Operating Manual Axio Lab.A1 Knowledge of this manual is essential for the operation of the instrument. Please familiarize yourself with the contents of this manual and pay special attention to instructions concerning safe operation of the instrument.

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

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1 INTRODUCTION

1.1 Notes on instrument safety

Axio Lab.A1 microscopes have been designed, produced and tested in compliance with DIN EN 61010-1 (IEC 61010-1) and IEC 61010-2-101 safety requirements for electrical measuring, control and laboratory instruments.

These instruments meet the requirements of EC Directive IVDD 98/79/EC (In Vitro Diagnostic); they carry the CE mark.

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

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



CAUTION

This symbol indicates a potential hazard to the user.



CAUTION *Hot surface!*



CAUTION

UV radiation emitted!



CAUTION

Disconnect the instrument from the power supply before opening!



CAUTION

This symbol indicates a potential hazard to the instrument or system.



NOTE

This symbol denotes a warning to which special attention must be paid.

Axio Lab.A1 microscopes, including original accessories, may only be used for the microscopy methods described in this manual.

The following instructions must be carefully observed:



The manufacturer is unable to assume any liability for any other use, including individual modules and individual components. This also applies to all service and repair work not performed by authorized service personnel. Furthermore, all warranty rights will be forfeited.



The socket into which the power cable is plugged must be earthed. The grounding effect must not be made ineffective by an extension cable that does not have a protective ground wire.



Should it be determined that protective measures are no longer effective, the instrument must be taken out of service and secured against unintended use. Please contact a Zeiss service agency or the Carl Zeiss Microscopy Service to repair the instrument.



Axio Lab.A1 microscopes are equipped with a power supply unit incorporated in the stand, which allows line voltages ranging from 100 V to 240 V \pm 10 % (50/60 Hz) to be used without requiring any change of voltage on the unit.



Always pull the mains plug before opening the instrument or replacing a fuse!

Only fuses for the rated power may be used. The use of provisional fuses or short-circuiting the fuse-holder is prohibited.



Axio Lab.A1 microscopes are not equipped with any special devices for protection from substances that are corrosive, potentially infectious, toxic, radioactive, or other substances that could be hazardous to health. Make sure to observe all legal regulations, particularly the relevant national accident prevention regulations when handling such substances.



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



Obstruction or covering of ventilation slits may result in heat build-up which may damage the instrument and in extreme cases cause fire. Always keep the ventilation slits clear and ensure that no objects enter the instrument through the ventilation slits.



Instruments may only be operated by trained personnel. The latter must be aware of the possible hazards involved with microscopy and the respective application. Axio Lab.A1 microscopes are high-precision instruments whose functional capabilities may be impaired or destroyed when handled improperly.



Instruments should not be operated in an explosive environment. Instruments should only be operated on a solid, non-combustible surface.



LED Risk Group 2 to IEC 62471, LED radiation will be emitted.

Never look into the LED beam of the illuminating device – either with or without optical instruments. Failure to observe this precaution may results in eye injuries!



Combustible and easily inflammable materials should not be held close to the light beam.

R

Be sure to read the safety data sheets for the Immersol 518 $N^{\mbox{\scriptsize B}},$ Immersol 518 $F^{\mbox{\scriptsize B}}$ and Immersol $W^{\mbox{\scriptsize B}}.$



Immersol 518 $N^{\ensuremath{\$}}$ immersion oil irritates the skin. Avoid any contact with skin, eyes and clothing.

In the case of inadvertent skin contact, wash the oil off with plenty of water and soap.

In the case of inadvertent eye contact, immediately rinse the eye with plenty of water for at least five minutes. Seek medical attention if irritation persists.



Proper disposal of immersion oil 518 $N^{\circledast}\!\!\!\!$: do not discharge into the surface water or sewerage system.



internal work instructions.

Do not put defective microscopes into the household waste; they must be disposed of in accordance with WEEE Directive 2002/96/EC. Samples must likewise be properly disposed of in accordance with statutory provisions and

Warning labels on Axio Lab.A1 stands





Warning label: Hot surface!

Affixed to all stands with transmitted light illumination.

Fig. 1-1

"RADIATION" and "LED APERTURE" warning labels on Axio Lab.A1 for transmitted and reflected light fluorescence



Fig. 1-2 "Hot surface below" warning label on Axio Lab.A1 for reflected light

1.2 Notes on ergonomics of the microscope

The Axio Lab.A1 light microscope was developed and designed in cooperation with occupational health physicians and the TÜV Rhineland to meet the most exacting demands with regard to ergonomics at the microscope workstation. This first light microscope worldwide is available with a special ergonomic configuration and bears TÜV certificate ID:0000025994 "Ergonomically tested".

Particularly laboratory microscopes in the Axio Lab.A1 device class are used continuously for many routine applications (e.g. haematological, histological and cytological examinations) over a period of several hours. With light microscopes which have not been ergonomically designed, such regular, extended periods of use could cause health problems in terms of posture control of the user. This health risk can be significantly reduced by careful ergonomic design and arrangement of the control elements, well-conceived and individual eyepiece adjustment options, together with correct set-up of the entire microscope workstation.

This results in improved working conditions, greater comfort of employees and higher work productivity. More and more countries are also introducing stringent workplace regulations for microscope workstations, particularly in the medical field. In addition, the regulations of employers' liability insurance associations increasingly place obligations on employers to provide ergonomic workplaces and microscopes.

TÜV certificate ID:000025994 "Ergonomically tested" prescribes the distance of control elements from the table, user and between instruments. Furthermore, it defines a broad adjustment range for the eyepiece to cater for the different body heights of male and female microscope users worldwide. For this purpose the ergonomic tube or ergo tube must be vertically and angle-adjustable. This enables it to be adjusted to suit different body heights (static ergonomics) and occasionally varied by the user during extended periods of use (dynamic ergonomics). The following workplace standards apply as a basis for this TÜV certificate of ergonomics:

_	DIN 58959-4:	Quality management in medical microbiology – Part 4:
		Requirements for investigations using light microscopes
_	DIN EN 1335-1:	Office work chairs – Part 1: Dimensions – Determination of dimensions
_	DIN EN 12464-1:	Lighting of work places – Part 1: Indoor work places
_	DIN EN 12665:	Basic terms and criteria for specifying lighting requirements
_	DIN EN 13150:	Workbenches for laboratories – Safety requirements and test methods
_	DIN EN ISO 15189:	Medical laboratories – Particular requirements for quality and competence
_	DIN EN ISO 9241-11:	Ergonomic requirements for office work with visual display terminals –
		Guidance on usability
_	DIN EN ISO 60601-1-6:	Medical-electrical equipment – General requirements for basic safety and essential performance

and the following ergonomic standards:

- DIN 33402-2: Ergonomics Human body dimensions Part 2: Values
- DIN 33406: Workplace dimensions in production
- DIN 33408: Body templates for seats
- DIN 33411: Human physical strength
- DIN 68877: Swiveling work chair Safety requirements, testing
- DIN EN 614-1: Ergonomic design principles Part 1: Terms and general principles

_	DIN EN 894-1:	Ergonomic requirements for the design of displays and control actuators – Part 1: General principles for human interaction with displays and control actuators
_	DIN EN 894-3:	Ergonomic requirements for the design of displays and control actuators – Part 3: Control actuators
_	DIN EN 62079:	Preparation of instructions – Structuring, content and presentation
_	DIN EN ISO 7250:	Basic human body measurements for technological design
-	DIN EN ISO 14738:	Safety of machinery – Anthropometric requirements for the design of workstations at machinery
_	ISO 11226:	Ergonomics – Evaluation of static working postures
_	SEMI S8-0307:	Safety guidelines for ergonomics engineering of semiconductor manufacturing equipment

For a further explanation of TÜV certificate of ergonomics and basic ergonomic setting and operation of the Axio Lab.A1 microscope please refer to Section 3.5.

1.3 Notes on warranty

The manufacturer guarantees that the instrument has no material or production defects when delivered. Any defects occurring must be reported without delay; every effort must be made to minimize damage. Once a defect has been notified, the manufacturer shall have the right to choose whether to repair the instrument or deliver a replacement in perfect condition. No warranty is provided for normal attrition (in particular of wearing parts) or improper use.

The instrument manufacturer shall not be liable for damage caused by faulty operation, negligence or any other tampering with the instrument, particularly the removal or replacement of instrument components, or the use of accessories from other manufacturers. Such actions will render any warranty claims invalid.

With the exception of the work specified in this manual, no maintenance or repair of microscopes may be carried out. Repairs are only to be performed by Carl Zeiss service or personnel specifically authorized by Carl Zeiss. Should a malfunction of the instrument occur, please contact the Carl Zeiss Microscopy Service in Germany (see page 112) or the responsible Carl Zeiss representative abroad.

DESCRIPTION OF THE INSTRUMENT 2

2.1 Intended use

Axio Lab.A1 microscopes were designed as all-purpose microscopes for biological and medical applications, as well as material analyses.

Depending on the microscope stand selected, they may also be used as true transmitted or reflected light microscopes or as combined transmitted/reflected light fluorescence microscopes.

Typical biomedical applications of Axio Lab.A1 microscopes include:

- Medical examinations in laboratories, clinics and medical practices
- Science and research (colleges, universities) in the fields of medicine and biology
- Industrial applications (pharmacology, food technology)
- Analysis of human blood and tissue samples.

Typical material analysis applications of Axio Lab.A1 microscopes include:

- Metallographic laboratories
- Automotive industry
- Microsystems engineering
- Geoscientific institutes
- Exploration industry

Depending on the instrument configuration, the following microscopy and contrasting techniques are possible:

Transmitted light

- Brightfield (H)
- Darkfield (D)
- Phase contrast (Ph)
- Polarization (Pol)
- Polarization (conoscopy)

- **Reflected light**
 - Brightfield (H)

 - Polarization (Pol)
 - Fluorescence (FL)
 - Differential Interference Contrast (DIC)

Polarization (C-Pol)

The binocular photo tubes and suitable adapters permit one microscope camera, one reflex camera or one digital/video camera to be attached for documentation purposes.

The Axio Lab.A1 was specially developed and designed for ergonomic use in lengthy routine applications, e.g. haematological, histological and cytological laboratory examinations.

Darkfield (D)

The ergonomic design elements of the Axio Lab.1 are:

- Vertically adjustable, swivel-type and swivel/vertically adjustable ergo tubes
- Skin-friendly surfaces on binocular section of the tubes, control elements and stand
- Ergo table with fixed stage drive
- Vertical and friction adjustable stage drives
- Optional use of fine focusing knobs in standard form or as a jog/shuttle knob
- Special, ergonomic arrangement of the three main control elements: focusing drive, stage drive and brightness control

A TÜV certificate of ergonomics was prepared for the light microscope in cooperation with occupational health physicians and the TÜV Rhineland and granted for the following basic configuration:

- Transmitted light brightfield stand with ergo table and comfortable ergo tube

2.2 System overview





Carl Zeiss

DESCRIPTION OF THE INSTRUMENT System overview











2.3 Technical data

Dimensions (width x depth x height)

Axio Lab.A1 basic microscope stand

without tube (430037-9000-000)approx. 219 mm x 410 mm x 3395 mm The other stand types differ slightly in depth and significantly in height, depending on the tube used. An overview of viewing heights (eyepoint heights) of the various tubes can be found in Section 2.3.1.

An estimate of the height of the stand with the respective tube can be obtained by adding:

- 10 mm to the viewing height in the lower position of binocular section
- for tubes with a fixed viewing angle
- 10 mm to the viewing height of the upper limit for ergo tubes

Weight

weight	
Axio Lab.A1 microscope stand (depending on version and	accessories) approx. 8 to 20 kg
Ambient conditions	
Shipping (in packaging):	
Permissible ambient temperature	-40 to +70 °C
Storage:	
Permissible ambient temperature	+10 to +10 °C
Permissible humidity (without condensation)	
•	
Operation:	
Permissible ambient temperature	
Permissible relative humidity (without condensation)	
Highest permitted altitude of use	
Air pressure	
Degree of pollution	2
Operational specifications	
Operational area	Classed rooms
•	
Protective class	
Protection type	
Electrical safety	acc. to DIN EN 61010-1 (IEC 61010-1)
in conformance with CSA and UL regulations	
Overvoltage category	
RFI suppression	
Noise immunity	
Mains voltage Axio Lab.A1	
	Conversion of mains voltage unnecessary!
Mains frequency	
Power consumption Axio Lab.A1	110 VA
Fuses to IEC 127	
Microscope stand Avia Lab A1	2 v T 2 15 A/U 5v20 mm

Light sources

LED transmitted light
Power consumption max. 3 W
Adjustment of light source 0.5 to 12 V
Halogen lighting transmitted light
Power consumption max. 35 W
Halogen lighting reflected light
Power consumption
Adjustment of light source
LED lighting reflected light fluorescence with replaceable LED modules
Wavelengths optional 615, 625 nm or neutral white (540 - 580 nm)
LED classification LED Risk Group 2 to IEC 62471

Axio Lab.A1:

Stand with manual stage focusing	
Coarse focusing	approx. 4 mm/revolution
Fine focusing	approx. 0.4 mm/revolution; 4 µm scale interval
Lifting range	
Height stop	pre-set at works
Optional condenser 0.9/1.25 H with or without mode	ulator disk for
	Brightfield, darkfield and phase contrast 1, 2, 3
Manual objective change Manual reflector module change	

2.3.1 Viewing height and tube angle

Order No.	Binocular tube	Viewing angle	Adjustment	Viewing height* in mm
425522-9000-000	Binocular tube 30°/20	30°	- none -	434 / 470
425522-9010-000	Binocular photo tube 30°/20 (50:50)	30°	- none -	434 / 470
425522-9020-000	Binocular ergo tube 8-38°/20	8-38°	Angle	407 - 534
425522-9030-000	Binocular ergo photo tube 838°/20 (50:50)	8-38°	Angle	407 - 534
425522-9040-000	Binocular ergonomic tube 8-38°/22	8-33°	Angle Height	412 - 603
425520-9000-000	Binocular tube 30°/23	30°	- none -	449 / 485
425520-9010-000	Bin. photo tube 30°/23 (50:50)	30°	- none -	449 / 485
425520-9020-000	Bin. photo tube 30°/23 (100:100) Bio	30°	- none -	449 / 485
425520-9030-000	Binocular photo tube 20°/23 (100:100)	20°	- none -	442 / 481
425520-9040-000	Bin. ergo tube (100/100), angle- adjustable, upright image	5-30°	Angle	395 - 537
425520-9050-000	Bin. ergo tube 15°/23 (50/50), telescopic, height, upright image	15°	Height telescopic	410 - 509
425520-9090-000	Binocular tube 20°/23	20°		442 / 481
425520-9100-000	Bin. photo tube 20°/23 Pol (100:100)	20°		442 / 481
425511-0000-000	Binocular ergo tube 20°/23, 44 mm height	20°	Height	457 - 574
425512-0000-000	Bin. ergo tube 20°/23 (100/100), reverse image, 44 mm height	20°	Height	457 - 574
425514-0000-000	Bin. ergo tube 20°/23 (100/100), upright image, 44 mm height	20°	Height	457 - 574

* Viewing heights:

Tubes with fixed viewing angle without ergo function:

Binocular part lower/upper e.g. 442 / 481 \rightarrow 442 to 481 mm

Angle- and vertically adjustable ergo tubes: Binocular part lower/upper $\,$ e.g. 457 / 574 $\,\rightarrow\,$ 457 to 574 mm $\,$

All specifications are for an interpupillary distance of 65 mm.

2.3.2 Assignment of dust covers, intermediate plate and base plate

Cat. No.	Binocular tube	Transmitted light 430037-9000-000 430037-9010-000 430037-9030-000	Conoscopy 430037-9040-000	Reflected light 430037-9020-000 430037-9050-000
425522-9000-000	Binocular tube	Small	Small	<u> </u>
	30°/20 Bio			
425522-9010-000	Binocular photo tube	Small	Small	
	30°/20 (50:50)			
425522-9020-000	Binocular ergonomic tube	Small	\land /	
	8-38°/20			
		<u>M*</u>		
425522-9030-000	Binocular photo tube	Medium		
	8-38°/20 (50:50)			
		<u>M*</u>		
425522-9040-000	Binocular ergonomic tube	Medium	Medium	Medium
	8-33°/22	Spacer	Spacer	
		<u>M*</u>	<u>M*</u>	<u>M*</u>
425520-9000-000	Binocular tube	Small	Small	Small
	30°/23 Bio	Spacer	Spacer	
425520-9010-000	Binocular photo tube	Medium	Medium	Medium
	30°/23 (50:50) Bio	Spacer	Spacer	
		М	М	М
425520-9020-000	Binocular photo tube 30°/23	Medium	Medium	Medium
	(100:100) Bio	Spacer	Spacer	
		Μ	М	М
425520-9030-000	Binocular photo tube	Medium	Medium	Medium
	20°/23 (100:100) upright image	Spacer	Spacer	
		Μ	М	М
425520-9040-000	(100/100), angle-adjustable, upright image	Medium	Medium	Medium
		Spacer	Spacer	
		M	Μ	Μ

Cat. No.	Binocular tube	Transmitted light 430037-9000-000 430037-9010-000 430037-9030-000	Conoscopy 430037-9040-000	Reflected light 430037-9020-000 430037-9050-000
425520-9050-000	Binocular ergo tube 15°/23	Medium	Medium	Medium
	(50/50), telescopic, height, upright image	Spacer	Spacer	
		M	M	M
425520-9090-000	Binocular tube	Small	Small	Small
	20°/23 Mat (analog 9030 without camera output)	Spacer	Spacer	
425520-9100-000	Binocular photo tube 20°/23 Pol (100/100)	Medium	Medium	Medium
		Spacer	Spacer	
		М	М	М
425511-0000-000	Binocular ergo tube 20°/23, reverse image, 44 mm height	Medium	Medium	Medium
		Spacer	Spacer	
		Μ	M	Μ
425512-0000-000	Binocular ergo tube 20°/23	Medium	Medium	Medium
	(100/100), 44 mm height	Spacer	Spacer	
		M	M	M
425514-0000-000	Binocular ergonomoic tube 20°/23 (100/100), upright image, 44 mm height	Medium	Medium	Medium
		Spacer	Spacer	
		M	M	Μ

* Included in shipping contents

Explanation of table:

Dust cover	Small: 415510-1901	Medium: 459306-0000-000
Intermediate plate for tubes (423732-9040-000)	Intermediate plate: necessary	unnecessary
Base plate (430037-910-000)	<u>M: Mandatory</u>	M: recommended unnecessary

2.4 Control and functional elements on microscope

2.4.1 Stand models

Five stand models are available in the delivery program:

- 1. Transmitted light stand for biomedical applications in brightfield, darkfield and phase contrast
- 2. Transmitted light stand for biomedical applications in brightfield, darkfield, phase contrast and polarization
- 3. Transmitted and reflected light stand for biomedical applications in brightfield, darkfield, phase contrast, polarization (transmitted light) and fluorescence (reflected light)
- 4. Reflected light stand for material applications in brightfield, darkfield, phase contrast, polarization and C-DIC
- 5. Transmitted light stand for material applications in brightfield, darkfield, phase contrast, polarization, conoscopy

In addition, the delivery program also includes two TÜV certificates, "Ergonomically tested" stand models.

A TÜV certificate of ergonomics was prepared for the light microscope in cooperation with occupational health physicians and the TÜV Rheinland and granted for the following two basic configurations:

- Transmitted light stand with ergo stage and comfortable ergonomic tube
- Reflected light fluorescence stand with ergo stage and comfortable ergo tube

2.4.2 Stand for transmitted light

Key to Fig. 2-1:

- 1 Basic stand
- 2 Stage carrier for mechanical stages
- 3 Light intensity control
- 4 Focusing drive fine adjustment (right side, finger wheel)
- **5** Focusing drive coarse adjustment (right side)
- 6 Drive knob for mechanical stage adjustment in X direction
- 7 Drive knob for mechanical stage adjustment in Y direction
- 8 Drive knob for vertical adjustment of condenser (right side)
- 9 Centering screw for condenser (right side)
- **10** Luminous-field diaphragm
- **11** Condenser with aperture diaphragm (optional with modulator disk)
- 12 Mechanical stage 75x30 (optionally for right- or left-hand operation or with ergo-drive right) with specimen holder
- 13 Nosepiece 5x H
- 14 Slot for 6x20 slider
- 15 Eyepieces
- **16** Binocular section of tube
- **17** Binocular tube/photo tube
- **18** Carrying handle
- **19** Centering screw for condenser (left side)
- 20 Drive knob for vertical adjustment of condenser (left side)
- **21** Focusing drive coarse adjustment (left side)
- 22 Focusing drive fine adjustment (left side)
- 23 ON/OFF switch
- 24 Transmitted light lamp in stand base
- **25** Tool flap/cable holder



Fig. 2-1 Axio Lab.A1, stand transmitted light

2.4.3 Stand for transmitted light polarization

Key to Fig. 2-2:

- Basic stand 1
- 2 Stage carrier for rotary stages (also suitable for mechanical stages)
- 3 Light intensity control
- Focusing drive fine adjustment (right side, finger wheel) Focusing drive coarse adjustment (right side) 4
- 5
- Drive knob for vertical adjustment of condenser (right side) 6
- Centering screw for condenser (right side) 7
- Luminous-field diaphragm 8
- Locking screw for rotary table (arrests rotation) 9
- Condenser with aperture diaphragm (optional with modulator disk) 10
- Rotary stage lock in stage carrier 11
- Rotary stage Pol with specimen guide 12
- Nosepiece 4x H Pol (3 eyes centering, 1 eye fixed) 13
- 14 Slot for 6x20 slider
- 15 Eyepieces
- Binocular section of tube 16
- Binocular tube/photo tube 17
- 18 Carrying handle
- Centering screw for condenser (left side) 19
- Drive knob for vertical adjustment of condenser (left side) 20
- Focusing drive coarse adjustment (left side) 21
- Focusing drive fine adjustment (left side) 22
- 23 ON/OFF switch
- 24 Tool flap/cable holder
- 25 Transmitted light lamp in stand base



Fig. 2-2 Axio Lab.A1, stand transmitted light polarization

2.4.4 Stand for transmitted light and reflected light fluorescence

Key to Fig. 2-3:

- Reflected light intensity control 1
- FL/TL switch (FL reflected light fluorescence; TL transmitted light) 2
- Push-pull rod for switching between LED 1 and LED 2 3
- 4 Cover flap for LED reflected light illumination in top part of stand
- 5 Basic stand
- Nosepiece 5x H FL-LED 6
- Stage carrier for mechanical stages 7
- Transmitted light intensity control 8
- Base plate for microscope stand 9
- 10 Focusing drive – fine adjustment (right side, finger wheel)
- Focusing drive coarse adjustment (right side) 11
- Drive knob for mechanical stage adjustment in X direction 12
- Drive knob for mechanical stage adjustment in Y direction 13
- Drive knob for vertical adjustment of condenser (right side) 14
- Centering screw for condenser (right side) 15
- Luminous-field diaphragm 16
- Condenser with aperture diaphragm (optional with modulator disk) 17
- Slot for 6x20 slider 18
- Mechanical stage 75x30 (optionally for right- or left-hand operation or with ergo-drive right) with specimen holder 19
- 20 Reflector turret 4x
- Eyepieces 21
- Binocular section of tube 22
- 23 Binocular comfortable ergo tube
- 24 Centering screw for condenser (left side)
- Drive knob for vertical adjustment of condenser (left side) 25
- Focusing drive coarse adjustment (left side) Focusing drive fine adjustment (left side) 26
- 27
- 28 ON/OFF switch
- Tool flap/cable holder 29
- 30 Transmitted light illumination in stand base



Fig. 2-3 Axio Lab.A1, stand transmitted and reflected light fluorescence

2.4.5 Stand for reflected light

Key to Fig. 2-4:

- **1** Reflected light illumination
- 2 Luminous-field diaphragm (centered)
- **3** Aperture diaphragm (centered)
- 4 Basic stand
- 5 Nosepiece 5x H HD
- **6** Stage carrier for mechanical stages
- 7 Light intensity control
- 8 Focusing drive fine adjustment (right side, finger wheel)
- **9** Focusing drive coarse adjustment (right side)
- **10** Drive knob for mechanical stage adjustment in X direction
- **11** Drive knob for mechanical stage adjustment in Y direction
- **12** Mechanical stage 75x30 A with specimen holder A for reflected light
- **13** Slot for 6x20 slider
- **14** Reflector turret 4x
- 15 Eyepieces
- **16** Binocular section of tube
- **17** Binocular tube/photo tube
- **18** Focusing drive coarse adjustment (left side)
- **19** Focusing drive fine adjustment (left side)
- 20 ON/OFF switch
- 21 Filter slide reflected light
- 22 Tool flap/cable holder



Fig. 2-4 Axio Lab.A1, stand reflected light

2.4.6 Stand for transmitted light conoscopy

Key to Fig. 2-5:

- **1** Rotary knob **A**: Swiveling analyzer in/out
- 2 Rotary knob BL: Swiveling Bertrand lens in/out
- 3 Basic stand
- 4 Nosepiece 4x H Pol (3 eyes centering, 1 eye fixed)
- 5 Stage carrier for rotary stages (also suitable for mechanical stages)
- 6 Light intensity control
- 7 Focusing drive fine adjustment (right side)
- 8 Focusing drive coarse adjustment (right side)
- 9 Drive knob for vertical adjustment of condenser (right side)
- **10** Centering screw for condenser (right side)
- **11** Luminous-field diaphragm
- **12** Locking screw for rotary stage (arrests rotation)
- **13** Condenser with aperture diaphragm (optional with modulator disk)
- **14** Rotary stage Pol with specimen holder
- **15** Slot for 6x20 slider
- **16** Setting wheel for direction of oscillation of analyzer
- 17 Setting wheel for focusing of Bertrand lens
- **18** Eyepieces
- **19** Binocular section of tube
- **20** Binocular tube/photo tube
- **21** Centering screw for condenser (left side)
- 22 Drive knob for vertical adjustment of condenser (left side)
- **23** Focusing drive coarse adjustment (left side)
- 24 Focusing drive fine adjustment (left side)
- 25 ON/OFF switch
- 26 Carrying handle
- 27 Storage compartments for two 6x20 slides
- **28** Transmitted light illumination
- **29** Tool flap/cable holder



CAUTION

The movements of rotary knobs **A** and **BL** (Fig. 2-5/1 and 2) and the respective setting wheels (Fig. 2-5/16 and 17) are coupled with one another. Only **one** control element should therefore be operated at a time and the movement of the other should not be inhibited or blocked. Mechanical damage may otherwise occur.



Fig. 2-5 Axio Lab.A1, stand transmitted light conoscopy

2.4.7 Ergo stands with TÜV certificate "Ergonomically tested"

Key to Fig. 2-6:

- **1** Binocular ergo tube 8-33°, 50 mm vertically adjustable
- 2 Mechanical stage 75x30 ergonomic with stationary drive
- **3** Stand for transmitted light and reflected light fluorescence



- Fig. 2-6 Axio Lab.A1 ergonomic stand with TÜV certificate "Ergonomically tested"
- Further information on the ergonomically correct setting of the microscope and its ergonomic operation can be found in Section 3.5.
2.5 Control and functional elements on optional components

2.5.1 Tubes/photo tubes

The appropriate adapters for reflex cameras, microscope cameras and video cameras may be plugged into the camera port (Fig. 2-7/1 or Fig. 2-8/1) of the binocular photo tubes.

Binocular photo tube 30°/20 with fixed graduation 50:50

50% of the light is directed to the eyepieces and camera port respectively (Fig. 2-7).



Fig. 2-7 Binocular photo tube 30°/20 with fixed graduation 50:50

Binocular photo tube 30°/23 (100:0/0:100)

The light can be directed using a shift knob to either the eyepieces or the mounted camera.

- Shift knob (Fig. 2-8/2) to front (eye symbol):
 100 % light to eyepieces.
- Shift knob (Fig. 2-8/2) to the rear (camera symbol):
 100 % light to camera
- Push-pull rod (Fig. 2-8/3) pushed in: Eyepiece shutter closed.
- Pull-push rod (Fig. 2-8/3) pulled out: Eyepiece shutter opened.
- Particularly for camera shots with extended exposure times it is recommended that possible residual light incidence through the eyepiece is prevented either by means of a tube shutter or eyepiece cover (included in dustguard set). If neither is available, remove the eyepiece and insert the supplied dust cap onto the eyepiece socket!



Fig. 2-8Binocular photo tube 30°/23 with
toggle graduation 100:0/0:100



Fig. 2-9 Setting the viewing height on the binocular tube

8 - 38°

Fig. 2-10 Binocular ergo photo tube 8-38°/20 with fixed graduation 50:50



CAUTION

The binocular ergo tube/ergo photo tube 8-38°/20 may only be used on the Axio Lab.A1 with the base plate (430037-9100-000) installed, as the microscope is otherwise liable to tip over, resulting in damage to the instrument or injury to the user.

Interpupillary distance and viewing height

In all tubes:

- The interpupillary distance can be varied by adjusting the eyepiece sockets relative to one another.
- The viewing height can be varied by swiveling the eyepiece sockets up (Fig. 2-9/A) or down (Fig. 2-9/B).
- For polarization microscopy we recommend the photo tube Pol with upright crosshairs.

Binocular ergo tube/ergo photo tube 8-38°/20

These tubes are designed for a field of view of 20.

The viewing angle can be continuously adjusted between 8° and 38° by swiveling the binocular section (Fig. 2-10/**1**).

The splitting ratio of the ergo photo tube is 50:50, i.e.: 50 % of the light is directed to the eyepieces and 50 % to the camera port.

Binocular comfortable ergo tube 8-33°/22 with vertical adjustment 50 mm

This comfortable ergo tube is designed for a field of view of 22.

The viewing angle can be continuously adjusted between 8° and 33° by swiveling the binocular section (Fig. 2-11/**3**) with the aid of the angle scale (Fig. 2-11/**2**).

The viewing height can be adjusted independently of the viewing angle. The latter can be continuously varied within a range of 0 mm to 50 mm with the aid of the rotary knobs (Fig. 2-11/1). The setting can be read off a vertical scale (Fig. 2-11/1).

In addition an even greater adjustment range can be used (depending on interpupillary distance) by swiveling the binocular section from the lower to the upper observation position.



CAUTION

The binocular comfortable ergo tube 8-33°/22 may only be used on the Axio Lab.A1 with the base plate (430037-9100-000) installed, as the microscope is otherwise liable to tip over, resulting in damage to the instrument or injury to the user.



Fig. 2-11 Binocular ergo tube 8-33°/20 with vertical adjustment 50 mm



Fig. 2-12Binocular ergo photo tube 20°/23
with vertical adjustment



Fig. 2-13 Binocular ergo photo tube 15°/23, telescopic with vertical adjustment

Binocular ergo tube/ergo photo tube 20°/23 and ergo photo tube 15°/23, each with continuous vertical adjustment

These ergo photo tubes are designed for field of view of 23. For use on the Axio Lab.A1 they are recommended for max. field of view of 22. The viewing angle is 20° or 15°.

Ergo tubes are continuously vertically adjustable in the range 0 mm to 44 mm.

In addition an even greater adjustment range can be used (depending on interpupillary distance) by swiveling the binocular section from the lower to the upper observation position.

- Continuous height adjustment is achieved by means of the rotary knobs (Fig. 2-12/1 and Fig. 2-13/1).
- The adjustment range of the ergo photo tube 20°/23 can be read off the lateral scale (Fig. 2-12/3).

The **ergo photo tube 20°/23** has two switch positions (Graduation: 100:0/0:100).

- Push-pull rod (Fig. 2-12/2) pushed in: 100 % to the eyepieces.
- Push-pull rod(Fig. 2-12/2) pulled out: 100 % to camera port.

The **ergo photo tube 15°/23** (Fig. 2-13) is only available with an upright image and fixed graduation (50:50).

The binocular part of the ergo photo tube 15°/23 is also continuously horizontally retractable up to 50 mm.

Axio Lab.A1

2.5.2 Microscope stages

Mechanical stage 75x30 R or L or mechanical stage 75x30 R ergonomic with stationary drive

- Mechanical stage (Fig. 2-14/7) for seating, positioning and securing specimens with specimen holder.
- Specimen holder (Fig. 2-14/2) for singlehanded operation or specimen holder for counting chambers (replaceable after loosening the two knurled screws, Fig. 2-14/1).
- Drive knobs for X (Fig. 2-14/6) and Y adjustment (Fig. 2-14/5).
 The drive knobs for X and Y can be adjusted to suit the personal needs of the user, both in height and friction. The required tools (Fig. 2-14/8) are located in the upper drive knob.
- Vernier scale for display of adjustment range in X (Fig. 2-14/3) and Y direction (Fig. 2-14/4).
- Drive knobs on right (R) or left (L) side depending on model.
- Furthermore, a mechanical stage (Fig. 2-15/1) with ergonomic, stationary X-Y drive (Fig. 2-15/2) is located on the righthand side.

Mechanical stage reflected light 75x30 R

- Mechanical stage (Fig. 2-16/2) for seating, positioning and securing specimens with specimen holding plate (Fig. 2-16/1) with spring clips.
- Drive knobs for X and Y adjustment on righthand side.
- Vernier scale for display of adjustment range in X and Y direction.
- Removable specimen holding plate (after loosening the two knurled screws).



Fig. 2-14 Mechanical stage 75x30 R with specimen holder



Fig. 2-15 Mechanical stage 75x30 R ergonomic with stationary drive



Fig. 2-16 Mechanical stage reflected light 75x30 R with specimen holding plate



Fig. 2-17 Rotary stage Pol



Fig. 2-18 Filter mount on luminous-field diaphragm operating ring for filter d=32x4 mm

Rotary stage Pol 360° with lock

- Rotary stage Pol (Fig. 2-17) for seating, positioning and securing specimens with specimen guide (Fig. 2-17/5) and specimen holder (Fig. 2-17/7).
- 360° rotation with lock using knurled screw (Fig. 2-17/6).
- Angle of rotation with vernier scale (Fig. 2-17/1) readable on angle scale.
- Specimen guide (Fig. 2-17/5) removable (after loosening clamp screw, Fig. 2-17/4; two cylindrical pins on the underside serve as orientation for mounting the specimen guide on the rotary stage).
- Specimen guide equipped with specimen holder which can be shifted in X and Y direction using drive knobs (Fig. 2-17/3 and 2). The shift in X and Y direction can be read off via the two vernier scales on the respective scale.

Filter mount on luminous-field diaphragm operating ring for filter 32x4 mm

- Place the filter (Fig. 2-18/2) on the luminousfield diaphragm operating ring (Fig. 2-18/3).
- To secure the filter insert the filter clamp (Fig. 2-18/1) on the luminous-field diaphragm operating ring.
- To replace the filter, grip the recesses of the filter clamp and pull it off the luminous-field diaphragm operating ring.

2.5.3 Condensers

Condenser 0.9/1.25 H, D, Ph1, Ph2, Ph3

Condenser 0.9/1.25 H (Fig. 2-19/**1**) with aperture diaphragm (Fig. 2-19/**4**) with modulator disk (Fig. 2-19/**3**) for:

- Brightfield (H)
- Darkfield (D)
- Phase contrast Ph 1, Ph 2, Ph 3

Position adjustment of modulator disk by turning knurled ring (Fig. 2-19/**2**).

This condenser is also available without modulator disk, i.e. only brightfield.



Fig. 2-19 Condenser 0.9/1.25 H, D, Ph1, Ph2, Ph3 with modulator disk

Condenser 0.9/1.25 H

Condenser 0.9 H (Fig. 2-20/1) with aperture diaphragm (Fig. 2-20/2) for brightfield

This condenser is also available with a modulator disk.



Fig. 2-20 Condenser 0.9/1.25 H



Fig. 2-21 Overview fixture

Overview fixture 2.5x–4x

The overview fixture is for full display field illumination when using an objective with a weak magnification factor (2.5x-4x) in combination with the Abbe condenser 0.0/1.25 H (424227-9000-000).

It can be centered and remains swiveled into the beam path for as long as the respective objective is in use.

Swivel overview fixture (Fig. 2-21/2) into/out of the beam path with the aid of a handle (Fig. 2-21/1). Ensure that the overview fixture snaps in securely when swiveled in.

The illumination of weak objective magnifications can be centered with the centering screws. For this purpose, the condenser should be centered on the other objectives without the overview fixture.



With the overview fixture attached it can be mounted on the luminous-field diaphragm operating unit, provided the condenser carrier is shifted as far down as possible!

Polarizer D, 90° rotatable, switchable (Fig. 2-22/3)

- Polarizer can be swiveled in/out using handle (Fig. 2-22/1)
- Polarizer with lever (Fig. 2-22/2), 90° rotatable

Polarizer, fixed with lambda plate, rotatable, (Fig. 2-22/4)

- Polarizer can be swiveled in/out using handle (Fig. 2-22/6)
- Lambda plate can be swiveled in/out using handle (Fig. 2-22/7)
- Lambda plate with lever (Fig. 2-22/5), rotatable

Fig. 2-22 Polarizers



With the polarizer attached it can be mounted on the luminous-field diaphragm operating unit, provided the polarizer carrier is shifted as far down as possible!

2.5.4 Reflector turret 4x

The 4x reflector turret is equipped with reflector positions P&C.

The reflector position is adjusted by turning the knurled ring (Fig. 2-23/1). The marking (Fig. 2-23/3) on the knurled ring shows the reflector position in the beam path.

The stickers supplied can be used to identify the reflector modules employed. The stickers can be applied to the areas provided (Fig. 2-23/**2**).



Fig. 2-23 4-position reflector turret

Nosepiece with objectives

- Nosepiece 4x or 5x depending on stand type with M27 threaded insert for four or five objectives.
- Quick change of objectives by turning nosepiece on knurled ring (Fig. 2-24/2).
- Incorporates slot (Fig. 2-24/3) for 6x20-slide (compensators, analyzers, quarter plates).
- Stand for transmitted light polarization and stand for transmitted light conoscopy with 4x nosepiece, of which 3 positions can be centered with the aid of two screws each (Fig. 2-24/1).



CAUTION

Do not overtighten the screws (Fig. 2-24/**1**) on the stop.

Filter slide for reflected light stand

- Filter slide for reflected light with two positions for filter d=25 mm (neutral and color filter, white balance filter)
- Insert the filter slide from the left and operate (Fig. 2-4/22)



Fig. 2-24 Nosepiece of the transmitted light polarization stand with mount for compensators

3 START-UP

The Axio Lab.A1 microscope can be independently installed, converted and started up by the customer. On request, the microscope can also be installed or converted by Zeiss Service at an extra charge.

Before installing and starting-up the microscope, be sure to carefully read the **Notes on instrument safety** (see Section 1.1).



Fig. 3-1Setting up the microscope



Fig. 3-2 Placing tools in the storage compartment

The activities described in the following are illustrated for the most part by way of examples for one microscope stand type. However, they apply analogously to other microscope stand models. Special features are described separately.

3.1 Installing standard components

3.1.1 Unpacking and setting up microscope stand

- Remove all components from the packaging and check that all components described on the delivery note are present.
- Set up microscope stand (Fig. 3-1/1) on a vibration-free, level, hard and non-combustible surface.
- Keep the original packaging for storage or for returning the instrument to the manufacturer, or dispose of it properly.
- Tools (Fig. 3-2/1) required for set-up and adjustment of the microscope are located in the storage compartment (Fig. 3-2/2) on the rear side of the stand. Press the underside of the cover flap at PRESS to open.

The following tools are included in the shipping contents:

- Angled socket wrench SW 3
- Two socket wrenches SW 1.5 for adjusting the phase contrast diaphragms in the respective condenser positions.

• For shipping the power cord (Fig. 3-3/1) can be rolled up and stowed in the open cover flap (Fig. 3-3/2).



Fig. 3-3 Stowing power cord in cover flap for shipping

3.1.2 Mounting base plate for use of larger tubes



The base plate must be mounted on Axio Lab.A1 stands in order to increase stability during operation for the majority of tubes/photo tubes/ergo tubes, or this is at least recommended. Please refer to the corresponding notes in the system overview, Section2.2.

- The base plate is not required for binocular tube or photo tube 30°/20 (425522-9000-000 and 425522-9010-000) or binocular tubes 20°/23 and 30°/23 (425520-9090-000 and 425520-9000-000).
- Lay the stand on its rear side.
- Unscrew or pull the rear rubber feet (Fig. 3-4/3) out of the holes in the stand.
- Set the base plate (Fig. 3-4/2) on the underside of the stand and secure with two screws. (Fig. 3-4/1).
- Set the stand in an upright position.
- Save the rubber feet of the microscope stand for use at a later date.



Fig. 3-4 Mounting the base plate

3.1.3 Attaching the binocular tube/photo tube

All binocular tubes listed in the system overview (refer to Section 2.2) can be mounted on the microscope stand as described below. Regardless of the stand type and tube used, in some cases an intermediate plate must also be mounted (see Section 2.2).

For tubes mounted without an intermediate plate, proceed as follows:

- Loosen the hexagonal socket head screw (Fig. 3-5/3) with a SW 3 socket wrench. Remove dust caps (Fig. 3-5/2, 5) from the underside of the tube and the dovetail ring mount on the stand side.
- Hold the binocular tube/photo tube (Fig. 3-5/1) at an angle, insert it with the dovetail ring into the stand mount (Fig. 3-5/4) and turn into a horizontal position. Rotate the binocular tube into the desired observation position and re-tighten the hexagonal socket head screw with the ball-headed screwdriver.



Fig. 3-5 Attaching the binocular tube

For tubes mounted **with** an intermediate plate, proceed as follows:

- Loosen the hexagonal socket head screw (Fig. 3-5/6) with a SW 3 socket wrench. Remove dust caps (Fig. 3-5/8, 11) from the underside of the tube and the dovetail ring mount on the stand side.
- Insert the intermediate plate (Fig. 3-5/10) with its dovetail ring into the stand mount (Fig. 3-5/7) and tighten hexagonal socket head screw (Fig. 3-5/6).
- Insert the binocular tube/photo tube (Fig. 3-5/12) into the intermediate plate, align and tighten the hexagonal socket head screw (Fig. 3-5/9) with the ball-headed screwdriver.

3.1.4 Installing eyepieces or auxiliary microscope or diopter

- Remove both dust caps (Fig. 3-6/1 and 5) from the binocular tube.
- Remove the two eyepieces (Fig. 3-6/2) from their cases and insert them into the binocular tube as far as they will go.
- Before inserting Pol eyepieces with tubes without upright reticle, the orientation screw on the reverse side of the eyepieces must be unscrewed. The eyepieces cannot otherwise be fully inserted.
- The auxiliary microscope (Fig. 3-6/**3**) can be used in place of the eyepiece in one of the binocular sockets for observing aperture, phase and darkfield stops or for centering phase



Fig. 3-6 Installing the eyepieces

stops. Focusing on these apertures is possible using the adjustable eye lens.

• The auxiliary microscope (Fig. 3-6/3) or diopter (Fig. 3-6/4) can be used for observing conoscopic images.

Installing the eyepiece reticle

Eyepiece reticles (Fig. 3-7/**3**) can be used with eyepieces with a red dot.

The slight image shift caused by the additional path through glass is taken into account on the diopter scale by the fact that the zero point position is indicated not by the white dot, but the red dot.

Make sure the line figure always faces the field stop.

The eyepiece reticles must be inserted under dust-free conditions. This should only be carried out by Carl Zeiss Service.



Fig. 3-7 Installing the eyepiece reticle

Inserting reversible eyecups

The eyepieces have rubber protection rings to avoid scratches on the eyeglasses. These may be replaced by reversible eyecups as desired.

• For this purpose remove the eyeglass protection rings (Fig. 3-7/2) from the eyepieces and mount the eyecups (Fig. 3-7/1).

Sometimes the eyeglass protection rings are seated very tightly in the eyepiece groove, so you may need a blunt object (wooden stick) to prod them off.



Fig. 3-8 Screwing in objectives

3.1.5 Screwing in objectives

- Move mechanical stage with stage carrier to lower stop.
- Remove the dust caps (Fig. 3-8/**5**) from the openings in the nosepiece.
- Remove objectives (Fig. 3-8/4) from the case and screw them into the nosepiece (Fig. 3-8/1) starting with the lowest magnification (clockwise rotation).
- Instead of an objective, the specimen marker (Fig. 3-8/3) with a spacer ring W0.8/M27 (Fig. 3-8/2) can be screwed on in any desired nosepiece position. If the specimen marker is not to be used for an extended period, apply the protective cap to prevent it from drying out.
- Always replace the dust caps on any empty positions on the nosepiece.
- Spacer ring W0.8/M27 is required when using W0.8 objectives.

3.1.6 Fitting and removing push&click modules in the reflector turret

The reflector turret 4x stand transmitted and reflected light (BioMed) and the reflected light stand (Material) is firmly installed.

The modules must be inserted and removed from the front after removing the cover cap.

Inserting a module:

- Remove cover cap (Fig. 3-9/4) from the stand towards the front.
- Insert the module (Fig. 3-9/2) as illustrated together with the retaining brackets on the right and left (Fig. 3-9/3) diagonally from below into the upper spring clips (Fig. 3-9/1) of the reflector turret.
- Then apply pressure to the module from below until it also securely engages with the lower spring clips of the reflector turret. The position number of the P&C module is shown on the right hand side of the reflector turret adjacent to the position of the respective P&C module.
- Apply the supplied stickers with the filter combination data of the respective module to the corresponding field of the cover cap (Fig. 3-9/**5**, position 1 to 4).

Removing a module:

- Slightly tilt the module in order to detach it from the lower spring clips, then from the upper spring clips of the reflector turret.
- Once the reflector modules have been installed / removed, refit the cover cap. The cover cap should be fitted onto the stand as straight as possible to avoid the knurled ring of the reflector turret becoming jammed and damaged.
- Apply pressure to the cover cap until the retaining brackets have engaged.



Fig. 3-9 Replacing the reflector module



Fig. 3-10 Replacing a mechanical stage

3.1.7 Mounting a mechanical stage

Axio Lab.A1 stands are fitted with the respective mechanical stage at the factory according to customer requirements.

The friction torque of the drive knobs is set at an average value at the factory.

Should the stage need to be replaced or the stage settings changed, proceed as follows:

3.1.7.1 Dismantling a stage

- Remove the four fastening screws (Fig. 3-10/4) on the stage carrier (Fig. 3-10/2) using an SW 3 (Fig. 3-10/3) Allen wrench.
- Remove stage (Fig. 3-10/1) upwards from the stage carrier.

3.1.7.2 Installing the stage

- Place stage (Fig. 3-10/1) onto stage carrier (Fig. 3-10/2) in such a manner that the threaded holes on the bottom of the stage (Fig. 3-10/6) are positioned above the stage carrier openings (Fig. 3-10/5).
- Insert four fastening screws (Fig. 3-10/4) through the stage carrier from below and screw them into the bottom of the stage.
- Turn the stage to orient it in an XY direction and tighten the fastening screws.

3.1.7.3 Setting drive length on stage drive

The length of the X and Y drives can be changed by shifting the respective drive knob (Fig. 3-11/4 or 1) axially within a range of approx. 15 mm.

3.1.7.4 Setting friction torque of drive knobs for X/Y adjustment of the mechanical stage

(1) X drive

- Push drive knob for X adjustment (Fig. 3-11/4) right to the bottom.
- Remove supplied adjusting pin (Fig. 3-11/5) from the drive knob for Y adjustment (Fig. 3-11/1) and insert into one of the holes of the lower hole nut(Fig. 3-11/3).
- Hold the drive knob for X adjustment (Fig. 3-11/4) and turn the hole nut with the adjusting pin in a clockwise direction (small friction torque: -) or counter-clockwise (large friction torque: +) until the desired freedom of movement has been achieved (see Fig. 3-11).
- It should not be shifted more than **one** revolution.



Fig. 3-11 Setting friction torque

(2) Y drive

- Push drive knob for Y adjustment (Fig. 3-11/1) right to the top.
- Insert the supplied adjusting pin (Fig. 3-11/5) into the hole of the upper hole nut (Fig. 3-11/2).
- Hold the drive knob for Y adjustment (Fig. 3-11/1) and turn the hole nut with the adjusting pin in a clockwise direction (small friction torque: –) or counter-clockwise (large friction torque: +) until the desired freedom of movement has been achieved.
- It should not be shifted more than **one** revolution.
- Re-insert the adjusting pin into the drive knob for Y adjustment (Fig. 3-11/1).
- Set the friction torque on the mechanical stage with ergonomic, stationary X-Y drive analogously. No tool is required for the purpose. The lock-nut (silver) of the respective drive can be adjusted manually; hold the drive knob tight.



Fig. 3-12 Replacing snap-in rotary stage Pol, detachable specimen guide Pol and stage clips

3.1.8 Mounting rotary stage Pol

3.1.8.1 Removing rotary stage pole

- Loosen screw cap (Fig. 3-12/6) from the spring housing (about three rotations).
- Press rotary stage Pol (Fig. 3-12/4) to the front against spring-loaded pin (Fig. 3-12/7), lift it off the stage carrier (Fig. 3-12/5) from the back and remove it upwards.
- Re-tighten screw cap (Fig. 3-12/6).

3.1.8.2 Attaching rotary stage Pol

- Where necessary, loosen screw cap (Fig. 3-12/6) of spring housing with approx. three rotations.
- Place rotary stage Pol with the groove of the dovetail (stage bottom) on spring-loaded pin (Fig. 3-12/**7**).
- Attach the rotary stage with the clamp screw (Fig. 3-12/8) pointing to the front right.
- Press the rotary stage Pol to the front against the spring-loaded pin and lower it towards the back into the stage carrier (Fig. 3-12/5), then release it.
- Re-tighten screw cap (Fig. 3-12/6).
- The rotary stage must be mounted so that the vernier scale is on the left side and clamp is on the right side.

3.1.8.3 Dismantling the detachable specimen guide and mounting stage clips

- Loosen the knurled screw (Fig. 3-12/1) on the specimen guide Pol. Withdraw the specimen guide Pol (Fig. 3-12/2) upwards.
- Insert the stage clips (Fig. 3-12/9) into the holes provided.

3.1.8.4 Removing the stage clips and mounting the detachable specimen guide Pol

- Remove the stage clips (Fig. 3-12/9) from the rotary stage Pol.
- Insert the specimen guide Pol (Fig. 3-12/2) with the two cylindrical pins on the underside into the holes provided (Fig. 3-12/3) and tighten the clamp screw (Fig. 3-12/1).

3.1.8.5 Centering rotary stage Pol

With high-power objectives centering can be exact only for one selected objective.

All stages are factory-precentered, i.e. while rotating the stage the specimen feature set to the center will remain in the center. If the specimen feature moves off the center of the field of view (Fig. 3-13/**5**), while rotating the stage, the stage should be re-centered by following this procedure:

- The KÖHLER illumination on the microscope must be adjusted before centering the stage (see Section 4.1.1).
- Turn the nosepiece to swing the non-centring objective mount.
- For centering the stage, use a contrasting specimen and an eyepiece with crossline reticle.
- Loosen the stage clamping screw (Fig. 3-13/1) and screw cap on the stage carrier (Fig. 3-13/3).
- Rotate the stage to determine the position of maximum offset of the specimen feature (Fig. 3-13/**5**, origin of arrow) from the center of the eyepiece reticle.



Fig. 3-13 Centring rotary stage Pol

- Reset the two centering screws on the stage carrier (Fig. 3-13/2) using a SW 1.5 socket wrench (Fig. 3-13/4) to move the specimen detail by half an arrow length in the direction of the crossline center. Check whether the specimen detail moves when the stage is rotated again; repeat the procedure, when required.
- The SW 1.5 socket wrenches are located in the storage compartment on the rear side of the microscope stand.
- When centering is finished, re-tighten screw cap (Fig. 3-13/3).



Fig. 3-14 Centering objectives

3.1.8.6 Centring objectives of the polarization stand

The nosepiece 4x Pol is equipped with one fixed and three centerable objective positions.

Stage centering of the non-centering objective mount is necessary to ensure that a specimen feature located in the center of the field of view does not drift out while rotating the stage. By centering of the remaining objectives, the specimen feature remains in the center of the field of view even after changing the objective.

- The KÖHLER illumination on the microscope must be adjusted before centering the stage (see Section 4.1.1).
- For centering the stage, use a contrasting specimen and an eyepiece with crossline reticle.
- First turn the nosepiece to swing the noncentering objective mount. Center the rotary stage for the non-centering objective mount as described under 3.1.8.5.
- Turn the nosepiece to move a centering objective mount into the light path.
- Rotate the stage to determine the position of maximum offset of the specimen feature (Fig. 3-14/3, origin of arrow) from the center of the eyepiece reticle.
- Reset the two centering screws on the stage carrier (Fig. 3-14/2) using one SW 1.5 Allen screw-driver (Fig. 3-14/1) each to move the specimen detail by half the arrow length in the direction of the crossline center. Check whether the specimen detail moves when the stage is rotated again; repeat the procedure, when required.
- Center the other two objectives in the same manner.
- To maintain this centering accuracy, when replacing it is important not to hold the objective itself, but the knurled ring of the nosepiece to rotate the latter.

3.1.9 Attaching condenser

• Move the stage carrier with focusing drive to the higher stop position.



CAUTION

The objectives should not collide with other parts.

- Swivel out front lens (if shiftable) on condenser using lever (Fig. 3-15/**7**).
- Remove both centering screws (Fig. 3-15/**5**) on the condenser carrier until their ends are no longer visible.
- Push the condenser carrier (Fig. 3-15/3) with drive knob for vertical adjustment (Fig. 3-15/2) down as far as it will go.
 If using an overview fixture, make sure that this does not come to rest on the luminous-field diaphragm.



Fig. 3-15 Attaching condenser

- Insert the condenser (Fig. 3-15/8 or 9) between the condenser carrier (Fig. 3-15/3) and the stage carrier (Fig. 3-15/1). In doing so, align the screwed stud bolt on the underside of the condenser with the groove (Fig. 3-15/6).
- Press the condenser with the dovetail ring against the mainspring (Fig. 3-15/4) of the condenser carrier until the condenser sits horizontally on the condenser carrier.
- Position the condenser on the carrier so that the screwed stud bolt sits against the groove (Fig. 3-15/6) at the front.
- Insert the centering screws until they engage with the dovetail ring of the condenser.

For mounting other types of condensers, proceed analogously.



Fig. 3-16 Removing the cover



Fig. 3-17 Removing the LED lamp



Fig. 3-18 Inserting the LED lamp

3.1.10 Installing or replacing 35 W halogen bulb or 3 W white light LED lamp

If desired, Axio Lab.A1 transmitted light stands can be equipped with a 3 W white light LED lamp with spectrum daylight or warm light.

To insert or replace the halogen/LED lamp, proceed as follows:

- Switch off the microscope, remove the power cord on the microscope and allow it to cool down at least 15 min.
- Press the clamping jaw (Fig. 3-16/1) on the cover (Fig. 3-16/2) downwards. Swing the cover down, remove from the retaining channel (Fig. 3-17/4) on the stand and set aside.
- Remove the lamp plug (Fig. 3-17/2) from the lighting unit (Fig. 3-17/3).
- Press the loops (Fig. 3-18/1) on the securing clip (Fig. 3-17/1) of the lamp holder together and swing it out to the front.
- When changing the lamp remove the lighting unit (Fig. 3-17/**3**).
- Position the new lighting unit/LED lamp (Fig. 3-18/1) with the lower front edge between the contact surface and securing clip. Set the light fitting on the securing clip.
- Lift the securing clips (Fig. 3-18/2) of the lamp holder with the lighting unit until the latter is completely enclosed in the lamp holder. In doing so, slightly press the ends of the securing clips together and guide past the two upper retaining elements. Release the pressure until the clamping bracket opens and engages on both sides in the retaining elements.

- Check that the lighting unit is seated correctly and insert the plug (Fig. 3-19/2) onto the pins of the lighting unit (Fig. 3-19/1). Ensure that it engages properly to avoid bending the pins.
- Insert the cable of the lamp plug into the stand so that it is not damaged when the cover is mounted.
- Insert the lower edge of the cover (Fig. 3-16/2) into the retaining channels (Fig. 3-17/4) of the stand and swing upwards until the clamping jaw (Fig. 3-16/1) locks in.
- Reconnect the power cord.







Fig. 3-20 Removing the cover



Fig. 3-21 Removing the 12V 50W halogen bulb



Fig. 3-22 Installing the 12V 50W halogen bulb

3.1.11 Installing or changing the 12 V 50 W halogen lamp

Each Axio Lab.A1 reflected light stand is equipped with a 12 V 50 W halogen lamp. To insert or replace a halogen lamp, proceed as follows:

- Switch off the microscope, remove the power cord on the microscope and allow it to cool down at least 15 min.
- Loosen the screw (Fig. 3-20/2) in the cover.
- Swing the cover (Fig. 3-20/1) slightly upwards and apply pressure from below to remove it from the stand.
- Remove lamp plug (Fig. 3-21/1) from the halogen lamp (Fig. 3-21/3).
- Press the securing clips (Fig. 3-21/**2**) on both sides of the lamp holder and swing the latter downwards.
- When changing the lamp remove the lighting unit (Fig. 3-21/**3**).
- Position the halogen lamp (Fig. 3-22/**2**) on the contact surface of the lamp holder (lamp will be held secure by the groove).
- Press the securing clip (Fig. 3-22/1) on both sides of the lamp holder and swing it upwards until the securing clip is seated against the halogen lamp. Slowly release the securing clip so that it opens and engages in the retaining elements to the right and left.
- Check that the halogen lamp is correctly seated and insert the plug (Fig. 3-22/**3**) on the pins of the latter. Ensure that it engages properly to avoid bending the pins.
- Insert the cable of the lamp plug into the stand so that it is not damaged when the cover is mounted.
- Insert the cover (Fig. 3-20/1) at a slant from below into the upper holding elements of the stand, swivel down and press into place. Tighten screw (Fig. 3-20/2).
- Reconnect the power cord.

3.1.12 Installing or replacing LED modules

Axio Lab.A1 transmitted/reflected light stands may be equipped for transmitted light applications with a 35 W halogen lamp or an 3 W LED lamp (see Section 3.1.10) and for reflected light fluoresce applications with up to two LED modules from the delivery program (see also Section 2.2). To insert or replace LED modules, proceed as follows:

- Switch off the microscope, disconnect the power cord.
- Loosen the screw (Fig. 3-23/2) in the cover.
- Swing the cover (Fig. 3-23/1) slightly upwards and apply pressure from below to remove it from the stand.
- Use the push-pull rod (Fig. 3-24/4) to swivel the placement position in the center (beam path).
- Remove the plug (Fig. 3-24/1) of the LED module to be replaced (Fig. 3-24/2) from the connecting socket while pressing the notched lever on the plug. Remove the securing clip (Fig. 3-24/3) of the module holder upwards and pull out the LED module (Fig. 3-24/2).
- Press the securing clip (Fig. 3-25/**5**) upwards and push in the replacement LED module (Fig. 3-25/**3**) until it locks into the LED holder. When the securing clip is release, it will secure the LED module in its slot.
- Insert the plug (Fig. 3-25/**2**) of the LED module into the respective connecting socket until it engages.
- Use the pull-push rod (Fig. 3-24/**4**) to swivel the other placement position into the beam path and insert or replace the second LED module (Fig. 3-25/**1**) in the same way.
- Insert the cover (Fig. 3-23/1) at a slant from below into the upper holding elements of the stand, swivel down and press into place. Tighten screw (Fig. 3-23/2).
- Record the data of the LED modules on the supplied stickers and apply at the positions intended on the upper part of the stand.
- Reconnect the power cord.



Fig. 3-23 Removing the cover



Fig. 3-24 Removing the LED module



Fig. 3-25 Installing the LED module

3.2 Mounting optional components



The microscope must be switched off and unplugged before starting work.

Upon completion of work the respective modules must be restored to function (see Sections 3.1 to 3.4).

3.2.1 Mounting the light intensive co-observer unit

The light-intensive co-observer unit is mounted on the Axio Lab.A1 with a main observer and one or two co-observers in accordance with the separate instructions for use for multi-discussion facilities (Order No. 425145-7144-001).



Fig. 3-26 Mounting the polarizer D

3.2.2 Mounting polarizer D or filter holder

- Lift the condenser carrier together with its drive knob upwards as far as it will go.
- If necessary, unscrew locking and holding pins together with the overview fixture from the condenser carrier.
- Hold the polarizer or filter holder (Fig. 3-26/4) parallel to the underside of the condenser carrier (Fig. 3-26/1) and screw the holding pin (Fig. 3-26/2) of polarizer (Fig. 3-26/4) with the angled adjusting lever (Fig. 3-26/3) into the front left threaded hole below the condenser carrier as far as it will go.
- Screw the locking pin (Fig. 3-26/6) with adjusting lever (Fig. 3-26/5) as far as it will go into the rear threaded hole of the condenser carrier.

The other components shown in the system overview at this point must be mounted analogously.

3.2.3 Mounting and centering the overview fixture

- Lift the condenser carrier together with its drive knob upwards as far as it will go.
- Remove the polarizer or filter holder from the condenser carrier as applicable.
- Hold the overview fixture or filter holder (Fig. 3-27/2) parallel to the underside of the condenser carrier (Fig. 3-27/1) and screw the holding pin (Fig. 3-27/4) of overview fixture with the angled adjusting lever (Fig. 3-27/6) into the front left threaded hole below the condenser carrier as far as it will go.
- Screw the locking pin (Fig. 3-27/8) with adjusting lever (Fig. 3-27/7) as far as it will go into the rear threaded hole of the condenser carrier.
- Swivel the overview fixture into the beam path. Ensure that it is securely locked into place.
- Completely open the aperture and luminous-field diaphragm.
- Use the SW 1.5 hexagonal socket wrench to set the two adjusting screws (Fig. 3-27/**3** and **5**) until the field of view is optimally illuminated.



Fig. 3-27 Mounting the overview fixture

The overview fixture should only be mounted on condenser 0.9/1.25.



Fig. 3-28 Modulator disk in condenser 0.9 H Pol

3.2.4 Inserting modulator disk in condenser 0.9 H Pol

• Remove the condenser (Fig. 3-28/1) from the condenser carrier (see Section 3.1.9).

If the condenser carrier cannot be lowered sufficiently, e.g. when the overview fixture is fitted, the latter must be detached to enable the condenser to be pushed down as far as it will go and removed.

- Loosen the clamp screw (Fig. 3-28/**5**) of the condenser scale segment (Fig. 3-28/**3**) with a SW 3 screwdriver and pull the scale segment out to the front.
- Insert the modulator disk (Fig. 3-28/4) with the two-pronged fork opening at the front into the condenser so that the internal guide is enclosed on both sides and serves as a stop for the modulator disk. At the same time it must be ensured that the pin of the clamp screw of the modulator disk is located in the orientation groove of the condenser.
- Tighten the clamp screw of the modulator disk with an SW 3 screwdriver.
- Re-insert the condenser into the condenser carrier (see Section 3.1.9).

3.3 Connecting to the power supply

In all stand models the power supply of the Axio Lab.A1 is located in the rear side of the instrument.

- Connect microscope (Fig. 3-29/1) to the mains power supply via a power cord and mains socket.
- The Axio Lab.A1 can be connected to a line voltage of 100 to 240 VAC, 50/60 Hz. The power unit is set **automatically** to the line voltage available.



Fig. 3-29 Mains power supply on rear side of stand

3.4 Switching the microscope on/off

- Switch the microscope on/off using (Fig. 3-30/1) the mains switch.
- Adjust image brightness (Fig. 3-31/3) using the brightness control.
 To do this, take hold of the recessed grips of the rotary knob with the fingertips and turn it

Only transmitted light and reflected light fluorescence stand:

to the desired position.

- Turn the **FL/TL** toggle switch (Fig. 3-31/**1**) to the desired position (FL = fluorescence lighting for reflected light; TL = lighting for transmitted light).
- Depending on the position of the FL/TL toggle switch, the image brightness is adjusted using the controller for transmitted light TL (Fig. 3-31/3) or for reflected light fluorescence FL (Fig. 3-31/2).



Fig. 3-30 Mains switch on left side of microscope



Fig. 3-31 Light intensity control and FL/TL toggle switch

3.5 Basic setting of the microscope from an ergonomic point of view

3.5.1 Configuration of an ergonomic microscope workplace

The Axio Lab.A1 light microscope was developed and designed in cooperation with occupational health physicians and the TÜV Rhineland to meet the most exacting demands with regard to ergonomics at the microscope workstation. This first light microscope worldwide is available with a special ergonomic configuration and bears TÜV certificate ID:0000025994 "Ergonomically tested". Besides this special TÜV-certified ergonomic configuration, the Axio Lab.A1 offers many other product characteristics and ergonomic components for configuring the microscope workstation according to ergonomic aspects.

Particularly laboratory microscopes in the Axio Lab.A1 device class are used continuously for many routine biomedical applications (e.g. haematological, histological and cytological examinations) over a period of several hours. With this regular use of the light microscope for extended periods it is particularly important to keep the burden on the user's musculoskeletal system to a minimum. The ergonomic design and arrangement of the control elements on the microscope, individual eyepiece adjustment and correct configuration of the entire microscope workstation, including lighting, chair and workbench permits a significant reduction in this burden for the user.

This results in improved working conditions, greater comfort of microscope users and higher work productivity. More and more countries are also introducing stringent workplace regulations for microscope workstations, particularly in the medical field. In addition, the regulations of employers' liability insurance associations increasingly place obligations on employers to provide ergonomic workplaces and microscopes.

The following sections of this user manual contain advice for the correct basic setting of the Axio Lab.A1, particularly from an ergonomic point of view. They also include notes on ergonomic configuration of the overall microscope workstation.

3.5.2 TÜV certificate ID:0000025994 "Ergonomically tested"

TÜV certificate ID:0000025994 "Ergonomically tested" prescribes the distance of control elements from the table, the user and each other. Furthermore, it defines a broad adjustment range for the eyepiece to cater for the different body heights of male and female microscope users worldwide. For this purpose the ergonomic tube or ergo tube must be vertically and angle-adjustable. This enables it to be adjusted to suit different body heights (static ergonomics) and occasionally varied by the user during extended periods of use (dynamic ergonomics). Furthermore, the TÜV ergonomic certificate is only fully valid when the remaining microscope workstation is ergonomically designed in terms of lighting, height-adjustable chair and table.

A series of fundamental workplace standards, as listed in Section 1.2 Notes on ergonomics of the microscope, serve as a basis for this TÜV ergonomic certificate. The TÜV certificate (Fig. 3-32) is located on the comfort ergo tube of the special ergonomically certified configuration.

Details of this certificate can be found online at: *www.tuv.com* upon entering ID:0000025994.

Besides this Axio Lab.A1 configuration explicitly distinguished with the TÜV ergonomic certificate it is also possible to enhance the ergonomic friendliness of the microscope workstation in stages by selecting an adjustable ergo tube or special ergonomically designed table with fixed stage drive.



Fig. 3-32 TÜV certificate "Ergonomically tested"

3.5.3 Ergonomic configuration of the microscope workstation

In addition to the use of an ergonomically designed microscope, e.g. the Axio Lab.A1, the microscope workstation must exhibit further ergonomically relevant aspects in terms of lighting conditions, air temperature, humidity, general workplace configuration, height-adjustable chair and table surface. These aspects are described in more detail in the following; references to standards with additional information in square brackets. It is recommended that the Axio Lab.A1 be used in a sitting position.

Lighting requirements are determined by the visual comfort and acuity of the user. Visual comfort gives the user a feeling of well-being, thus contributing to a high level of productivity. Visual acuity enables the user to perform visual tasks even under difficult conditions over extended periods of time. Specifically, this means that the workstation should not be in direct sunlight or reflected light from other sources. For fluorescence microscopy the workstation must be capable of darkening [EN 58959, 1997] with an illumination level of less than 50 lux [EN 12464-1].

Air temperature, humidity, air speed and ambient radiation temperature are variables which influence the heat exchange of the human body with the surroundings. Well-being, health and efficiency of the user can only be guaranteed if the above variables remain within a certain range. Target values are, e.g. a room temperature of 20 °C and relative humidity of approx. 60 % [DIN 33403-2].

The microscope workstation must be separate from the general laboratory area to enable uninterrupted work, in particular for medium and long periods of work. The workstation must be free of dust and acidic vapors which could impact the proper performance of the microscope, Each microscope workstation must be configured to permit adequate storage of the materials required for the examination and work regulations. Laboratory workbenches must be installed so as to enable vibration-free work on the microscope [EN 58959, 1997; EN 12464-1]. Minimum leg space must be provided beneath the workbench [see Section 6 in EN 527-1:2000; DIN EN 13150].

Individual adjustments must be possible at sitting workplaces with a fixed working height at least by means of height-adjustable chairs with foot supports [DIN 33406: Workplace dimensions in production]. A height-adjustable workbench is even better.

The sitting position should adjusted so that the underarms rest horizontally on the work surface and the upper arms are relaxed. It should not be necessary to raise the shoulders or bend over. The upper part of the body should be as upright as possible [DIN EN 1335-1: Office work chairs]. The seat and backrest of the office swivel chair should satisfy ergonomic requirements and support dynamic sitting postures [TÜV 2PfG974: Ergonomic requirements for office swivel chairs]. These requirements must be satisfied for users of different sizes by means of a height-adjustable chair.



Fig. 3-33 Ergonomic adjustment of the microscope

3.5.4 Ergonomic adjustment of the microscope

Individual adjustment of the microscope, in particular the eyepiece, to different body sizes must be possible. This is referred to as "static ergonomics". In particular, the correct eyepiece height must be achieved with a height-adjustable tube which supports an ergonomic sitting position. The fine adjustment of the viewing height may also be achieved in part by swiveling the eyepiece sockets. Ideally, however, it should be a combination of continuous height adjustment and swiveling range.

To relieve the neck and shoulder muscles the forward inclination of the head should not exceed approx. 30° from the horizontal. On the other hand, the angle should not be less than 8°, as this corresponds to the natural head posture and viewing direction of the eyes in a relaxed state.

Individual adjustment of the viewing angle and/or height also permits dynamic variation of the working posture. This is referred to as "dynamic ergonomics". For example, in the TÜV-certified ergonomic configuration, including the comfortable ergo tube (425522-9040-000), the eyepiece viewing height and angle are continuously variable to cover the range from the 5th percentile female to the 95th percentile male. When using other ergo tubes from the Axio Lab.A1 program the coverage of the setting range is somewhat smaller.

In all cases it is important that the user can adjust his/her sitting position and eyepiece viewing height according to individual needs. The sitting position should adjusted so that the underarms rest horizontally on the work surface and the upper arms are relaxed. It should not be necessary to raise the shoulders or bend over. The upper part of the body should be as upright as possible [DIN EN 1335-1: Office work chairs]. The seat and backrest of the office swivel chair should satisfy ergonomic requirements and support dynamic sitting postures [TÜV 2PfG974: Ergonomic requirements for office swivel chairs]. Accordingly, eyepiece viewing should be achieved by swinging the binocular part up or down for fixed observation tubes or by continuous adjustment of an inclinable eyepiece and/or viewing angle for ergo tubes. The viewing angle should in this case be between 8° and approx. 30°. The eyepiece viewing height should be selected to enable the user to assume a relaxed, upright posture. The static muscle work of the user's musculoskeletal system should be reduced as far as possible, to minimize the risk of muscle strain in the back and neck. In addition, insofar as he/she is working on the variable ergo tube, the user should slightly vary the settings from time to time in order to reduce muscle strain caused by extended periods of work.

The TÜV ergonomically certified configuration of the Axio Lab.A1 provides optimum conditions. Other Axio Lab.A1 configurations, in particular those equipped with the ergo stage and/or ergo tube, likewise enable a wide range of adjustments to maximize ergonomics at the microscope workstation. These ergonomic design considerations should always be taken into account when setting up a microscope workstation in the laboratory. The longer the user works continuously at a microscope workstation, the more important these considerations are.



Fig. 3-34 Setting the interpupillary distance on the binocular tube



Fig. 3-35 Setting the viewing height on the binocular tube

3.5.5 Setting interpupillary distance on the binocular tube

• Adjusting individual interpupillary distance by symmetrical swiveling of the two eyepiece sockets against each other (Fig. 3-34).

The correct interpupillary distance has been set when the observer sees only **one** round image when looking into the eyepiece!

3.5.6 Setting the viewing height

• The viewing height can be adjusted to individual requirements by swiveling the eyepiece sockets up (Fig. 3-35/**A**) or down (Fig. 3-35/**B**).

This individual height adjustment in two stages (upper and lower position) is basically possible with all tubes of the Axio Lab.A1 program. The viewing height difference thus achieved depends on the selected interpupillary distance and the tube viewing angle, which may be stationary or variable, depending on the model. With an interpupillary distance of 65 mm and a tube viewing angle of 30°, this adjustable height difference is approx. 40 mm.

Binocular ergo (photo) tubes (425511-0000-000, 425512-0000-000, 425514-0000-000, 425520-9050-000) are provided with continuous height adjustment in the range 44 mm or 50 mm.

The binocular part of the ergo photo tube 425520-9050-000 is also continuously horizontally retractable up to 50 mm.

The eyepoint angle of binocular ergo (photo) tubes (425522-9020-000 and 425522-9030-000) is continuously adjustable in the range 8° to 38°.

The comfortable binocular ergo tube (425522-9040-000) is continuously height-adjustable by up to 50 mm and angle-adjustable from 8° to 33°. This is the ergo tube which received the highest distinction and recommendation in terms of ergonomics at the microscope workstation within the scope of the TÜV certificate of economics.

3.5.7 Adjusting for ametropia when using eyepiece reticles

The prerequisite for correct use of an eyepiece reticle is two adjustable eyepieces to compensate for different degrees of ametropia of the user.

- Focus on the line figure of the eyepiece reticle with the focusable eye lens of the adjustable eyepiece.
- Focus on the microscopic image of a loaded specimen with the focusing drive while observing with the eyepiece containing the eyepiece reticle.
- When both the microscopic image and the eyepiece reticle are in focus, the image for the second eye is brought into focus with the focusable eye lens of the second eyepiece.

Both microscopic images including the eyepiece reticle are thus in focus. Focusing work should now be carried out only by means of the focusing drive.

4 OPERATION

4.1 Lighting and contrasting method in transmitted light

4.1.1 Adjusting the transmitted light brightfield according to KÖHLER

(1) General principle

Transmitted light brightfield microscopy is the most common optical microscopy method, as highcontrast or colored specimens (e.g. blood smears) can be viewed quickly and easily.

For a true-to-object imaging, indirect ray bundles, i.e. ray bundles diffracted and scattered on the specimen details, are of major importance in addition to the so-called direct ray bundles. The higher the portion of indirect bundles of rays (aperture), the more realistic the microscopic image according to ABBE will be.

To make use of the microscope's full optical performance, and in particular that of the objective, the condenser, luminous-field diaphragm and aperture diaphragm should be adjusted in line with the requirements for KÖHLER illumination. These fundamental rules of microscope adjustment are described in detail below in Section 4.1.1 (3) "Setting transmitted light brightfield according to KÖHLER".

(2) Instrumentation for transmitted light brightfield

The equipment of every Axio Lab.A1 microscope, except the stand for reflected light, allows the transmitted light brightfield method to be used.

All available condensers, except special condensers such as darkfield condensers, can be used for the transmitted light brightfield.

(3) Adjusting the transmitted light brightfield according to KÖHLER

- The Axio Lab.A1 has been started up correctly (see Section3).
- The Axio Lab.A1 is switched on.
- Adjust image brightness with controller for light intensity (Fig. 4-1/2) on microscope stand.
- Insert a high-contrast specimen into the specimen holder of the mechanical stage.
- If condensers with a swiveling front lens are used, swivel these in with ≥ 10x objectives and set the condenser with drive head for height adjustment (Fig. 4-1/3 or Fig. 4-2/2) to the upper stop. The stop must be adjusted so that the specimen is not lifted by the condenser (for setting the condenser stop see Section 4.1.1 (4)).
- When using condensers with a turret/modulator disk and knurled ring (Fig. 4-2/3) set position H (brightfield).
- Swivel in 10x objective on nosepiece (Fig. 4-1/6) and focus with drive knob (Fig. 4-1/2) on the specimen.
- Close luminous-field diaphragm (Fig. 4-1/**5**) until it is visible (even if not in focus) in the field of view (Fig. 4-1/**A**).
- Turn the vertical control of the condenser drive to lower the condenser until the edge of the luminous-field diaphragm appears in focus (Fig. 4-1/**B**).
- Center the luminous-field diaphragm using the two centering screws (Fig. 4-1/**4**) on the condenser carrier (Fig. 4-1/**C**) and then open the luminous-field diaphragm until the edge of the diaphragm just disappears from the field of vision (Fig. 4-1/**D**).
- For aperture diaphragm adjustment (contrast) remove an eyepiece from the tube socket and look into the socket with the naked eye. Set the aperture diaphragm with the adjusting lever (Fig. 4-2/4) to approx. 2/3 ... 4/5 of the diameter of the exit pupil of the objective (Fig. 4-1/E). In most applications, this aperture diaphragm setting provides optimum contrast at almost ideal resolution, and is therefore the best compromise for the human eye.
- Reinsert the eyepiece into the tube socket.



g. 4-1 Microscope settings in transmitted light brightfield

Every change of objective will result in a change in specimen field size and objective aperture, together with a possible slight change in centering, so that for optimum results the luminous-field and aperture diaphragm adjustments must be repeated.

With objectives < 10x the front lens of the condenser (if swivelable) must be folded out and the aperture diaphragm completely opened. For better contrast with such large object fields, the luminous-field aperture can be used by reducing its aperture by a certain amount. Overclosing should be avoided so as not to impair the uniformity of the illumination of the field of view.



Fig. 4-2 Setting the height stop on the condenser carrier

(4) Setting the height stop on the condenser carrier

- Loosen the fastening screw (Fig. 4-2/1) of the height stop using a SW 3 ball-headed screwdriver.
- Use focusing drive to focus on the specimen.
- Close luminous-field diaphragm and focus it by turning the vertical control (Fig. 4-2/**2**) of the condenser.
- Carefully raise the condenser slightly without lifting the specimen.
- Tighten fastening screw (Fig. 4-2/1) of height stop.

4.1.2 Adjusting the transmitted light darkfield according to KÖHLER

(1) General principle

Due to their transparency, unstained biological specimens, such as bacteria or living cell cultures, are often barely or not at all visible in transmitted light bright field. This is radically changed when such specimens are observed in the transmitted light darkfield. In principle, the specimen is exposed to light from an illumination aperture which is larger than that of the objective used.

In dark field, only the diffracted and scattered light components, which are important for imaging, reach the objective, while the direct unchanged light bundles are directed past the objective. This is one of the reasons why even fine structures can be resolved, which are sometimes below the resolving power of the light microscope and which appear very bright on a dark background.

(2) Instrumentation

All Axio Lab.A1 microscopes, except stands for reflected light, are suitable for darkfield applications. Condenser with darkfield stop in position **D** e.g.:

- Condenser 0.9/1,25 H with modulator disk H, D, Ph 1, Ph 2, Ph 3,
- Condenser, achrom.-aplan. 0.9 H D Ph DIC,
- Darkfield condenser with dry darkfield,
- Ultra condenser.

(3) Adjusting the transmitted light darkfield

- Adjust the brightness according to KÖHLER analogously to the method for the transmitted light brightfield. Instead of the 10x objective, however, swivel in the objective with the highest aperture which does not exceed the limit aperture for the darkfield with the condenser used.
- Position the turret/modulator disk of the condenser at **D** and swivel in the condenser front lens (if existing).
- Remove the eyepiece from the tube (or replace with auxiliary microscope) and check the centering of the darkfield diaphragm in the exit pupil of the objective. If the central darkfield stop D in the universal condenser is partly outside of or de-centered to the exit pupil of the objective, and if the exit pupil is not homogeneously dark, the darkfield stop must be re-centered.
- To center the darkfield stop (not possible with all condensers), use the two SW 1.5 Allen screwdrivers (Fig. 4-3/1 and 4) to turn the two centering screws (Fig. 4-3/2 and 3) until the exit pupil of the objective appears homogeneously dark. After centering, remove both SW 1.5 screwdrivers from the condenser.
- Since the apertures of objectives with an integrated aperture iris stop are too high for transmitted light dark field, the aperture iris stop must at least be closed to the limit aperture of 0.65.

The performance criterion for the darkfield method is always the darkest possible background of the field of view.



Fig. 4-3 Center darkfield stop on condenser, achromatic-aplanatic 0.9 H D Ph DIC

- Reinsert the eyepiece into the tube.
- If the height of the darkfield condenser is set correctly and sensitively, it is possible to reduce any brightening in the field of view left, and the luminous-field diaphragm image appears almost perfectly in focus.
- Finally, match the size of the luminous-field diaphragm to the size of the field of view.

Dark field microscopy requires specimens to be considerably cleaner than in other techniques. In particular fingerprints, dirt or dust particles have a negative effect, as they brighten the background of the field of view and decrease the contrast of the object image.

4.1.3 Setting the transmitted light phase contrast

(1) General principle

The phase contrast technique is ideal for examining thin, unstained specimens such as cultured cells. Generally, the human eye is unable to perceive phase differences (index and thickness differences) between the different cell components.

The phase contrast technique uses "phase stop and phase ring" optical modulators and interference procedures in forming the intermediate image in order to transform small phase differences into differences in intensity and color which are visible to the human eye.

High-intensity, direct light components are attenuated with the optically defined ring channel "phase stop and phase ring" and given a constant phase shift. The indirect light components diffracted at different cell components, however, bypass this optical channel and are influenced in phase by the refractive index and the thickness differences in the specimen.

Interference in the intermediate image level occurs due to the differently influenced partial beams, and these strengthen or weaken according to phase position. This interference results mainly in image contents displaying differences in intensity which can be perceived by the human eye.

(2) Instrumentation

All Axio Lab.A1 microscopes, except stands for reflected light, are suitable for phase contrast applications.

- Phase contrast objectives with phase rings Ph 1, Ph 2 or Ph 3 for different average numerical apertures which can also be used in the brightfield.
- Condenser with turret/modulator disk containing centering phase stops Ph 1, Ph 2 and Ph 3 for different average numerical apertures.
- The phase stop used on the condenser must correspond to the label on the objective used, e.g. Ph 1.

(3) Setting transmitted light phase contrast

- Swivel the phase contrast objective, e.g. labeled **Ph 1**, into the beam path.
- On the turret disk of the condenser, swivel in the phase stop to match the phase contrast objective, e.g. 1.
- To check the centering and the 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. Using the centering telescope's correction facility, focus on the phase stop and the phase ring in the exit pupil of the objective.
- If congruence is not perfect (Fig. 4-4/A), use the two SW 1.5 hexagonal screwdrivers (Fig. 4-3/1 and 4) to turn the two centering screws (Fig. 4-3/2 and 3) to recenter the bright phase stop until complete congruence with the dark phase ring is achieved (Fig. 4-4/B).
- Finally, remove the auxiliary microscope from the tube and reinsert the eyepiece.

To enhance the image contrast, an interference wide-band filter, green 32×4 , can be inserted on the luminous-field diaphragm or in the color glass holder (if existing).

Perfect phase contrast is only achieved if the bright phase stop (in the condenser) and the dark phase plate (in the objective) are precisely congruent in the illumination beam path (Fig. 4-4/**B**).



Fig. 4-4 Centering the phase stop (bright ring in condenser) to the phase ring (dark ring in objective)

4.1.4 Setting transmitted light polarization

4.1.4.1 Detecting birefringence

(1) Application

The transmitted light polarization method is used for specimens which change the state of polarization of light. These specimens, such as crystals, minerals or polymers, are referred to as birefringent. When these birefringent substances are viewed between crossed polarizers (polarizer \perp analyzer), they appear bright while their surroundings remain dark.

Birefringent substances are identified in that they show four bright and four dark positions when rotated through 360° between crossed polarizers. Depending on birefringence, thickness and orientation of the specimen, interference colors ranging from gray (mostly with biological specimens) to white, yellow, red and blue appear in this process. These interference colors can be of the first or any higher order.

(2) Instrumentation

Polarization methods can be used in the transmitted light on Axio Lab.A1 microscopes for transmitted light polarization and conoscopy.

- Tension-free objectives
- Rotary stage Pol
- Polarizer D (rotatable or fixed)
- Analyzer slide D, fixed, or lambda or lambda/4 compensator
- Depolarizer (for screwing into Axio Lab.A1 tubes) to avoid undesirable polarization effects

The depolarizer is already incorporated in the Axio Lb.A1 stand for conoscopy.

A depolarizer (quartz depolarizer) should be installed in all microscopes used for examining mineral/geological specimens.

A depolarizer suppresses undesirable polarization effects which may occur after the analyzer (e.g. on prism surfaces in the tube), or shifts these to higher orders.

(3) Setting the microscope

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1 (3)).
- Center rotary stage Pol (Fig. 4-5/1) (see Section 3.1.8.5) and objectives (see Section 3.1.8.6).
- Swivel polarizer (Fig. 4-5/**3**) into the light path and, if it is rotatable, position it at 0°.
- Insert the analyzer slider (Fig. 4-5/2) into the slit for compensators (if tube does not already have an analyzer). The field of view will appear dark due to the crossed polarizers. With screw-on analyzers, care must be taken to ensure that they are aligned with polarizer D (i.e. crossed position).
- Move the specimen into the field of view and turn it with the rotary stage. As a rule, while being rotated between crossed polarizers, birefringent (anisotropic) specimens will now display the variations in color and intensity described above. However, optically anisotropic substances may also remain dark, if an isotropic direction, e.g. of optically uniaxial or biaxial crystals, is oriented parallel to the direction of observation.



Fig. 4-5 Components for transmitted light polarization

Determination of gout and pseudogout 4.1.4.2

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1 (3)).
- Then swivel the polarizer, rigidly fixed to the lambda plate (445226-0000-000) (Fig. 4-5/3) into the beam path.
- Insert the analyzer slider (Fig. 4-5/2) into the slit for compensators.
- The field of view will appear dark due to the crossed polarizers.
- Swivel the rotatable lambda plate into the beam path and set the metal adjusting lever of the lambda plate to 45°.

The gamma direction is orthogonal to the position of the level and is indicated by a white line on the top of the lambda plate.

The 45° position is at the third white marking on the scale. The scale graduation from one R S marking to the next is 15°. The 45° position is at the third marking, calculated from the 0° marking. As a further reference point for the correct position of the lever, the Greek letter λ has been engraved on the upper side of the lambda plate, likewise at the 45° position.



Fig. 4-6 Gamma direction

• Select crystals which are oriented in the gamma direction (Fig. 4-6).

Evaluation:

If the crystal needles parallel to the gamma direction are yellow and those perpendicular to the gamma direction are blue, they are monosodium urate crystals (gout).

If the crystal needles parallel to the gamma direction are blue and those perpendicular to the gamma direction are yellow, they are calcium pyrophosphate crystals (pseudogout).

Alternatively, a combination of fixed polarizer (427701-0000-000) and analyzer with fixed 13 lambda plate, 45° (453681-0000-000) can also be used. This offers the advantage that the lambda plate is pre-set to 45°, precluding the risk of incorrect setting. The evaluation is performed in the same way as described above.

4.1.4.3 Determining the direction of oscillation n_γ

(1) Application

The determination of the direction of oscillation of $n\gamma$ and $n\gamma'$ (direction of oscillation with the absolutely or relatively highest refractive index) and n_{α} and $n_{\alpha'}$ (direction of oscillation with the absolutely or relatively lowest refractive index) in relation to the morphological directions, e.g. of crystal surfaces, crystal needles or fibers, provides an important criterion for recognition. It is also employed for the diagnosis of biocrystals (e.g. gout, pseudogout).



Fig. 4-7 Determining the direction of oscillation $n\gamma$ using a synthetic fiber as an example

(2) Instrumentation

- Eyepiece with crossline reticle
- Tension-free objectives
- Rotary stage Pol (Fig. 4-5/1)
- Polarizer D (rotatable or fixed)
- Screw-in fixed analyzer slide D or lambda/lambda4 compensator combined with analyzer (in Axio Lab.A1 tubes)
- Alignment specimen for polarization microscope (453679-0000-000)

(3) Setting the microscope

- Set the microscope as for the transmitted light brightfield (see Section 4.1.1), taking care to ensure the correct interpupillary distance in the binocular tube (see Section 3.5.5).
- Center rotary stage Pol (Fig. 4-5/1) and objectives (see Sections 3.1.8.5 and 3.1.8.6).
- Swivel polarizer (Fig. 4-5/3) into the light path and, if it is rotatable, position it at 0°.
- Insert the analyzer slider (Fig. 4-5/2) into the slit for compensators (if tube does not already have an analyzer). The field of view will appear dark due to the crossed polarizers. If not, align the analyzer in the tube or the intermediate plate.
- Set the alignment specimen Pol on the microscope stage and turn to the dark position of the alignment specimen.
- Remove the analyzer and align the crossline reticle with the cracks in the specimen.
- Subsequently reinsert the analyzer and remove the alignment specimen. The pass directions of the polarizer and analyzer will now be parallel to the crossline reticle (polarizer EW, analyzer NS).

R

An adjustment of the crossline reticle is not necessary when working with the intermediate plate and the binocular photo tube Pol (425520-9100-000).

• Rotate the rotary stage Pol with the specimen, e.g. a synthetic fiber, until the specimen appears as dark as possible. In this position, the fiber extends parallel to one of the two directions of the crossline reticle.

Do not change the interpupillary distance on the binocular tube, as the the angle of the crossline reticle to the fiber will be changed.

- Now turn the stage on by 45° so that the longitudinal axis of the fiber is oriented NE-SW (Fig. 4-8). The specimen will display the greatest brightness here (diagonal position). In this position the specimen may have any color.
- Insert the compensator λ (only possible if used with screw-in analyzer in tube or intermediate plate).

Like the specimen, the compensator λ is a birefringent object, albeit with a defined path difference of 550 nm and the principal direction of oscillation n_y definitely oriented in a NE-SW direction.

By moving compensator λ into the light path, the specimen changes its color. The type of color change depends on the orientation of the specimen (NE-SW or NW-SE).

The changes in color are attributable to optical interference. The interference colors (path differences) in both diagonal positions (NE-SW and NW-SE) of the specimen must be compared in this connection.

The path difference results from the superposition (interference) of the direction of oscillation of the specimen with the direction of oscillation of the compensator λ .

The greater path difference occurs, if the direction of oscillation of the specimen with the absolutely or relatively highest refractive index (n_{γ} or $n_{\gamma'}$) is parallel to the principal direction of oscillation of the compensator λ . The specimen will then appear, for instance, in greenish-blue (Fig. 4-7/**2**).

The smallest path difference occurs, if the direction of oscillation of the specimen with the absolutely or relatively lowest refractive index (n_{α} or $n_{\alpha'}$) is perpendicular to the direction of oscillation of the compensator λ . The specimen will then appear, for instance, in yellow (Fig. 4-7/**3**).

R

(4) Conclusions

The grayish-white color appearing first in the bright position in the above example (Fig. 4-7/1) corresponds to a path difference of 150 nm according to the Michel-Lévy color chart (Fig. 4-8).

When the compensator λ is brought into the light path, the non-birefringent "surroundings" of the synthetic fiber appear in a dark red color, which corresponds to the path difference of the compensator of 550 nm (1st order interference color for the path difference of 550 nm corresponds to 1 λ).

If the direction of oscillation $(n_{\gamma} \text{ or } n_{\gamma'})$ of the birefringent specimen to be examined is parallel to the principal direction of oscillation (n_{γ}) of the compensator λ , i.e. in NE-SW direction, the path difference of the specimen (e.g. grayish-white: 150 nm) and the path difference of the compensator λ (red: 550 nm). This results in a color change of the specimen from grayish white to greenish-blue (resulting path difference = 700 nm).



If the direction of oscillation of the specimen to be examined is perpendicular to the principal direction of oscillation of the compensator λ , i.e. in NW-SE direction, the path difference of the compensator λ (e.g. grayish-white: 150 nm) is subtracted from the path difference of the compensator (red: 550 nm). In this case, the interference color of the specimen visibly changes from grayish-white to orange (resulting path difference = 400 nm).

Color charts according to Michel-Lévy are available under Cat. No. 42-312.

4.1.4.4 Measuring path differences

The measurement compensators are required for exact measurement of path differences. These return, i.e. compensate, the path difference created by the specimen to zero (black of the first order).

Whereas in the above-described methods the addition or subtraction position was of interest, **solely** the subtraction position is of interest in the measurement.

Path differences in the specimen can assume very small values (1/50 λ or 10 nm) and very great values (more than 10 λ or approx. 5500 nm and more) and with that determine the compensator appropriate for the measurement.

The suitable compensator is determined as follows:

- Set the microscope as for the transmitted light brightfield (see Section 4.1.1), taking care to ensure the correct interpupillary distance in the binocular tube (see Section 3.5.5).
- Accurately position the specimen to be examined on the center of the crosshairs.

- Limit to aperture to a value of about 0.2.
- Turn the rotary stage Pol until the specimen is almost obliterated, i.e. **completely dark**, and set the 45° locking position.
- Rotate the stage **once** (by 45°) so that the specimen is in a diagonal position (bright).

The interference intensity or color leads to the following conclusion:

 If more or less strong interference colors appear on the specimen, the path difference ranges approximately between 1/2 λ and 5 λ. The suitable compensator is:

Tilting compensator **B O E 1**

Tilting compensator B 0-5 λ .

- If the specimen-side color changes from light gray/white to a strong interference color, when a compensator λ (473704-0000-000) is inserted in the compensator slot, the path difference is (1/4 ...1/2) λ .
- A prerequisite for occurrence of the color change effect may be the evaluation in two specimen positions rotated at an angle of 90° from one another, plus a centered stage.

The suitable compensator is:

Tilting compensator B 0-5 λ or the compensation method according to DE SENARMONT up to 1 λ using the Senarmont compensator 546/4 nm.

- The compensation method according to DE SENARMONT requires the use of the rotatable analyzer.
- After insertion of the compensator λ and rotation of the object by 90°, the interference color remains white; in this case, however, it is a "higher-order white" and thus the path difference is > 5 λ . The suitable compensator is:

Tilting compensator K 0-30 λ (Accessory 000000-1115-698)

 A dark gray as appearing interference color suggests very small path differences (λ/10 or 54.6 nm). The suitable compensator is:

Brace–Köhler rotary compensator λ/10 (Accessory 000000-1115-703).

• Insert the compensator into the slit as far as it will go.

The accompanying instructions must be observed for measurement preparation and procedure.

4.1.4.5 Circular polarization contrast

(1) Application

Unlike standard polarization contrast, circular polarization contrast does not show any dark (extinction) positions that depend on the angle of rotation (azimuth) of the specimen relative to polarizer or analyzer. This means that when the stage is rotated the same image impression remains, as the light/dark positions are omitted. With optical anisotropy, all transparent specimens display the characteristic interference colors.

(2) Instrumentation

- Tension-free objectives
- Rotary stage Pol
- Circular polarizer D (no polarizers may be adapted on the condenser) including corresponding $\lambda/4$ plate.
- Stationery analyzer slide D or screw-in analyzer (in Axio Lab.A1 tubes).

(3) Setting the microscope

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1).
- Center rotary stage Pol or objective (if this has not already been done see Section 3.1.8.5 or 3.1.8.6).
- Initially do **not** use a specimen for the further settings.
- Swivel in the lower part of circular polarizer D (Fig. 4-9/2) into the beam path until it engages and evaluate the extinction (darkening) of the field of view without the specimen at full light intensity. If this is not optimum, align the analyzer in the tube or intermediate plate as necessary.
- Insert the respective slide 6x20 with $\lambda/4$ plate (Fig. 4-9/1) as far as it will go into the slot for compensators above the nosepiece.
- Then swivel the upper part of circular polarizer D (Fig. 4-9/4) into the beam path.
- Rotate the lever of the $\lambda/4$ plate of the circular polarizer D (Fig. 4-9/3) until the extinction is maximum (dark-gray field of view) (lever points 45° to the right).



- **1** Slide 6x20 with $\lambda/4$ plate
- 2 Lower section of circular polarizer
- **3** Lever for rotating $\lambda/4$ plate
- 4 $\lambda/4$ plate in upper part of the circular polarizer
- 5 Adjustment slits

Fig. 4-9 Components for circular polarization contrast

- An (anisotropic) specimen should not be observed until after the above adjustment.
- Reinsert the specimen to be examined.

In their interference color – dependent on material, specimen thickness and orientation – the specimens appear constant and independent of stage rotation.

For a high-contrast image with higher objective enlargements (from approx. 20x) the illumination aperture must be reduced to a value between 0.15 and 0.20, i.e. the aperture diaphragm must be closed accordingly.

The effect of the $\lambda/4$ plate (Fig. 4-9/4) can be undone by either swiveling it out of the light path or turning it with lever (Fig. 4-9/3) into one of its two click-stop positions.

4.1.5 Setting transmitted light polarization with the conoscopy stand

4.1.6 Determining the optical character of crystals

For the classification (and thus identification) of crystalline material – instead of the observation of the specimen itself – the analysis of an interference image in the objective pupil provides the more valuable information. This image is visible in the eyepiece when an additional lens (so-called Bertrand lens) is switched on. Alternatively, the auxiliary microscope or a diopter may be used to view the interference image.

In contrast to orthoscopy, this is referred to as conoscopy, because the illumination is ideally provided by a wide open cone. In practice this means that the aperture diaphragm is fully open and the objective should likewise have a high aperture.

(1) Application

The crystal diagnosis is for determining the optical character of transparent and weakly absorbent crystals. This method is also referred to as conoscopy.

Its main application is the classical mineral microscopy. However, synthetic crystals, industrial minerals and plastics (e.g. films) can also be identified and characterized.

(2) Instrumentation

Conoscopic viewing is preferably carried out on the Axio Lab.A1 microscope for transmitted light conoscopy.

- Tension-free objectives; recommended:

N-Achroplan 50x/0.8 Pol objective or EC Plan-Neofluar 40x/0.9 Pol objective

- Rotary stage Pol
- Polarizer D (rotatable or fixed)
- 0.9 Pol condenser

(3) Setting the microscope for conoscopy

In the case of uniaxial crystals, the most favorable orientation for conoscopic viewing is obtained with those specimen features (e.g. of a thin section) that in orthoscopic viewing change the brightness as little as possible. In this case, the direction of viewing and the optical axis are parallel. The same applies to biaxial crystals if viewed in or approximately in the direction of one of the two optical axes.



Fig. 4-10 Axio Lab.A1 for transmitted light conoscopy

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1).
 - Place the specimen on the stage and focus on it.
- Swivel the analyzer into the beam path (**on** position) with rotary knob **A** (Fig. 4-10/**2**). The direction of oscillation can be changed using the setting wheel (Fig. 4-10/**4**) of the analyzer.



CAUTION

The movements of rotary knobs **A** and **BL** and the respective setting wheels are coupled with one another. Only **one** control element should therefore be operated at a time and the movement of the other should not be inhibited or blocked. Mechanical damage may otherwise occur.

If rotary knob **BL** is set to the **on** position, if it is not already at the **on** position rotary knob **A** is automatically carried.

If, on the other hand, rotary knob **A** is set to the **off** position, if it is not already at the **off** position rotary knob **BL** is automatically carried.

- Place a selected crystal in the center of the crossline reticle.
- Swivel in objective N-Achroplan 50x/0.8 Pol or EC Plan-Neofluar 40x/0.9 Pol and focus with the focusing drive.
- If necessary, close the luminous-field aperture to avoid superimposition of the axial figure by axial figures of neighboring crystals. The smallest crystal range that can be faded out is 170 µm.
- Switch on Bertrand lens **BL** (Fig. 4-10/**1**) (Position **on**). The axial figure will appear in the field of view.
- Bring the axial figure into focus with setting wheel (Fig. 4-10/5).

(4) Evaluation

Crystalline anisotropic specimens can be separated into optical uni- and biaxial, in each case with "optically positive" or "negative" character.

Uniaxial crystals display a **black cross** when the optical axis is parallel to the direction of view. **Depending on the size of the birefringence and specimen thickness**, concentrically arranged colored **interference rings** (so-called isochromes) may appear (see also Fig. 4-11 second row).

This cross remains closed when the stage is rotated. Depending on the section it may lie within or outside the displayed objective pupil.

With **optically biaxial** crystals, the cross resolves into two **dark hyperbola branches** (the so-called isogyres) **depending on stage rotation**, which are surrounded by colored interference patterns depending on the amount of birefringence and specimen thickness (suggestive of the figure "8").

Inserting a compensator λ (473704-0000-000) or $\lambda/4$ (473714-0000-000) or a wedge compensator 0-4 λ (000000-1140-663) in the compensator slot with the initial state of the axial figure being as illustrated in Fig. 4-11 results in the following changes in color shown schematically (blue and yellow areas) to the axial figure, thus allowing differentiation in "optically positive" and "optically negative".

	Optically	uniaxial	Opticall	y biaxial	
	Positive	Negative	Positive	Negative	
λ -Plate (white \rightarrow blue \rightarrow yellow)	+ + +	+ - +		+	+ = blue – = yellow
Quartz wedge (Direction of motion at insertion)					Direction ofmovement
λ/4 plate (position of black spots)					

Fig. 4-11 Determining optical character

In the case of less favourable sections in which the cross-hair center is optically uniaxial or the isogyres are optically biaxial specimens outside the objective pupil, an assessment is possible as follows:

- If the black isogyres are **straight** and they run parallel to the pupil (in relation to the cross-hairs), the specimen is **optically uniaxial**.
- If the black isogyres are **curved lines** which wander on a circular path through the pupil, the specimen is **optically biaxial**.

Paying appropriate attention, also such axial figures not illustrated in Fig. 4-11 can be interpreted.

- **C** Axial figures can often be better displayed with circular polarization. Particularly the axis angle of optically biaxial specimens (quasi distance between the isogyres) can be determined more clearly. The optical character can also be determined. For this purpose the compensator λ (6 x 20), arranged in the compensator slot, is used.
- Two storage compartments for 6x20 slides are located on the reverse side of the conoscopy stand.

4.1.6.1 Demonstrating birefringence with the Axio Lab for conoscopy

(1) Application

The transmitted light polarization method is used for specimens which change the state of polarization of light. These specimens, such as crystals, minerals or polymers, are referred to as birefringent. When these birefringent substances are viewed between crossed polarizers (polarizer \perp analyzer), they appear bright while their surroundings remain dark.

Birefringent substances are identified in that they show four bright and four dark positions when rotated through 360° between crossed polarizers. Depending on birefringence, thickness and orientation of the specimen, interference colors ranging from gray (mostly with biological specimens) to white, yellow, red and blue appear in this process. These interference colors can be of the first or any higher order.

(2) Instrumentation

On the Axio Lab.A1 microscope for transmitted light conoscopy:

- Tension-free objectives
- Rotary stage Pol
- Polarizer D (rotatable or fixed)
- Compensator lambda or lambda/4

A depolarizer (quartz depolarizer) should be installed in all microscopes used for examining mineral/geological specimens.

A depolarizer suppresses undesirable polarization effects which may occur after the analyzer (e.g. on prism surfaces in the tube), or shifts these to higher orders.

The depolarizer is already incorporated in the Axio Lb.A1 stand for conoscopy.

(3) Setting the microscope

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1 (3)).
- Center rotary stage Pol (Fig. 4-12/**1**) (see Section 3.1.8.5) and objectives (see Section 3.1.8.6).
- Swivel polarizer (Fig. 4-12/**3**) into the light path and, if it is rotatable, position it at 0°.
- Swivel the analyzer into the beam path and adjust on the setting wheel until the field of view is dark.(Fig. 4-12/2)
- Move the specimen into the field of view and turn it with the rotary stage. As a rule, while being rotated between crossed polarizers, birefringent (anisotropic) specimens will now display the variations in color and intensity described above. However, optically anisotropic substances may also remain dark, if an isotropic direction, e.g. of optically uniaxial or biaxial crystals, is oriented parallel to the direction of observation.



Fig. 4-12 Components for transmitted light polarization on conoscopy stand

4.1.6.2 Determination of gout and pseudogout

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1 (3)).
- Swivel the polarizer, rigidly fixed or rotatable (Fig. 4-12/**3**) into the beam path. Set the rotatable polarizer to the 0° position.
- With the Axio Lab for polarization push the analyzer (453681-0000-000) into the 6x20 slider mount. (Fig. 4-12/**2**)
- With the AxioLab for conoscopy swivel the analyzer into the beam path and bring it into a crossed position using the setting wheel. Additionally, insert compensator 6x20 (473704-0000-000) into the slider mount 6x20.
- The field of view will appear dark due to the crossed polarizers.
- Select crystals which are oriented in the gamma direction (Fig. 4-13).



Fig. 4-13 Gamma direction

Evaluation:

If the crystal needles parallel to the gamma direction are yellow and those perpendicular to the gamma direction are blue, they are monosodium urate crystals (gout).

If the crystal needles parallel to the gamma direction are blue and those perpendicular to the gamma direction are yellow, they are calcium pyrophosphate crystals (pseudogout).

4.1.6.3 Determining the direction of oscillation n_γ

(1) Application

The determination of the direction of oscillation of n_{γ} and $n_{\gamma'}$ (direction of oscillation with the absolutely or relatively highest refractive index) and n_{α} and $n_{\alpha'}$ (direction of oscillation with the absolutely or relatively lowest refractive index) in relation to the morphological directions, e.g. of crystal surfaces, crystal needles or fibers, provides an important criterion for recognition. It is also employed for the diagnosis of biocrystals (e.g. gout, pseudogout).



Fig. 4-14 Determining the direction of oscillation $n_{\gamma'}$ using a synthetic fiber as an example

(2) Equipment configuration for Axio Lab for conoscopy

- Eyepiece with crossline reticle
- Tension-free objectives
- Rotary stage Pol (Fig. 4-12/1)
- Polarizer D (rotatable or fixed)
- Compensator lambda or lambda/4 as required
- Alignment specimen for polarization microscope (453679-0000-000)

(3) Setting the microscope

- Set the microscope as for the transmitted light brightfield (see Section 4.1.1), taking care to ensure the correct interpupillary distance in the binocular tube (see Section 3.5.5).
- Center rotary stage Pol (Fig. 4-5/1) and objectives (see Sections 3.1.8.5 and 3.1.8.6).
- Swivel polarizer (Fig. 4-5/3) into the light path and, if it is rotatable, position it at 0°.
- Swivel the analyzer into the beam path and bring it into a crossed position using the setting wheel (Fig. 4-5/2). The field of view will appear dark due to the crossed polarizers.
- Set the alignment specimen Pol on the microscope stage and turn to the dark position of the alignment specimen.
- Swivel out the analyzer and align the crossline reticle with the cracks in the specimen.
- Subsequently swivel the analyzer back in and remove the alignment specimen. The pass directions of the polarizer and analyzer will now be parallel to the crossline reticle (polarizer EW, analyzer NS).

- Rotate the rotary stage Pol with the specimen, e.g. a synthetic fiber, until the specimen appears as dark as possible. In this position, the fiber extends parallel to one of the two directions of the crossline reticle.
- Do not change the interpupillary distance on the binocular tube, as the the angle of the crossline reticle to the fiber will be changed.
- Now turn the stage on by 45° so that the longitudinal axis of the fiber is oriented NE-SW (Fig. 4-15). The specimen will display the greatest brightness here (diagonal position). In this position the specimen may have any color.
- Inserting compensator λ (473704-0000-000).

Like the specimen, the compensator λ is a birefringent object, albeit with a defined path difference of 550 nm and the principal direction of oscillation n_{γ} definitely oriented in a NE-SW direction.

By moving compensator λ into the light path, the specimen changes its color. The type of color change depends on the orientation of the specimen (NE-SW or NW-SE).

The changes in color are attributable to optical interference. The interference colors (path differences) in both diagonal positions (NE-SW and NW-SE) of the specimen must be compared in this connection.

The path difference results from the superposition (interference) of the direction of oscillation of the specimen with the direction of oscillation of the compensator λ .

The greater path difference occurs, if the direction of oscillation of the specimen with the absolutely or relatively highest refractive index (n_{γ} or $n_{\gamma'}$) is parallel to the principal direction of oscillation of the compensator λ . The specimen will then appear, for instance, in greenish-blue (Fig. 4-14/**2**).

The smallest path difference occurs, if the direction of oscillation of the specimen with the absolutely or relatively lowest refractive index (n_{α} or $n_{\alpha'}$) is perpendicular to the direction of oscillation of the compensator λ . The specimen will then appear, for instance, in yellow (Fig. 4-14/**3**).

An adjustment of the crossline reticle is not necessary when working with the intermediate plate and the binocular photo tube Pol (425520-9100-000).



Fig. 4-15 Schematic diagram of the color charts according to Michel-Lévy

(4) Conclusions

The grayish-white color appearing first in the bright position in the above example (Fig. 4-14/**1**) corresponds to a path difference of 150 nm according to the Michel-Lévy color chart (Fig. 4-15).

When the compensator λ is brought into the light path, the non-birefringent "surroundings" of the synthetic fiber appear in a dark red color, which corresponds to the path difference of the compensator of 550 nm (1st order interference color for the path difference of 550 nm corresponds to 1 λ).

If the direction of oscillation $(n_{\gamma} \text{ or } n_{\gamma'})$ of the birefringent specimen to be examined is parallel to the principal direction of oscillation (n_{γ}) of the compensator λ , i.e. in NE-SW direction, the path difference of the specimen (e.g. grayish-white: 150 nm) and the path difference of the compensator λ (red: 550 nm). This results in a color change of the specimen from grayish white to greenish-blue (resulting path difference = 700 nm).

If the direction of oscillation of the specimen to be examined is perpendicular to the principal direction of oscillation of the compensator λ , i.e. in NW-SE direction, the path difference of the compensator λ (e.g. grayish-white: 150 nm) is subtracted from the path difference of the compensator (red: 550 nm). In this case, the interference color of the specimen visibly changes from grayish-white to orange (resulting path difference = 400 nm).

R

Color charts according to Michel-Lévy are available under Cat. No. 42-312.

4.1.6.4 Measuring path differences with the Axio Lab for conoscopy

The measurement compensators are required for exact measurement. These return, i.e. compensate, the path difference created by the specimen to zero (black of the first order).

Whereas in the above-described methods the addition or subtraction position was of interest, **solely** the subtraction position is of interest in the measurement.

Path differences in the specimen can assume very small values (1/50 λ or 10 nm) and very great values (more than 10 λ or approx. 5500 nm and more) and with that determine the compensator appropriate for the measurement.

The suitable compensator is determined as follows:

- Set the microscope as for the transmitted light brightfield (see Section 4.1.1), taking care to ensure the correct interpupillary distance in the binocular tube (see Section 3.5.5).
- Accurately position the specimen to be examined on the center of the crosshairs.

- Limit to aperture to a value of about 0.2.
- Turn the rotary stage Pol until the specimen is almost obliterated, i.e. **completely dark**.
- Rotate the stage **once** (by 45°) so that the specimen is in a diagonal position (bright).

The interference intensity or color leads to the following conclusion:

- If more or less strong interference colors appear on the specimen, the path difference ranges approximately between 1/2 λ and 5 λ . The suitable compensator is:

tilting compensator B 0-5 λ .

- If the specimen-side color changes from light gray/white to a strong interference color, when a compensator λ (473704-0000-000) is inserted in the compensator slot, the path difference is (1/4 ...1/2) λ .
- A prerequisite for occurrence of the color change effect may be the evaluation in two specimen positions rotated at an angle of 90° from one another, plus a centered stage.

The suitable compensator is:

tilting compensator B 0-5 λ or the compensation method according to DE SENARMONT up to 1 λ using the Senarmont compensator 546/4 nm.

- The compensation method according to DE SENARMONT requires the use of the rotatable analyzer.
- After insertion of the compensator λ and rotation of the specimen by 90°, the interference color remains white; in this case, however, it is a "higher-order white" and thus the path difference is > 5 λ .

The suitable compensator is:

tilting compensator K 0-30 λ (Accessory 000000-1115-698)

- A dark gray as appearing interference color suggests very small path differences (λ /10 or 54.6 nm). The suitable compensator is:

Brace-Köhler rotary compensator λ/10 (Accessory 000000-1115-703).

• Insert the compensator into the slit as far as it will go.

The accompanying instructions must be observed for measurement preparation and procedure.

4.1.6.5 Circular polarization contrast with Axio Lab for conoscopy

(1) Application

Unlike standard polarization contrast, circular polarization contrast does not show any dark (extinction) positions that depend on the angle of rotation (azimuth) of the specimen relative to polarizer or analyzer. This means that when the stage is rotated the same image impression remains, as the light/dark positions are omitted. With optical anisotropy, all transparent specimens display the characteristic interference colors.

(2) Instrumentation

- Tension-free objectives
- Rotary stage Pol
- Circular polarizer D (no polarizers may be adapted on the condenser) including corresponding $\lambda/4$ plate.

(3) Setting the microscope

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1).
- Center rotary stage Pol or objective (if this has not already been done see Section 3.1.8.5 or 3.1.8.6).
- Initially do **not** use a specimen for the further settings.
- Swivel the analyzer into the beam path.
- Swivel in the lower part of circular polarizer D (Fig. 4-16/2) into the beam path until it engages and evaluate the extinction (darkening) of the field of view without the specimen at full light intensity. If this is not optimum, align the analyzer as necessary.
- Insert the respective slide 6x20 with $\lambda/4$ plate (Fig. 4-16/1) as far as it will go into the slot for compensators above the nosepiece.
- Then swivel the upper part of circular polarizer D (Fig. 4-16/4) into the beam path.
- Rotate the lever of the $\lambda/4$ plate of the circular polarizer D (Fig. 4-16/3) until the extinction is maximum (dark-gray field of view) (lever points 45° to the right).



- **1** Slide 6x20 with $\lambda/4$ plate
- 2 Lower section of circular polarizer
- **3** Lever for rotating $\lambda/4$ plate
- 4 $\lambda/4$ plate in upper part of the circular polarizer
- **5** Adjustment slits

Fig. 4-16 Components for circular polarization contrast on conoscopy stand

- An (anisotropic) specimen should not be observed until after the above adjustment.
- Reinsert the specimen to be examined.

In their interference color – dependent on material, specimen thickness and orientation – the specimens appear constant and independent of stage rotation.

For a high-contrast image with higher objective enlargements (from approx. 20x) the illumination aperture must be reduced to a value between 0.15 and 0.20, i.e. the aperture diaphragm must be closed accordingly.

The effect of the $\lambda/4$ plate (Fig. 4-16/**4**) can be undone by either swiveling it out of the light path or turning it with lever (Fig. 4-16/**3**) into one of its two click-stop positions.

4.1.7 Setting transmitted light polarization for conoscopic observation – determining the optical character of crystals

For the classification (and thus identification) of crystalline material – instead of the observation of the specimen itself – the analysis of an interference image in the objective pupil provides the more valuable information. This image is visible in the eyepiece when an additional lens (so-called Bertrand lens) is switched on. Alternatively, the auxiliary microscope or a diopter may be used to view the interference image.

In contrast to orthoscopy, this is referred to as conoscopy, because the illumination is ideally provided by a wide open cone. In practice this means that the aperture diaphragm is fully open and the objective should likewise have a high aperture.

4.1.7.1 Application

The crystal diagnosis is for determining the optical character of transparent and weakly absorbent crystals. This method is also referred to as conoscopy.

Its main application is the classical mineral microscopy. However, synthetic crystals, industrial minerals and plastics (e.g. films) can also be identified and characterized.

(1) Instrumentation

Conoscopic viewing is preferably carried out on the Axio Lab.A1 microscope for transmitted light conoscopy.

- Tension-free objectives; recommended: N-Achroplan 50x/0.8 Pol objective or EC Plan-Neofluar 40x/0.9 Pol objective
- Rotary stage Pol
- Polarizer D (rotatable or fixed)
- 0.9 Pol condenser

(2) Setting the microscope for conoscopy

In the case of uniaxial crystals, the most favorable orientation for conoscopic viewing is obtained with those specimen features (e.g. of a thin section) that in orthoscopic viewing change the brightness as little as possible. In this case, the direction of viewing and the optical axis are parallel. The same applies to biaxial crystals if viewed in or approximately in the direction of one of the two optical axes.

- Set the microscope as in the transmitted light brightfield according to KÖHLER (see Section 4.1.1).
- Swivel polarizer (Fig. 4-12/**3**) into the light path and, if it is rotatable, position it at 0°.
- Swivel the analyzer into the beam path and bring into a crossed position with the setting wheel. (The field of view will now appear dark)
- Place the specimen on the stage and focus on it.
- Swivel the analyzer into the beam path (**on** position) with rotary knob **A** (Fig. 4-17/**2**). The direction of oscillation can be changed using the setting wheel (Fig. 4-17/**4**) of the analyzer.



Fig. 4-17 Axio Lab.A1 for transmitted light conoscopy



CAUTION

The movements of rotary knobs **A** and **BL** and the respective setting wheels are coupled with one another. Only **one** control element should therefore be operated at a time and the movement of the other should not be inhibited or blocked. Mechanical damage may otherwise occur.

If rotary knob **BL** is set at the **on** position, rotary knob **A** is automatically carried if it is not already in the **on** position.

If, on the other hand, rotary knob **A** is set to the **off** position, if it is not already at the **off** position rotary knob **BL** is automatically carried.

- Place a selected crystal in the center of the crossline reticle.
- Swivel in objective N-Achroplan 50x/0.8 Pol or EC Plan-Neofluar 40x/0.9 Pol and focus with the focusing drive.
- If necessary, close the luminous-field aperture to avoid superimposition of the axial figure by axial figures of neighboring crystals. The smallest crystal range that can be faded out is approx. 170 µm.
- Switch on Bertrand lens **BL** (Fig. 4-17/**1**) (Position **on**). The axial figure will appear in the field of view.
- Bring the axial figure into focus with setting wheel (Fig. 4-17/5).

4.1.7.2 Evaluation

Crystalline anisotropic specimens can be separated into optical uni- and biaxial, in each case with "optically positive" or "negative" character.

Uniaxial crystals display a **black cross** when the optical axis is parallel to the direction of view. **Depending on the size of the birefringence and specimen thickness**, concentrically arranged colored **interference rings** (so-called isochromes) may appear (see also Fig. 4-11 second row).

This cross remains closed when the stage is rotated. Depending on the section it may lie within or outside the displayed objective pupil.

With **optically biaxial** crystals, the cross resolves into two **dark hyperbola branches** (the so-called isogyres) **depending on stage rotation**, which are surrounded by colored interference patterns depending on the amount of birefringence and specimen thickness (suggestive of the figure "8").

Inserting a compensator λ (473704-0000-000) or $\lambda/4$ (473714-0000-000) or a wedge compensator 0-4 λ (000000-1140-663) in the compensator slot with the initial state of the axial figure being as illustrated in Fig. 4-18 results in the following changes in color shown schematically (blue and yellow areas) to the axial figure, thus allowing differentiation in "optically positive" and "optically negative".

	Optically	v uniaxial	Opticall	y biaxial	
	Positive	Negative	Positive	Negative	
λ -Plate (white \rightarrow blue \rightarrow yellow)	+	+ - +		+	+ = blue - = yellow
Quartz wedge (Direction of motion at insertion)					Direction ofmovement
λ/4 plate (position of black spots)					

Fig. 4-18 Determining the optical character

In the case of less favorable sections in which the cross-hair center is optically uniaxial or the isogyres are optically biaxial specimens outside the objective pupil, an assessment is possible as follows:

- If the black isogyres are **straight** and they run parallel to the pupil (in relation to the cross-hairs), the specimen is **optically uniaxial**.
- If the black isogyres are **curved lines** which wander on a circular path through the pupil, the specimen is **optically biaxial**.

Paying appropriate attention, also such axial figures not illustrated in Fig. 4-18 can be interpreted.

- Axial figures can often be better displayed with circular polarization. Particularly the axis angle of optically biaxial specimens (quasi distance between the isogyres) can be determined more clearly. The optical character can also be determined. For this purpose the compensator λ (6 x 20), arranged in the compensator slot, is used.
- Two storage compartments for 6x20 slides are located on the reverse side of the conoscopy stand.

4.2 Lighting and contrasting method in reflected light

4.2.1 Adjusting the reflected light brightfield according to KÖHLER

(1) Application

Reflected light brightfield microscopy is the simplest and most common optical microscopy method for examining opaque samples or specimens, e.g. material sections or wafers.

For a true-to-object imaging, indirect ray bundles, i.e. ray bundles diffracted and scattered on the specimen details, are of major importance in addition to the so-called direct ray bundles. The higher the portion of indirect bundles of rays (aperture), the more realistic the microscopic image according to ABBE will be.

The bundled light emitted by the reflected light unit is reflected on a color-neutral beam splitter and then passes through the objective which focuses the beam on the surface of the sample (so-called condenser function). The objective collects the light reflected by the specimen and together with the tube lens it generates the microscopic intermediate image which can then be visually observed or objectively documented.

(2) Instrumentation

Reflected light brightfield viewing is only possible with the stand for reflected light.

- Reflector module brightfield ACR P&C for reflected light in the reflector turret

(3) Setting the reflected light brightfield

- The microscope has been started up correctly as described in Section 3.
- The microscope is switched on.
- Adjust the light intensity by turning the regulator (Fig. 4-19/4).
- Position a high-contrast reflected light specimen on the microscope stage.
- Swivel in the 10x objective on the nosepiece (Fig. 4-19/3).
- On the nosepiece (Fig. 4-19/6) swivel in the position with the reflector module brightfield.
- Bring the specimen into focus with the focusing drive (Fig. 4-19/**5**). If possible, always focus away from the specimen in order to avoid a collision between the objective and the specimen.
- Set the knurled wheel of aperture diaphragm **A** (Fig. 4-19/1) in the middle position (about half open/closed).
- Adjust (reduce) the knurled wheel of luminous-field aperture **F** (Fig. 4-19/**2**) until the luminous-field aperture is visible in the field of vision.
- Use the focusing drive to adjust the focus on the edge of the luminous-field aperture.
- Now open the luminous-field aperture until the latter just disappears behind the edge of the field of view.
- For aperture diaphragm adjustment (image contrast) remove an eyepiece from the tube socket and look into the socket with the naked eye, or use the auxiliary microscope instead of the eyepiece. This functions only with sufficiently reflecting specimens.

• For specimens with medium contrast characteristics, set the aperture diaphragm with the knurled wheel (Fig. 4-19/1) to about 2/3 to 4/5 of the exit pupil diameter of the objective.

In most applications, this aperture diaphragm setting provides optimum contrast at almost ideal resolution, and is therefore the best compromise for the human eye.

- Then reinsert the eyepiece, adjust the focus with the coaxial coarse and fine focusing knob and adjust the brightness to the reflected light specimen.
- Never use the aperture diaphragm for controlling image brightness. Use the adjusting knob (Fig. 4-19/4) for illumination intensity!



- 1 Knurled wheel of aperture diaphragm A
- 2 Knurled wheel of luminous-field diaphragm F
- 3 Nosepiece
- 4 Light intensity control
- **5** Focusing drive
- 6 Reflector turret



4.2.2 Adjusting the reflected light darkfield

(1) Application

The reflected light darkfield method is used for examining incompletely reflecting surfaces with different degrees of reflectivity (ideal reflected light brightfield specimens), i.e. with scratches, ruptures, pores or other disruptions to the even surface. All these light-scattering details light up brightly in the darkfield, whereas the even surface remains dark.

(2) Instrumentation

Observations in the reflected light darkfield can only be made on Axio Lab.A1 microscopes for reflected light.

- Epiplan-Neofluar, EC Epiplan-Neofluar, Epiplan objectives with the additional designation "HD"
- Reflector module darkfield ACR P&C for reflected light

The stand for reflected light is equipped with a built-in darkfield stop.

(3) Adjusting the reflected light darkfield

- Adjust the microscope as described in Section 4.2 for the reflected light brightfield. In order to avoid reflexes, the displayed luminous-field aperture should be located slightly beyond the edge of the field of view.
- If used, remove the 6x20 compensator slide.
- Swivel in the objective position with darkfield objective (HD) on the nosepiece.
- If necessary, swivel in the reflector module darkfield on the reflector turret.
- Completely open the aperture diaphragm and switch off or remove the neutral filter as necessary.
- Place the specimen on the stage and sharpen the image.

4.2.3 Adjusting reflected light polarization – Proof of bireflexion and reflexionpleochroism

(1) Application

Reflected light polarization is a further contrasting method for cut surfaces of mineral ore, coal, ceramic products, certain metals and alloys, as depending on the orientation of the crystals and object details, the cut surfaces often react differently when reflected in linear polarized light

The illumination light is linear polarized by the polarizer before passing through the objective onto the specimen surface, where it is reflected. Here the beam parts experience phase differences depending on the structure and polarization optical rotations which, when passing through the analyzer, are displayed in different shades of grey. The grey can be converted into a color contrast with the aid of a compensator with λ -plate.

With objectives of very low magnification, a rotatable $\lambda/4$ plate arranged in front of the objective (Antiflex cap) permits the reflections to be eliminated even with "dark" specimen surfaces, which otherwise would be unavoidable.

(2) Instrumentation

Observations in the reflected light darkfield can only be made on Axio Lab.A1 microscopes for reflected light.

- Rotary stage Pol
- Epiplan-Neofluar Pol, EC Epiplan-Neofluar Pol, Epiplan Pol objectives
- C DIC/DIC/TIC ACR P&C or DIC/Pol ACR P&C or DIC Red I ACR P&C reflector module or Pol ACR P&C reflector module in reflector turret
- Analyzer slide D, fixed or compensator Lambda, 6x20 or Lambda/4, 6x20

(3) Adjusting reflected light polarization

- Adjust the microscope as described in Section 4.2 for the reflected light brightfield.
- Swivel reflector module P&C (for DIC or Pol) on the reflector turret into the beam path and insert the analyzer slide (or lambda, lambda/4 compensator) into the 6x20 compartment.
- Insert a specimen, adjust the desired magnification level, focus and observe the specimen in the polarization contrast now present while turning the rotary stage Pol.

A specimen is bireflective when the details display differences in brightness and color which change when the stage is rotated.

For samples with low bireflexion it is advisable to use the analyzer with lambda plate, rotatable.

Pleochroism can be detected when the color of the specimen changes when the stage is rotated (reflected light polarizer turned on, analyzer turned off).

4.2.4 Adjusting reflected light fluorescence

(1) General principle

The reflected light fluorescence method is used to show fluorescent substances in typical fluorescence colors in high contrast. The light originating from a high-performance illuminator in a reflected light fluorescence microscope passes through a heat protection filter to an excitation filter (bandpass). The filtered, short-wavelength excitation beam is reflected by a dichroic beam splitter and is focused on the specimen above the objective. The specimen absorbs the short-wave radiation before emitting longer-wave fluorescence radiation (Stokes' Law). This radiation is then captured from the image side by the objective and passes through the dichroic beam separator. Finally, the beams pass through a band elimination filter (longpass/bandpass) which only permits the long-wave radiation emitted by the specimen to pass through.

The spectra of the excitation and the band-elimination filters must match very closely. They must be inserted in a reflector module FL P&C together with the respective dichroic beam splitter.

Only powerful LED are supplied as FL excitation light sources in the Axio Lab.A1 program.

(2) Instrumentation

Observations in reflected light fluorescence can only be made on Axio Lab.A1 microscopes for reflected light and reflected light fluorescence.

- Recommended objectives: EC Plan-Neofluar or Fluar (UV excitation)
- LED modules for FL excitation (maximum two configurable)
- Reflector modules FL P&C equipped with respective filter sets
- Fluorescence protection shield

(3) Adjusting reflected light fluorescence

The adjustment of reflected light fluorescence is facilitated by starting with an objective of average magnification, e.g. EC Plan-Neofluar 20x/0.50, and a specimen of high fluorescence. Demonstration samples can also be used for the start-up.

- If the compensator λ used for the transmitted light polarization method is still in its compartment above the nosepiece, it must be removed before adjusting reflected light fluorescence.
- Slide the fluorescence protection shield (Fig. 4-20/8) into the compensator compartment above the nosepiece.
- Swivel in the 20x/0.50 EC Plan-Neofluar objective on the nosepiece (Fig. 4-20/4).
- Initially set the FL/TL toggle switch (Fig. 4-20/2) to position **TL** (transmitted light).
- If necessary, turn the condenser turret (Fig. 4-20/**7**) to position **H** transmitted light-brightfield (or phase contrast if using a Ph objective) and seek the specimen detail to be examined.
- Adjust the light intensity by turning the regulator (Fig. 4-20/5) and focus (Fig. 4-20/6).

- On the reflector turret (Fig. 4-20/9), select reflector module FL P&C with the desired fluorescence filter combination (depending on excitation mode) and switch on.
- Use the push-pull rod (Fig. 4-20/1) to swivel the desired LED (1 or 2) into the beam path.



When switching between the two LEDs the current brightness setting is adopted.



To avoid dazzling when switching between the LEDs, the brightness should be reduced slightly beforehand.

- Set the FL/TL toggle switch to position FL (reflected light fluorescence).
- Adjust the light intensity for reflected light by turning the regulator (Fig. 4-20/3).
- Finally, sharpen the image of the specimen.



- **1** Push-pull rod for swiveling in LED 1/LED 2.
- 2 FL/TL toggle switch (reflected light fluorescence/transmitted light)
- **3** Reflected light intensity control
- 4 Nosepiece
- **5** Transmitted light intensity control
- **6** Focusing drive
- 7 Modulator disk
- 8 Fluorescence protection shield
- 9 Reflector turret

Fig. 4-20 Components for reflected light fluorescence

5 CARE, FUSE REPLACEMENT AND SERVICE

5.1 Instrument care

The only care required for the Axio Lab.A1 is as follows:

- Switch the device off each time after use and apply the protective cover (protects against dust and moisture).
- Do not set the instrument up in a moist environment (max. humidity \leq 75 %).
- Cover all open tubes with dust caps.
- Remove dust and loose dirt on visible, optical surfaces by means of a fine brush, blower brush, cotton wool bud, optical paper or cotton cloth.
- Remove water-soluble dirt (coffee, cola, etc.) by breathing on it and wiping with a dust-free cotton cloth or a moistened cloth. A mild cleaning agent may be added to the water.
- Remove stubborn, oily or greasy dirt (immersion oils, finger prints) using cotton wool buds or a dust-free cotton cloth and optical cleanser L. This cleaning agent is manufactured from 85 vol% gasoline and 15 vol% isopropyl alcohol (IPA). The components are also known by the following names: Gasoline: rubbing alcohol, light petroleum, Isopropanol: 2-propyl alcohol, dimethylcarbinol, 2-hydroxypropane

Clean the optical surfaces with circular movements from the middle to the edge. Exert only slight pressure on optical surfaces.



The front lens of the condenser Pol should not be cleaned with acetone.

Please observe the following guidelines if the microscope is to be used in a hot and humid climate:

• Keep the instrument in bright, dry and well-aired rooms; humidity \leq 75 %; especially sensitive parts such as objectives and eyepieces should be kept in special dry closets.

Precision optical instruments are always susceptible to mould if they are kept and used under following conditions:

- Relative humidity > 75 % over periods exceeding three days at temperatures between +15 °C and +35 °C.
- Set up in dark rooms without sufficient ventilation.
- Dust and finger prints on optical surfaces.

5.2 Instrument maintenance

5.2.1 Checking the instrument

- Ensure that the prescribed line voltages are observed.
- Check the power cable and the plug for possible damage.
- If any damage is observed, turn the instrument off and secure immediately. Call in a qualified professional to remedy the problem.



Fig. 5-1 Replacing the fuses in the stand

5.2.2 Replacing the fuses in the stand



Always disconnect the instrument from the power supply before replacing fuses.

If the fuses fail, the reason must first of all be ascertained and technical problems properly remedied.

The fuse box is located on the rear side of the microscope. It is combined with the supply plug and contains two type **T 3.15 A/H /250 V** fuses.

- Disconnect from the power supply
- Remove the fuse holder (Fig. 5-1/**2**) by pulling it to the front. Use a small screwdriver for the purpose if necessary.
- Remove the fuses from the fuse holder and replace with new fuses.
- Push the fuse holder back into the fuse box (Fig. 5-1/1) until it engages.
- Connect the power plug.

5.3 Troubleshooting

Problem	Cause	Troubleshooting
Shadows or inhomogeneous image brightness in the field of view; the field is not entirely	The vis/fot push-pull rod/shift knob on the photo tube is not in the correct position (intermediate position).	Move the vis/fot push-pull rod/shift knob to the correct position (end position).
visible.	Nosepiece with objective not engaged in click-stop.	Push in nosepiece with objective until it engages.
	Condenser not correctly adjusted.	Set the condenser correctly (adjustment, centering); see p. 72 ff.
	Aperture diaphragm not correctly adjusted.	Set the aperture diaphragm correctly (opening); see p. 72 ff.
	Luminous-field aperture not correctly adjusted.	Set the luminous-field diaphragm correctly (aperture); see p. 72 ff.
	The filter has not been inserted correctly in the filter mount.	Insert filter correctly in the filter mount.
Low resolving power and poor image contrast.	Aperture diaphragm not correctly adjusted.	Set the aperture diaphragm as per 2/3 rule or the specimen features; see p. 72 ff.
	The condenser has not been correctly focused and front lens 0.9 not swiveled in/out correctly.	Focus the condenser and swivel front lens 0.9 in or out correctly; see p. 72ff.
	Wrong frame glass thickness for transmitted light objectives corrected for 0.17 mm frame glass thickness.	Use standard frame glass with a thickness of 0.17 mm.
	Specimen slide placed upside down.	Turn the specimen slide over; the specimen side should be on top.
	Use of no or non-specified immersion oil with immersion objectives.	Use immersion oil 518 N or 518 F from Carl Zeiss
	Air bubbles in immersion oil.	Repeat oiling procedure with fresh oil.
	Immersion oil on the front lens of a dry objective.	Clean the front lens of the dry objective
	Correction setting is not set to the proper thickness of the frame glass.	Adjust the correction setting ring to the correct frame glass thickness.
	Dirt or dust on the optical surfaces of objectives, eyepieces, condensers or filters.	Clean the respective optical components.

Problem	Cause	Troubleshooting
Asymmetric image sharpness, e.g. one side is sharp, one is side	Condenser is not correctly adjusted.	Adjust the condenser correctly; see p. 72 ff.
blurred.	Nosepiece is not engaged in its locking position.	Engage the nosepiece in its locking position (click-stop).
	The specimen has not been fixated on the mechanical stage.	Insert and fixate correctly in the specimen holder.
Distinct focus differences when changing the objective.	Focusable eyepieces are not adjusted correctly or a Pol eyepiece was used in a binocular tube without upright reticle.	Adjust the focusable eyepieces according to the vision defect, see p. 70.
	Objective is not screwed in all the way.	Screw the objective in to the stop.
	Tube lens is not mounted, or mounted unnecessarily.	Mount the tube lens or remove it, as appropriate.
Left and the right field of view cannot be brought together in one image.	Eyepiece distance (interpupillary distance) is not adjusted correctly.	Adjust the interpupillary distance correctly; see p. 70.
	Focusable eyepieces are not adjusted correctly.	Adjust the focusable eyepieces according to the vision defect, see p. 70.
Eye fatigue when using the microscope.	Eyepiece distance (interpupillary distance) is not adjusted correctly.	Adjust the interpupillary distance correctly; see p. 70.
	Focusable eyepieces are not adjusted correctly.	Adjust the focusable eyepieces according to the vision defect, see p. 70.
	Image brightness is unacceptable.	Adjust the lamp voltage or insert a conversion filter.
	Binocular tube is optically/mechanically misaligned.	Call in service personnel for check/repair.
Dirt or dust in the field of view.	The condenser has not been correctly focused and front lens 0.9 not swiveled in/out correctly.	Focus the condenser and swivel front lens correctly in or out; see p. 72ff.
	Opening of the aperture diaphragm is too small.	Set the aperture diaphragm as per 2/3 rule or the specimen features; see p. 72 ff.
	Dirt or dust on optical surfaces of objectives, eyepieces, condensers, filters or specimens.	Clean the optical surfaces of the soiled components, see p. 107.

Problem	Cause	Troubleshooting
LED/halogen lamp does not light up although the switch in the on position.	Power plug is not plugged into the mains outlet.	Insert the plug into the mains outlet. Ensure that the outlet and instrument have the same voltage.
	Lamp is not installed.	Install lamp, see p. 58.
	Lamp is defective.	Replace lamp, see p. 58.
	Fuses are defective.	Replace fuses, see p. 108.
	Installed electrical equipment may be defective.	Call in the service personnel to check components and replace if necessary, see p. 112.
	No voltage from the power socket.	Use a different mains power socket.
LED/halogen lamp flickers, illumination intensity is not stable.	Halogen lamp is reaching the end of its life span.	Replace the halogen lamp, see p. 58.
	Power cable is not installed properly or is damaged.	Install the power cable properly or replace it.
	Pins of the LED/halogen lamp are not properly inserted in the socket.	Insert the pins of the lamp correctly, see p. 58.

5.4 Service

Repairs of mechanical, optical or electronic components inside the instrument and electrical components of Axio Lab.A1 microscopes may only be performed by Carl Zeiss service staff or specially **authorized** personnel.

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

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

If servicing is required, please contact your local representative or

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6 ANNEX

6.1 List of abbreviations

- AC Alternating current
- BL Bertrand lens
- Br. Suitability for spectacle wearers
- CSA Canadian Standards Association
- D Frame glass thickness
- D Darkfield
- d Diameter (e.g. filter)
- DIC Differential Interference Contrast
- DIN Deutsches Institut für Normung (German Standards Institute)
- EC European Community
- EN Euronorm (European standard)
- Ergo Ergonomic
- FL Fluorescence
- foc. focusable
- fot photographic
- H brightfield
- IEC International Electrotechnical Commission
- IP Internal protection (through housing)
- ISO International Standardization Organization
- L Left (Drive knob left on mechanical stage)
- LED Light emitting diode
- Ph Phase contrast
- PL Plan
- Pol Polarization
- P&C Push&click
- R Right (drive knob to right of mechanical stage)
- SLR Single lens reflex
- SW Width across flats
- T Delayed action (fuse type)
- TIC Total interference contrast in circular polarized light
- TL Transmitted light
- UL Underwriter Laboratories
- UV Ultraviolet
- VAC Volt AC
- vis visual

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6.3 Industrial property rights

Instruments, instrument components or methods described in this manual are protected by the following patents:

- see label on microscope stand