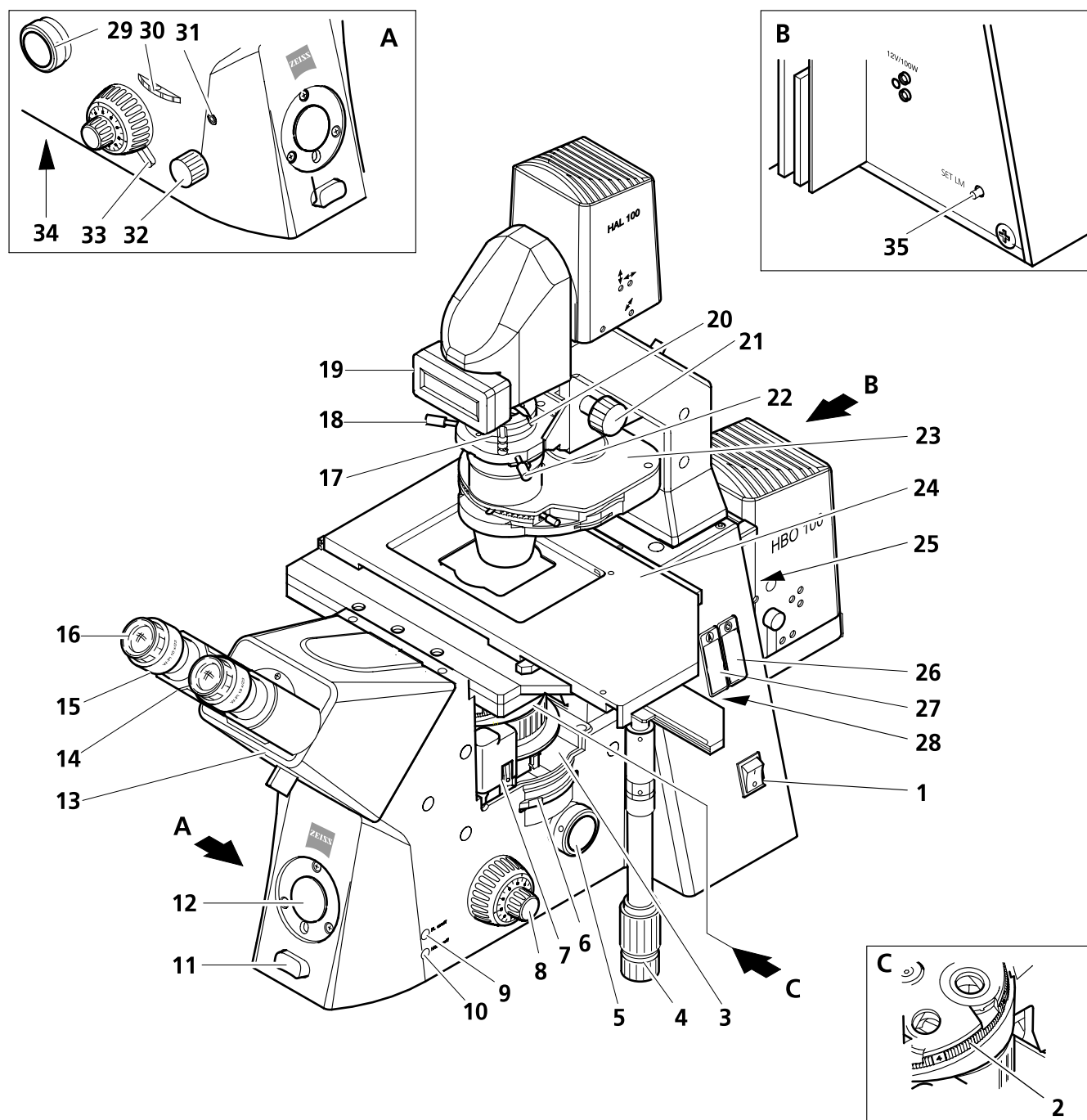


Fig. 3-1 Operation and function controls on the Axiovert 200 (manual)

Key to Fig. 3-1:

- 1 On / Off switch
- 2 Nosepiece
- 3 Compartment for analyzer slider (can be equipped from the right or left)
- 4 Drive controls for XY-movement of the mechanical stage
- 5 Sideport right
- 6 Setting wheel for Optovar turret (max. 3 positions)
- 7 Reflector turret
- 8 Focusing drive coarse / fine
- 9 FL key on / off for fluorescence shutter
- 10 HAL on / off switch
- 11 Toggle switch for illumination intensity
- 12 Frontport
- 13 Binocular tube
- 14 Binocular component of the tube
- 15 Setting ring of eyepiece
- 16 Eyepiece
- 17 Polarizer D with 2-position filter changer
- 18 Centering screw for condenser
- 19 LCD display
- 20 Adjusting lever of luminous-field diaphragm
- 21 Setting knob for vertical adjustment of condenser
- 22 Centering screw for condenser
- 23 Condenser
- 24 Microscope stage
- 25 HBO / XBO adjusting aid
- 26 Compartment for 3-position filter slider (dia. 25 mm)
- 27 Compartment for slider with iris stop as aperture diaphragm reflected light
- 28 Compartment for slider with iris stop as luminous-field diaphragm reflected light
- 29 Sideport left
- 30 Setting wheel for Sideport right / Sideport left / vis
- 31 Clamping screw for Frontport
- 32 Setting knob for Frontport / Baseport / vis
- 33 Stop for focusing drive
- 34 Baseport
- 35 Key "Set LM"

On / Off switch (3-1/1)

- position 0 = instrument switched off
- position I = instrument switched on, switch lights up

Key HAL on/off (3-1/10)

- Brief pressing switches the halogen illuminator on or off alternately.
- Long pressing (> 1 s) automatically sets the brightness to 3200K for color photography.

Toggle switch for brightness control of the halogen illuminator (3-1/11)

- Controls the brightness of the halogen illuminator in 2 steps.
 - step 1 – slow brightness change: switch pressed half down until first pressure point:
 - switch to the left – brightness reduction
 - switch to the right – brightness increase
 - step 2 – fast brightness change: switch pressed down until stop (second pressure point)
 - switch to the left – brightness reduction
 - switch to the right – brightness increase
- Variable voltage range between 0 and 12 V. When the limit values are reached, a warning beep is emitted.
- At 10.5 V, the halogen lamp provides light with the color temperature of 3200 K for color photography. To make this setting easier for you, the fast increase of the lamp voltage stops at this point and a beep is emitted. To further increase the lamp voltage, keep the toggle switch pressed for more than 1 sec **or** release it briefly and then press it again.
- If the toggle switch is pressed down for more than 3 sec while the halogen illuminator is switched off, the halogen illuminator will automatically switch on again.

Focusing drive (3-1/8)

- Coarse adjustment (big knob):
Focusing knob for coarse adjustment on both sides of the instrument: 1 coarse rotation = 5 mm
Overall lift: approx. 10 mm
- Fine adjustment (small knob):
Focusing knob for fine adjustment available on both sides of the instrument: 1 fine rotation = 0.1 mm

Clamping lever for vertical stop of the focusing drive (3-1/33)

- Setting of vertical stop for stage focusing.
- Turn clamping lever for stop upwards towards the stopping pin. Use the focusing drive to move the stage to the uppermost admissible position where collisions with the stage or the microscope slide are no longer possible. Then press the clamping lever downwards to clamp it in the stop position.

Nosepiece with objectives (3-1/2)

- 6-position nosepiece H, DIC (3-2/1) with compartments for DIC slider (3-2/2) in all objective positions.
- Fast change of objectives through rotation of the nosepiece via the setting ring (3-2/4).



When the heating stage is used, the nosepiece must first be moved to the lowest position via the focusing drive before a new objective is swung in, since otherwise collision between the objective and the heating stage might occur.

Compartment for analyzer slider (3-1/3 and 3-2/3)

- For fixed analyzer slider with two dia. 32 mm filter positions, or analyzer slider $\pm 30^\circ$ for DIC.

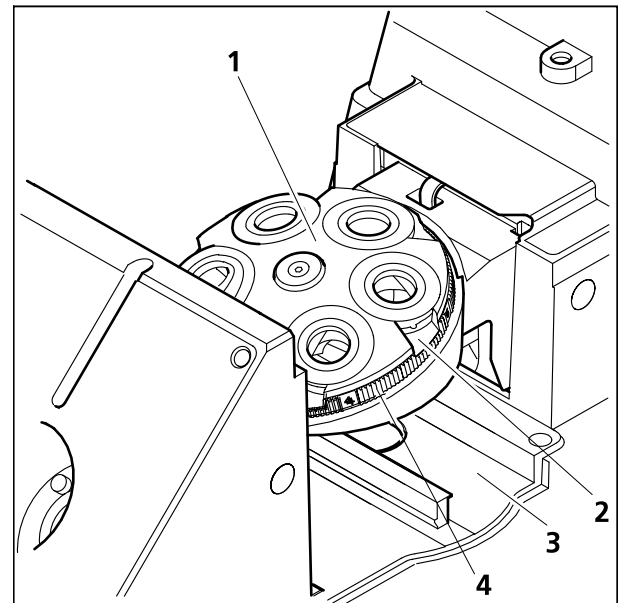


Fig. 3-2 **Nosepiece with mounts for DIC slider**

Drive controls for XY-adjustment of the mechanical stage or the object guide if specimen stage 250x230 (3-1/4) is attached.

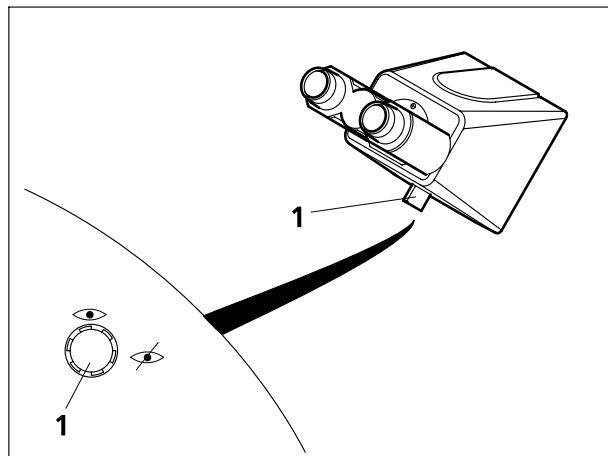
- Upper drive knob: adjustment in Y direction.
- Lower drive knob: adjustment in X direction.

Setting wheel for Optovar turret (3-1/6)

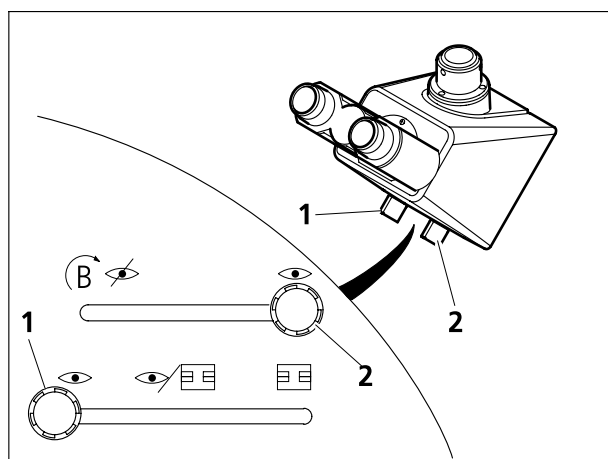
- Maximum of 3 switching positions with:
 - tube lens 1x;
 - optovar lens 1.6x;
 - optovar lens 2.5x
- In the case of equipment with less than 3 lenses, the empty positions are blocked.

Binocular tubes (3-1/13)

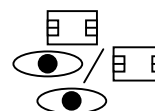
- The binocular tubes offered permit the individual setting of the interpupillary distance and the viewing height within defined limits.

**Fig. 3-3 Binocular tube 45°/23****Binocular tube 45°/23 with manual shutter vis (3-3)**

- Shutter switched on / off via rotary knob (3-3/1):
100 % vis
0 % vis (light shutter)

**Fig. 3-4 Binocular phototube 45°/23****Binocular phototube 45°/23 with sliding prism for vis / doc, Bertrand lens and manual shutter vis (3-4)**

- Shutter switched on / off via rotary / slider knob (3-3/1):
100 % vis
0 % vis (light shutter)
Bertrand lens
- Focusing of Bertrand lens through activation of rotary / slider knob, with the Bertrand lens being switched on
- Switching of beam path (sliding prism vis / doc) via slider knob (3-3/2):
0 % vis: 100 % doc
50 % vis: 50 % doc
100 % vis: 0 % doc



Binocular component of the tubes (3-1/14)

- The eyepiece distance is matched to the individual interpupillary distance by swinging the eyepiece tubes symmetrically towards one another (3-5/A and 3-5/B).
- Two vertical adjustments through rotation of the binocular component around 180°.

Eyepiece / setting ring of eyepiece (3-1/16 and 15)

Both eyepiece models permit compensation of the user's ametropia, and also accept eyepiece reticles (see chapter 2).

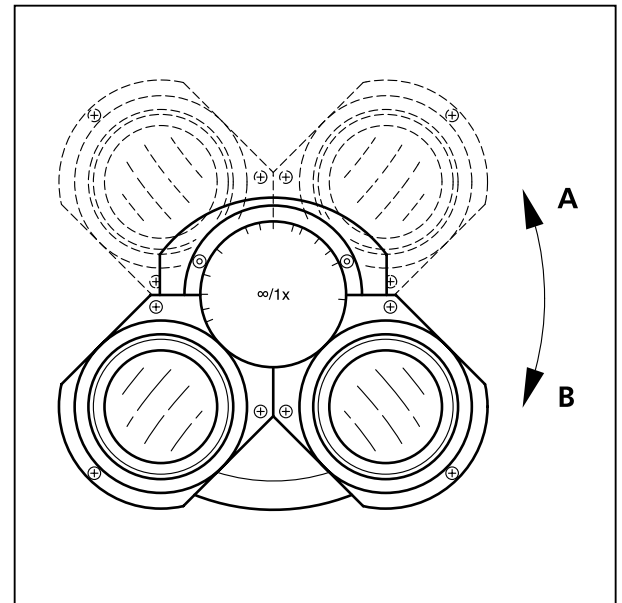


Fig. 3-5 Setting of eyepiece distance on the binocular tube

LCD display (3-1/19)

- Two-line display with 16 characters each attached to the carrier for transmitted-light illumination / condenser.
- Display of objective position (3-6/1), magnification (3-6/2) and contrasting technique (3-6/3) in the upper line.
- **FL** display (3-6/4) on the right of the top line if the fluorescence shutter is opened.
- Display of lamp brightness in a line scale (3-6/6) and display of the relevant voltage (3-6/5) in the lower line. If the color temperature (10.5 V) has been set, the line scale displays the value **3200 K**. One interval corresponds to 0.4 V. If the halogen illuminator is switched off, **HAL off** is displayed.
- The LCD background illumination can be switched on or off as required. Activation / deactivation is performed by pressing the **FL** key for more than 2 seconds.
- The LCD display is also used for the configuration of the microscope (Set mode).

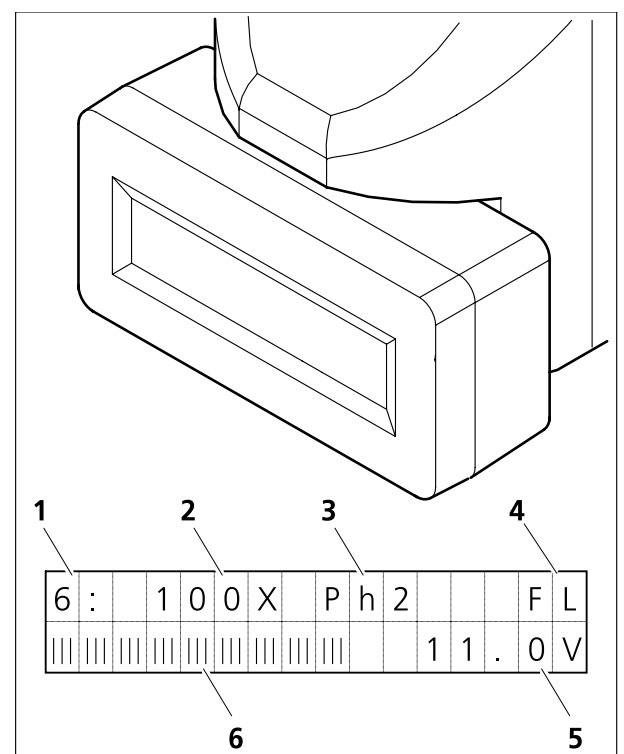


Fig. 3-6 LCD display

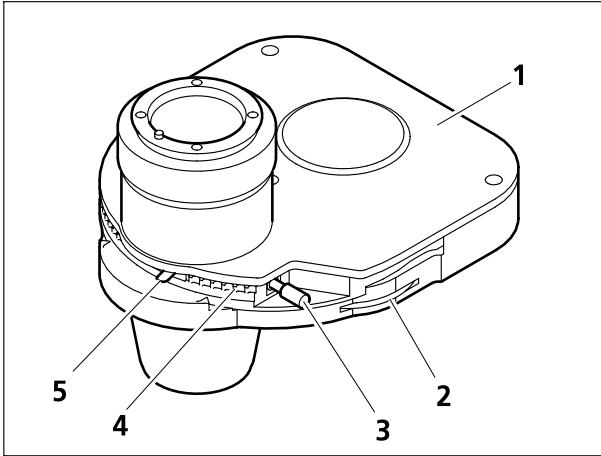


Fig. 3-7 LD condenser 0.55, 6-position,
H, Ph1, Ph2, Ph3, Var1/2

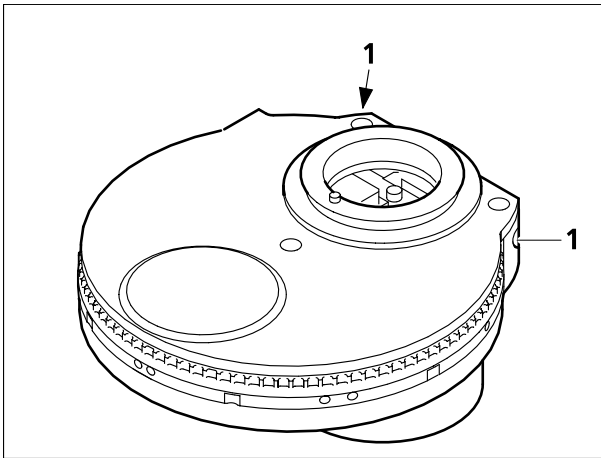


Fig. 3-8 LD condenser 0.35, 6-position,
H, Ph0, Ph1, Ph2, DIC, DIC

Condensers (3-1/23)

Depending on their type, condensers (3-7/1) are equipped as follows:

- 5- or 6-position turret for:
 - brightfield: H
 - phase contrast: Ph0, Ph1, Ph2, Ph3 with centerable stops
 - interference contrast: DIC
 - Varel contrast: Var1, Var2
- Aperture diaphragm (iris stop). Use knurled knob (3-7/2) to open and close the aperture diaphragm.
- Turn the turret wheel (3-7/4) to swing the brightfield insert or the contrast stops into the beam path.
- The brief description of the set turret position (e.g. **H**) is displayed to the user at the front.
- Condensers for Varel contrast are equipped with a knurled knob (3-7/3) which allows the position of the Varel stop to be changed when the Varel turret position has been set. The lever (3-7/5) permits switching between Var1 and Var2.
- The condensers 0.35 and 0.55 for phase contrast require the use of one SW 1.5 Allen screwdriver each (plugged in at 3-8/1) to center the phase stops.



The condenser turrets feature the so-called automatic diaphragm mechanism, i.e. the aperture diaphragm (iris stop) is opened completely when a phase stop position is switched on. When a new turret position is switched on, the aperture diaphragm is automatically reset to its last opening.

Centering screw for condenser (3-1/18 and 22)

- Screws for condenser centering attached to both sides of the carrier for transmitted-light illumination / condenser.

Lever for luminous-field diaphragm, transmitted light (3-1/20)

- Lever attached to the carrier for transmitted-light illumination / condenser which opens and closes the transmitted-light luminous-field diaphragm to set KÖHLER illumination
- Lever in front position: luminous-field diaphragm fully opened
- Lever in rear position: luminous-field diaphragm closed

Lever for vertical adjustment of the condenser (3-1/21)

- Setting knob attached to the carrier for transmitted-light illumination / condenser which raises and lowers the condenser to set KÖHLER illumination

Polarizer D with 2-position filter changer (3-1/17)

- The polarizer and two filter positions can be swung in and out separately. Stop position in swung-in status.

Microscope stages with specimen holder (3-1/24)

- The specimens are mounted, positioned and fixed in position using the specimen holder.
- Equipped as follows, depending on the instrument configuration:
 - specimen stage 250x230 with object guide and mounting frame for object guide (M)
 - mechanical stage 250x230 and mounting frame for mechanical stage (K)
 - scanning stage
 - heating stage
 - gliding stage Z

Key "Set LM" (3-1/35)

- Stores the Light Manager values and enters the configuration mode.

Frontport (3-1/12)

- Port for the connection of documentation equipment.

Clamping screw for Frontport (3-1/31)

- Clamping screw for fixation of the camera / video adapter to the Frontport.

Baseport (3-1/34)

- Port for the connection of documentation equipment below the Axiovert 200.

Setting knob for Frontport / Baseport / vis (3-1/32)

- Selects the beam splitting ratio for Frontport, Baseport and visual observation.
- 3 switching positions:

100 % vis (free light path);	
100 % Frontport;	
100 % Baseport;	

Sideport right (3-1/5)

- Port for the connection of documentation equipment.
- Different splitting ratios for Sideport right, Sideport left and visual observation (vis), depending on the instrument configuration.

Sideport left (3-1/29)

- Port for the connection of documentation equipment.
- Different splitting ratios for Sideport right, Sideport left and visual observation (vis), depending on the instrument configuration.

Setting wheel for Sideport right / Sideport left / vis (3-1/30)

- Selection of beam splitting for Sideport (doc) right, Sideport (doc) left and visual (vis) observation.
- 2 or 3 switching positions with different beam splitting ratios.
- 2 switching positions with instrument configuration with Sideport 60 left:

100 % vis: 0 % doc;	
20 % vis: 80 % doc left;	
- 3 switching positions with instrument configuration with Sideport 60 left:

100 % vis: 0 % doc;	
0 % vis: 100 % doc left;	
50 % vis: 50 % doc left	
- 3 switching positions with instrument configuration with Sideport 60 right:

100 % vis: 0 % doc;	
0 % vis: 100 % doc right;	
50 % vis: 50 % doc right	
- 3 switching positions with instrument configuration with Sideport 60 left and right:

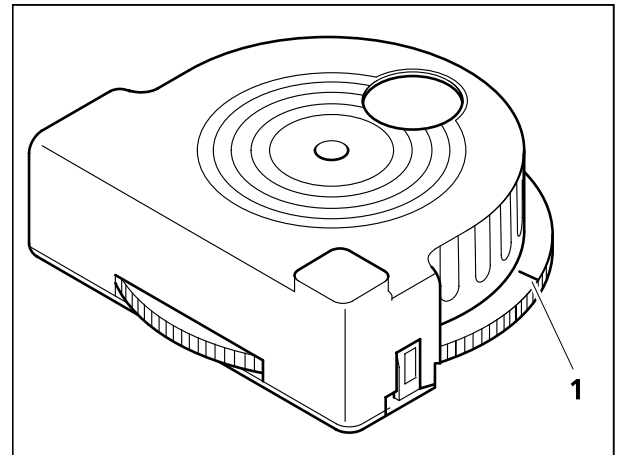
100 % vis: 0 % doc;	
0 % vis :100 % doc left;	
20 % vis: 80 % doc right	

5-position reflector turret (3-1/7)

- Accepts the maximum of 5 reflector modules for epi-fluorescence.
- Fast change of reflector position by turning the setting ring of the reflector turret.
- The activated reflector position is marked with a line (3-9/1) on the right of the reflector turret.

FL key on/off (3-1/9)

- Switches the fluorescence shutter on or off alternately. **FL** in the LCD display, if available, indicates that the shutter has been opened.

**Fig. 3-9 5-position reflector turret****HBO/XBO adjusting aid for lamp adjustment (3-1/25)**

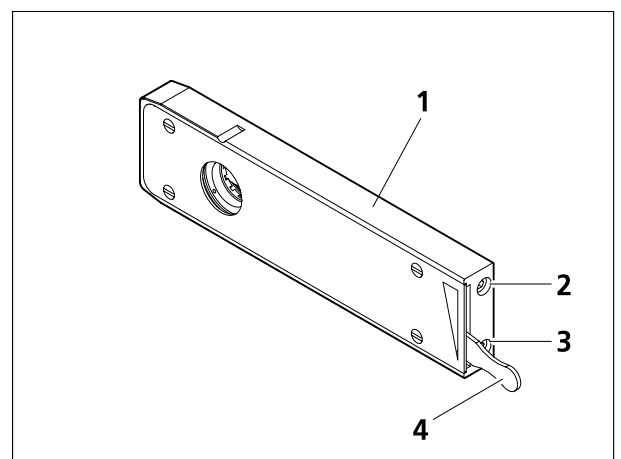
- The adjusting aid is used to adjust and center the N HBO103 mercury vapor short-arc lamp.
- The adjusting aid is activated / deactivated by pulling out / pushing in the cylinder until stop.

Compartment for 3-position filter slider (3-1/26)

- for 3-position, dia. 25 mm filter slider
- Push in filter slider until the required stop position, with the labeling being visible from the front.

Compartment for iris stop slider, reflected light (3-1/27 and 28)

- Accepts the slider with iris stop (3-10/1) as aperture or luminous-field diaphragm for the setting of KÖHLER illumination.
- Push slider into the compartment until stop. Symbol for stop opening (wedge) points to the user.
- Use lever (3-10/4) of the slider to open or close the iris stop (lower position).
- The two centering screws (3-10/2 and 3; socket head SW 3) permit the stop to be centered in the beam path.

**Fig. 3-10 Iris stop slider**

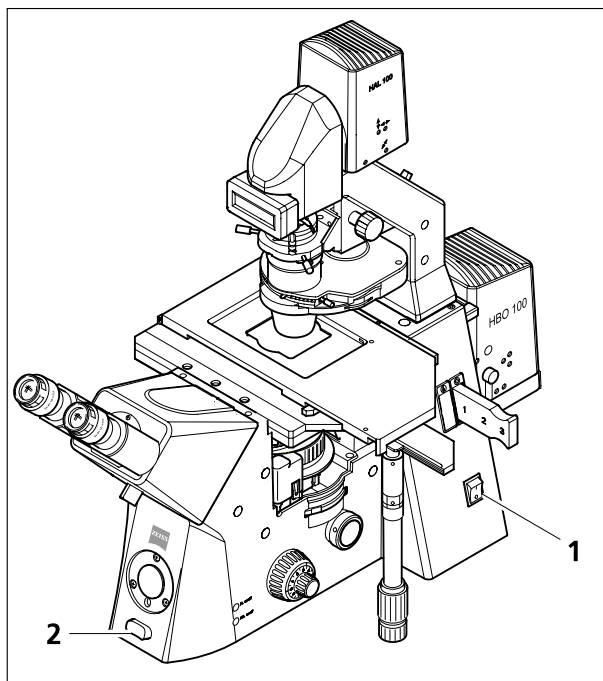


Fig. 3-11 On / Off switch and toggle switch

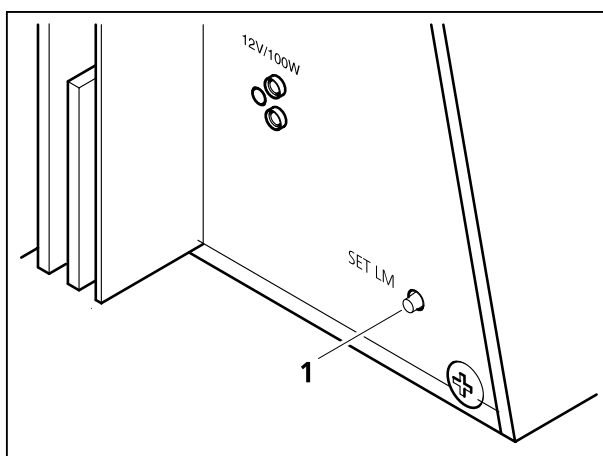


Fig. 3-12 Key "Set LM"

3.1.2 Switching on and basic settings on the Axiovert 200 (manual)

- Switch on the microscope with the on/off switch on the right side (3-11/1).

The indicator light in the switch lights up. The microscope is ready for operation after a few seconds.

3.1.2.1 Configuration (Set mode)

If the microscope is equipped with the LCD display, the correct objective names must be assigned to the objective positions first after the microscope has been switched on or when objectives have been changed.

The objective positions are entered in the Set mode. In the case of the first entry, a question mark (?) appears in the upper line of the LCD display instead of the objective name.

- To activate the Set mode, keep the **Set LM** key (3-12/1) on the instrument rear pressed for approx. 1 second until a double beep is emitted. The objective name in the LCD display starts blinking.
- Now set the magnification and contrasting technique one after the other for each nosepiece position by using the toggle switch (3-11/2) on the instrument front to quickly or slowly scroll through a list of all possible combinations in forward or backward direction.

- To exit the Set mode and return to the standard mode, briefly press the **Set LM** key on the instrument rear. The permanent storage of all the settings is confirmed by a beep. **LightManager Settings stored** appears in the LCD display.



If no adjustment is performed for more than 60 seconds, the Set mode is exited automatically.

3.1.2.2 Light Manager

The Light Manager is automatically activated each time the Axiovert 200 is switched on.

The Light Manager automatically sets the illumination intensity of the halogen illuminator last saved for each individual objective position. Therefore, the correct brightness is immediately available in almost all cases.

During the objective change, the lamp voltage is reduced automatically to avoid glare and to permit the required brightness to be achieved sooner.

FL key on / off for fluorescence shutter

If this key is pressed briefly, the optional fluorescence shutter (reflected light) can be opened or closed. Opening is confirmed by a beep. The Light Manager remembers the setting of the halogen illuminator separately for the opened and closed shutter and resets it automatically after a changeover, i.e. the correct lamp brightness is always available immediately after any change between reflected and transmitted light.



This function of the fluorescence shutter key is also beneficial for pure transmitted-light operation to change quickly and easily between two different settings of the lamp brightness, e.g. in the following cases:

- change between the brightfield and darkfield contrasting technique,
- change between differently absorbing filters in the beam path,
- change between visual observation and documentation using a camera.

Programming of switching status

The individual settings of the Light Manager can be saved permanently so that they are always available immediately when the instrument is switched on.

- To save the Light Manager parameters, briefly press the **Set LM** key on the instrument rear. Saving as switching status is confirmed by a beep. **LightManager Settings stored** appears in the LCD display.

3.2 Axiovert 200 M (motorized)

3.2.1 Operation and function controls on the Axiovert 200 (motorized)

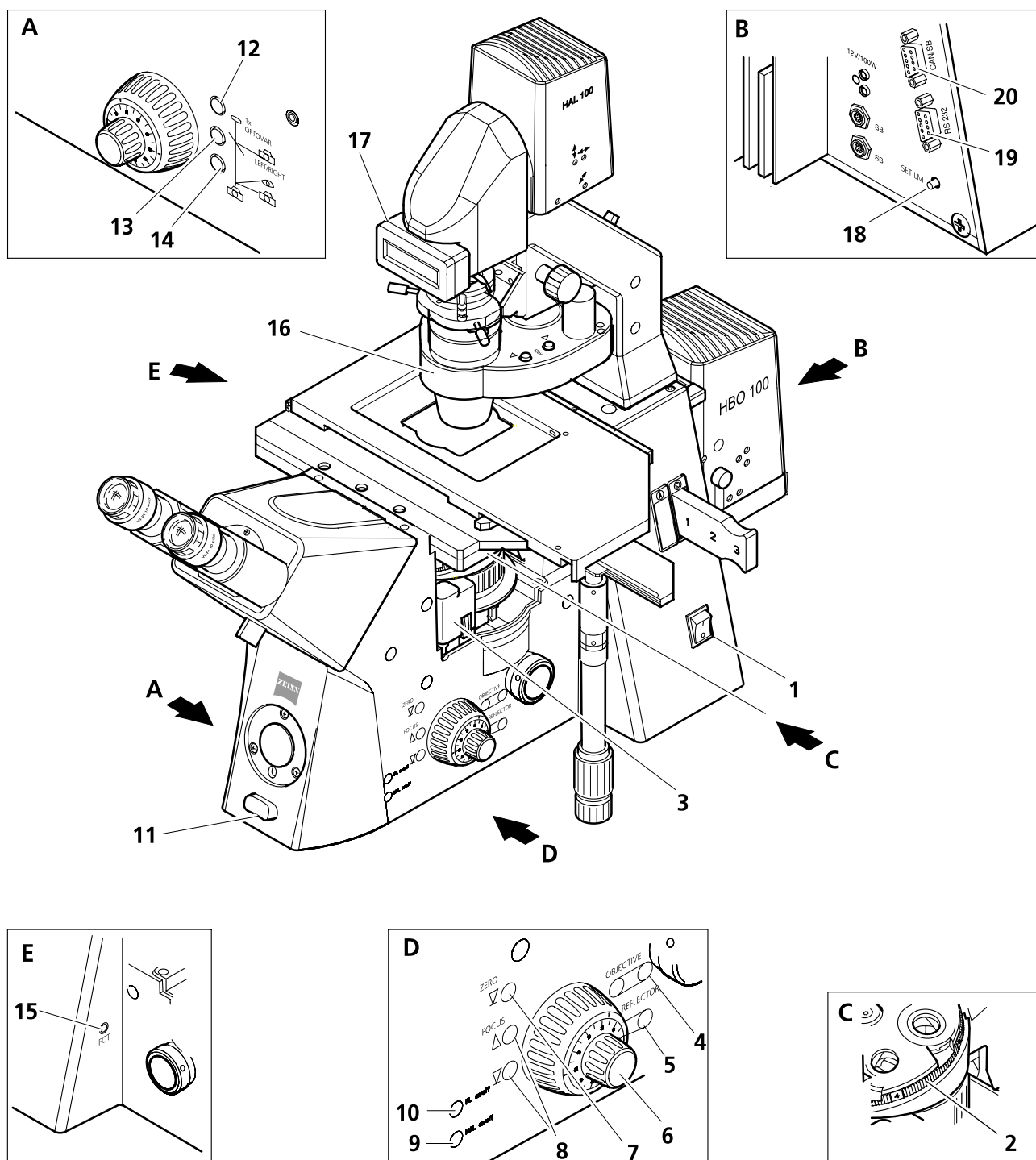


Fig. 3-13 Operation and function controls on the Axiovert 200 M (motorized)

Key to Fig. 3-13:

- 1 On / Off switch
- 2 Nosepiece (motorized)
- 3 Reflector turret (motorized)
- 4 OBJECTIVE keys (forward and backward)
- 5 REFLECTOR keys (forward and backward)
- 6 Focusing drive coarse / fine (motor-supported)
- 7 ZERO key
- 8 FOCUS keys (Work / Load)
- 9 HAL on / off key
- 10 FL key on / off (fluorescence shutter)
- 11 Toggle switch for illumination intensity and programming
- 12 1x OPTOVAR key (maximum of 3 positions)
- 13 Left / Right key for Sideport switching
- 14 Key for switching between visual observation, Frontport, Baseport
- 15 FCT key – programmable function key
- 16 Condenser (motorized)
- 17 LCD display
- 18 Key "Set LM"
- 19 RS 232 connector
- 20 CAN / SB connector (CAN-Bus, Sub-bus)

On / Off switch (3-13/1)

- position 0 = instrument switched off
- position I = instrument switched on, switch lights up

HAL key on/off (3-13/9)

- Brief pressing switches the halogen illuminator on or off alternately.
- When the unit is switched on again, the voltage present at the time of switching off is automatically set again.
- When the unit is switched on, the lower line of the LCD display shows the voltage in a scale and the lamp voltage value (nominal value). When the unit is switched off, the message **HAL OFF** appears on the LCD display.
- Long pressing (> 1 s) automatically sets the brightness to 3200K for color photography.

Motorized focusing drive (3-13/6)

- Focusing knob available on both sides of the instrument.
- Coarse adjustment - big knob
- Fine adjustment - small knob

FOCUS key - Work / Load (3-13/8)

- **Load ▼** key Brings the nosepiece to the lowest position.
 Load Position appears in the lower line of the LCD display. The lower LCD line blinks every second as a warning sign.
- **Work ▲** key pressed briefly (< 0.9 s): travel to work position, if in load position.
 pressed long (> 2 s): setting of focus limit.

ZERO ▼ key (3-13/7)

- Key pressed briefly: current focus position is shown in the LCD display;
 activation of measuring mode
- key pressed long (> 2 s): Z-position is zeroed.
- As soon as the Z-drive is moved, the current focus position is displayed in µm with one digit after the comma: negative sign for lowering of nosepiece from the zero position, positive sign for moving upwards from zero position.
- Exiting of measuring mode after objective change or Optovar change.

Motorized nosepiece with objectives (3-13/2)

- Motorized 6-position nosepieces H, DIC with compartments for DIC sliders in all objective positions.
- Fast change of objectives through pressing the key on the right of the stand (3-13/4). The nosepiece can also be operated manually.



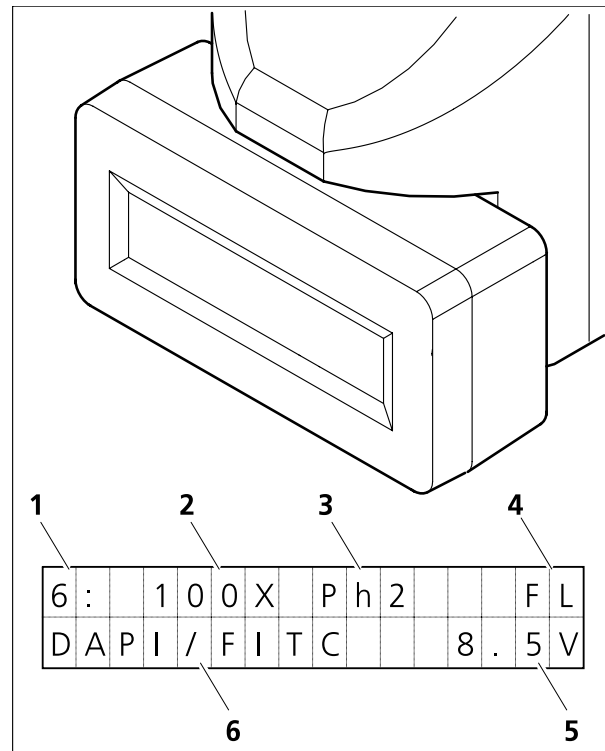
When the heating stage is used, the nosepiece must first be moved to the lowest position via the focusing drive before a new objective is swung in, since otherwise collision between the objective and the heating stage might occur.

OBJECTIVE ◀▶ key (3-13/4)

- Switches the nosepiece forwards or backwards by one position. The nosepiece can also be operated manually.
- Before turning the nosepiece to the next position, move it to its lower end position. After the change, the previous Z-position is approached again and the previous lamp voltage value is reset. Lowering is not required if the nosepiece is turned manually.
- The position number, magnification and contrasting technique of the current position is shown in the upper line of the LCD display

LCD display (3-13/17)

- Two-line display with 16 characters each attached to the carrier for transmitted-light illumination / condenser.
- Display of objective position (3-6/1), magnification (3-6/2) and contrasting technique (3-6/3) in the upper line.
- **FL** display (3-6/4) on the right of the top line if the fluorescence shutter is opened.
- Display of lamp brightness in a line scale and display of the relevant voltage (3-6/5) in the lower line. One interval corresponds to 0.4 V.
- The LCD background illumination can be switched on or off as required. Activation / deactivation is performed by pressing the **FL** key for approx. 1 second.
- Brightness adjustment of the LCD display is by pressing/holding the **FL** key + the toggle switch.
- The LCD display is also used for the configuration of the microscope (Set mode).

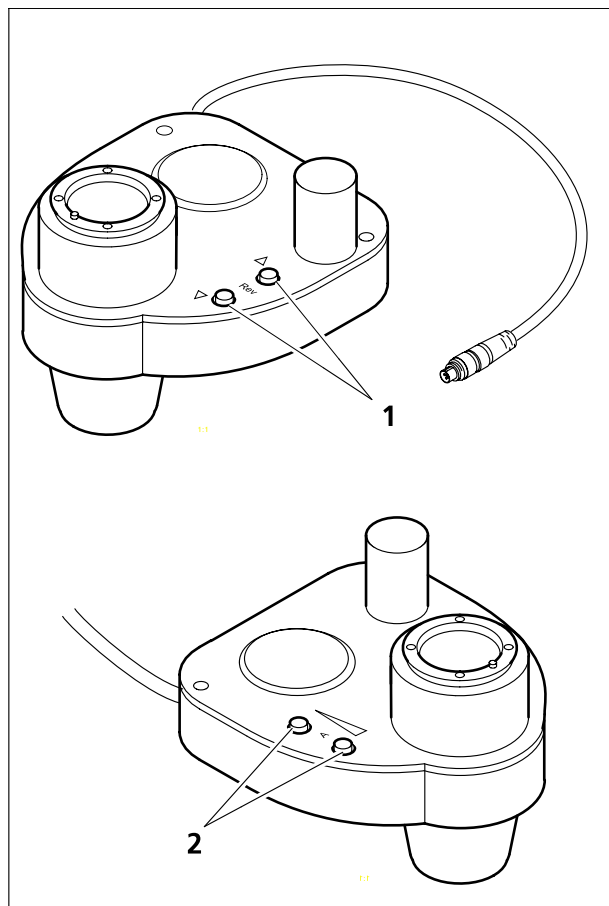
**Fig. 3-14 LCD-Display**

Instead of the line scale for the lamp brightness, the following can be displayed in the lower line (3-6/6) of the LCD display:

- **HAL off** when the halogen illuminator is switched off.
- **3200K** when the lamp voltage has been set to the color temperature (10.5 V).
- Description of the swung-in filter set on the reflector turret (e.g. **DAPI/FITC**), provided that the fluorescence shutter has been opened. If no filter set is contained in the reflector turret, the reflector turret position (e.g. **Refl. 2**) is displayed instead.

The currently set position appears in the LCD display for 4 seconds during performance of the following key functions: Both lines of the display are used.

- **1x Optovar**: display of the Optovar magnification factor (**1.6x**) and the overall magnification (**Total Mag.**) in the intermediate image
- **LEFT/RIGHT** and **Slider VIS/Frontport/Baseport**: display of the actual intensities at Sideport, Frontport, Baseport or VIS, depending on the selected positions of both control elements, e.g. **SP: Right 20%** (upper line of the LCD display) and **Binocular 80%** (lower line).
- **FOCUS - Load**: **Load Position** appears in the lower line (display blinks every second).
- **ZERO**: display of the current focus position in mm (e.g. **Zdis: +0.0µm**)
- If a motorized condenser is connected, the currently set condenser turret position appears in the lower line (e.g. **Cond. DICIII**). If the aperture is changed by pressing the key on the condenser, the value of the stop opening is displayed (e.g. **Cond. NA: 0.55**). When a phase stop position is set, the aperture diaphragm is always entirely open.
- Display function during the instrument configuration (SET mode).



**Fig. 3-15 Condenser 0.55, 6-position
H, Ph1, Ph2, Ph3, DIC, DIC**

Motorized condensers (3-13/16)

Motorized condenser, equipped with:

- 6-position turret for:
brightfield: H
phase contrast: Ph1, Ph2, Ph3 with centerable stops
interference contrast: DIC I, DIC II
- If a motorized condenser is recognized by the Sub-bus of the stand, the current condenser turret position automatically appears in the lower line of the LCD display. The condenser display is always available.
- Motorized adjustment of the turret by pressing key **Rev** Δ ∇ (forward and backward, 3-15/1) on the right side of the condenser.
- Motorized adjustment of the aperture diaphragm by pressing key **A** (open and close, 3-15/2) on the left side of the condenser. If a phase stop is contained in the beam path, the aperture diaphragm always fully opens automatically (NA = 0.55).
- The set turret position appears in the lower line of the LCD display (e.g. **Cond. DICII**). **Cond. NA: 0.55** is displayed in a phase stop position, and the key pair **A** is without function.



The motorized condenser can only be used with the motorized stand, or with a manual stand which is equipped with an interface PC-board.

1x OPTOVAR key for the motorized change of Optovar settings (3-13/12)

- Key for the selection of the Optovar magnification; switching sequence 1x, 1.6x, 2.5x
- Key pressed briefly: current position is shown in the LCD display for 4 s (or until the next press of the key);
without LCD display, the position is approached immediately
- key pressed again: target position is displayed and approached
- In the LCD display, the selected Optovar magnification and the overall magnification are shown.

- briefly pressed: Light Manager values are saved
- pressed long (> 2 s): activation of Set mode

Motorized 5-position reflector turret (3-13/3)

- Accepts the maximum of 5 reflector modules for epi-fluorescence.
- Fast change of reflector position through pressing the key (forward or backward) on the right of the stand (3-13/5).
- The activated reflector position is marked with a line on the right of the reflector turret. The reflector turret can also be operated manually.

Key REFLECTOR ◀▶ (3-13/5)

- Switches the reflector turret forwards or backwards by one position. The reflector turret can also be operated manually.
- Key pressed briefly: current position is shown in the LCD display for 4 s (or until the next press of the key);
 without LCD display, the position is approached immediately
- key pressed again: target position is displayed and approached
- If a position with a filter set is in the beam path, the filter set is shown in the lower line of the LCD display. The voltage of the halogen illuminator is displayed only as a digit. If the halogen illuminator is switched off, **0 V** is displayed. If no filter set has been configured for the reflector position, the reflector position is displayed. However, the filter set and the reflector position are only displayed if the FL fluorescence shutter is open (**FL** is displayed in the upper line of the LCD display).

FL key on/off (3-13/10)

- Switches the fluorescence shutter on or off alternately.
- pressed briefly (< 1 s): FL shutter open (**FL** appears in the LCD display) or closed.
- pressed long (> 2 s): background illumination of the LCD display is switched on or off.
- If the key is kept pressed when the microscope is switched on, the Light Manager is deactivated. The Light Manager is activated automatically when the microscope is switched on the next time.

3.2.2 Switching on and basic settings on the Axiovert 200 M (motorized)

- Switch on the microscope with the on/off switch on the right side.

The indicator light in the switch lights up.

Initialization of the instrument follows.

After initialization, the LCD display shows the positions of all the stand components cyclically. The display remains visible until a key is pressed.

3.2.2.1 Configuration (Set mode)

The microscope must be configured after it has been switched on, i.e. the equipment of the nosepiece and the reflector turret, the Z-focus factors and the parfocal offset of the objectives to each other must be set. Setting is performed in the Set mode with the aid of the LCD display.

The microscope is already configured in the factory in accordance with the equipment ordered by the customer.

When the equipment in the nosepiece and the reflector turret is changed, the configuration must be updated accordingly.

Activation of Set mode

- To activate the Set mode, keep the **Set LM** key (3-12/1) on the instrument rear pressed for approx. 2 seconds until a double beep is emitted.

The objective name (default) or the reflector name in the LCD display starts blinking.



If no adjustment is performed for more than 60 seconds, the Set mode is exited automatically.

Setting of nosepiece equipment

- Press one of the two **OBJECTIVE** keys.

Change to nosepiece programming is made, and **SET Objective** is displayed in the upper line of the LCD display. The set nosepiece position, the magnification and the contrasting technique of the objective are displayed in the lower LCD line.

- Activation of the toggle switch on the front of the stand permits a list of objectives to be scrolled through upwards or downwards.
- If the correct objective for the relevant nosepiece position appears in the LCD display, the next nosepiece position can be swung in by pressing one of the two **OBJECTIVE** keys and the relevant objective can be assigned.
- Repeat the procedure for all nosepiece positions. Then change to the next programming item or exit the Set mode.

Setting of reflector turret equipment

- Press one of the two **REFLECTOR** keys.

Change to reflector turret programming is made, and **SET Reflector** is displayed in the upper line of the LCD display. The set reflector turret position and the selected filter set are shown in the lower LCD line.

- Activation of the toggle switch on the front of the stand permits a list of filter sets to be scrolled through upwards or downwards.
- If the correct filter set for the relevant reflector turret position appears in the LCD display, the next turret position can be swung in by pressing one of the two **REFLECTOR** keys and the relevant filter set can be assigned.
- Repeat the procedure for all reflector turret positions. Then change to the next programming item or exit the Set mode.

Setting of Z focus factors and parfocality

The matching of focus positions (parfocality) and the adaptation of the focus speed (Z-focus factors) to the objective magnification are normally only performed when the microscope is used for the first time or after the change of objectives in the nosepiece.

The parfocality values are automatically saved during setting of the Z focus factors. To correctly save the parfocality values, the objective with the highest magnification must be used to focus on a specimen before the Set mode is entered.

- Place a specimen on the stage. Swing in dry objective with the highest magnification.
- Focus on the specimen via the fine adjustment.
- Activate Set mode by pressing key **Set LM** (for more than 2 seconds).
- Press one of the two **FOCUS** keys.

Change to focus programming is made, and **SET FOC SPEED 6** is displayed in the upper line of the LCD display. The number **6** stands for the focus factor currently set. The set nosepiece position and the objectives in the nosepiece are shown in the lower LCD line.

- Activation of one of the two **FOCUS** keys permits the focus factor to be changed individually for the swung-in objective.

Key FOCUS ▲ : the drive moves faster

Key FOCUS ▼ : the drive moves slower

- Then focus on the specimen again and swing in the objective with the next lowest magnification.

While the nosepiece is being moved to the next position, the focus position and focus speed values set for the previous objective are saved in the Set mode.

- Use the current objective to set the focus speed, focus exactly on the specimen and move the nosepiece to the next position to store the settings.
- Repeat the above settings for all objectives. The last settings should always be made with the oil immersion objectives.
- Finally, the nosepiece must be turned by one further position to permit the values to be saved.
- To save the entire setting, exit the Set mode by briefly pressing the **Set LM** key.

Resetting of Z focus factors and parfocality to default values

- Activate Set mode.
- Press and hold FOCUS ▲ and FOCUS ▼ keys simultaneously for approx. 1 s until you hear a beep.

Deactivation of Set mode and saving of settings

The Set mode can be exited after conclusion of the settings. The settings / changes performed are automatically stored when the mode is exited.

- To exit the Set mode and return to the standard mode, briefly press the **Set LM** key on the instrument rear.

The permanent storage of all the settings is confirmed by a beep. **Config. & Focus Settings Stored** appears in the LCD display.



If no operation is performed in the Set mode for 60 seconds, the microscope is automatically reset to the standard mode. The settings made until then will not be stored.

If the instrument is switched off in the Set mode, the settings performed will not be stored either.

3.2.2.2 Setting of focus limit (software limit)

In addition to the recognition of the hardware end positions, the software also permits the setting of the focus limit, i.e. the upper limit of the travel range of the focusing drive.

This limit is an absolute focus position which applies to all nosepiece positions and which is only corrected by the amount of parfocality alignment of the relevant objective.

In the factory alignment of the Axiovert 200 M, the focus limit lies above the upper end position switch and is therefore not effective.

Activation of focus limits

- Press **Focus Up ▲** key for more than 2 seconds.

The current position of the focusing drive is stored as software limit (topmost Z-position).

FOC-Limit set appears in the LCD display for 4 seconds.

Shifting of focus limit

- Keep the **Work ▲** key pressed and simultaneously move to the required position via the focusing drive.

The new focus limit is stored after the key is released.

3.2.2.3 Light Manager

The Light Manager is applied with the Axiovert 200 M (storage of set values) in the same way as with the manual version.

However, the functionality of the Light Manager is considerably greater in the Axiovert 200 M on account of the coding / motorization of the major microscope components.

The Light Manager is automatically activated each time the Axiovert 200 is switched on. However, there is the possibility of deactivating the Light Manager temporarily.

Temporary deactivation is achieved by keeping the key **FL on / off** pressed after the instrument is switched on until a double beep is emitted. The Light Manager is then deactivated until the microscope is switched on the next time. **LM off** appears in the LCD display.

In **transmitted light**, the following parameters are stored with each nosepiece position:

- illumination intensity of the halogen illuminator
- condenser turret position (i.e. one contrasting technique, e.g. H, Ph, DIC, per nosepiece position)
- aperture stop opening

The intensity value is stored for each Optovar turret position (in combination with an objective), i.e. the illumination intensity is always suitable after a change of the Optovar magnification. The condenser turret position does not influence the illumination intensity.

In **reflected light**, the position of the fluorescence shutter is also taken into consideration.

The position of the reflector turret switches the halogen illuminator and the fluorescence shutter. If a fluorescence filter set is swung in on the reflector turret, the halogen illuminator is switched off and the fluorescence shutter is opened. If no fluorescence filter set (brightfield) is swung in or configured, the halogen illuminator is switched on and the fluorescence shutter is closed.

If the halogen illuminator shall be used parallel to the reflected-light illuminator, it can be switched on via the **Hal on / off** key, the illumination intensity can be matched, and this setting can also be stored by briefly pressing the **Set LM** key (instrument rear).

Standard settings are performed and stored in the factory for all components integrated into the Light Manager function.

The settings of the Light Manager can be manually adapted to the available conditions any time.

If manually changed settings shall be stored permanently, this is possible by briefly pressing the **SET LM** key. A brief beep after release of the key confirms that storage has been performed.

3.3 Illumination and contrasting techniques

3.3.1 Setting of transmitted-light brightfield for KÖHLER illumination

3.3.1.1 General principle

Transmitted-light brightfield microscopy is the most usual of all the optical techniques, since it allows the easy and fast viewing of high-contrast or stained specimens (e.g. blood smears).

In addition to so-called direct beam bundles, indirect bundles which are diffracted and scattered at the specimen details, are of major importance for an image as true to the object as possible. The greater the portion of the indirect bundles (aperture), the more the microscope image will be true to the object, according to ABBE.

To make use of the entire optical performance of the microscope and the objective in particular, the condenser, the luminous-field diaphragm and the aperture diaphragm should be set in accordance with the rules of KÖHLER illumination. These basic rules of microscope setting are described in detail in the following section 3.3.1.3, "Transmitted-light brightfield setting according to KÖHLER on the Axiovert 200 (manual)".

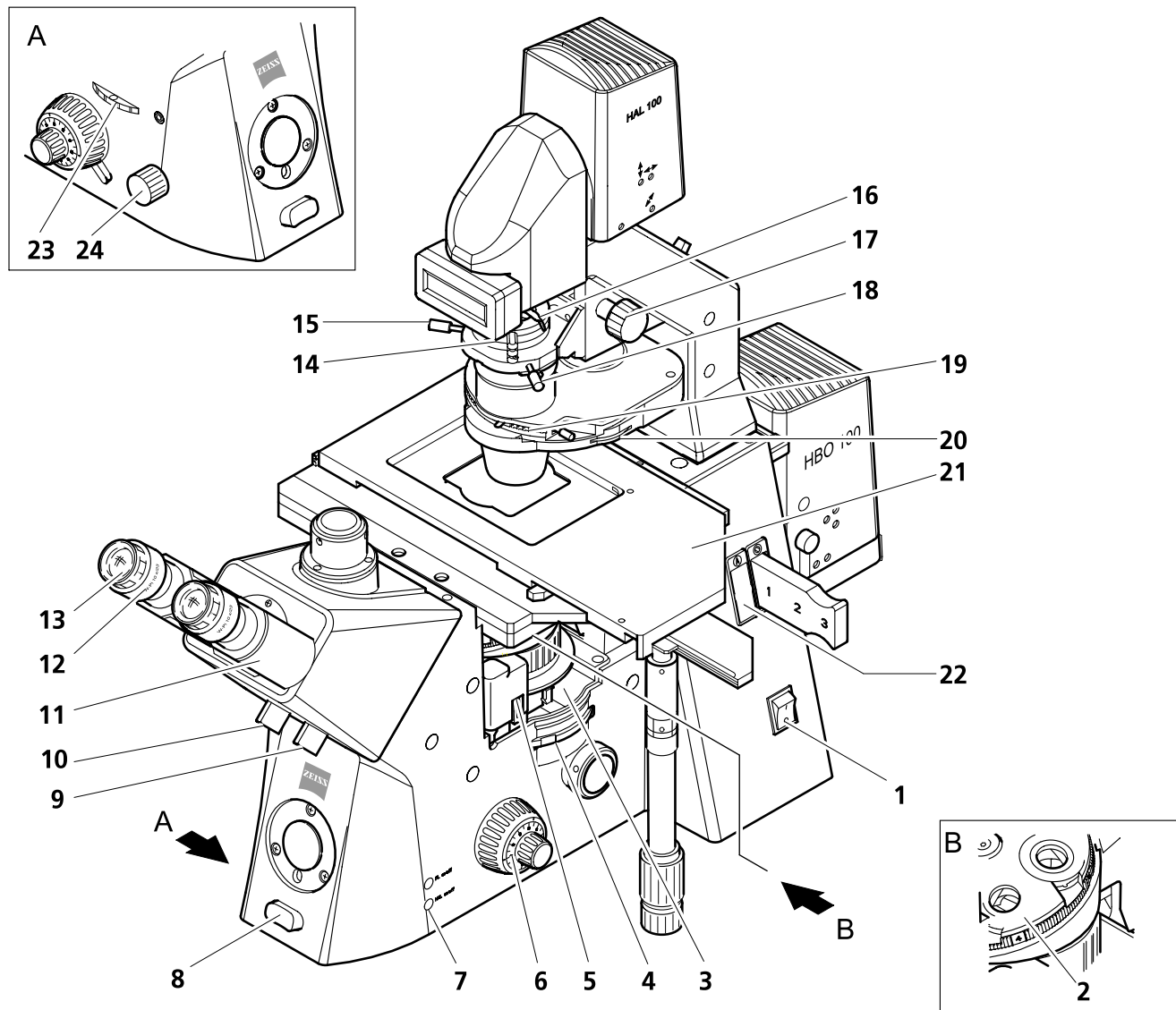
3.3.1.2 Transmitted-light brightfield configuration of the Axiovert 200 manual

Each manual Axiovert 200 microscope can be configured to permit the transmitted-light brightfield technique.

3.3.1.3 Transmitted-light brightfield setting according to KÖHLER on the Axiovert 200 (manual)

- Put the Axiovert 200 (manual) in operation as described in chapter 2.
- Switch on the Axiovert 200 (manual).

- Select the objective with the lowest magnification (e.g. 10x objective with the yellow ring) on the nosepiece (3-16/2) and ensure the correct stop position.
- Set factor 1x on the setting wheel (3-16/4) of the Optovar turret; ensure the correct stop position.
- Open the luminous-field diaphragm completely by pulling the lever (3-16/16) on the carrier for transmitted-light illumination to the front until stop.
- Open the aperture diaphragm completely by turning the setting wheel (3-16/20) on the condenser to the front until stop.
- Turn the setting ring (3-16/19) to move the condenser turret in position **H** for brightfield (if not available, to position **DIC**).
- Turn the setting ring to move the reflector turret (3-16/5, if available) in a position without filter combination and ensure the correct stop position.
- If required, remove analyzer slider from the compartment (3-16/3) or switch to free light path; ensure the correct stop position.
- Turn setting wheel for Sideport right / Sideport left / vis (3-16/22) to position 100 % vis (visual).
- Turn setting knob for Frontport / Baseport / vis (3-16/23) to position 100 % vis.
- Set beam splitting ratio to 100 % vis (3-16/10) on the binocular (photo)tube. Switch off the Bertrand lens (if available). Move combined rotary / slider knob (3-16/9) to position 100 % vis.



- | | | |
|--|---|---|
| 1 On / Off switch | 10 Rotary or slider knob for Bertrand lens and manual shutter | 18 Centering screw for condenser |
| 2 Nosepiece | 11 Binocular component of the tube | 19 Turret of condenser |
| 3 Compartment for slider analyzer | 12 Setting ring of eyepiece | 20 Setting wheel for aperture stop on the condenser |
| 4 Setting wheel for Optovar turret | 13 Eyepiece | 21 Microscope stage |
| 5 Reflector turret | 14 Polarizer D with 2-position filter changer | 22 Compartment for aperture stop slider |
| 6 Focusing drive coarse / fine | 15 Centering screw for condenser | 23 Setting wheel for Sideport |
| 7 HAL on/off key | 16 Adjusting lever of luminous-field diaphragm | 24 Setting knob for Frontport / Baseport |
| 8 Toggle switch for illumination intensity | 17 Setting knob for vertical adjustment of the condenser | |
| 9 Rotary or slider knob for vis / doc beam splitting | | |

Fig. 3-16 Axiovert 200

- Swing out 3-position filter changer (3-16/14).
- Place a high-contrast specimen on the microscope stage (3-16/21).
- Match the eyepiece distance (interpupillary distance) to the user's individual interpupillary distance: For this purpose, pull apart or push together the binocular component (3-16/11) of the tube.
- Set the zero point of ametropia compensation on the setting ring (3-16/12) of the eyepieces (3-16/13) to the white dot if no eyepiece reticle is available, to the red dot if eyepiece reticle is available.
- If required, optimize focus of the selected object detail via the setting ring of the relevant eyepiece for ametropia compensation.
- Use the coarse / fine focusing drive (3-16/6) to focus on the selected detail of the specimen. If no light is visible in the eyepieces, check whether light exits the housing of the halogen illuminator. If this is not the case, switch on the halogen illuminator by pressing the **HAL on/off** key (3-16/7).
- Use the toggle switch (3-16/8) to set the light intensity to a comfortable brightness. Adjustment is made in small steps when the key is pressed half down (pressure point), and in big steps when the key is pressed down completely.
- Close luminous-field diaphragm (3-16/16) until it is visible in the field of view, even if not in focus (3-17/A).
- Focus on the edge of the luminous field diaphragm (3-16/B) by moving the condenser (3-17/17) vertically.
- Center (3-16/C) luminous-field diaphragm using centering screws (3-17/15 and 18) and open it until the edge of the diaphragm just disappears from the field of view (3-17/D).
- For aperture diaphragm setting, remove one eyepiece from the eyepiece tube and set aperture diaphragm (3-16/20) to approx. 2/3 of the diameter of the objective exit pupil (3-17/E). Optimum contrast setting is dependent on the respective specimen.
- Insert eyepiece again and, if required, refocus on the specimen via fine drive.
- Match the light intensity via the toggle switch.

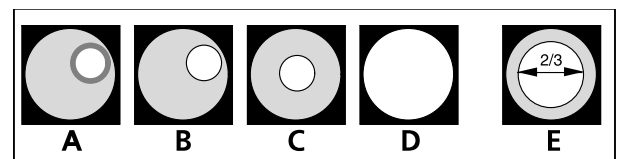


Fig. 3-17 Diaphragm settings for KÖHLER illumination in transmitted-light brightfield



The field size and the objective aperture change after every objective change, i.e. the luminous-field diaphragm and aperture diaphragm settings must be repeated to ensure optimum results.

3.3.1.4 Transmitted-light brightfield configuration of the Axiovert 200 M

Each Axiovert 200 M microscope can be configured to permit the transmitted-light brightfield technique.

3.3.1.5 Transmitted-light brightfield setting according to KÖHLER on the Axiovert 200 M

- Put the Axiovert 200 M in operation as described in chapter 2.
- Configure the instrument in the Set mode.
- Switch on the instrument and activate the Light Manager (standard setting).

The illumination intensity, the reflector turret setting (brightfield position), the condenser settings (position of the turret and aperture diaphragm opening) and the status "transmitted light on or off" are stored by pressing the **Set LM** key.

Motorized microscope components must be activated via the relevant control elements.

- Set KÖHLER illumination as described in section 3.3.1 for the Axiovert 200 (manual).
- After the last setting item, briefly press the **Set LM** key on the instrument rear to store the settings. A brief beep is emitted after release of the key to confirm that the setting has been stored.
- The above diaphragm settings must be repeated for all objectives, since the field size and the objective aperture are changing.

3.3.2 Setting of transmitted-light phase contrast

3.3.2.1 General principle

The phase contrast technique is ideal for examinations of thin, unstained specimens, e.g. culture cells. The human eye is unable to recognize phase differences (differences in refractive index and thickness) between the various cell components.

The phase contrast technique uses the optical modulators "phase stop and phase ring" and the interference procedures during the formation of the intermediate image to change the small phase differences in intensity and color differences which are visible to the human eye.

The high-intensity, direct light components are attenuated using the annular channel optically defined as "phase stop and phase ring", and a constant phase shift is applied. The indirect light components diffracted at various cell components, however, by-pass this optical channel and are influenced by the in-phase refractive index and the thickness differences of the specimen.

In the intermediate image plane, the differently influenced partial beams interfere and are enhanced or attenuated – depending on the phase position. This interference results in image contents displaying intensity and color differences which can be recognized by the human eye.

3.3.2.2 Configuration of the Axiovert 200 (manual) and Axiovert 200 M

- Phase contrast objectives with phase rings Ph 0, Ph 1, Ph 2 or Ph 3 for different average numeric apertures which can also be used in brightfield without any restriction.
- Condenser with turret containing centering phase stops Ph 0, Ph 1, Ph 2 and Ph 3 for various medium numeric apertures.
- The activated phase stop on the condenser must match with the relevant description on the objective, e.g. Ph 1.

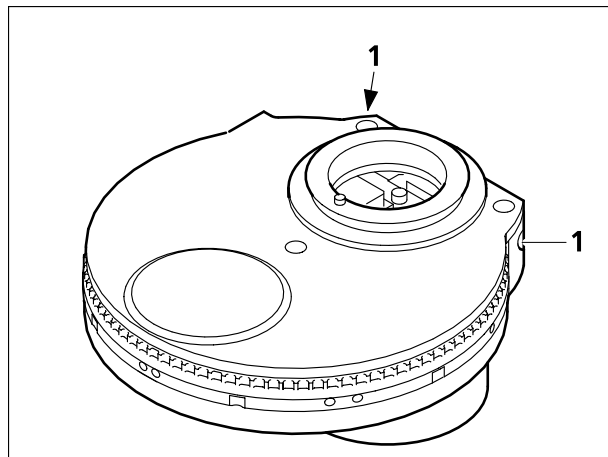


Fig. 3-18 Centering the phase stop on the condenser

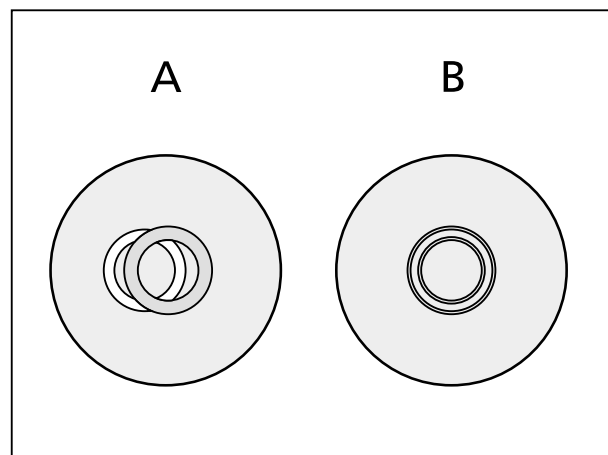


Fig. 3-19 Centering of phase stop (bright in condenser) with phase ring (dark in objective)

3.3.2.3 Setting of transmitted-light phase contrast on the Axiovert 200 / Axiovert 200 M

- Swing phase contrast objective, e.g. Ph 1, into the beam path.
- Switch phase stop with the same description as the phase contrast objective (e.g. Ph 1) in the turret of the condenser.
- To check the centering and congruence of the bright phase stop (in the condenser) with the dark phase ring (in the objective), remove one eyepiece from the tube and replace it with the centering telescope. Use the correction facility of the centering telescope to focus on the phase stop and the phase ring in the objective exit pupil.

When the phototube is used, the Bertrand lens can also be switched on for observation of the objective exit pupil. When the Bertrand lens is used, the factor 1x must be set on the setting wheel of the Optovar turret.

- If congruence is not perfect (3-19/A), two SW 1.5 Allen keys (3-18/1) must be used to recenter the bright phase stop until complete congruence with the dark phase ring has been achieved (3-19/B).
- Finally, remove the centering telescope from the tube and replace it with an eyepiece, or switch off the Bertrand lens.

However, centering is normally not required, since the phase stops are centered in the factory.

To enhance the image contrast, an interference wide-band filter, green 32 x 4, can be inserted in the filter changer. Complete phase contrast is only achieved if the bright phase stop (in the condenser) and the dark phase ring (in the objective) are exactly congruent in the illumination beam path (3-19/B).



Adjustment of the phase rings must be performed for all phase contrast objectives used. When liquid objects are examined in small vessels, the beam path must be aligned to the center of the vessel, since liquids on the edge of a vessel have the same effect as lenses and impair the microscope image.

3.3.3 Setting of differential interference contrast (DIC) in transmitted light

3.3.3.1 General principle

The transmitted-light DIC technique permits high-contrast 3D images of transparent specimen details.

Light which has been linearly polarized by a polarizer is split into two partial beams in a birefringent prism. These partial beams pass two neighboring specimen areas at a small distance and experience different path differences on account of different refractive indices and specimen thicknesses. Both beams are then united in a second birefringent prism and feature the same vibration direction after passing the analyzer. Therefore, both partial beams can interfere in the intermediate image, with the path differences being changed to different gray values (intensities).

3.3.3.2 Configuration of the Axiovert 200 (manual) and Axiovert 200 M

- Objectives offered with the DIC equipment, e.g. Plan-Neofluar
- DIC slider, suitable for the objectives used
- Condenser with turret equipped with DIC prisms (DIC I, DIC II, DIC III)
- Polarizer, e.g. model D with 2-position filter changer
- Analyzer, e.g. fixed analyzer slider or analyzer slider $\pm 30^\circ$ (de Sénarmont)

3.3.3.3 Setting of transmitted-light DIC on the Axiovert 200 (manual) and Axiovert 200 M

- Swing in the suitable DIC objective. Push appropriate DIC slider (3-20/3) into the compartment on the nosepiece. Make sure that the DIC slider engages in the stop position.
- Swing in the suitable DIC prism I, II or III (digit on condenser turret).
- Push analyzer slider (3-20/4) into the stand. Ensure the correct stop position.

(1) Transmitted-light DIC with fixed analyzer slider

- Switch on polarizer (3-20/1) on the carrier for transmitted-light illumination. Ensure the correct stop position.
- Place a specimen on the stage.
- Set luminous-field diaphragm and aperture diaphragm on the condenser (3-20/2) to KÖHLER illumination.
- Use knurled screw on the DIC slider to set optimum contrast. The symmetric adjustment of the DIC slider around its center position permits specimen details to be shown in 3D as if they were raised or lowered.

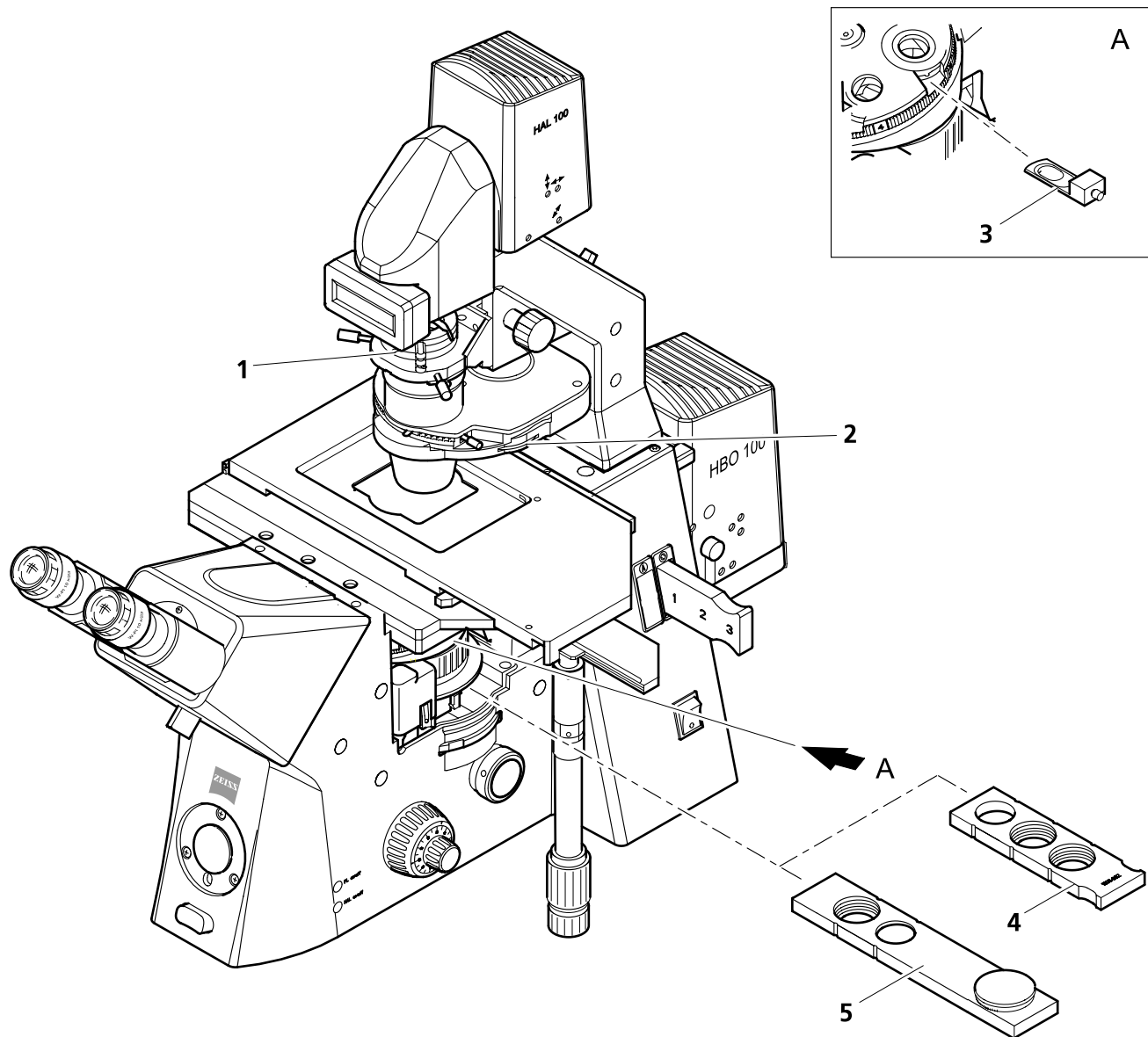
(2) Transmitted-light DIC with analyzer slider $\pm 30^\circ$ (de SENARMONT)

When the analyzer slider $\pm 30^\circ$ is used, the DIC slider must be brought to the center position first.

- Swing in the polarizer and bring analyzer $\pm 30^\circ$ (3-20/5) in 0° -position (dark position) (polarizer and analyzer are crossed).
- Swing out the DIC prism on the condenser turret (use brightfield or phase contrast position, for example).
- Remove one eyepiece and replace it with the centering telescope (or switch on Bertrand lens on the phototube).
- If the field is viewed using the centering telescope (or Bertrand lens), a diagonal black line of the DIC slider becomes visible (from the left top to the right bottom).
- Adjust the knurled screw on the DIC slider to move the diagonal black line to the center of the field of view.
- Remove the centering telescope and insert the eyepiece again (or switch off Bertrand lens).
- Swing in DIC position on the condenser.
- Place a specimen on the stage.
- Use the setting wheel to turn the analyzer out of the 0° -position and to set the optimum contrast in this way.



Since the DIC technique uses polarized light, it is impaired if birefringent components, e.g. foils sometimes used with histological sections, are positioned between polarizer and analyzer. The same applies to Plexiglas culture chambers if the chamber bottom is made of plastic. In such cases, it is recommended to use chambers with glass bottoms to avoid the loss of optical performance.



- 1 Polarizer D (fixed, optional: rotary)
- 2 Condenser
- 3 DIC slider
- 4 Analyzer slider, fixed
- 5 Analyzer slider $\pm 30^\circ$

Fig. 3-20 Components for the transmitted-light DIC technique on the Axiovert 200

3.3.4 Setting of VAREL contrast in transmitted light

3.3.4.1 General principle

VAREL contrast provides a relief-like image of objects and can be used as an alternative to phase contrast. VAREL contrast can also be used on curved surfaces, e.g. 96-well microtiter plates, for which no contrast can be achieved with the phase contrast technique (no flush ring positioning!).

3.3.4.2 Configuration of the Axiovert 200 (manual) and Axiovert 200 M

- Condenser with turret equipped with VAREL stops.
- Objectives which are suitable for VAREL contrast, e.g. A-Plan Ph1Var1 or LD A-Plan Ph2Var2.

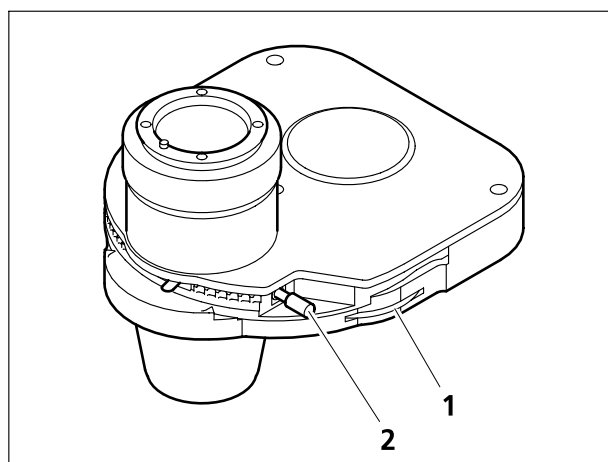


Fig. 3-21 Setting of VAREL contrast

3.3.4.3 Setting of VAREL on the Axiovert 200 (manual) and Axiovert 200 M

- Open the aperture diaphragm (3-21/1) completely.
- Swing VAREL stop on the turret of the condenser into the beam path (**Var** position).
- Swing in the required VAREL objective via the nosepiece.
- Use the setting screw (3-21/2) to move the Varel diaphragm opening until optimum VAREL contrast is achieved (relief-like impression).



Microtiter plates:

Select the opposite VAREL ring of the diaphragm for illumination at the edge of the cavity; in the center of the cavity, the right or left VAREL ring can be used.

The specimen field with the relevant pupil image of the VAREL diaphragm is shown in Fig. 3-22. In the pupil image, the diaphragm appears rotated by 180°.

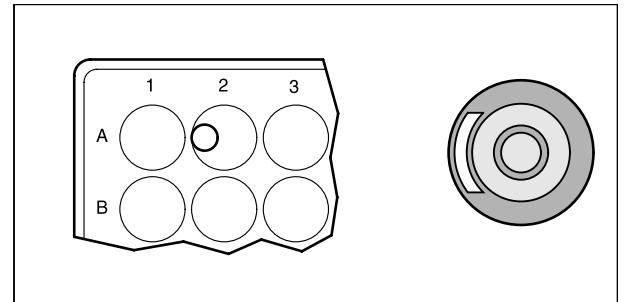


Fig. 3-22 VAREL contrast with microtiter plates



- 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.

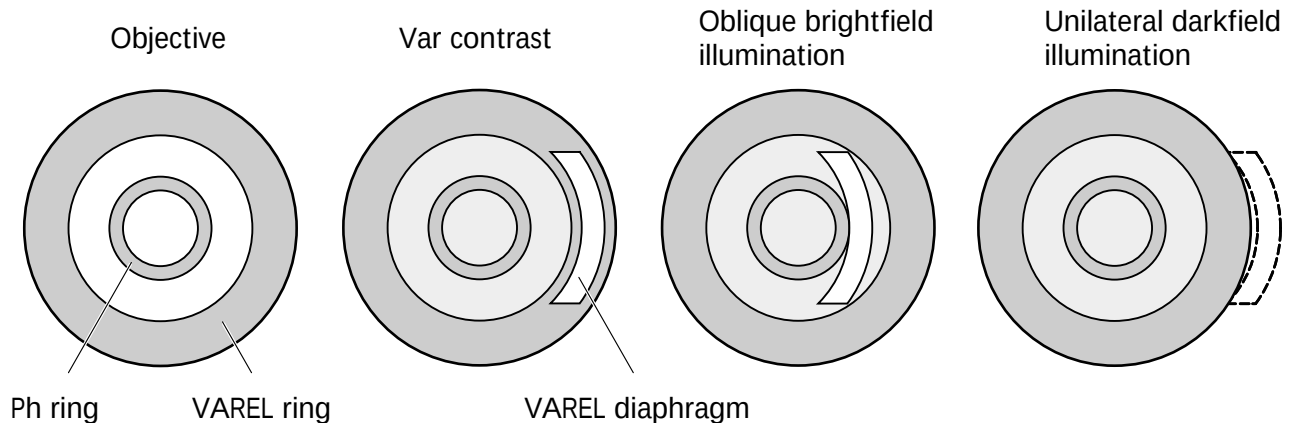


Fig. 3-23 Pupil images in VAREL contrast

3.3.5 Setting of fluorescence contrast in reflected light

3.3.5.1 General principle

The epi-fluorescence technique permits high-contrast images of fluorescent substances in typical fluorescence colors. In the epi-fluorescence microscope, the light generated by a high-performance illuminator reaches the excitation filter via a heat-reflecting filter. The filtered, short-wave excitation emission is reflected from a dichroic beam splitter and is focused on the specimen via the objective. The specimen absorbs the short-wave emission and then emits long wave fluorescence (STOKE's law) which is now gathered by the objective and transmitted by the dichroic beam splitter. Finally, the beams pass a barrier filter which only allows the long-wave emission from the specimen to be transmitted.

Excitation and barrier filters, which are positioned in the FL reflector module together with the appropriate dichroic beam splitter, must be perfectly matched.

3.3.5.2 Configuration of the Axiovert 200 (manual) and Axiovert 200 M

- Recommended objectives: brightfield objectives
- FL reflector module in the reflector turret
- N HBO 103 fluorescence illuminator or HBO 50 for reflected-light illumination
- HAL 100 halogen illuminator for transmitted-light illumination

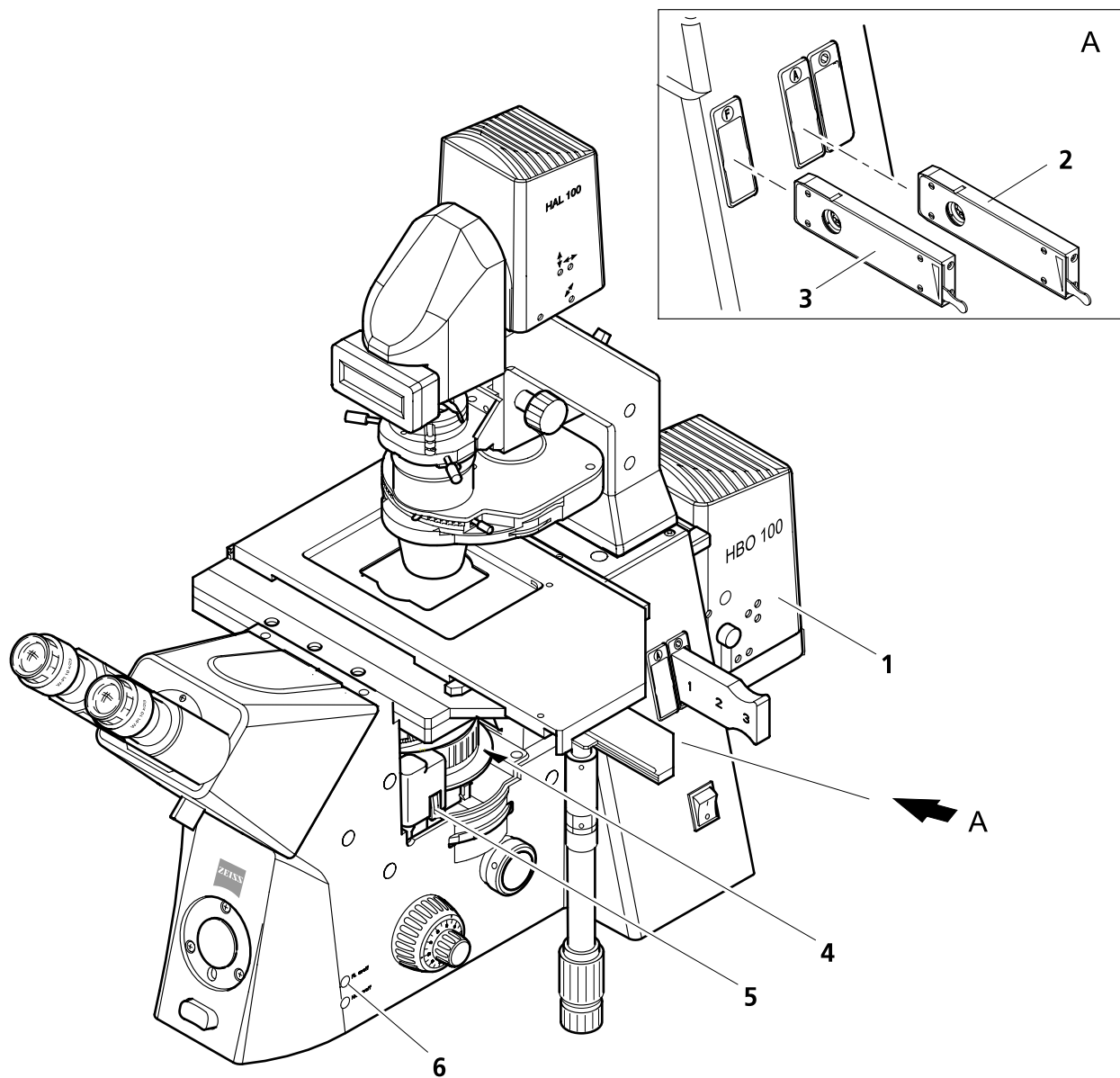


Before the epi-fluorescence technique is applied, it is absolutely necessary to adjust the mercury vapor short-arc lamp in accordance with sections 2.12.2 through 2.12.4 by using the adjusting aid. If required, re-adjustment must be performed depending on the operation time.

3.3.5.3 Setting of epi-fluorescence on the Axiovert 200 (manual) and Axiovert 200 M

The first epi-fluorescence setting is considerably facilitated if the Plan-Neofluar objective 20x/0.50 and a specimen featuring pronounced fluorescence are used. It is also possible to use demonstration specimens first.

- Switch on the HAL 100.
- Swing in suitable objective, e.g. Plan-Neofluar 20x/0.50, via the nosepiece (3-24/4).
- Move condenser turret to position H, transmitted-light brightfield (or also phase contrast), and then move to the specimen area to be examined.
- Use focusing drive for focusing.
- Keep light path in the reflected-light part blocked at first using the fluorescence shutter by pressing the FL on / off key (3-24/6).



- 1 HBO 103 fluorescence illuminator
- 2 Iris stop slider (aperture diaphragm)
- 3 Iris stop slider (luminous-field diaphragm)
- 4 Nosepiece
- 5 Reflector turret
- 6 FL on / off key

Fig. 3-24 Components for epi-fluorescence on the Axiovert 200

- Switch on the NHBO 103 fluorescence illuminator (3-24/1) via the power supply and allow it to heat to operating temperature for approx. 15 minutes.
- Select the FL reflector module in the reflector turret containing the required fluorescence filter combination (depending on the excitation) and switch it on.
- Press the **FL on/off** key (3-24/6) to open the fluorescence shutter. The opening of the shutter is confirmed by a beep.

For the epi-fluorescence technique, one slider each with iris stop is used as aperture and luminous-field diaphragm. However, since the aperture diaphragm is not visible in reflected light, the aperture diaphragm slider must first be centered in the compartment of the luminous-field diaphragm and then be inserted in the compartment for the aperture diaphragm.

The centering for the aperture diaphragm and the luminous-field diaphragm is performed in the same way:

- Insert an iris stop slider into the compartment (3-24/3) for the luminous-field diaphragm until stop.
- Use the lever to close the diaphragm until it becomes visible in the field of view.
- Then center the diaphragm using the two adjusting screws in the slider (with SW 3 mm ball-headed screwdriver). Open the diaphragm until the entire field is uncovered.
- Push the slider centered in this way into the compartment for the aperture diaphragm (3-24/2) until stop.
- Insert an additional iris stop slider into the compartment (3-24/3) for the luminous-field diaphragm.
- Close the luminous-field diaphragm until it becomes visible in the field of view.
- Use the two centering screws to center the luminous-field diaphragm in the edge of the field of view.
- Either open the luminous-field diaphragm until it just disappears behind the edge of the field of view or, if there is a risk of specimen bleaching, reduce it until it is visible in the field of view.
- Finally, refocus on the specimen and optimize the collector position of the fluorescence illuminator. For this purpose, use the knurled knob on the fluorescence illuminator to set the collector in such a way that illumination of the field of view provided by the short-wave excitation reflector module is as homogeneous as possible. Correction of the collector position is not required when modules with long-wave excitation are used.

3.4 Documentation

Depending on the instrument configuration, the Axiovert 200 (manual) and Axiovert 200 M microscopes can be equipped with up to five documentation ports.

- Frontport for the connection of a SLR camera, a video or digital camera (e.g. AxioCam from Zeiss) via a special video or camera adapter.
- Sideport (right or left) for the connection of documentation equipment via the 60 mm interface for LSM and FCS connections please see page 1-9).
- Baseport (bottom) for the connection of documentation equipment via the 60 mm interface.
- Binocular phototube with 60 mm interface.

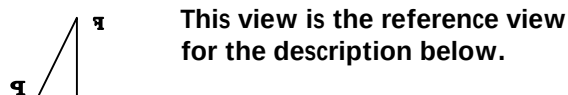
3.4.1 Image orientation of camera ports

The following table gives a detailed overview of the image orientation on the Axiovert 200 camera ports.

The descriptions are based on an object, such as a stage micrometer, with readable figures or letters to illustrate the orientation:



As shown above, the object is placed on the microscope stage with the readable side facing the objective and is then seen from top by the microscope user as follows:



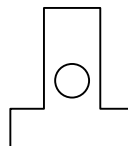
Viewed from top, too, a movement of the specimen stage in Y direction to the back (thick arrow) and in X direction (thin arrow) appears like this:



For a given viewing direction related to the respective stand component, the table below provides the following information:

- Intermediate image of the object and the image on the monitor as captured by the camera as well as
- direction of motion of the object, when moving the stage.

Order No. / Description	Viewed with / Switch position	Intermediate / image / Monitor	Direction of motion of object	Viewing direction, schematic
000000-1005-827 Binocular tube	Eyepieces (100% vis)			
000000-1005-828 Binocular phototube	100% vis : 0% doc 50% vis : 50% doc 0% vis : 100% doc	 	 	
000000-1005-829 Binocular ergotube	Eyepieces (100% vis)			
000000-1005-815 Sideport 60, left, 2 switching positions	20% vis : 80% doc			
000000-1005-816 Sideport 60, left, 3 switching positions	50% vis : 50% L 0% vis : 100% L			
000000-1005-817 Sideport 60, right, 3 switching positions	50% vis : 50% R 0% vis : 100% R			
000000-1005-818 Sideport 60, left and right, 3 switching positions	0% vis : 100% L 20% vis : 80% R	 	 	
000000-1069-228 Beam path switching	100% Frontport			
000000-1069-229 Beam path switching mot	100% Frontport			

Order No. / Description	Viewed with / Switch position	Intermediate / image / Monitor	Direction of motion of object	Viewing direction, schematic
000000-1005-824 Baseport	100% doc			

Camera adapters working without intermediate imaging do not change the orientation of the image. This also applies to the Frontport with adapters V200 T2 2.5x for SLR used on Frontport 000000-1005-869 and video adapter V200 C 2/3" 0.63x used on Frontport 000000-1071-171.

The use of Optovars 000000-1005 and 000000-1005-856, too, does not change image orientation.

The above description also applies to any other specimen.

3.4.2 Photomicrography with SLR camera

The Axiovert 200 (manual) and Axiovert 200 M microscopes permit the beam path to be switched to 100% vis (visual observation through eyepieces and an open position for activation of the Sideport to the right or left), 100% Frontport (port pointing to the front for photomicrography) or 100% Baseport (port pointing downwards).

Since this is a 100% switching position, simultaneous visual observation during photography is not possible.

The 2.5x camera adapter and the camera-specific T2 adapter permit commercially available 35 mm SLR cameras to be connected to the microscope.

- Remove dust cover or camera lens from the camera housing (3-25 /1).
- Attach appropriate T2 adapter (3-25/2) to the camera housing.
- Remove dust cover from the 2.5x camera adapter (3-25/3).
- Screw 2.5x camera adapter into the T2 adapter via the thread.
- Remove dust cover from the camera port.

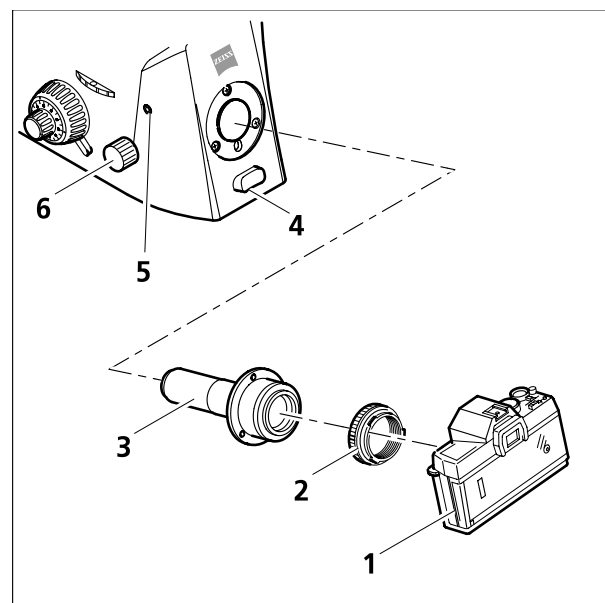


Fig. 3-25 Connection of a SLR camera

- Attach the premounted camera system to the Frontport, align it horizontally and tighten clamping screw (3-25/5) using the SW 3 ball-headed screwdriver.
- Switch on the microscope and, when using a color film (artificial light), set the toggle switch for illumination intensity (3-25/4) to 10.5 V (corresponds to the color temperature of 3200 K). The voltage value can be directly read from the LCD display. To make this setting easier for you, the increase of the lamp voltage stops at 10.5 V when the toggle switch is pressed down completely, and a beep is emitted.
Should the illumination intensity be too high for the specimen, an attenuation filter must be used.

- Select the object area to be photographed via the binocular tube.



To determine the image section and for exact focusing of the image, the adjusting eyepiece should be equipped with the dia. 26 MC 10x photo reticle (see section 2.2.2).

- After switching the beam path (3-25/6) from visual observation to the Frontport, 100 % of the light is now available for the camera.
- To operate the camera system, please observe the manufacturer's manual.



The magnification on the film is the product of objective magnification, Optovar magnification and the factor 2.5 of the camera adapter.

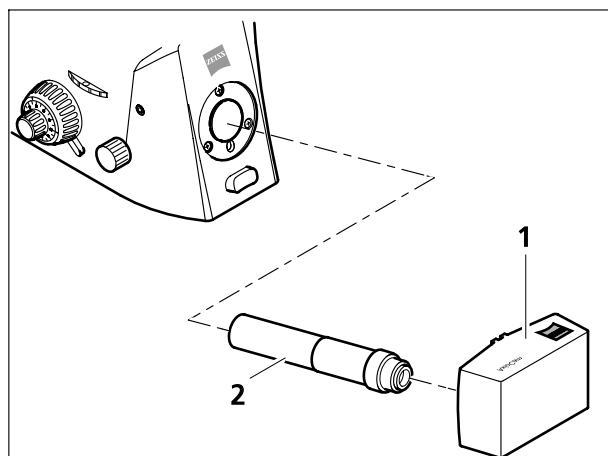


Fig. 3-26 Video attachment

3.4.3 Photomicrography using a digital camera and videomicroscopy

- The Axiovert 200 and Axiovert 200 M permit the connection of one video camera or one digital camera (e.g. AxioCam digital camera from Zeiss, (3-26/1) to the Frontport via the 0.63/C-mount video adapter (3-26/2).
- Attachment of the video adapter is performed in the same way as the camera adapter (described in section 3.4.2).

CARE, MAINTENANCE, TROUBLESHOOTING AND SERVICE

Contents

4	CARE, MAINTENANCE, TROUBLESHOOTING AND SERVICE.....	4-3
4.1	Care	4-3
4.2	Maintenance	4-4
4.2.1	Performing checks	4-4
4.2.2	Changing the fuses on the microscope	4-4
4.2.3	Changing the fuses on the ebq 100 dc power supply	4-5
4.3	Service.....	4-6

List of illustrations

Fig. 4-1	Changing the fuses.....	4-4
Fig. 4-2	Changing the fuses on the power supply.....	4-5

4 CARE, MAINTENANCE, TROUBLESHOOTING AND SERVICE

4.1 Care

Care of the instruments is limited to the following operations:

- Cover the instrument with the dust cover after every use.
- Do not install instrument in a damp room.
- Cover open tubes with dust protection caps.
- Remove dust and dirt from visible optical surfaces using a brush, airblower, Q-tips, optics cleaning paper or a cotton cloth.
- Remove water-soluble dirt (coffee, Coke, etc.) after breathing on it and wiping it off with a dust-free cotton cloth or a moistened cloth. A mild cleaning agent can also be added to the water.
- Remove stubborn, oily or greasy dirt (immersion oils, finger prints) with a wad of cotton or a dust-free cotton cloth dipped in the optics cleaning mixture L.

This cleaning mixture is produced of 90 Vol% benzoline and 10 Vol% isopropanol (IPA). The various components are also known under the following synonyms:

benzoline: medical alcohol, petroleum ether

Isopropanol: 2-propanol, dimethyl carbinol, 2-hydroxypropane

Clean the optical surface by moving in circles starting in the middle. Slight pressure should be exerted on the optics during cleaning.

When using the Axiovert 200 in humid climatic zones, proceed as follows:

- Store the microscope in bright, dry and well ventilated rooms with a humidity of less than 65%; store particularly sensitive components and accessories, such as objectives and eyepieces, in a dry closet.
- When the equipment is stored in closed cases for a longer period of time, the growth of fungus can be avoided by including cloths soaked in fungicide in the cases.



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

- relative humidity of more than 75% and temperatures between +15° C and +35° C for more than three days.
- installation in dark rooms without air ventilation, and
- dust deposits and fingerprints on optical surfaces.

4.2 Maintenance

4.2.1 Performing checks

The following checks should be performed regularly every six months.

General

- Check line cables and plugs for defects.
- If any damage is visible, switch off the instrument. Have defects repaired by Zeiss service staff.
- Make sure that the maximum operation time of the halogen and HBO / XBO lamps is not exceeded (once a week).

Illumination

- Check the correct setting of the halogen and HBO / XBO lamps.
- Check contacts of the electrical connection points of the illuminators.

Optics

- Visual checking of cleanliness of objectives and eyepieces

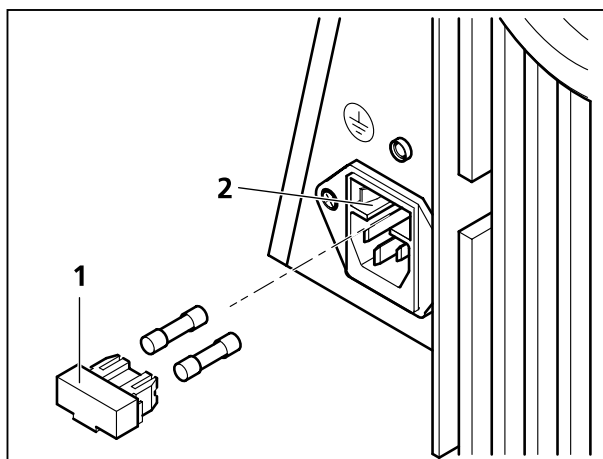


Fig. 4-1 Changing the fuses

4.2.2 Changing the fuses on the microscope

The fuses for the instrument input voltage are positioned on the rear of the microscope stand. The fuse compartment, which contains two **T4.0 A/H 250 V 5x20 mm** fuses, is combined with the instrument socket.

If fuses blow, the cause must be found out first and any technical defect must be efficiently eliminated.



Always pull the power plug before changing the fuses.

- Remove fuses from the holder and insert new fuses.
- Insert fuse holder back in the fuse compartment (4-1/2) until stop. Plug in power cords.

4.2.3 Changing the fuses on the ebq 100 dc power supply

The fuse holder for the fuses F1 and F2 is positioned on the rear of the power supply. It is combined with the instrument socket and contains two **T 3, 15A** fuses.

If fuses blow, the cause must be found out first and any technical defect must be efficiently eliminated.



Always pull the power plug before changing the fuses.

- Disconnect the instrument from the line.
- Pull fuse holder (4-2/1) out of the fuse compartment (4-2/2) in forward direction.
- Replace defective fuse.
- Then push fuse holder back into the fuse compartment until click-stop.
- Plug in power cords.

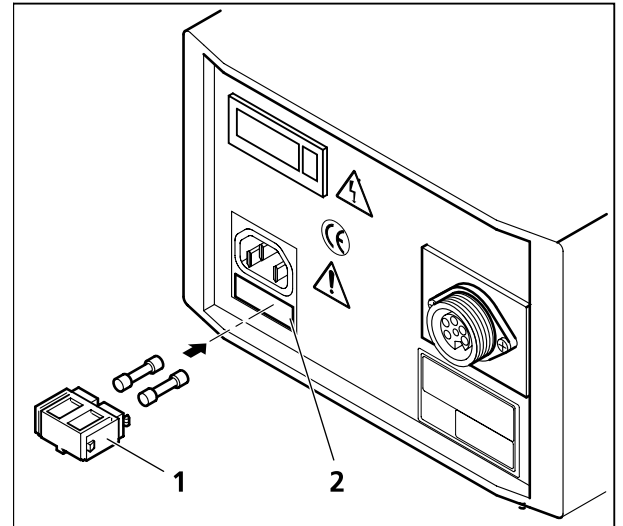


Fig. 4-2 Changing the fuses on the power supply

4.3 Service

All repairs of optical or electronic components inside the instrument or any repair of the power supply may only be performed by service staff or specially **authorized** personnel.

If service is required, please get in touch with your local agency or with

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Telefax: ++49 551 5060 464
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Annex:

List of abbreviations	A-3
List of key words.....	A-5
Certification in accordance with DIN ISO 9001 / EN 46001	
EC Declaration of Conformity	

List of abbreviations

a	Free working distance
AC	Alternating Current
A-Plan	Achromatic objectives featuring improved image flatness (ICS line)
Br.	suitable for eyeglass wearers
CAN bus	Controller Area Network Communication Bus
CSA	Canadian Standards Association
DC	Direct Current
D	transmitted light / cover slip thickness
d	diameter:
DIC:	Differential Interference Contrast
DIN	German standards association
doc	documentation
EG	European Community
EN	European standards
EMV	electromagnetic compatibility
EWG	European Economic Community
foc.	focusing
H	brightfield
HAL	halogen illuminator
HBO	mercury vapor short-arc lamp
HD	brightfield / darkfield
ICS	Infinity Color-Corrected System
IEC	International Electrotechnical Commission
IP	International Protection (protection class)
ISO	International Organization for Standardization
LCD	Liquid Crystal Display
LD	Long Distance
M, mot.	motoriziert
N. A.	numerical aperture

Ph	phase contrast
PL	flatfield
SB	Sub-Bus
SLR	Single Lens Reflex
SW	wrench opening
T	slow-blow (fuse type)
TV	Television
UL	Underwriter Laboratories
VIS	visual
XBO	Xenon short-arc lamp

List of key words

	Page
3	
3.200K	3-6, 3-17
A	
adjusting aid	3-13
ametropia	2-7
analyzer	3-7
aperture diaphragm	3-32, 3-42
attachment of tube	2-6
B	
Baseport	3-12
basic settings	3-14, 3-16
beam splitter	2-16
binocular component	3-9
binocular tubes	3-8
brightfield	3-28
brightness control of halogen illuminator	3-6
C	
care	4-3
changing fuses	4-4, 4-5
color photography	3-6, 3-17
condenser	2-13, 3-10, 3-11
condenser, mot.	3-20
configuration	3-23
contrasting technique	3-28
D	
DIC prisms	2-14
DIC differential interference contrast	3-35
diffusion disk	2-22
documentation	3-43
E	
Equipotential bonding terminals	2-19
eyepiece reticle	2-7
eyepieces	2-6, 2-7, 3-9
F	
FCT	3-21
filter changer	3-11
filter slider	3-13
FL key on/off	3-13, 3-22
FL reflector module	2-16

F

fluorescence contrast	3-40
fluorescence illuminator	3-42
fluorescence shutter	3-15
FOCUS	3-18
focus limit	3-26
focusing drive	3-6, 3-17
Frontport	3-11

H

HAL on/off	3-17
------------------	------

I

Illuminator N HBO 103	2-23
image orientation	3-43
installation	2-5
iris stop slider	3-13

K

key HAL on/off	3-6
KÖHLER	3-11, 3-13

L

LCD display	3-9, 3-19
LEFT/RIGHT	3-21
Light Manager	3-15, 3-27
line connection	2-18
LOAD	3-18
luminous field diaphragm, transmitted light	3-11
luminous-field diaphragm	3-42

M

maintenance	4-4
microscope stage	2-11, 3-11
mounting frame	2-11

N

nosepiece	2-9, 3-7, 3-18, 3-25
-----------------	----------------------

O

object guide	2-11
OBJECTIVE	3-18
Objectives	2-9
On / Off switch	3-6, 3-17
OPERATION	3-3
Operation and function controls	3-4, 3-16
OPTOVAR	3-20
Optovar turret	3-7

P

parfocality	3-25
performing checks	4-4
phase contrast	3-33
photomicrography	3-45, 3-46
polarizer	3-11

R

reflected light:	3-40
REFLECTOR	3-22
reflector turret	2-15, 3-13, 3-20

S

Service	4-6
Set LM	3-11, 3-21
Set mode	3-14, 3-23
Sideport left	3-12
Sideport right	3-12
specimen holder	3-11
specimen stage	2-11
Start-up	2-5
stop for focusing drive	3-6
switch on / off	2-19

T

toggle switch	3-6
transmitted light	3-28, 3-32, 3-33, 3-35, 3-38
transmitted-light illumination	2-8
Tube	2-6

U

unpacking	2-5
-----------------	-----

V

VAREL contrast	3-38
vertical adjustment of condenser	3-11
videomicroscopy	3-46

W

WORK	3-18
------------	------

X

XY-adjustment	3-7
---------------------	-----

Z

Z focus factors	3-25
ZERO	3-18

