

Operating Manual
Axio Observer
Inverted microscope

Knowledge of this manual is required to operate the instrument. You should therefore familiarize yourself with the contents of this manual and pay special attention to instructions concerning the safe operation of the instrument.

Technical specifications are subject to change; this operating manual will not be updated.

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CONTENTS

	Page
1 INTRODUCTION	8
1.1 Safety notes	8
1.2 Notes on warranty	12
1.3 Stand types (overview)	13
2 DESCRIPTION	14
2.1 Designation and intended use	14
2.2 Description and main features	15
2.3 Equipment and compatibility table	16
2.4 System overview	19
2.5 Objectives	23
2.6 Eyepieces	25
2.7 Condensers	25
2.8 Specimen stages	26
2.9 Binocular tubes	26
2.10 Technical specifications	27
3 SETTING UP THE INSTRUMENT	29
3.1 Unpacking and installing the microscope	29
3.2 Attaching the binocular (photo) tube	30
3.2.1 Inserting the eyepieces and centering telescope	30
3.2.2 Inserting the eyepiece reticle	31
3.3 Fitting the transmitted light illuminator	32
3.3.1 Fitting the 100 W transmitted light illuminator carrier	32
3.3.2 Fitting the 35 W transmitted light illuminator carrier	32
3.4 Screwing in the objectives	33
3.5 Fitting the microscope stages	34
3.5.1 Fitting the mechanical stage 130x85 R/L and mounting frame K for the mechanical stage ...	34
3.5.2 Fitting a scanning stage	35
3.5.3 Attaching the specimen stage 250x230 , object guide and mounting frame M for the object guide	36
3.5.4 Fitting the heating stage	37
3.5.5 Fitting the gliding stage Z	38
3.6 Attaching the condensers	38
3.6.1 Condensers for the Axio Observer	38
3.6.2 Condensers from the Axio Imager range	39
3.6.3 Changing the DIC prism in the condenser turret	40
3.7 Reflector turret	41
3.7.1 Fitting the reflector turret	41
3.7.2 Loading the reflector turret	41
3.7.3 Changing the filter set in the FL P&C reflector module	42
3.7.4 Changing the beam splitter in the FL P&C reflector module	43
3.8 Fitting the TFT display to the Z1 motorized stand	45

3.9	Fitting the TFT display to the docking station	45
3.10	Connecting the microscope to the mains power supply	46
3.11	Sockets on the rear of the Axio Observer	47
3.11.1	A1 stand	47
3.11.2	D1 stand	47
3.11.3	Z1 stand	48
3.12	Switching the microscope and the power supply for the HBO 100 on and off	49
3.13	HAL 100 halogen illuminator	50
3.13.1	Attaching the HAL 100 halogen illuminator	50
3.13.2	Adjusting the HAL 100 halogen illuminator	51
3.13.3	Fitting and removing the diffuser for fine adjustment of the halogen illuminator	52
3.13.4	Replacing the HAL 100 halogen bulb	53
3.14	HBO 100 illuminator	54
3.14.1	Inserting the HBO 103 W/2 mercury vapor short-arc bulb	54
3.14.2	Attaching the HBO 100 illuminator	54
3.14.3	Adjusting the HBO 100 illuminator	55
3.15	Fitting the Aqua Stop II	56
3.16	Attaching the mounting adaptor for Laserport to the stand	57
4	OPERATION	58
4.1	Overview of components and controls	58
4.1.1	Axio Observer.A1 (manual)	58
4.1.2	Axio Observer.D1 (coded, semi-motorized)	60
4.1.3	Axio Observer.Z1 (motorized)	62
4.2	Description of components and controls	64
4.3	Basic settings for the D1 stand	75
4.3.1	Configuring the D1 stand	75
4.3.2	Options during operation (when the status display is active)	76
4.4	Light Manager on the Axio Observer.D1 and .Z1	77
4.4.1	Light Manager mode: OFF	78
4.4.2	Light Manager mode: CLASSIC	78
4.4.3	Light Manager mode: SMART	79
4.4.4	Selecting Light Manager mode on the D1 stand	79
4.4.5	Selecting and configuring Light Manager mode on the Z1 stand	80
4.5	Contrast Manager on the Axio Observer.Z1	81
4.6	TFT display touchscreen on the Axio Observer.Z1	82
4.6.1	Screen layout	82
4.6.2	Menu overview	84
4.6.3	Home page	85
4.6.4	Microscope	86
4.6.5	Settings	94
4.6.6	Display	103
4.7	Illumination and contrast techniques	104
4.7.1	Setting transmitted light bright field KÖHLER illumination	104
4.7.2	Setting up transmitted light phase contrast	107
4.7.3	Setting up differential interference contrast (DIC) for transmitted light	109
4.7.4	Setting up transmitted light PlasDIC contrast	112

4.7.5	Setting up transmitted light VAREL contrast	113
4.7.6	Setting up epifluorescence contrast	115
4.8	Documentation	118
4.8.1	Image orientation for camera outputs	118
5	CARE, MAINTENANCE, TROUBLESHOOTING AND SERVICE.....	121
5.1	Care.....	121
5.2	Maintenance	122
5.2.1	Checking the instrument.....	122
5.2.2	Replacing the microscope fuses.....	122
5.2.3	Replacing the fuses in the power supply for the HBO 100.....	123
5.3	Servicing	123
6	ANNEX.....	124
6.1	List of abbreviations	124
6.2	Index.....	126
6.3	Patent rights	129

LIST OF ILLUSTRATIONS

Fig. 2-1	System overview (sheet 1)	19
Fig. 2-2	System overview (sheet 2)	20
Fig. 2-3	System overview (sheet 3)	21
Fig. 2-4	System overview (sheet 4)	22
Fig. 3-1	Installing the microscope.....	29
Fig. 3-2	Attaching the binocular tube.....	30
Fig. 3-3	Inserting the eyepieces	30
Fig. 3-4	Inserting the eyepiece reticle	31
Fig. 3-5	Fitting the 100 W transmitted light illuminator carrier.....	32
Fig. 3-6	Fitting the 35 W transmitted light illuminator carrier.....	32
Fig. 3-7	Screwing in the objectives	33
Fig. 3-8	Fitting the mechanical stage 130x85	34
Fig. 3-9	Fitting the mounting frame K	34
Fig. 3-10	Scanning stage 130x85 mot. CAN, underside.....	35
Fig. 3-11	Scanning stage 130x85 mot. CAN, upper side.....	35
Fig. 3-12	Scanning stage 130x85 mot. CAN, connections on the underside	36
Fig. 3-13	Attaching the specimen stage 250x230.....	36
Fig. 3-14	Fitting the object guide and mounting frame.....	37
Fig. 3-15	Fitting the heating stage	37
Fig. 3-16	Attaching the condenser	38
Fig. 3-17	Fitting the condenser carrier.....	39
Fig. 3-18	Changing the DIC prism.....	40
Fig. 3-19	Fitting the reflector turret.....	41
Fig. 3-20	Fitting the reflector modules	41
Fig. 3-21	Changing the filter set in the FL P&C reflector module.....	42
Fig. 3-22	Installing the filter and beam splitter	43
Fig. 3-23	Changing the beam splitter.....	43
Fig. 3-24	Changing the beam splitter.....	44
Fig. 3-25	Beam splitter designation	44

Fig. 3-26	Fitting the TFT display.....	45
Fig. 3-27	Fitting the TFT display to the docking station.....	45
Fig. 3-28	Axio Observer.A1 (rear view).....	46
Fig. 3-29	Power supply for HBO 100 (rear).....	46
Fig. 3-30	Axio Observer.A1 (rear view).....	47
Fig. 3-31	Axio Observer.D1 (rear view).....	47
Fig. 3-32	Axio Observer.Z1 (rear view).....	48
Fig. 3-33	VP231 Power Supply.....	48
Fig. 3-34	D1 stand and power supply for HBO 100.....	49
Fig. 3-35	Attaching the HAL 100 halogen illuminator.....	50
Fig. 3-36	Adjusting the HAL 100 halogen illuminator.....	51
Fig. 3-37	Fitting and removing the diffuser.....	52
Fig. 3-38	Replacing the halogen bulb.....	53
Fig. 3-39	Attaching the HBO 100 illuminator.....	54
Fig. 3-40	Power supply for HBO 100.....	54
Fig. 3-41	Adjusting the HBO 100.....	55
Fig. 3-42	Fitting the Aqua Stop II.....	56
Fig. 3-43	Fitting the Laserport adaptor.....	57
Fig. 4-1	Axio Observer.A1 components and controls (manual).....	59
Fig. 4-2	Axio Observer.D1 components and controls (coded, semi-motorized).....	60
Fig. 4-3	Axio Observer.Z1 components and controls (motorized).....	62
Fig. 4-4	Nosepiece with slot for DIC slider and PlasDIC slider; analyzer slider slot.....	65
Fig. 4-5	LD condenser 0.55, 6-position H Ph 1, Ph 2, Ph 3, Var 1/2.....	66
Fig. 4-6	LD condenser 0.35; 6-position H, Ph 0, Ph 1, Ph 2, DIC, DIC.....	66
Fig. 4-7	Condenser 0.55, 6-position H, Ph 1, Ph 2, Ph 3, DIC, DIC.....	67
Fig. 4-8	Iris stop slider, manual.....	68
Fig. 4-9	FL attenuator, motorized.....	68
Fig. 4-10	6-position reflector turret.....	69
Fig. 4-11	Binocular tube 45°/23.....	70
Fig. 4-12	Binocular phototube 45°/23.....	70
Fig. 4-13	Setting the binocular tube eyepiece distance.....	71
Fig. 4-14	Vertical stop for focus drive.....	72
Fig. 4-15	LCD display.....	72
Fig. 4-16	Control ring, right (view from rear).....	73
Fig. 4-17	Control ring, left (view from rear).....	73
Fig. 4-18	Docking station with TFT display, control ring and focus drive.....	74
Fig. 4-19	Illumination intensity looking into the eyepieces, changing only the objective (starting from 20x).....	78
Fig. 4-20	Illumination intensity looking into the eyepieces, changing only the objective (starting from 20x). Light Manager settings for each objective have been saved previously.....	78
Fig. 4-21	Illumination intensity looking into the eyepieces, changing only the objective (starting from 20x). Settings for one objective have been saved previously.....	79
Fig. 4-22	Selecting Light Manager mode.....	79
Fig. 4-23	Selecting and configuring Light Manager mode.....	80
Fig. 4-24	Main areas of the TFT display.....	82
Fig. 4-25	Controls area of the TFT display.....	82
Fig. 4-26	Menu overview.....	84
Fig. 4-27	Home page.....	85
Fig. 4-28	STOP button.....	85
Fig. 4-29	Motorized focus drive limit reached.....	85
Fig. 4-30	Page Microscope -> Control -> Objectives.....	86
Fig. 4-31	Page Microscope -> Control -> Reflector.....	87
Fig. 4-32	Page Microscope -> Control -> Optovar.....	87

Fig. 4-33	Page Microscope -> Control -> Light path	88
Fig. 4-34	Page Microscope -> Control -> FluoArc	89
Fig. 4-35	Page Microscope -> Control -> F/A	89
Fig. 4-36	Page Microscope -> XYZ -> Position	90
Fig. 4-37	Page Microscope -> XYZ -> Save position	91
Fig. 4-38	Page Microscope -> XYZ -> Measure	92
Fig. 4-39	Page Microscope -> Incubation -> Incubation	93
Fig. 4-40	Incubation channel window	93
Fig. 4-41	Page Microscope -> Incubation -> Y-Module	93
Fig. 4-42	Page Microscope -> Settings -> Components -> Objectives	94
Fig. 4-43	Page Microscope -> Settings -> Components -> Objectives	94
Fig. 4-44	Page Microscope -> Settings -> Components -> Reflector	95
Fig. 4-45	Page Microscope -> Settings -> Components -> Reflector	95
Fig. 4-46	Page Microscope -> Settings -> Components -> Focus	96
Fig. 4-47	Page Microscope -> Settings -> Components -> Focus	97
Fig. 4-48	Page Microscope -> Settings -> Components -> Cameraports	97
Fig. 4-49	Page Microscope -> Settings -> Components -> Stage	97
Fig. 4-50	Page Microscope -> Settings -> Components -> Misc	98
Fig. 4-51	Field of view eyepiece	98
Fig. 4-52	Illumination type	98
Fig. 4-53	Config. reflected light	98
Fig. 4-54	Page Microscope -> Settings -> User -> Mode	99
Fig. 4-55	Page Microscope -> Settings -> User -> Buttons left	99
Fig. 4-56	Page Microscope -> Settings -> User -> Mode	100
Fig. 4-57	Page Microscope -> Settings -> User -> Stand type	100
Fig. 4-58	Page Microscope -> Settings -> Extras -> Light manager	101
Fig. 4-59	Page Microscope -> Settings -> Extras -> Oil stop	101
Fig. 4-60	Page Microscope -> Settings -> Extras -> Dazzle protect	101
Fig. 4-61	Page Microscope -> Settings -> Extras -> Ethernet	102
Fig. 4-62	Page Microscope -> Settings -> Extras -> Misc	102
Fig. 4-63	Page Microscope -> Settings -> Info -> Firmware	103
Fig. 4-64	Page Home -> Display	103
Fig. 4-65	Axio Observer.D1	105
Fig. 4-66	Setting the stops for transmitted light bright field KÖHLER illumination	106
Fig. 4-67	Centering the condenser phase stop	108
Fig. 4-68	Centering the phase stop (bright, in the condenser) with the phase plate (dark, in the objective)	108
Fig. 4-69	Components for transmitted light DIC on the Axio Observer	110
Fig. 4-70	Setting up VAREL contrast	113
Fig. 4-71	VAREL contrast for microtiter plates	113
Fig. 4-72	Pupil images with VAREL contrast	114
Fig. 4-73	Components for epifluorescence on the Axio Observer	116
Fig. 5-1	Replacing the fuse	122
Fig. 5-2	Replacing the power supply fuses	123

1 INTRODUCTION

1.1 Safety notes

Axio Observer microscopes have been designed, manufactured 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.

They meet the requirements of EU directive 98/79/EC (In-Vitro-Diagnostic) and are labeled with the **CE** marking.

This operating manual contains information and warnings which must be observed by persons operating the instrument.

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

**NOTE**

This symbol indicates an instruction which requires particular attention.

**CAUTION**

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

**CAUTION**

This symbol indicates a potential hazard to the user.

**CAUTION**

Hot surface!

**CAUTION**

UV radiation emission!

**CAUTION**

Disconnect the instrument from the power supply before opening!

Axio Observer microscopes and original accessories are to be used for the microscopy procedures described in this manual only.

Particular attention must be paid to the following notes:



The manufacturer cannot accept liability for any other applications, including applications making use of individual modules or components. The same applies to any service or repair work which is carried out by persons other than authorized service personnel. Such actions will also render any warranty claims invalid.



The socket into which the power cable is plugged must be earthed. The earthing protection must not be rendered ineffective by the use of an extension cable which is not earthed.

- The microscope must not be operated in areas with potential danger by explosion.
- Use the microscope only on rigid and non-combustible bases.
- Specimens have to be disposed in comply with legal requirements and internal labor standards.



If it is determined that the earth protection is no longer effective, the instrument must be removed from operation and measures taken to ensure that the instrument is not operated inadvertently. To repair the instrument, contact the Carl Zeiss microscopy service team in Germany (see page 123) or your local Carl Zeiss overseas representative.



Axio Observer microscopes are not equipped with any special equipment for protection from substances which are corrosive, potentially infectious, toxic, radioactive or otherwise hazardous to health. All legal requirements, in particular local accident prevention regulations, must be observed when handling such samples.




Defective microscopes are not to be disposed of as ordinary domestic waste. They should be disposed of in accordance with the relevant regulations.





Risk of squeeze between stage carrier and stand base when using stands with motorized focussing drives. Do not reach under the stage carrier when lowering the stage.





If reflector modules inserted in the reflector turret are equipped with neutral splitters or partial mirrors at the beam splitting mirror position and the HBO, X-Cite or HXP lamp is switched on, there will be the danger of glare damaging to health when looking into the eyepieces. This is especially the case if the specimen or the specimen holder has reflecting properties. To avoid damage to the eyes, suitable measures must be taken to attenuate the radiation (e.g. by inserting neutral filters).


 Axio Observer.A1 and .D1 (manual) are equipped with an integrated power supply. The power supply can be connected to a mains voltage of 100 to 127 V AC or 200 to 240 V AC $\pm 10\%$, 50/60 Hz. The power supply automatically adjusts to the corresponding voltage.


 The Axio Observer.Z1 (motorized) is supplied with power from the external power supply VP231. The power supply can be connected to a mains voltage of 100 to 127 V AC or 200 to 240 V AC $\pm 10\%$, 50/60 Hz. The power supply automatically adjusts to the corresponding voltage. The power supply units for HBO 100 (ebq 100 dc) and XBO 75 (ebx 75 isolated) respectively can be connected to a mains voltage of 100 to 240 V AC, 50/60 Hz. The power supply units automatically adjust to the corresponding voltage.


 Before switching on the power units for the HBO 50 / HBO 100, check that you are using the correct power unit for the local mains voltage.


 Always disconnect the instrument from the mains power supply before opening the instrument or replacing the fuse.

 Use only fuses with the correct current rating. Do not use temporary fuses or short-circuit the fuse carriers.

 Gas discharge lamps such as the HBO 100 emit ultraviolet radiation which may cause burns to eyes and skin. Never look directly into the light and avoid direct skin exposure. When using the microscope, always use the instrument's protective devices (e.g. special attenuation filters). Gas discharge lamps develop high internal pressure when hot. They should therefore only be replaced when cool. Protective gloves and eyewear should be worn (for detailed information, please see operating manual B 40-065 e).

 When using fluorescence filters, the heat filter, which provides protection from radiant heat emitted by the microscope illuminator, is not to be removed. Fluorescence filters are heat-sensitive and their function may be impaired if the heat filter is removed.

 Placing objects against or covering ventilation slits may lead to a build-up of heat which may damage the instrument and in extreme cases lead to fire. Keep ventilation slits clear and ensure that no objects are inserted into the instrument through the ventilation slits.

 Do not touch lamp housing when hot. Always disconnect the instrument from the power supply before replacing the bulb and leave the instrument to cool down for approx. 15 mins.



Dust and dirt may impair the performance of the instrument. The instrument should therefore be protected from dust and dirt and covered with the dust cover when not in use. Always check that the instrument is switched off before covering.



These instruments are to be operated by trained personnel only, who must have received training on possible hazards associated with microscopy and their specific area of use.



Axio Observer microscopes are precision optical and mechanical instruments, the function of which may be impaired if they are handled incorrectly.



Users must read the safety data sheet for Immersol 518 N[®].



Immersol 518 N[®] immersion oil is a skin irritant. Avoid contact with skin, eyes and clothing.

In the event of skin contact, rinse with plenty of water and soap.

In the event of eye contact, rinse the eye with plenty of water immediately for at least five minutes. If irritation persists, consult a physician.



Disposal (Immersol 518 N[®]):

Ensure that immersion oil does not enter surface water or the sewage system.

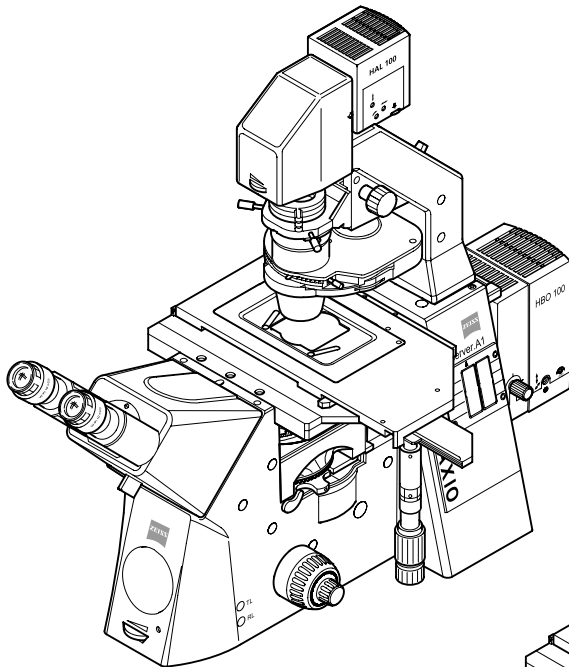
1.2 Notes on warranty

The manufacturer guarantees that the instrument is free of material or production defects when delivered. You must inform us immediately of any defects and take all reasonable steps to minimize any damage arising as a result. If the manufacturer is informed of such a defect, he is obliged to remedy it by, at his discretion, repairing the instrument or supplying an instrument free of any defect. No warranty is given for defects arising from normal wear and tear (in particular for wear parts) or improper use.

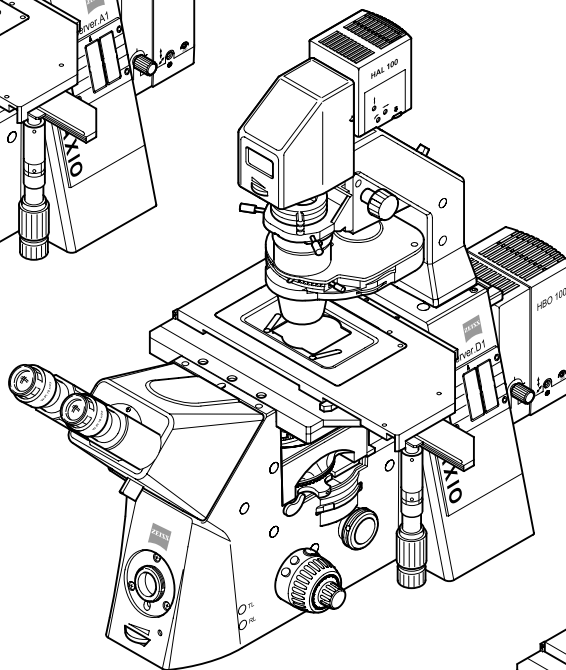
The instrument manufacturer is not liable for damage caused by incorrect operation, negligence or any other tampering with the instrument, in particular the removal or replacement of components of the instrument or the use of accessories supplied by other manufacturers. Such actions will render any warranty claims invalid.

With the exception of the work described in this manual, no maintenance or repair work is to be carried out on these microscopes. Repairs are only to be performed by Carl Zeiss service staff or personnel specifically authorized by Carl Zeiss. In the event of a problem with the instrument occurring, please contact the Carl Zeiss microscopy service team in Germany (see page 123) or your local Carl Zeiss overseas representative.

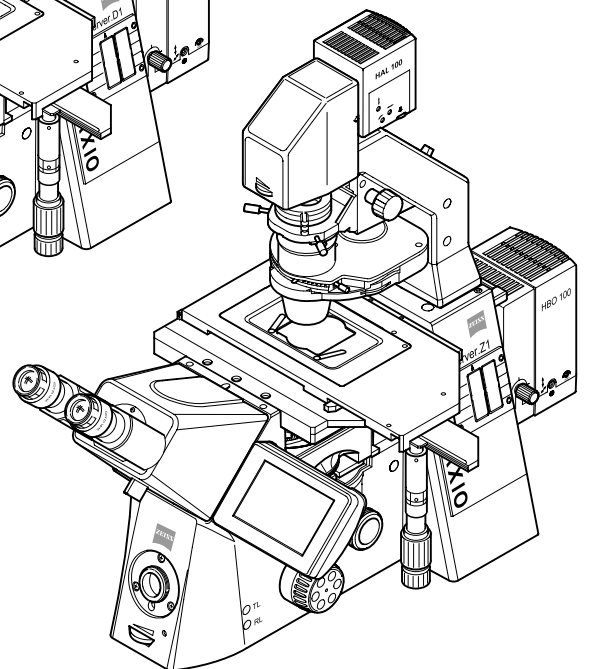
1.3 Stand types (overview)



**Overall view
Axio Observer.A1**



**Overall view
Axio Observer.D1**



**Overall view
Axio Observer.Z1**

2 DESCRIPTION

2.1 Designation and intended use

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

Short designation: Axio Observer.A1 (manual version)
 Axio Observer.D1 (coded / semi-motorized version)
 Axio Observer.Z1 (fully motorized version, including motorized Z-drive)

The Axio Observer microscopes' position in the family of inverted light microscope products is as follows:

Laboratory microscopes

- Axiovert 40 C
- Axiovert 40 CFL

Research microscopes

- **Axio Observer.A1 / .D1 / .Z1**

Axio Observer microscopes are inverted light microscopes for universal use. They are used primarily for examining cell and tissue cultures and sediments in culture flasks, Petri dishes and microtiter plates under transmitted and reflected light.

Axio Observer microscopes can be used for bright field, phase contrast, differential interference contrast, VAREL contrast and PlasDIC contrast transmitted light techniques and epifluorescence techniques.

Axio Observer microscopes form the basis for microscopy work on living cells.

- The robust stand allows the attachment of a variety of tools (micromanipulation), various light sources and incubation components.
- The microscope's inverted design, LD condensers and the use of fixed stages means that there is ample space for specimens and mounting frames. This allows experiments which would not be possible with upright microscopes to be performed.
- The design allows the simple attachment of cameras, lasers, custom stages, etc.

Typical fields of application:

Examination of human blood and tissue samples, observation of intracellular processes in living cell cultures, cell/cell interactions, motility, growth, measurement of potentials, drug detection, microinjection, IVF (in-vitro fertilization), toxicity studies, patch-clamp techniques, ion measurements, digital recordings, time lapse studies with automated processes, z-sectioning, deconvolution, visualization of molecular structures, Fura (Ca measurement), GFP, optical tweezers and scissors, single molecule detection, TIRF.



There are separate operating manuals for the temperature control and incubation units, and these must be observed when using these units.

2.2 Description and main features

The Axio Observer is supplied with three stand types - manual (.A1), semi-motorized (.D1) and fully motorized (.Z1). Accessory components have a modular design.

For the documentation of microscopy examinations, the Axio Observer can be equipped with up to five camera / TV ports as required.

The instrument's main features are (see also overview of equipment variations in section 2.3):

- ICS optics for image generation
- High thermal and mechanical stability
- High degree of flexibility for documenting results
- Improved ergonomics
- LCD display for instrument parameters
- Instrument parameters are displayed on and can be configured using a TFT display
- 23 mm field of view
- Light Manager, Contrast Manager
- Modular design for optimum adaptation for different applications
- 6-position nosepiece
- 6-position reflector turret - can be loaded *in situ* or removed for loading
- 5 or 6-position condenser turret
- 3-position Optovar turret
- Removable aperture stop and luminous-field stop sliders for reflected light
- Fluorescence shutter (internal standard shutter or external high-speed shutter)
- HAL 100, HBO 100, HBO 50 illuminators
- All essential microscope functions are motorized (.Z1)

2.3 Equipment and compatibility table

Equipment	Option	A1	D1	Z1
Stand	manual	+	+	-
	motorized	-	O*	+
Coding	readable by PC	-	+	+
Display	LCD display	-	O**	-
	TFT display	-	-	+
	Docking station	-	-	O
Ports	CAN	-	+	+
	RS 232	-	+	+
	USB	-	+	+
	TCP/IP	-	+	+
	Socket for external UNIBLITZ shutter	-	+	+
	Trigger socket (in/out) for shutter	-	+	+
Light Manager		-	+	+
Contrast Manager		-	-	+
Operation button at focus knob (control ring)	right	-	+	+
	left	-	-	+
Z-focus drive	manual (2mm / 0.2mm)	+	+	-
	motorized, stepper motor drive (z-step size 10 nm)	-	-	+
Adjustable limit stop for z focus left (focus stop)	manual	-	+	-
Automatic Component Recognition (ACR)	Nosepiece ACR	-	-	O
	Reflector turret ACR	-	O	O
Power supply	internal	+	+	-
	external	-	-	+
Z-drive operation (flat control knob)	right	O	-	O
	left	O	O**	O
Z-drive, 13 mm extended travel range	manual	O	O	-
	motorized	-	-	O

Equipment	Option	A1	D1	Z1
Nosepiece	6-pos. H DIC man. (3x H / 3x H DIC)	+	-	-
	6-pos. H DIC cod.	-	O**	-
	6-pos. H DIC mot.	-	-	O
	6-pos. H DIC mot. ACR	-	-	O
Tube lens mount, fixed / Optovar turret	1-pos. tube lens mount, fixed	+	O	O
	3-pos. optovar turret, encoded	-	O	-
	3-pos. optovar turret, motorized	-	-	O
Sideport (type)	2 or 3-pos. man. (exit to the left only)	+	-	-
	2 or 3-pos. man. L/R	-	+	-
	3-pos. mot. L/R	-	-	+
Sideport (accessory)	60N L, 2 switching positions (100% vis : 0% L / 20% vis : 80% L)	O	O	-
	60N L 100, 2 switching positions (100% vis : 0% L / 0% vis : 100% L)	O	O	-
	60N L, 3 switching positions (100% vis : 0% L / 0% vis : 100% L / 50% vis : 50% L)	O	O	O
	60N R, 3 switching positions (100% vis : 0% R / 0% vis : 100% R / 50% vis : 50% R)	-	O	O
	60N L/R, 3 switching positions (100% vis : 0% LR / 0% vis : 100% L / 20% vis : 80% R)	-	O	O
	60N L/R 100, 3 switching positions (100% vis : 0% LR / 0% vis : 100% L / 0% vis : 100% R)	-	O	O
Path deflection to the tube (VIS only)		+	O	O
Beam path switching (for VIS / front port / base port)	manual	-	O	-
	motorized	-	-	O
Baseport / Frontport		-	O	O
Scanning stages	Scanning stage 130x85 mot; CAN	-	O	O
	Scanning stage 130x85 mot; CAN and CAN – USB converter	O	-	-
	Scanning stage 120x100 STEP	O	O	O
Transmitted light illumination	35W HAL	O	-	O
	100W HAL	O	-	O
	100W HAL with LCD display	-	O**	-

Equipment	Option	A1	D1	Z1
Condensers	LD 0.35/0.55, manual	O	O	O
	LD 0.55, motorized	-	O	O
	Axiovert 40 LD 0.2/0.4/0.55	O	-	O
	Axio Imager 0.8/1.4	O	O	O
Shutter for transmitted-light	internal	-	O	O
	external, high-speed (with int. controller)	-	O	O
Reflected light illumination	manual	O	O	O
	motorized	-	O	O
Slider for reflected light illumination	manual	O	O	O
	motorized	-	O	O
Shutter for reflected light	Shutter FL, internal	O	O	O
	High-speed, external (with int. controller)	-	O	O
Port for custom laser		-	O	O
Reflector turret	6-pos. manual	O	O	O
	6-pos. encoded	-	O	O
	6-pos. motorized	-	O	O
	6-pos. motorized ACR	-	O	O
Excitation filter wheel (8 positions) mot. CAN	motorized	-	O	O
FluoArc control	via CAN (TFT or PC control)	-	-	O
	via CAN (PC control)	-	O	-
	via CAN – USB converter (PC control)	O	-	-
Laser safety upgradeable	TIRF	-	O	O
	LSM	-	-	O
ApoTome		O	O	O

+ = included in stand

O = optionally available

O* = optional: motorized reflector turret, reflected light illumination, LD condenser 0.55

O** = mandatory

- = not available

2.4 System overview

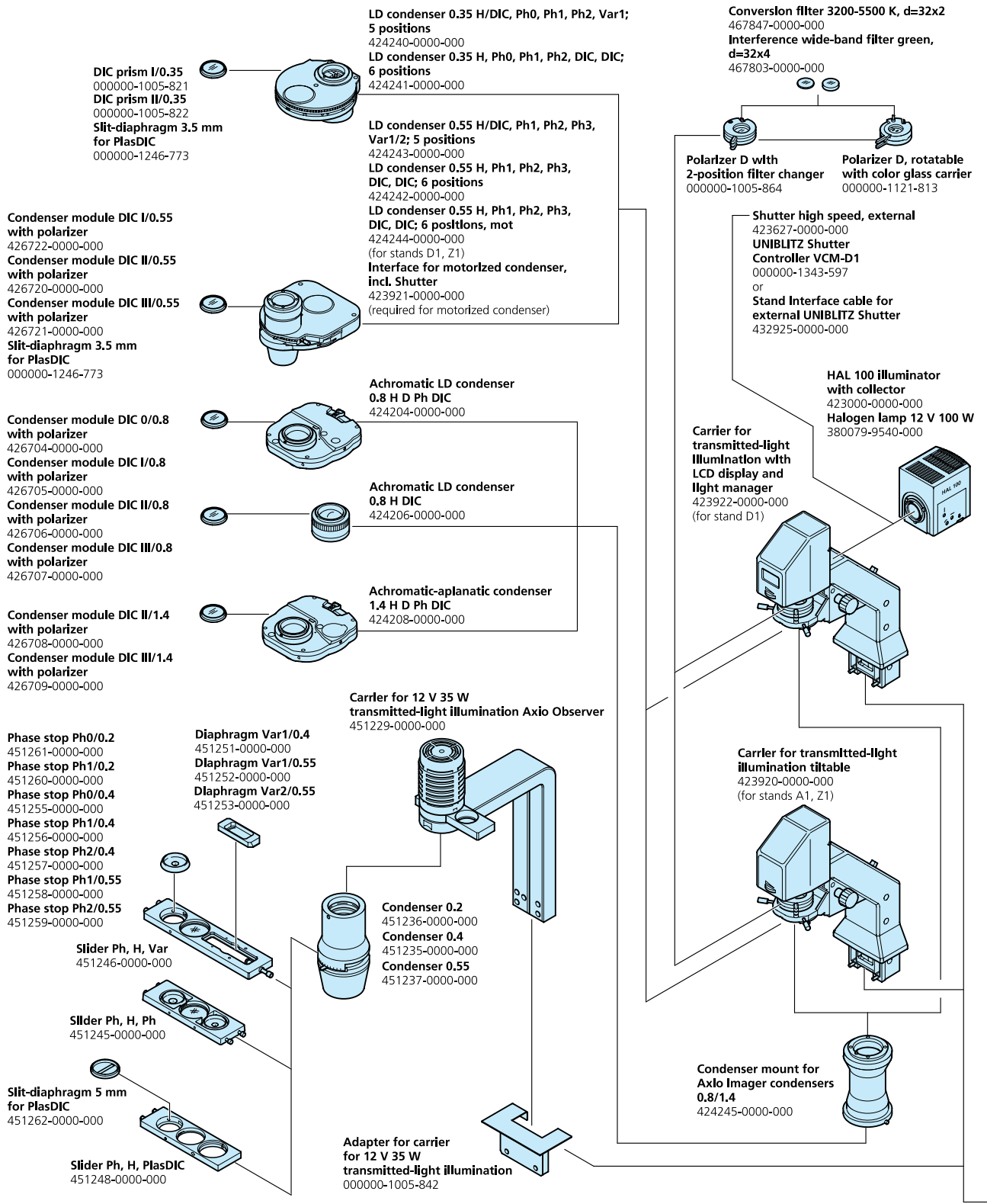


Fig. 2-1 System overview (sheet 1)

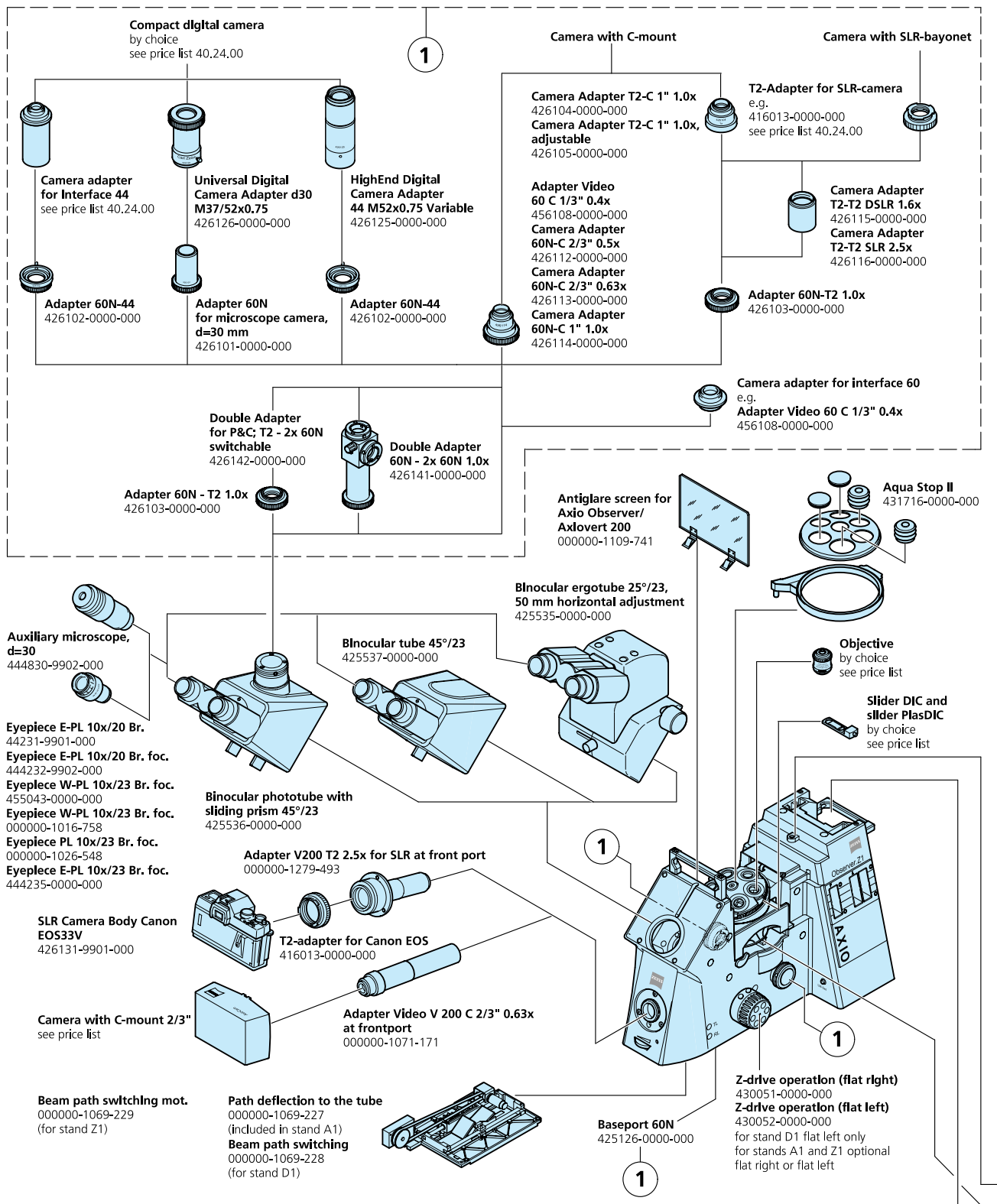


Fig. 2-2 System overview (sheet 2)

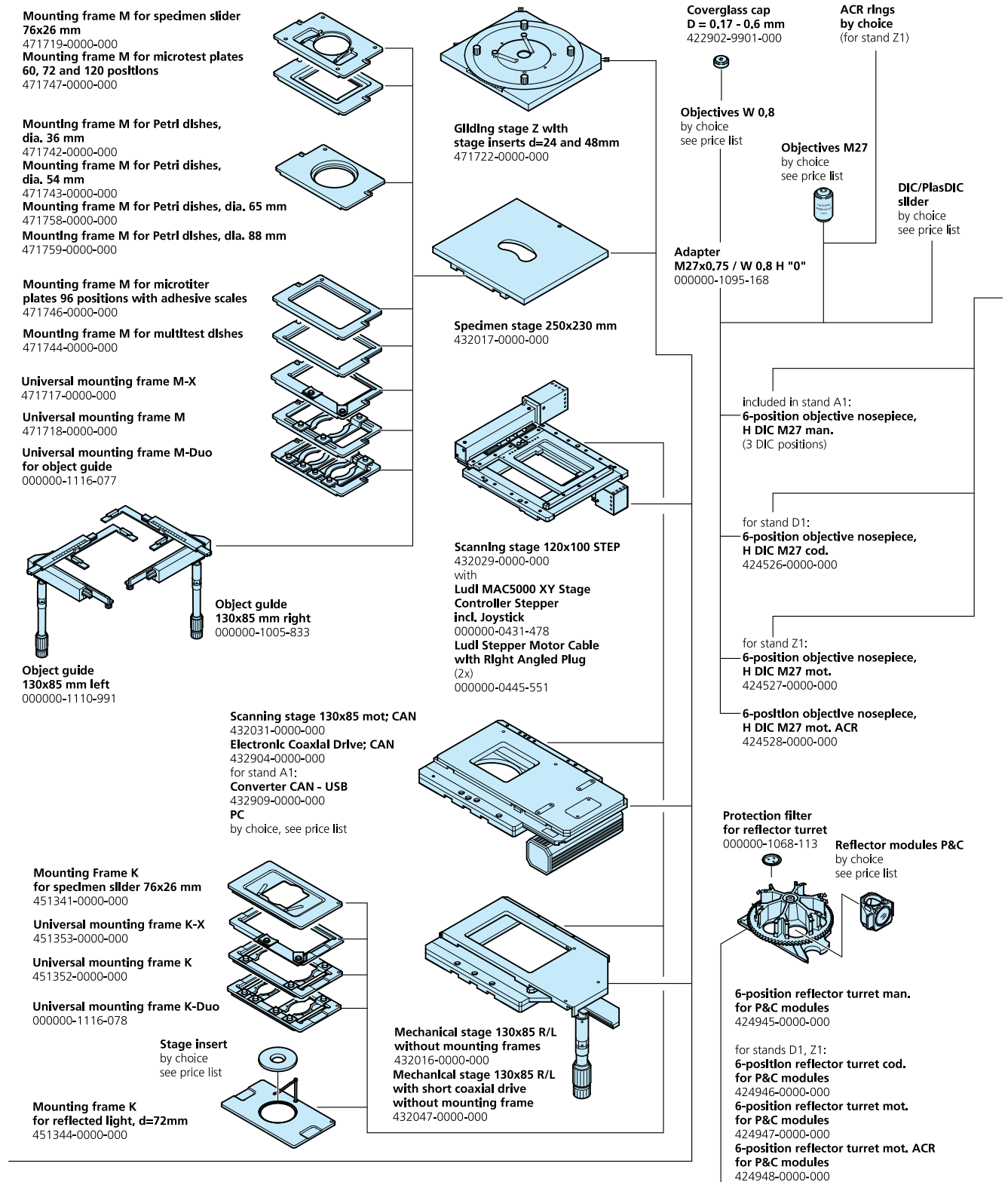


Fig. 2-3 System overview (sheet 3)

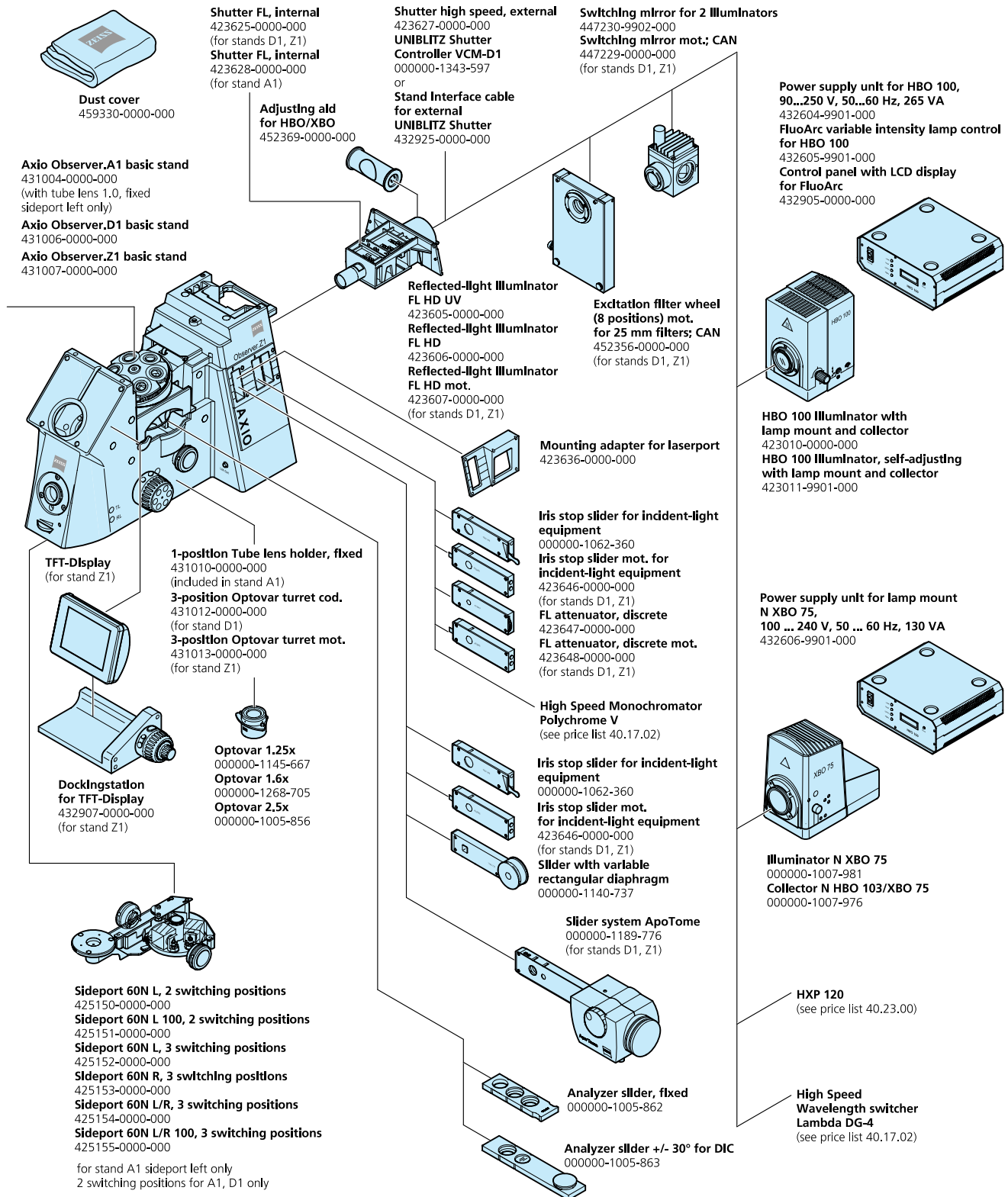


Fig. 2-4 System overview (sheet 4)

2.5 Objectives

The objectives form the core of the microscope's optical systems. An objective might include the following labeling:

A-Plan 10×/0.25 Ph1

Key:

- 10× objective magnification - each objective has a color coded ring indicating the magnification (Zeiss color coding)
- 0.25 numerical aperture
- ∞ infinite tube length
- can be used without a cover slip (D = 0 mm) or with a cover slip with thickness D = 0.17 mm
- or
- 0 can only be used without a cover slip (D = 0 mm)
- 0.17 can only be used with a cover slip with thickness D = 0.17 mm
- and
- Oil oil immersion objective
- Ph 1 phase contrast objective with objective labeling in green and Ph 1 phase plate

Magnification color coding:

Color ring on objective	black	brown	red	orange	yellow	green	light blue	dark blue	white
Magnification	1.0×; 1.25×	2.5×	4×; 5×	6.3×	10×	16×; 20×; 25×; 32×	40×; 50×	63×	100×; 150×

The overall visual magnification is determined by multiplying the objective magnification (e.g. 10×) by the eyepiece magnification (e.g. 10×) and the Optovar magnification (e.g. 1.6×), e.g., $10 \times 10 \times 1.6 = 160\times$.

The numerical aperture $\times 1000$, e.g. $0.20 \times 1000 = 200\times$, is the highest useful magnification. No further detail will be resolved above this limit.

For transmitted light applications, the higher the numerical aperture of the objective, the greater the necessity of using a cover slip of precisely 0.17 mm thickness. For this reason, certain objectives can be adjusted for different cover slip thicknesses using a correction collar. To correct for cover slip thickness, select an appropriate area of the specimen, and find the correction ring position which produces the optimum focus and image contrast (refocusing will always be necessary).

Immersion objectives are, on principle, not so sensitive to differences in cover slip thickness.

With immersion objectives, the air between the cover slip and the objective is replaced with a liquid, usually immersion oil.

The following objectives are available for the Axio Observer microscope:

Objective type	Magnification / num. aperture	Cover slip thickness D in mm	Working distance a in mm	Contrast	Order no.
A-Plan					
A-Plan	5x/0.12		a = 9.9	Ph 0	000000-1018-589
A-Plan	10x/0.25		a = 4.4	Ph 1 var 1	000000-1020-863
LD A-Plan *	20x/0.30	D = 0 - 2.0	a = 4.3	Ph 1	000000-1006-591
LD A-Plan *	20x/0.30	D = 0 - 2.0	a = 4.3	Ph 1 var 1	000000-1006-592
LD A-Plan *	32x/0.40	D = 0.6 - 1.4	a = 3.1	Ph 1	000000-1006-593
LD A-Plan *	32x/0.40	D = 0.6 - 1.4	a = 3.1	Ph 1 var 1	000000-1006-594
LD A-Plan *	40x/0.50	D = 0.7 - 1.3	a = 2.0	Ph 2	000000-1006-595
LD A-Plan *	40x/0.50	D = 0.7 - 1.3	a = 2.0	Ph 2 var 2	000000-1006-596
Cover slip cap		D = 0.17 - 0.6			422902-9901-000
Plan-Apochromat					
Plan-Apochromat	40x/0.95 corr	D = 0.13 - 0.21	a = 0.25	DIC	420660-9970-000
Plan-Apochromat	40x/1.3 oil	D = 0.17	a = 0.21	DIC	420762-9800-000
Plan-Apochromat	63x/1.40 oil	D = 0.17	a = 0.19		420780-9900-000
Plan-Apochromat	63x/1.40 oil	D = 0.17	a = 0.19	DIC	420782-9900-000
Plan-Apochromat	100x/1.40 oil	D = 0.17	a = 0.17	DIC	420792-9900-000
α -Plan-Apochromat	100x/1.46 oil	D = 0.17	a = 0.10	DIC	420792-9800-000
i Plan-Apochromat					
i Plan-Apochromat	63x/1.4 oil	D = 0.17	a = 0.18	DIC	420782-9900-720
EC Plan-Neofluar					
EC Plan-Neofluar	5x/0.16	D = 0.17	a = 18.5	Ph 1	420331-9911-000
EC Plan-Neofluar	10x/0.3	D = 0.17	a = 5.2	Ph 1	420341-9911-000
EC Plan-Neofluar	20x/0.5	D = 0.17	a = 2.0	Ph 2	420351-9910-000
EC Plan-Neofluar	40x/0.75	D = 0.17	a = 0.71	Ph 2	420361-9910-000
EC Plan-Neofluar	40x/1.30 oil	D = 0.17	a = 0.21	Ph 3	420461-9910-000
EC Plan-Neofluar	63x/1.25 oil	D = 0.17	a = 0.10	Ph 3	420481-9910-000
EC Plan-Neofluar (anti-reflection)	63x/1.25 oil	D = 0.17	a = 0.09	Ph 3	420489-9900-000
EC Plan-Neofluar	100x/1.30 oil	D = 0.17	a = 0.20	Ph 3	420491-9910-000
LD Plan-Neofluar					
LD Plan-Neofluar	20x/0.4 corr	D = 0 - 1.5	a = 8.4 - 7.4	Ph 2	421351-9970-000
LD Plan-Neofluar	20x/0.4 corr	D = 0 - 1.5	a = 8.4 - 7.4	Ph 1 Ph 2-	421351-9970-710
LD Plan-Neofluar	40x/0.6 corr	D = 0 - 1.5	a = 3.3 - 2.5	Ph 2	421361-9970-000
LD Plan-Neofluar	40x/0.6 corr	D = 0 - 1.5	a = 3.3 - 2.5	Ph 1 Ph 2-	421361-9970-710
LD Plan-Neofluar	63x/0.75 corr	D = 0 - 1.5	a = 2.2 - 1.2	Ph 2	421381-9970-000
LCI Plan-Neofluar					
LCI Plan-Neofluar	25x/0.8 Imm corr	D = 0 - 0.17	a = 0.21 for D = 0.17	DIC	420852-9972-000
LCI Plan-Neofluar	63x/1.3 Imm corr	D = 0.15 - 0.19	a = 0.17 for D = 0.17	DIC	420882-9970-000
i LCI Plan-Neofluar					
i LCI Plan-Neofluar	25x/0.8 Imm corr	D = 0 - 0.17	a = 0.21 for D = 0.17	DIC	420852-9972-720
i LCI Plan-Neofluar	63x/1.25 Imm corr	D = 0.15 - 0.19	a = 0.17 for D = 0.17	DIC	420882-9970-720
LD LCI Plan-Apochromat					
LD LCI Plan-Apochromat	25x/0.8 Imm corr	D = 0 - 0.17	a = 0.57	DIC	420852-9870-000

* a value based on D = 1

2.6 Eyepieces

The following eyepieces are recommended for the Axio Observer:

Eyepiece type	Field angle	Order no.
E-PL 10x/20 Br.		444231-9901-000
E-PL 10x/20 Br. foc.		444232-9902-000
W-PL 10x/23 Br. foc.	24.7°	455043-0000-000
W-PL 10x/23 Br. foc.	24.7°	000000-1016-758
PL 10x/23 Br. foc.		000000-1026-548
E-PL 10x/23 Br. foc.		444235-0000-000

Other suitable eyepieces can be found in the current price list.

2.7 Condensers

The following condensers are available for the Axio Observer:

Condenser type	Order no.	Comments
LD condenser 0.35 H/DIC, Ph 0, Ph 1, Ph 2, Var 1; 5-position	424240-0000-000	For objective magnifications from 2.5x to 100x (a = 70 mm)
LD condenser 0.35 H, Ph 0, Ph 1, Ph 2, DIC, DIC; 6-position	424241-0000-000	For objective magnifications from 2.5x to 100x (a = 70 mm)
LD condenser 0.55 H, Ph 1, Ph 2, Ph 3, DIC, DIC; 6-position	424242-0000-000	For objective magnifications from 4.0x to 100x (a = 26 mm)
LD condenser 0.55 H/DIC, Ph 1, Ph 2, Ph 3, Var 1/2; 5-position	424243-0000-000	For objective magnifications from 4.0x to 100x (a = 26 mm)
LD condenser 0.55 H, Ph 1, Ph 2, Ph 3, DIC, DIC; 6-position mot.	424244-0000-000	For objective magnifications from 4.0x to 100x (a = 26 mm)
Achromatic LD condenser 0.8 H D Ph DIC	424204-0000-000	For objective magnifications from 5.0x to 100x (a = 8.6 mm)
Achromatic LD condenser 0.8 H DIC	424206-0000-000	For objective magnifications from 5.0x to 100x (a = 8.6 mm)
Achromatic aplanatic condenser 1.4 H D Ph DIC	424208-0000-000	For objective magnifications from 20x to 100x (a = 0.4 mm)
Condenser adaptor for 0.8/1.4 Axio Imager condensers	424245-0000-000	
0.2 condenser	451236-0000-000	For objective magnifications from 5.0x to 20x (a = 90 mm)
0.4 condenser	451235-0000-000	For objective magnifications from 5.0x to 40x (VAREL contrast from 10x to 32x) (a = 53 mm)
0.55 condenser	451237-0000-000	For objective magnifications from 5.0x to 40x (VAREL contrast from 10x to 40x) (a = 31 mm)

Other suitable condensers and accessories can be found in the current price list.

2.8 Specimen stages

Axio Observer microscopes can be fitted with the following specimen stages:

Designation	Order no.
Specimen stage 250x230 mm	432017-0000-000
Object guide 130x85 right, can be fitted on either side, accepts various mounting frames	000000-1005-833
Object guide 130x85 left, can be fitted on either side, accepts various mounting frames	000000-1110-991
Mechanical stage 130x85 R/L excluding mounting frame	432016-0000-000
Mechanical stage 130x85 R/L with short coaxial drive, excluding mounting frame	432047-0000-000
Gliding stage Z	471722-0000-000
Scanning stage 120x100 STEP	432029-0000-000
Scanning stage 130x85 mot. CAN	432031-0000-000
Mounting frame	see section 2.4

2.9 Binocular tubes

The following binocular tubes are available for the Axio Observer series:

Designation	Order no.	Viewing angle / field number	Light distribution %
Binocular ergotube 25°/23, 50 mm vertical range, with Bertrand lens and manual shutter for VIS	425535-0000-000	25° / 23	100 %
Binocular phototube 45°/23, with sliding prism	425536-0000-000	45° / 23	100-0, 0-100, 50-50 %
Binocular tube 45°/23	425537-0000-000	45° / 23	100 %

2.10 Technical specifications**Dimensions (width x depth x height)**

Axio Observer stand..... approx. 295 x 805 x 707 mm

Weight

Axio Observer.A1..... approx. 27 kg

Axio Observer.D1..... approx. 30 kg

Axio Observer.Z1 (excluding external power supply)..... approx. 36 kg

Ambient conditions**Transport (in packaging):**

Permissible ambient temperature -40 to +70 °C

Storage:

Permissible ambient temperature +10 to +40 °C

Permissible humidity (non condensing)..... max. 75 % at 35 °C

Operation:

Permissible ambient temperature +5 to +40 °C

Permissible relative humidity (non condensing)..... max. 75 % at 35 °C

Altitude..... max. 2000 m

Air pressure..... 800 hPa to 1060 hPa

Pollution level..... 2

Operating specifications for Axio Observer.A1 and .D1, manual with integrated power supply or Axio Observer.Z1, motorized with external VP231 Power Supply

Area of use..... closed rooms

Electrical protection class I

Protection type IP 20

Electrical safety..... conforms to DIN EN 61010-1 (IEC 61010-1)
..... and CSA and UL regulations

Overvoltage category II

Radio interference suppression in accordance with EN 55011, Class B

Noise immunity..... in accordance with DIN EN 61326

Voltage 100 to 127, 200 to 240 VAC ($\pm 10\%$)

The voltage does not need to be set manually!

Supply frequency..... 50/60 Hz

Power consumption Axio Observer, manual max. 260 VA

Power consumption Axio Observer, motorized..... max. 280 VA

Power supply unit for HBO 100

Area of use.....	closed rooms
Electrical protection class.....	I
Protection type.....	IP 20
Voltage.....	100 VAC to 240 VAC
Supply frequency.....	50/60 Hz
Power consumption using the HBO 100.....	155 VA

Fuses in accordance with IEC 127

Axio Observer stand, manual.....	T 5 A/H / 250 V, 5x20 mm
Power Supply VP231 for Axio Observer, motorized.....	T 6.3 A/H / 250 V, 5x20 mm
Power supply unit for HBO 100.....	T 2.0 A/H / 250 V, 5x20 mm

Light sources

HBO 50 W/AC mercury vapor short-arc lamp	
Lamp voltage for lamp type L1 and L2.....	L1: 39 - 45 V / L2: 34 - 39 V
Power consumption.....	50 W
Average service life.....	100 h
HBO 103 W/2 mercury vapor short-arc lamp..... 100 W	

Optical and mechanical data

Stand with stage focusing.....	with coarse drive (2 mm / revolution) and fine drive (0.2 mm / revolution) Fine scaling 1 μ m scale Range of travel approx. 10 mm
Objective change.....	using 6-position nosepiece
Objectives.....	with M27x0.75 thread
Eyepieces with insertion diameter.....	30 mm
and.....	field number 23 mm

3 SETTING UP THE INSTRUMENT

Because of the complexity of the equipment and to ensure correct function, the Axio Observer will be installed and set up for use on site by your Carl Zeiss representative.

This includes the following services:

- Installation of the microscope, assembly and adjustment of all components (where these are not factory-adjusted)
- Connection of cables and power supply
- Training

If you wish to install the instrument yourself or to relocate the instrument, proceed as described below.

 Before installing and operating the microscope read carefully the safety instruction (see chapter 1).

3.1 Unpacking and installing the microscope

The basic instrument is delivered in a standard polythene case in cardboard packaging.

The package includes the stand, binocular tube, objectives, eyepieces, condenser, halogen illuminator, fluorescence illuminator and a number of small components such as filter and stop sliders, DIC slider, dust cover and tools.

Additional, optional accessories are delivered in a separate case.

- Remove all components from the packaging and check that all components described on the delivery note are present.
- Place the stand (3-1/1) on a flat, low-vibration work surface.
- Dispose of packaging properly, or retain for future storage or for returning the instrument to the manufacturer.
- Unscrew and remove the transport handle (3-1/2) using the 4 mm Allen key.

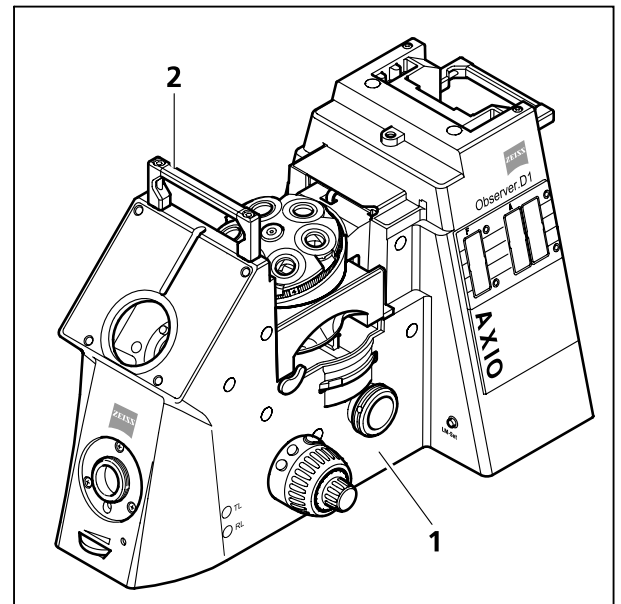


Fig. 3-1 Installing the microscope

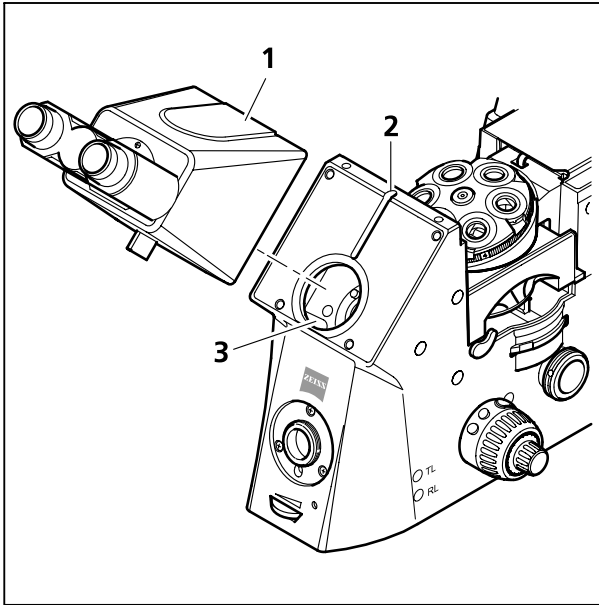


Fig. 3-2 Attaching the binocular tube

3.2 Attaching the binocular (photo) tube

All the binocular tubes listed in the system overview can be attached to the Axio Observer stand as described below.

Where no binocular tube is fitted, or to change the binocular tube proceed as follows:

- Loosen the Allen screw (3-2/2) using the 3 mm ball-end Allen key. If changing the binocular tube, hold the tube firmly when unscrewing and remove it in a forward direction.
- Remove the dust cap from the binocular tube which is to be attached.
- Insert the binocular tube fitting (3-2/1) into the tube adaptor (3-2/3) of the stand, align it with the stand and tighten the Allen screw (3-2/2) using the ball-end Allen key.

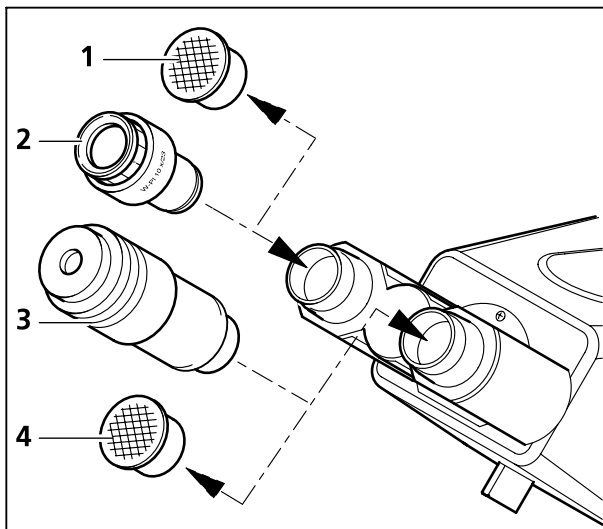


Fig. 3-3 Inserting the eyepieces

3.2.1 Inserting the eyepieces and centering telescope

- Remove the two dust caps (3-3/1 and 4) from the binocular tube.
- Remove the two eyepieces (3-3/2) from their cases and insert them into the binocular tube as far as they will go.
- The centering telescope (3-3/3), which is used to view the aperture stops and phase stops and to center the phase stops, can be inserted in one of the tubes in place of one of the eyepieces. The adjustable eyepiece lens can be used to focus on these stops.

3.2.2 Inserting the eyepiece reticle

Focussing eyepieces can be equipped with eyepiece reticles.

The slight image displacement caused by the additional glass is compensated for on the diopter scale in that the zero position is indicated by the red dot (3-4/**R**) rather than the white dot (3-4/**W**).

The eyepiece reticles (3-4/**1**) are glued to screw-in mounts (3-4/**2**) for easy fitting and removal.

To replace an eyepiece reticle, simply unscrew the screw-in mount (3-4/**2**) with eyepiece reticle (3-4/**1**) and insert the new screw-in mount with the required eyepiece reticle.

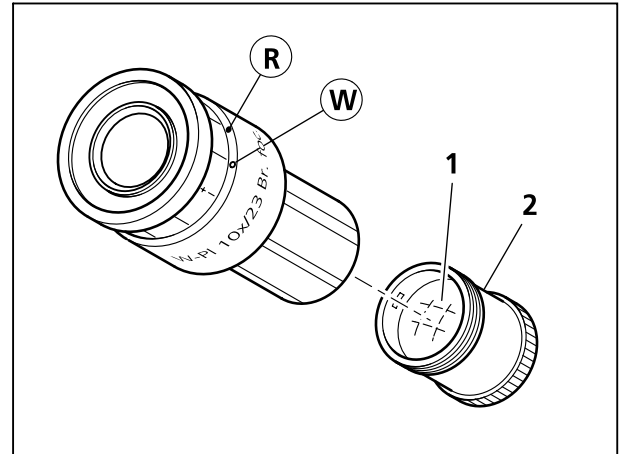


Fig. 3-4 Inserting the eyepiece reticle



If you wish to insert an eyepiece reticle into a mount, you should ensure that the labeling on the eyepiece is still visible in the correct position after the eyepiece has been reinserted.

Adjusting for ametropia when using eyepiece reticles

Correct use of an eyepiece reticle requires two adjustable eyepieces, so that the user can compensate for differences in visual acuity between their two eyes.

- Use the eyepiece focus control to bring the eyepiece reticle into focus. If no eyepiece reticle is used, focus on the edge of the field of view.
- With the eyepiece so adjusted, bring the image of the specimen into focus using the focus drive.
- Without moving the focus drive, adjust the second eyepiece to bring the microscope image into focus for the second eye.

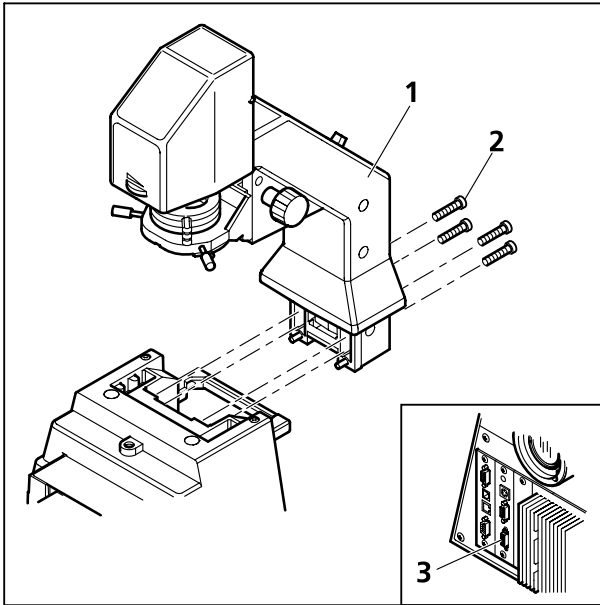


Fig. 3-5 Fitting the 100 W transmitted light illuminator carrier

3.3 Fitting the transmitted light illuminator

3.3.1 Fitting the 100 W transmitted light illuminator carrier

- Place the carrier (3-5/1) in position against the rear of the stand and screw into place using the four Allen screws provided (3-5/2) and the 4 mm Allen key.
- Insert the LCD display connector (D1 stand only) into the socket for the transmitted light illuminator carrier LCD display (3-5/3) on the rear of the D1 stand.

The 100 W carrier does not require any adjustment.

3.3.2 Fitting the 35 W transmitted light illuminator carrier

- Remove the HAL 100 and HBO 100 illuminators from the microscope.
- If required, remove the 100 W carrier by unscrewing the four 4 mm Allen screws.
- On the D1 stand, disconnect the LCD display connector from the rear of the stand.
- Screw the adapter plate (000000-1005-842, 3-6/1) to the rear of the stand using the four Allen screws.
- Fit the 35 W transmitted light illuminator carrier (3-6/2) to the adapter plate and screw into place using the three 4 mm Allen screws (3-6/3).

The 35 W carrier does not require any adjustment.

- Connect the power connector for the 35 W transmitted light illuminator carrier (3-6/5) to the 3 pin upper socket (3-6/4) on the rear of the stand.

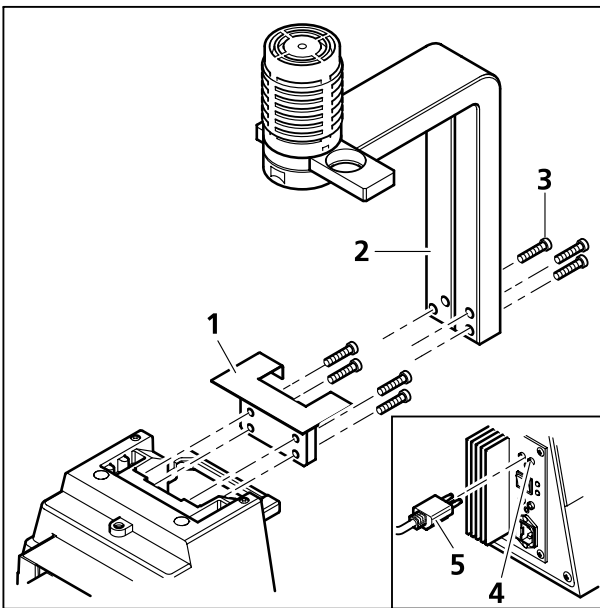


Fig. 3-6 Fitting the 35 W transmitted light illuminator carrier

3.4 Screwing in the objectives

- Remove the dust caps (3-7/1) from the openings in the nosepiece.
- Remove the objectives (3-7/2) from the case and screw them into the nosepiece (3-7/3), starting with position 1 (see engraved number), in increasing order of magnification. Ensure that the objectives are screwed in correctly and securely.



Always replace the dust caps on any empty positions on the nosepiece.

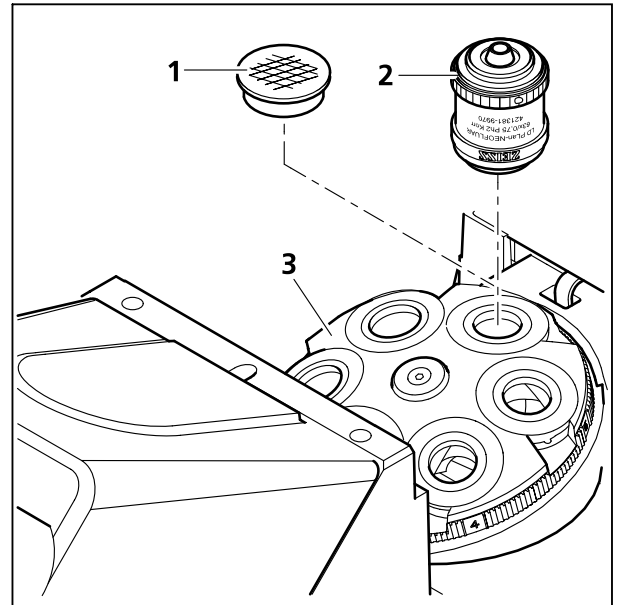


Fig. 3-7 Screwing in the objectives

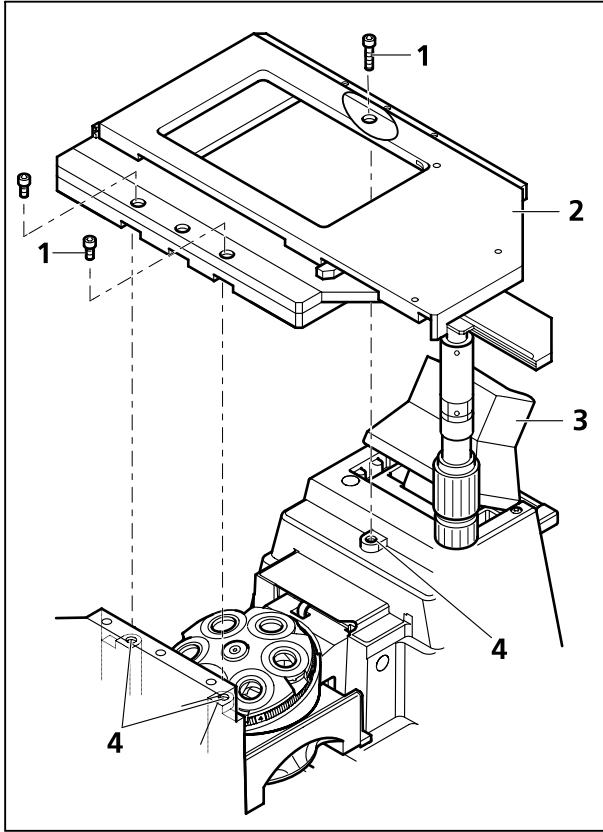


Fig. 3-8 Fitting the mechanical stage 130x85

3.5 Fitting the microscope stages

3.5.1 Fitting the mechanical stage 130x85 R/L and mounting frame K for the mechanical stage

The mechanical stage is fitted directly to the stand via three attachment points with drilled screw holes.

- To improve access during stage assembly, the transmitted light illuminator carrier (3-8/3) can be tilted backwards.
- Place the mechanical stage (3-8/2) onto the three attachment points (3-8/4) of the stand with the spacer disks in place and screw into place using three Allen screws (3-8/1), two at the front and one at the rear.

The mechanical stage 130x85 R/L has three countersunk holes on both the front and the rear to allow it to be fitted with the coaxial drive to either the left or the right.

- Insert the mounting frame K (3-9/1) into the mechanical stage, by placing the corner of the mounting frame marked with a red dot (3-9/2) at the point on the mechanical stage marked in red (3-9/3) and pushing the mounting frame diagonally downwards against the springs into the recess. Ensure that the mounting frame is seated correctly.

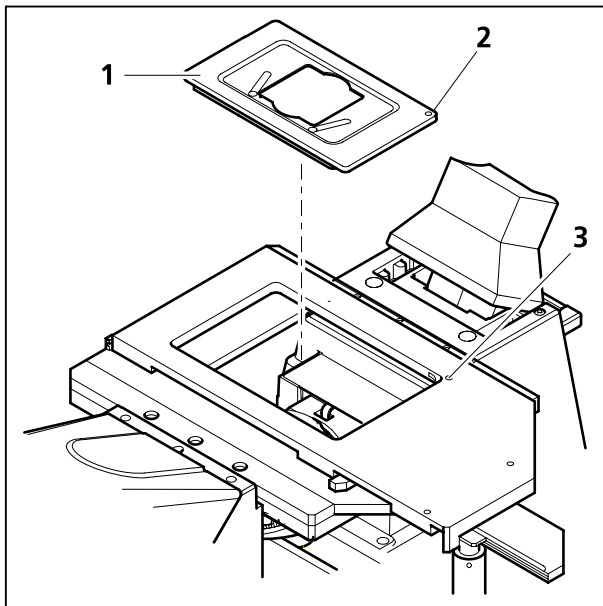


Fig. 3-9 Fitting the mounting frame K

3.5.2 Fitting a scanning stage

- Scanning stages are fitted in the same way as the mechanical stage. However, before fitting the scanning stage the three 4 mm disks included with the stand must be put in place.
- In the case of the scanning stage 120x100 STEP the cable connection to the separate controller Ludl MAC5000 must be established.



Because of the scanning stage 120x100 STEP's large range of travel, the frame of the stage may collide with the objectives at the end of the range of travel.

Supplementary notes for assembling and using the scanning stage 130x85 mot. CAN:

- After mounting the stage to the stand, the transport safety pin (3-10/1) on its underside must be unscrewed.



For further transports of the stage, the safety pin must be screwed in again.

If necessary, the travel ranges in X and Y direction of the scanning stage can be limited as follows:

X direction:

- To displace the right or left stop for the X direction, loosen the corresponding stop screw (3-10/2) on the underside of the stage, shift it as desired and tighten it again.

Y direction:

- To displace the Y direction stop at the front or at the back, unscrew first the screws (3-11/1) of the covers on the upper side of the stage and remove the covers (3-11/2).
- Loosen then the stop screws (3-11/3), shift them as desired and tighten them again.
- Finally, screw on the covers.

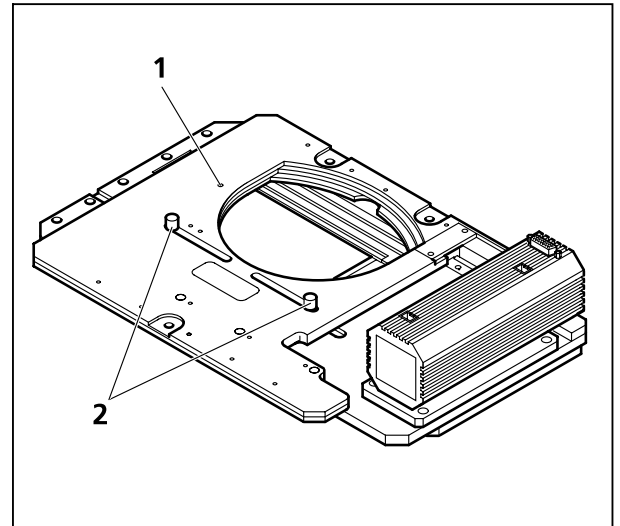


Fig. 3-10 Scanning stage 130x85 mot. CAN, underside

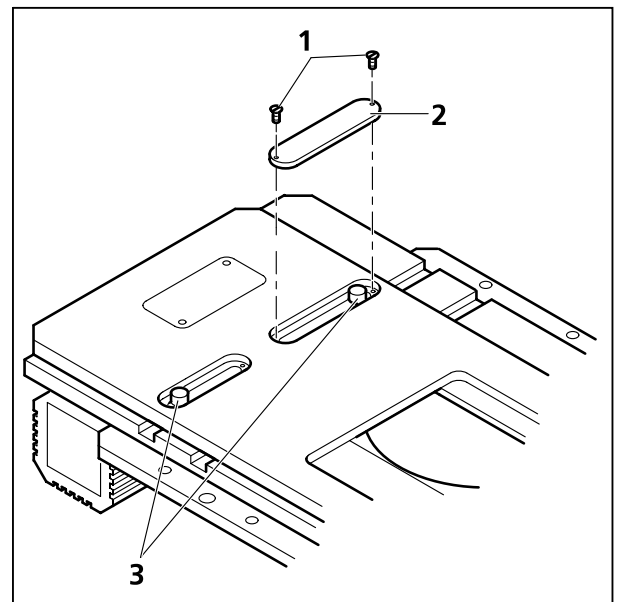


Fig. 3-11 Scanning stage 130x85 mot. CAN, upper side

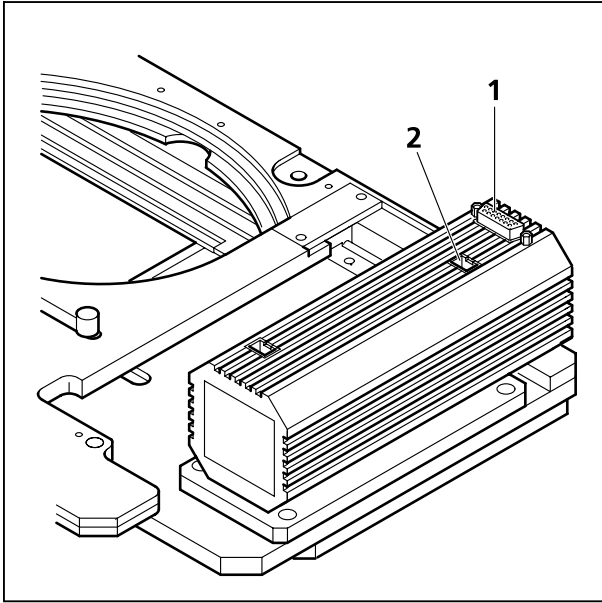


Fig. 3-12 Scanning stage 130x85 mot. CAN, connections on the underside

- After mounting the stage to the microscope stand, establish the cable connections to the XY drive (3-12/1) and to the stand (3-12/2).



To use the scanning stage 130x85 mot. CAN with the A1 manual stand, it must be connected directly to a PC using the CAN -USB converter.

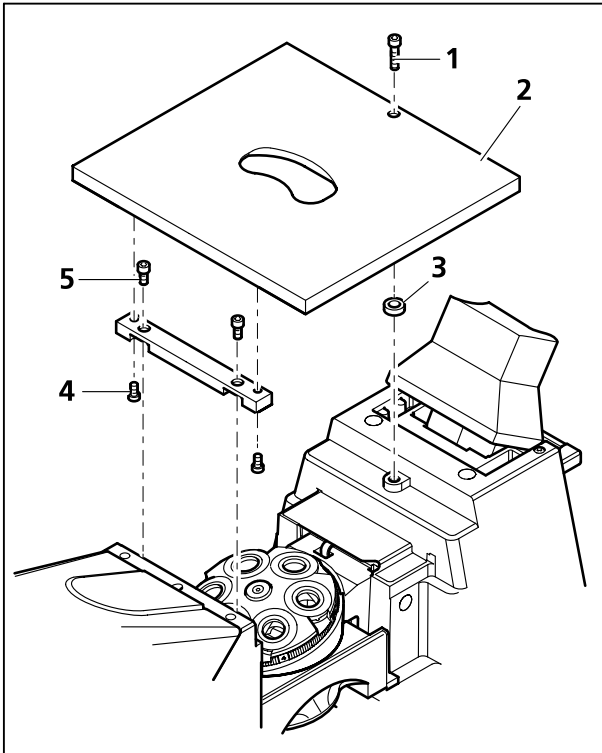


Fig. 3-13 Attaching the specimen stage 250x230

3.5.3 Attaching the specimen stage 250x230 , object guide and mounting frame M for the object guide

The specimen stage is fitted to the stand using a spacer bar and a spacer disk.

- Using the two shorter Allen screws, screw the spacer bar (3-13/5) to the two front attachment points.
- Place the spacer disk (3-13/3) onto the rear attachment point.

- Place the specimen stage (3-13/2) onto the stand and screw to the rear attachment point from above using the longer Allen screw (3-13/1). Ensure that the screw passes through the hole in the spacer disk.
- Screw the specimen stage to the spacer bar from below using two Allen screws (3-13/4), left and right.
- Tighten the rear screw (3-13/1).
- Attach the object guide (3-14/1) to the left or right side of the specimen stage and fix it in position from below using three Allen screws (3-14/2).
- Insert the mounting frame M for the object guide (3-14/3) under the two springs of the object guide from the front until it clicks into position.

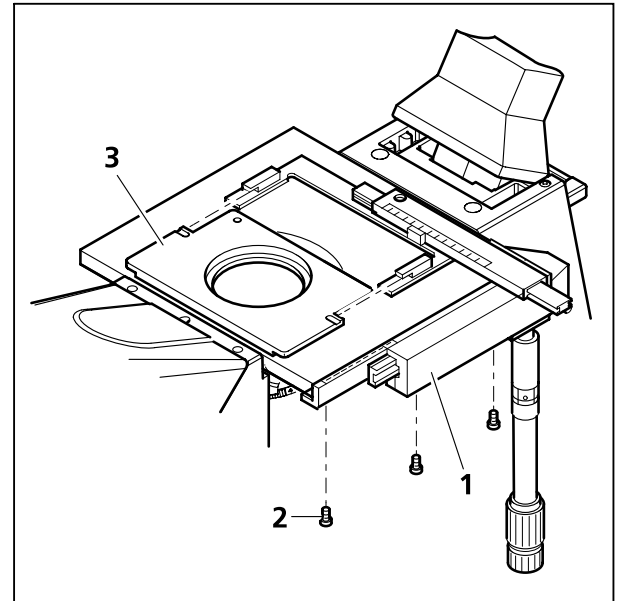


Fig. 3-14 Fitting the object guide and mounting frame

3.5.4 Fitting the heating stage

The heating stage is fitted to the attachment points of the stand using three spacer disks.

- If necessary, remove the microscope stage and additional mounting components.
- Place the spacer disks (3-15/2) on the three attachment points of the stand.
- Place the heating stage (3-15/1) onto the stand and screw into place from above using three Allen screws. Ensure that each screw passes through the hole in the relevant spacer disk.
- Connect the heating stage to the mains power supply as described in the separate operating manual.

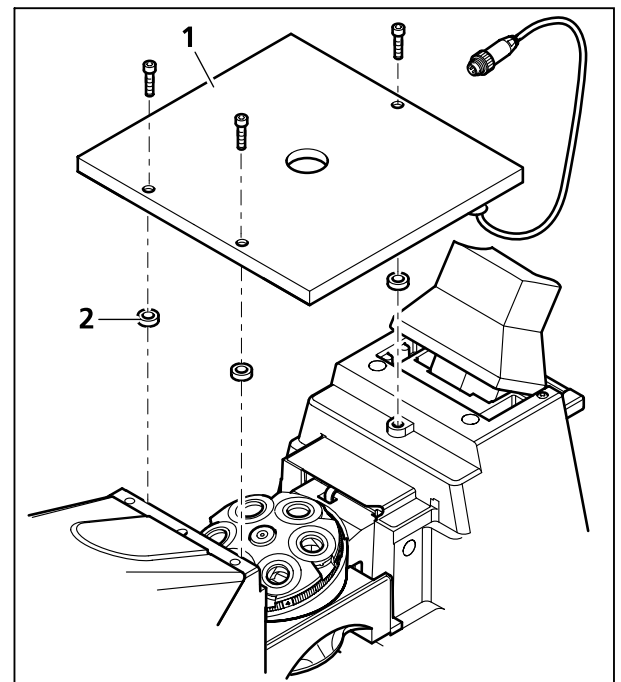


Fig. 3-15 Fitting the heating stage



If the heating stage is used, the nosepiece must be lowered fully using the focus drive before changing objectives - otherwise the objective may collide with the heated stage.

3.5.5 Fitting the gliding stage Z

The gliding stage is fitted in the same way as the heating stage using three spacer disks.

- Before attaching the gliding stage to the stand, the three support elements on the underside of the gliding stage must be unscrewed.
- Place spacer disks on the three attachment points of the stand.
- Place the gliding stage on the stand and screw in place using the three Allen screws from above. Ensure that each screw passes through the hole in the relevant spacer disk.



If the gliding stage is used, the nosepiece must be lowered fully using the focus drive before changing objectives - otherwise the objective may collide with the gliding stage.



Tilt the transmitted light illuminator carrier back into the working position.

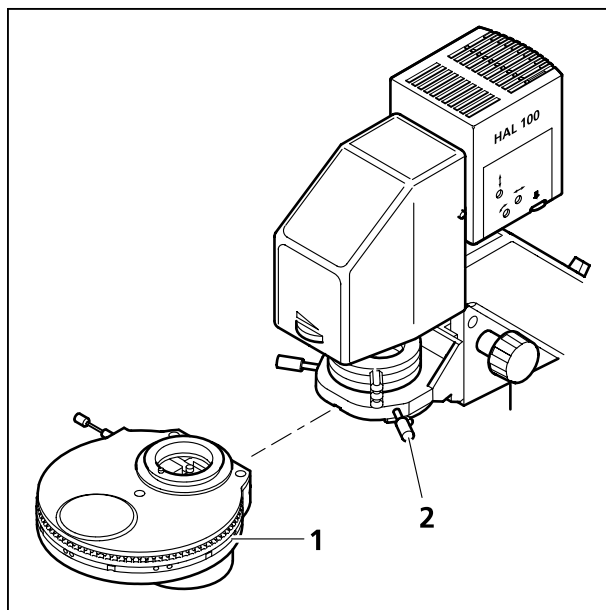


Fig. 3-16 Attaching the condenser

3.6 Attaching the condensers

3.6.1 Condensers for the Axio Observer

- Insert the condenser (3-16/1) into the condenser carrier on the transmitted light illuminator carrier with the fitting facing upwards. Ensure that the locating pin on the condenser is at the front and engages precisely with the guide groove on the condenser carrier.
- Fix the condenser in place using the locking screw (3-16/2).
- If motorized condensers are used, connect the cable to the socket on the left of the carrier.




WARNING

Where the incubator PM S1 or incubator micromanipulation S1 are used, there is a risk of glass breakage. Always fit the condenser with the transmitted light illuminator carrier tilted backwards. After fitting, tilt the mount carefully back into the forward position, taking care not to damage the glass of the incubator. If necessary, lift the condenser carrier to the highest position.

3.6.2 Condensers from the Axio Imager range

The following condensers from the Axio Imager range can be used:

- Achromatic LD condenser 0.8 H D Ph DIC (424204-0000-000)
- Achromatic LD condenser 0.8 H DIC (424206-0000-000)
- Achromatic aplanatic condenser 1.4 H D Ph DIC (424208-0000-000)

 The Axio Observer's inverted design means that these condensers must be fitted "backwards" (with the turret at the rear), such that the controls are at the back and the labeling is upside down.

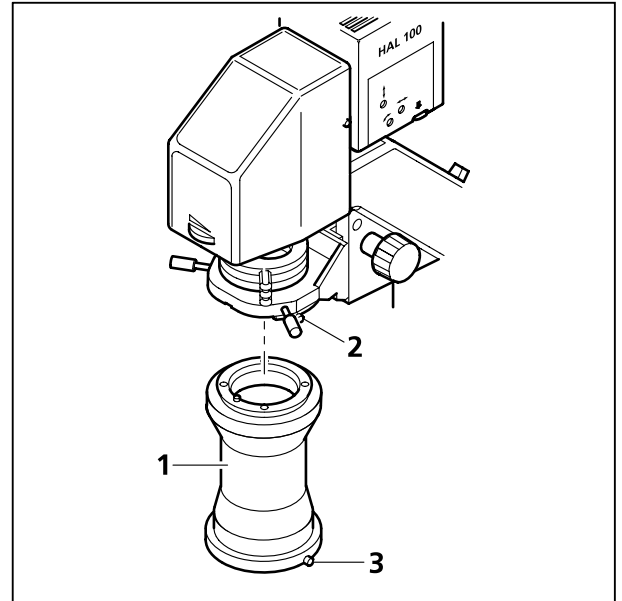



Fig. 3-17 Fitting the condenser carrier

- Tilt the transmitted light illuminator backwards and lift the condenser carrier to the highest position.
- Insert the condenser carrier (3-17/1) into the condenser carrier on the transmitted light illuminator carrier with the fitting facing upwards. Ensure that the locating pin on the condenser is at the front and engages precisely with the guide groove on the condenser carrier.
- Fix the condenser carrier in position using the locking screw (3-17/2).
- Insert the fitting of the required condenser into the condenser carrier, checking that it is correctly orientated, and fix into position using the locking screw (3-17/3).
- Tilt the transmitted light illuminator carrier (3-8/3) carefully back into the forward position, ensuring that the condenser does not collide with the specimen or mounting frame.
- Set up "KÖHLER" illumination.

 The use of condensers of the Axio Imager range is recommended for specimen on specimen slides only.

It is recommended to use with achromatic aplanatic condenser 1.4 exclusively mounting frames with clamping springs to fix the specimen slides.

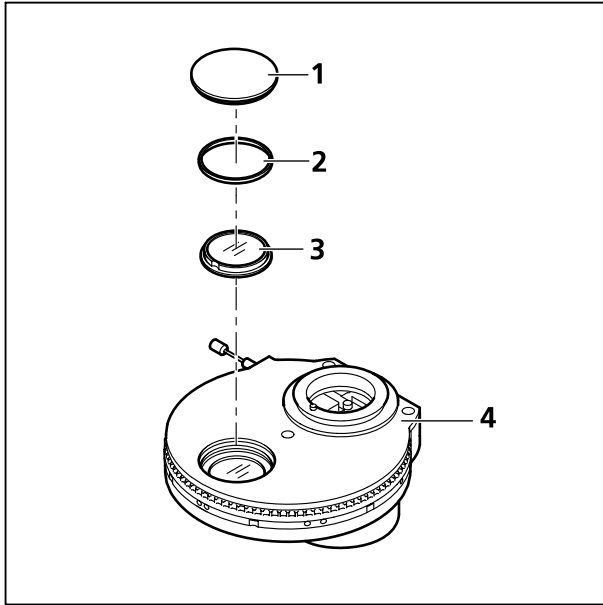


Fig. 3-18 Changing the DIC prism

3.6.3 Changing the DIC prism in the condenser turret



If a motorized condenser is used, switch off the device and pull off the connector plug. Only after that you may move the condenser turret manually, otherwise the condenser will be damaged.

- To change a DIC prism, remove the condenser and place it down on a stable surface.
- Remove the plastic cover (3-18/1) from the mounting hole on the condenser (3-18/4).
- Position the turret disk containing the DIC prism which is to be replaced in the mounting hole and hold it in position using the knurled ring.
- Unscrew the retaining ring (3-18/2) using the retaining ring tool from the tool set.
- Turn the condenser upside down and allow the DIC prism (3-18/3) to slide out onto a soft surface.

The new DIC prism is fitted in the reverse order:

- Carefully insert the new DIC prism into the mounting hole with the writing facing upwards. If necessary, use tweezers to hold the DIC prism carefully by its outer ring. Ensure that the DIC prism is correctly inserted into the mount (notch of the DIC prism must engage with the pin on the mount).
- Carefully reinsert the retaining ring and screw in place using the retaining ring tool.
- Replace the plastic cover.
- Ensure that the correct labeling is displayed on the knurled ring of the turret disk.
- Turn the condenser over and reinsert it into the transmitted light illuminator carrier.

3.7 Reflector turret

3.7.1 Fitting the reflector turret

The reflector turret (manual or motorized) is inserted into the stand from the right.



Switch the instrument off before inserting the motorized reflector turret. Close the RL shutter when changing the reflector turret to prevent the emission of refracted light.

- Rotate the locking lever (3-19/2) downwards and open the cover (3-19/3) upwards.
- Insert the loaded or unloaded reflector turret (3-19/1) into the opening beneath the nosepiece as far as it will go.
- Close the cover (3-19/3) and rotate the locking lever (3-19/2) upwards.

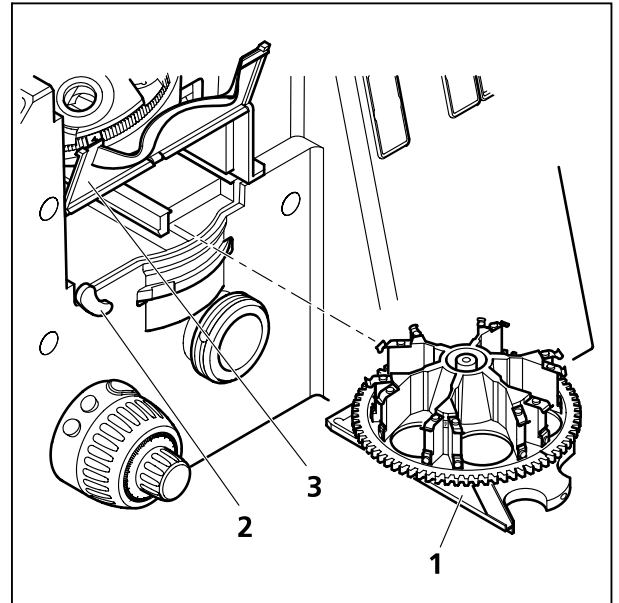


Fig. 3-19 Fitting the reflector turret

3.7.2 Loading the reflector turret

To load the reflector turret, it can be either half or completely retracted from the stand.

- Rotate the locking lever (3-19/2) downwards and open the cover (3-19/3) upwards.
- Pull the reflector turret (3-20/1) out of the stand to the first stop position (or remove from the stand completely and place on a suitable surface).
- Insert the reflector modules (3-20/4) in the appropriate reflector positions for the filter combination (see position marking 3-20/6), starting with position 1 (emission filter downwards). To insert the reflector modules, insert the retaining elements (3-20/5) on the left and right of the module into the two lower spring clips (3-20/3) diagonally from above, then push the module against the upper spring clips (3-20/2) from the front until it clicks into position.
- To remove a reflector module, pull it first out of the upper spring clips, then out of the lower clips.
- After loading, reinsert the reflector turret into the stand.
- Close the cover and rotate the locking lever upwards.

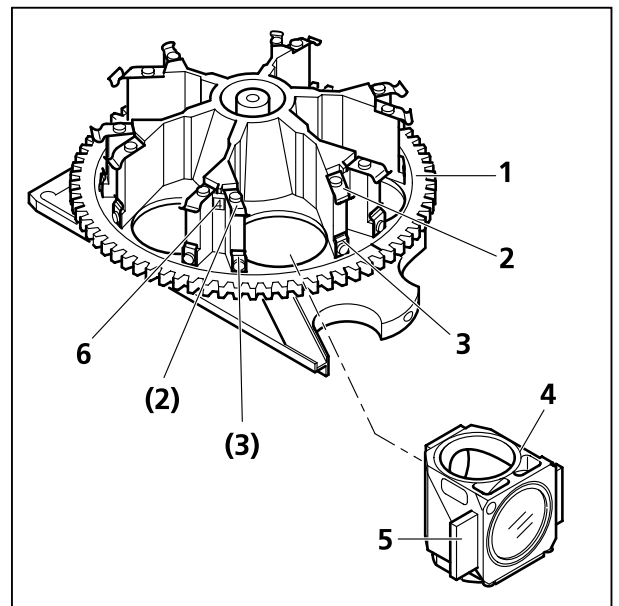



Fig. 3-20 Fitting the reflector modules

 If the protecting glass for the reflector turret was included in the first delivery, it is mounted already.

In case of retrofitting it will be built in by a technician of our Sales Department.

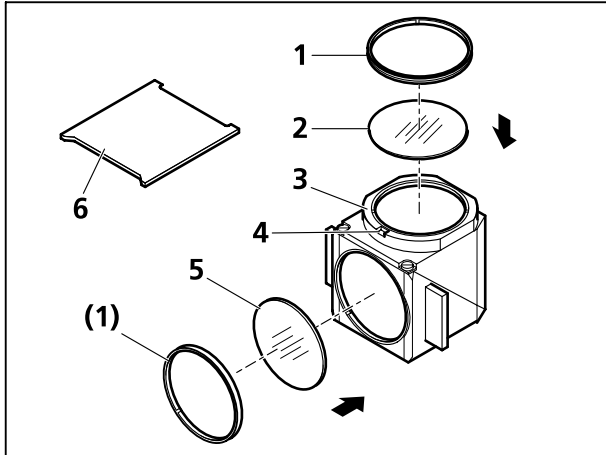


Fig. 3-21 Changing the filter set in the FL P&C reflector module

3.7.3 Changing the filter set in the FL P&C reflector module

The filter sets for the FL P&C reflector module can be combined and assembled individually by the customer. Filter sets or fully assembled FL P&C reflector modules can be ordered from Carl Zeiss.

- Remove FL P&C reflector module (3-21/3) from the reflector turret and set it down (also refer to Section 3.7.2).
- Use mounting device (2-43/6) from the tool set to unscrew retainer ring (3-21/1).
- Turn the reflector module around and allow the filter (3-21/2 or 5) to drop out on a soft surface.
- The emission filter is inserted at (3-21/2), the excitation filter at (3-21/5), and both are secured using the retainer ring (3-21/1).

An arrow and designation can be provided on the circumference of the emission filter and excitation filter. The arrow indicates the direction the particular filter is installed in the reflector module and must always point inwards (refer to arrows in Fig. 3-21).

An additional label can be provided on the emission filter to show the position of the wedge angle in order to minimize image movement during multiple fluorescence procedures.

The label should be aligned on the orientation groove (3-21/4) when the particular emission filter concerned is installed in the reflector modules used. This ensures that the wedge angle of the emission filter is in the same, defined position of the reflector modules, which minimizes the already minimal image shift when Zeiss filter sets are used.

If it is necessary to install filters without direction arrows, we recommend the following procedure:

Filters with reflective, dielectric layers need to be installed, so that the reflective layer (3-22/6) on the excitation filter (3-22/5) points outwards (in relation to the reflector module). With the emission filter (3-22/1), the reflective layer (3-22/2) points inwards (Fig. 3-22).

The reflective layer (3-22/4) of the beam splitter (3-22/3) should point downwards in its installation position.

The arrows (3-22/7) mark the illumination or imaging beam path.

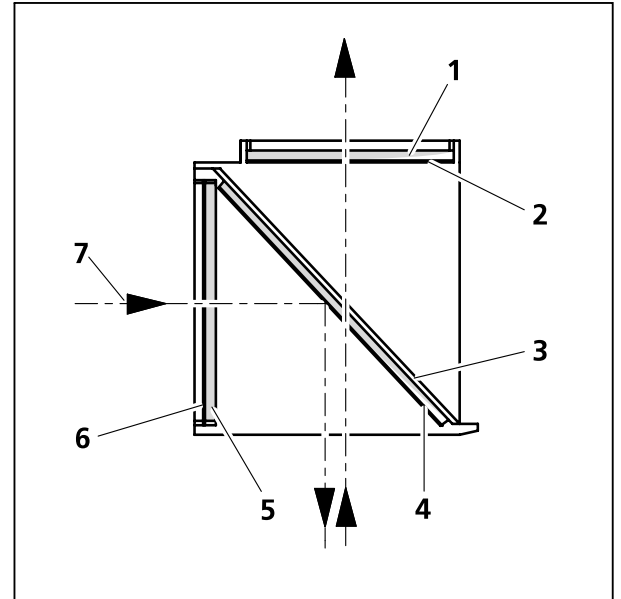


Fig. 3-22 Installing the filter and beam splitter

3.7.4 Changing the beam splitter in the FL P&C reflector module



Attachment of filters and the beam splitter requires utmost care to prevent damage to and contamination of the optical components.

We recommend ordering completely equipped FL P&C reflector modules, since changing the beam splitter is quite demanding.

However, should you choose to change the beam splitter, proceed as follows:

- Remove the FL P&C reflector module from the reflector turret (also refer to Section 3.7.2).
- Undo the two slotted screws (3-23/1) with a screwdriver.
- Hold both halves of the reflector module together (**emission** half (3-23/2) and **excitation** half (3-23/3)), turn in the opposite direction to the installation position and put it down.

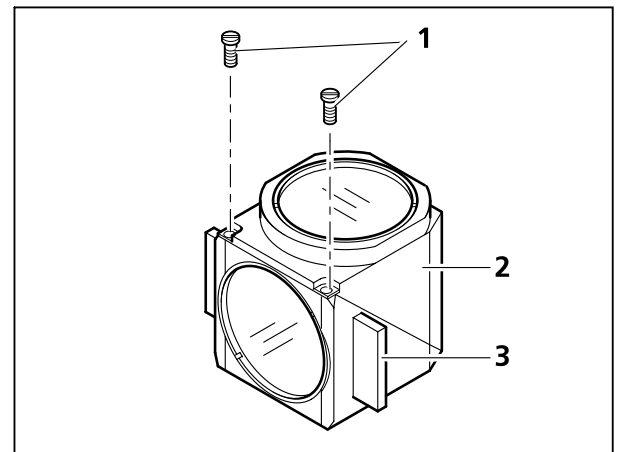


Fig. 3-23 Changing the beam splitter

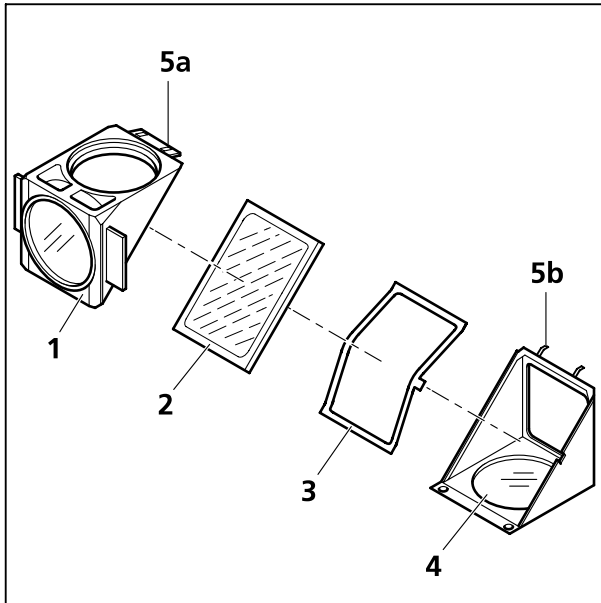


Fig. 3-24 Changing the beam splitter

- Tip up the **excitation** half of the module (3-24/1), which now is on top, and remove it from the retaining pins (3-24/5b) on the bottom half of the module (**emission**) (3-24/4).
- Remove the beam splitter (3-24/2) and spring frame (3-24/3) from the bottom half of the module.
- Remove the old beam splitter and carefully place the new one onto the spring frame (3-24/3) with the reflective side facing up and place both parts together into the bottom half of the module. Take care to ensure that the side latch of the spring frame is in the appropriate recess in the bottom half of the module.

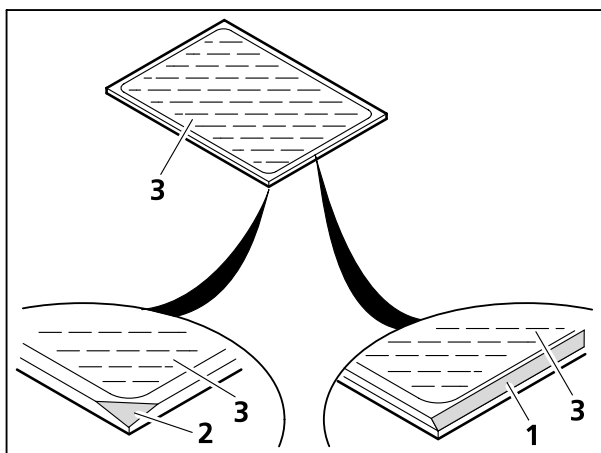


Fig. 3-25 Beam splitter designation



The reflective (layered) side (3-25/3) of the beam splitter has a slanted edge (3-25/1) or corner (3-25/2).

- Place the **excitation** half of the module (3-24/1) onto the **emission** half of the module (3-24/4) (the retaining pins 3-24/5b will grip the eyelets 3-24/5a). Hold both halves together and turn them back into the installation position.
- Re-insert the slotted screws and tighten them up.
- Finally, attach the adhesive label with the name of the filter combination to the side of the module.

3.8 Fitting the TFT display to the Z1 motorized stand



CAUTION

Switch off the microscope before fitting the TFT display.

- Prior to mounting the TFT display, insert the supplied spacer disks into the counter sunk holes of the stand.
- Place the TFT display (3-26/2) on the right side of the stand (3-26/1). Ensure that the pin contact is inserted precisely into the relevant opening.
- Screw the TFT display tight using the three screws (3-26/3).

The stand and TFT display are electrically connected via the pin contact.

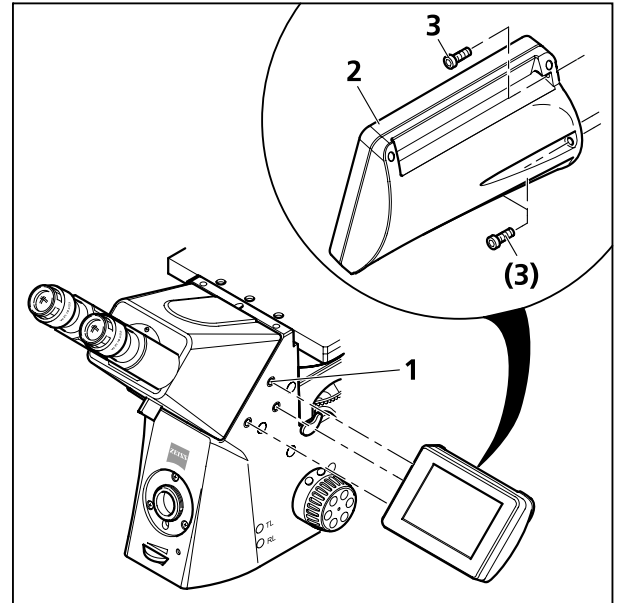


Fig. 3-26 Fitting the TFT display

3.9 Fitting the TFT display to the docking station



CAUTION

Switch off the microscope before fitting the TFT display and docking station.

- If the TFT display is fitted to the Z1 stand, remove it from the stand.
- After that, cover the screw holes and electrical contact openings on the stand with the cover supplied.
- Place the TFT display (3-27/1) on the docking station (3-27/2) and screw in place using the long Allen key supplied. Ensure that the pin contact is inserted precisely into the relevant opening.
- To connect the docking station to the Z1 stand, the appropriate expansion card must be installed at the rear of the stand (see 3-32/9).



The plug-in card has to be inserted by the salesman only.

- Insert the docking station cable (3-27/3) into the socket (3-32/9) on the rear of the stand.
- The angle of the TFT display can be adjusted using the two control wheels (3-27/4) on the rear of the docking station.

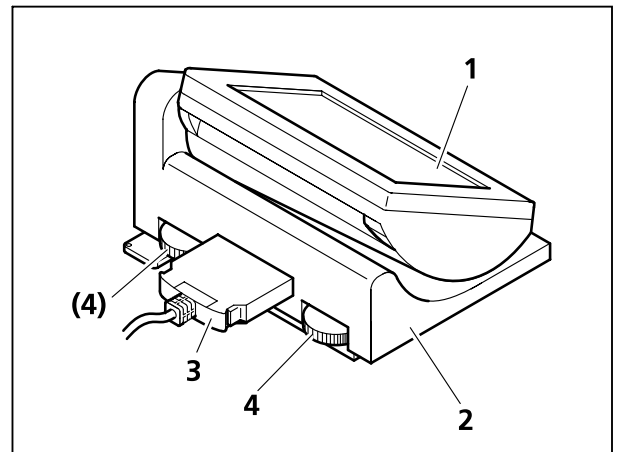


Fig. 3-27 Fitting the TFT display to the docking station

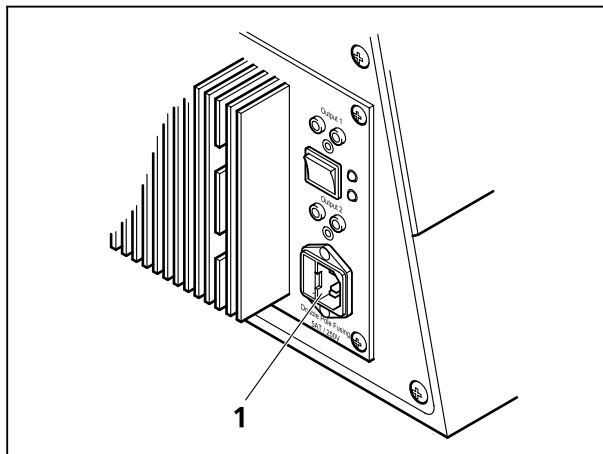


Fig. 3-28 Axio Observer.A1 (rear view)

The power supply can be connected to a mains voltage of 100 to 127 V AC or 200 to 240 V AC, 50/ 60 Hz. The power supply automatically adjusts to the corresponding voltage.

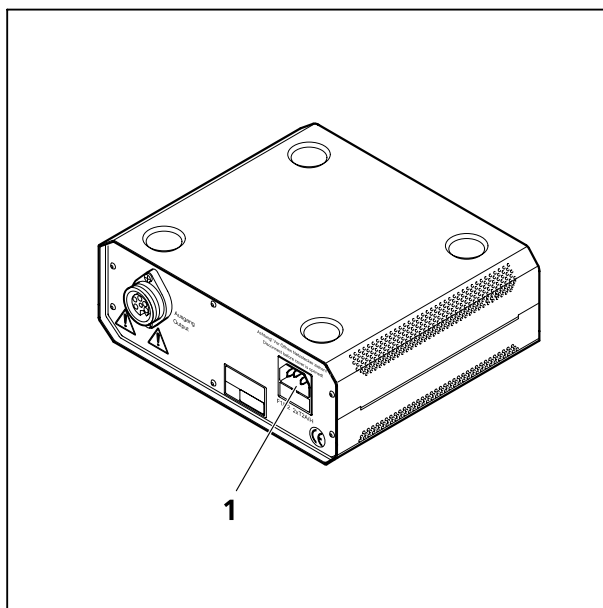


Fig. 3-29 Power supply for HBO 100 (rear)

3.10 Connecting the microscope to the mains power supply

- Connect the microscope's power input socket (3-28/1) (on the rear of the A1 and D1 stands) to a mains socket using the power cable.

The microscope can be connected to a mains voltage of 100 to 127 V AC or 200 to 240 V AC, 50/ 60 Hz. The microscope automatically adjusts to the corresponding voltage.

- Connect the power socket on the rear of the Z1 stand (3-32/1) to the external VP231 power supply unit.
- Connect the external VP231 power supply to a mains socket.

The HBO 100 illuminator (for epifluorescence) is powered by a separate power supply.

- The power supply's socket (3-29/1) should be connected to a mains socket (see also section 3.14.2 *Attaching the HBO 100 illuminator*).

The power supply is also equipped with a wide-spectrum power supply unit.

3.11 Sockets on the rear of the Axio Observer



Switch off the microscope before connecting any components.

3.11.1 A1 stand

Key to Fig. 3-30:

- 1 Socket for transmitted light halogen illuminator (Output 1)
- 2 Reflected/transmitted light (HAL) switch (Output 2)
- 3 Socket for reflected light halogen illuminator
- 4 Power input socket

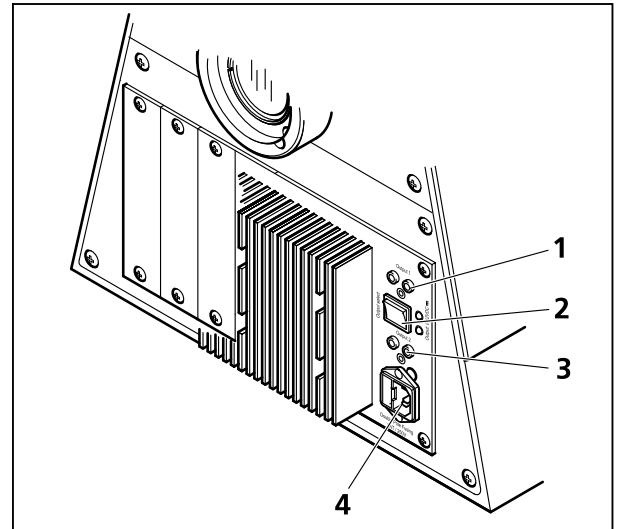


Fig. 3-30 Axio Observer.A1 (rear view)

3.11.2 D1 stand

Key to Fig. 3-31:

- 1 Socket for transmitted light halogen illuminator (Output 1)
- 2 Reflected/transmitted light (HAL) switch
- 3 Socket for reflected light halogen illuminator (Output 2)
- 4 Power input socket
- 5 Socket for LCD display transmitted light illumination carrier
- 6 RS-232 socket
- 7 Socket for transmitted light shutter
- 8 TCP/IP socket
- 9 USB port
- 10 CAN port
- 11 Trigger socket (IN/OUT) for shutter
- 12 Socket for external high speed shutter

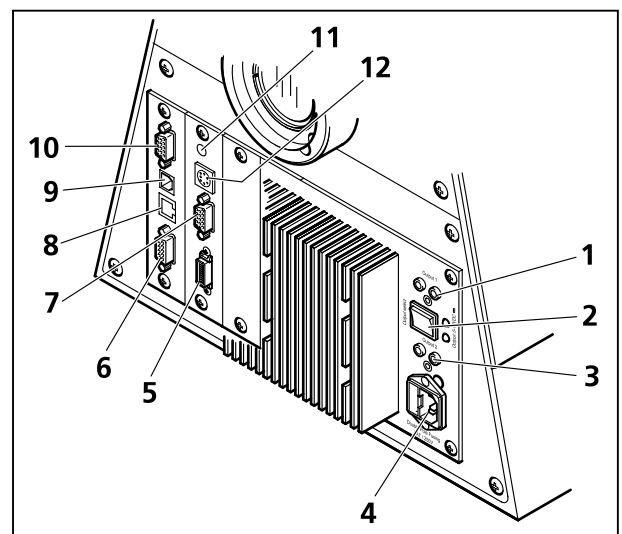


Fig. 3-31 Axio Observer.D1 (rear view)

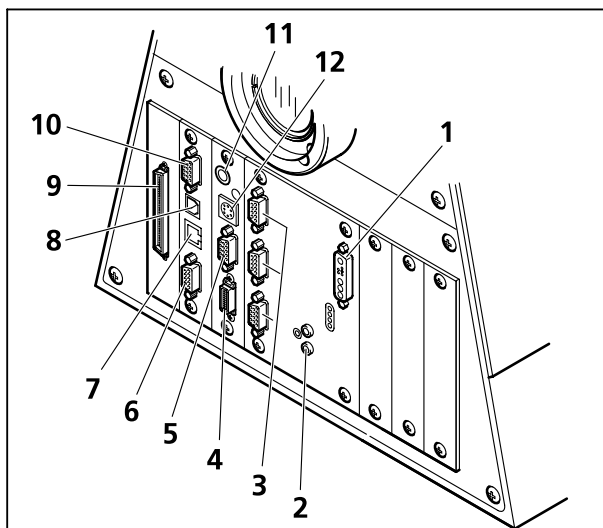


Fig. 3-32 Axio Observer.Z1 (rear view)

3.11.3 Z1 stand

Key to Fig. 3-32:

- 1 VP231 power supply socket
- 2 Socket for transmitted light halogen illuminator
- 3 CAN ports
- 4 not in use
- 5 Socket for transmitted light shutter
- 6 RS-232 socket
- 7 TCP/IP socket
- 8 USB port
- 9 Docking station socket (optional)
- 10 CAN port
- 11 Trigger socket (IN/OUT) for shutter
- 12 Socket for external high speed shutter

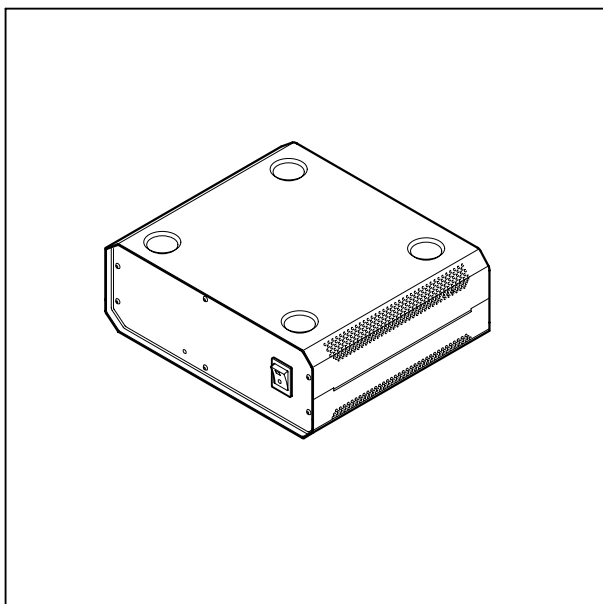


Fig. 3-33 VP231 Power Supply

3.12 Switching the microscope and the power supply for the HBO 100 on and off

A1 stand:

- Switch the microscope on and off using the power switch (on the left of the stand, equivalent position to 3-34/1).

D1 stand:

- Switch the microscope on and off using the power switch (3-34/1).

Z1 stand:

- Switch on the microscope's external power supply unit.
- Start up the microscope using the standby button (on the left of the stand, equivalent position to 3-34/1).
- To switch the microscope off, press the standby button then switch off the external power supply unit.



If the microscope is on, the power LED (3-34/3) at the front of the stand (A1, D1 and Z1) will be illuminated.

Power supply:

- If a fluorescence illuminator (e.g. HBO 100) is connected (see section, *found. Attaching the HBO 100 illuminator*), switch the power supply on (3-34/2) and off using the power switch.

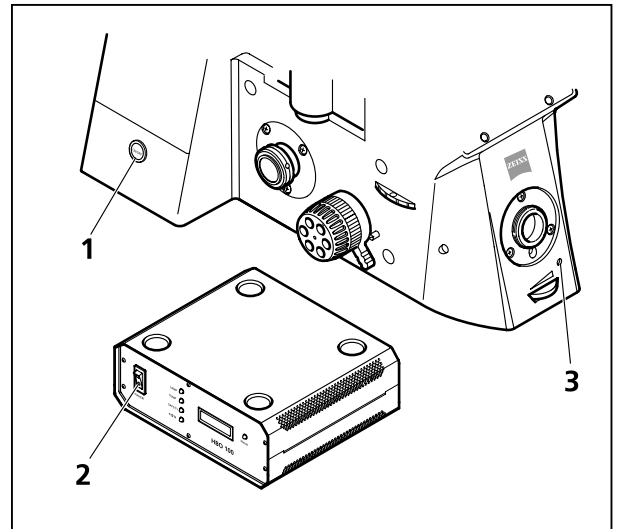


Fig. 3-34 D1 stand and power supply for HBO 100

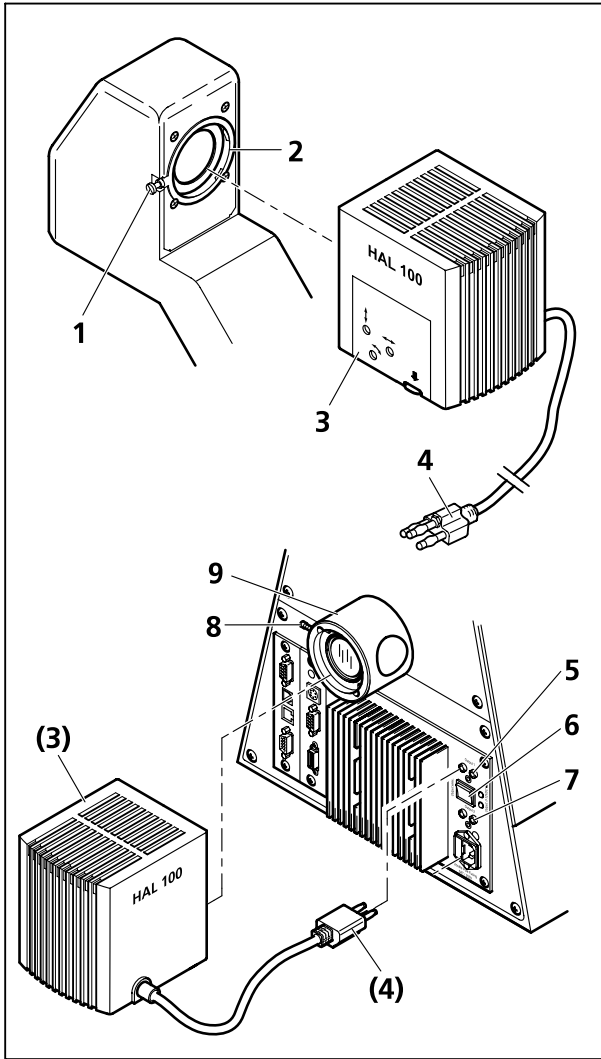


Fig. 3-35 Attaching the HAL 100 halogen illuminator

3.13 HAL 100 halogen illuminator

The HAL 100 halogen illuminator is used as a light source for transmitted light and reflected light techniques (excluding fluorescence) on the Axio Observer. The procedure for attaching the halogen illuminator to the reflected light and transmitted light mounts is essentially the same.

3.13.1 Attaching the HAL 100 halogen illuminator




Before using the halogen illuminator, the bulb replacement tool must be removed from the housing. Failure to do so may result in overheating and damage to the tool (see section 3.13.4).

- Remove the protective cap from the reflected light / transmitted light mount.
- Insert the dovetail of the lamp housing (3-35/3) into the socket (3-35/2 / 3-35/9) and tighten the locking screw (3-35/1 / 3-35/8) using the 3 mm ball-end Allen key.
- Insert the 3 pin plug (3-35/4) into the appropriate 3 pin socket (3-35/5 for transmitted light / 3-35/7 for reflected light) on the rear of the instrument.



Only **one** halogen illuminator can be directly connected to the Z1 stand.

- Switch the transmitted light / reflected light switch (3-35/6) to the required position.

 The Light Manager function depends on the position of the reflected light / transmitted light switch.

3.13.2 Adjusting the HAL 100 halogen illuminator

(1) Coarse adjustment

- Loosen the locking screw (3-35/1 / 3-35/8), and remove the halogen illuminator (3-36/3) from the microscope stand.
- Switch on the microscope.
- Direct the light beam onto a projection area (wall) at a distance of at least 3 m.



Do not look into the light emitted by the illuminator.

- Using a 3 mm ball-end Allen key adjust the adjusting screw (3-36/3) until the two filament images on the projection area are as sharply in focus as possible.
- Adjust the two adjusting screws (3-36/4 and 5) until one filament image precisely fills the gaps in the reflected image (3-36/1).

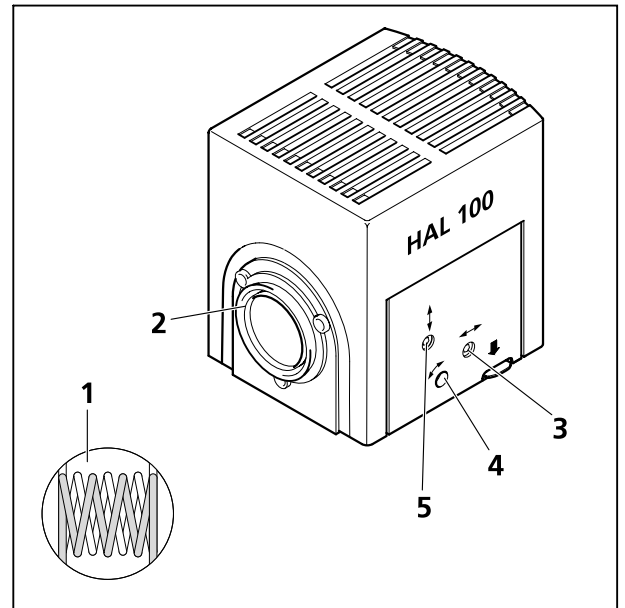


Fig. 3-36 Adjusting the HAL 100 halogen illuminator

(2) Fine adjustment

- Reattach the microscope illuminator to the microscope stand and lock it in place with the locking screw.
- Remove the diffuser (see section 3.13.3) and filter from the optical path.
- Focus on the specimen with a $\leq 40\times$ objective and find an empty area on the slide.
- Remove the eyepiece and center the image of the lamp filament and its reflection using the adjusting screws (3-36/4 and 5).
- Adjust the adjusting screws (3-36/3) until the illumination of the visible image is as homogeneous as possible.



To carry out fine adjustment of the halogen illuminator when mounted on the reflected light socket, it is helpful to use the adjusting aid (3-41/5). After pulling out the adjusting aid, the lamp filament and reflected image can be viewed directly in the viewing glass of the adjusting aid.

- Insert the diffuser and reactivate the filter.

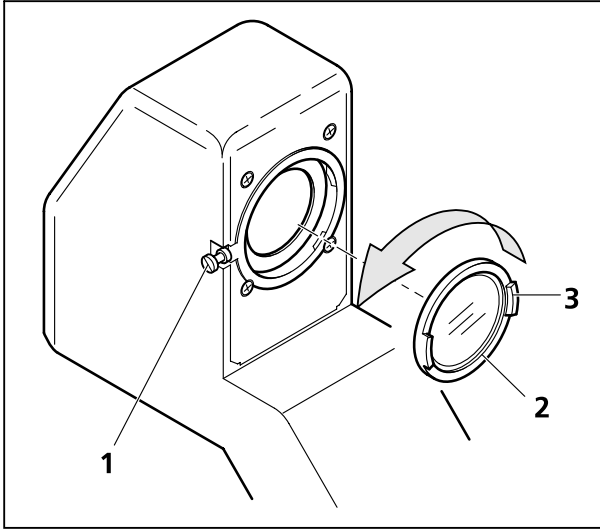


Fig. 3-37 Fitting and removing the diffuser

3.13.3 Fitting and removing the diffuser for fine adjustment of the halogen illuminator

The diffuser must be removed before carrying out fine adjustment:

- Unscrew the HAL 100 locking screw (3-37/1) and remove the illuminator from the transmitted light illumination mount.
- Manually unscrew the diffuser (3-37/2) from the adaptor (anti-clockwise). Hold the edge of the diffuser by the projections on the disk (3-37/3).
- Reattach the HAL 100 and tighten the locking screw.
- If there are any filters in the optical path, remove (deactivate) them.
- Focus on the specimen using a $\leq 40\times$ objective and find an empty area on the slide.
- Remove the eyepiece and center the image of the lamp filament and its reflection using the adjusting screws (3-36/4 and 5).
- Adjust the adjusting screws (3-36/3) until the illumination of the visible image is as homogeneous as possible.
- Once you have completed the adjustment, remove the HAL 100.
- Manually screw the diffuser into the adaptor.
- Reattach the HAL 100 and replace (activate) any filters.

3.13.4 Replacing the HAL 100 halogen bulb



CAUTION
Hot surface!



The lamp housing does not need to be removed from the stand in order to replace the halogen bulb. The bulb replacement tool (3-38/7) is **not** to be stored in the lamp housing when the illuminator is in use.
The spare bulb (3-35/8) can remain in place in the lamp housing.

- Switch off the microscope and disconnect the connector (3-35/4) from the 12 V/100 W socket (3-35/7 for reflected light / 3-35/5 for transmitted light). Allow to cool for about 15 min.
- Press the release button (3-38/3) on the HAL 100 halogen illuminator (3-38/1) downwards and remove the bulb holder (3-38/2) completely. Place it on a flat surface.
- Press down the two spring levers (3-38/5) and remove the old halogen bulb (3-38/6) by pulling upwards.
- Press down the two spring levers and insert the new bulb into the bulb socket (3-38/4). Release the spring levers. Always hold the halogen bulb by means of the bulb replacement tool (3-38/7), as traces of grease on the bulb may shorten bulb life.
- Press down the spring levers once more to center the bulb.
- Reinsert the bulb holder until you feel it click into place.

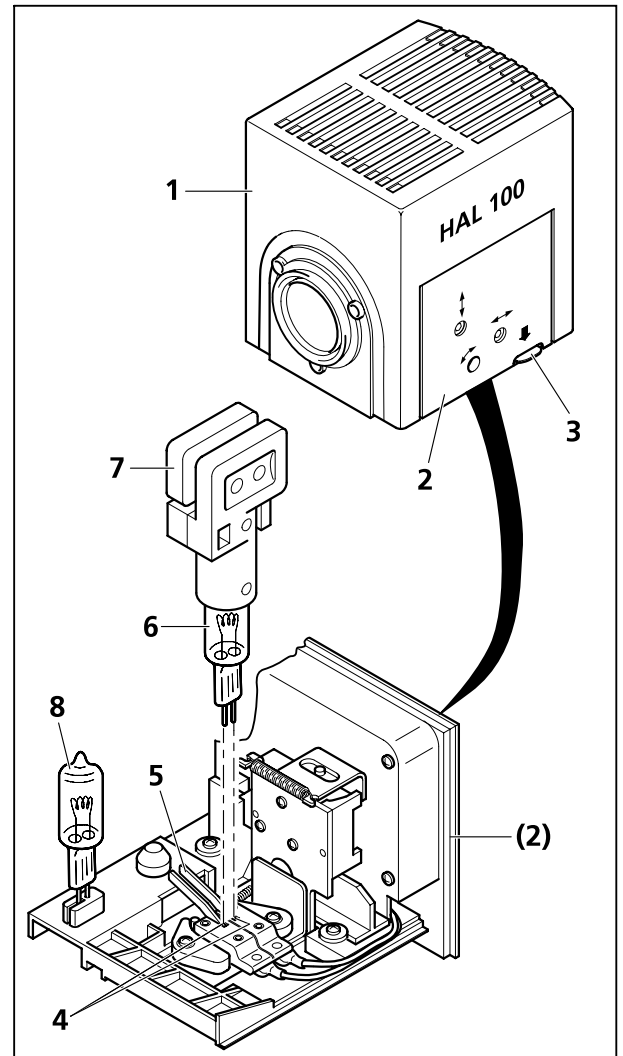


Fig. 3-38 Replacing the halogen bulb

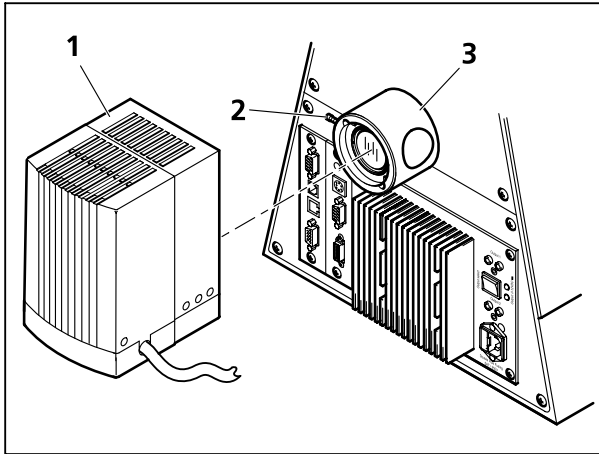


Fig. 3-39 Attaching the HBO 100 illuminator

3.14 HBO 100 illuminator

3.14.1 Inserting the HBO 103 W/2 mercury vapor short-arc bulb

For safety reasons, the HBO 100 illuminator and the HBO 103 W/2 mercury vapor short-arc bulb are packed separately.

The first step in setting up the illuminator is therefore to insert the HBO 103 W/2 bulb into the lamp housing.

Instructions on inserting or replacing the HBO 103 W/2 bulb can be found in the operating manual supplied with the illuminator.



CAUTION

An FL attenuator, discrete (manual or motorized) should be used to modify the transmission.

3.14.2 Attaching the HBO 100 illuminator

- Remove the cover from the reflected light mount (3-39/3).
- Insert the dovetail of the lamp housing (3-39/1) into the reflected light mount (3-39/3) on the rear of the instrument and tighten the locking screw (3-39/2) using the 3 mm ball-end Allen key.
- Insert the multi-pin plug of the HBO 100 illuminator into the device socket (3-40/1) on the HBO 100 power supply and secure with the coupling ring.
- Insert the power cable into the power socket (3-40/2) on the HBO 100 power supply, then insert the plug into a mains socket.

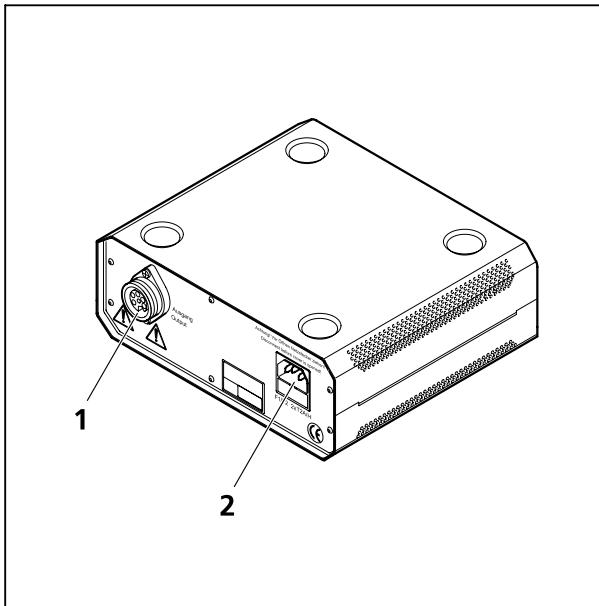


Fig. 3-40 Power supply for HBO 100

3.14.3 Adjusting the HBO 100 illuminator

Two versions (manual and automatic adjustment) of the HBO 100 illuminator are available.

The self-adjusting HBO 100 (423011-9901-000) adjusts the illumination automatically after the power supply is switched on.

The instructions below describe adjustment of the manually adjusted version of the HBO 100 illuminator (423010-0000-000) with and without the adjusting aid.

Adjustment with adjusting aid

- Switch on the HBO 100 illuminator (3-41/1) at the HBO 100 power supply (3-34/2) and allow to warm up to operating temperature.
- Pull out the adjusting aid (3-41/5) on the microscope stand. The HBO 103 W/2 bulb's brighter focal spot and the slightly darker reflected image will be visible in the smoked glass window of the adjusting aid.
- Using the knurled knob (3-41/4), adjust the collector to bring the brighter focal spot into sharp focus.
- Using the adjusting screws (3-41/2 and 3), adjust the darker, reflected focal spot so that it lies within the engraved circle, as shown in the focal spot illustration (3-41/6).
- Push the adjusting aid back in.



The HBO 103 W/2 bulb's two focal spots should be closely juxtaposed within the circle on the adjusting aid!

Adjustment without adjusting aid



CAUTION

Never look directly into the ignited lamp to avoid the danger of a possibly irreparable damage to the eyes. Put on protective glasses, e.g. a pair of sunglasses, to protect your eyes when observing the bright light spot.

- Unscrew an objective and control the image of the light source through the open position on a sheet of paper in the object plane (on the stage).
- Adjust the collector by means of the knurled knob (3-41/4) to bring the brighter arc into sharp focus.
- Position the arc image beside the arc (analogously to 3-41/6) centrally using the 3 mm ball-end Allen key and the adjustment screws for vertical (3-41/2) and horizontal adjustment (3-41/3), respectively.
- Objektiv wieder in Objektivrevolver einschrauben.

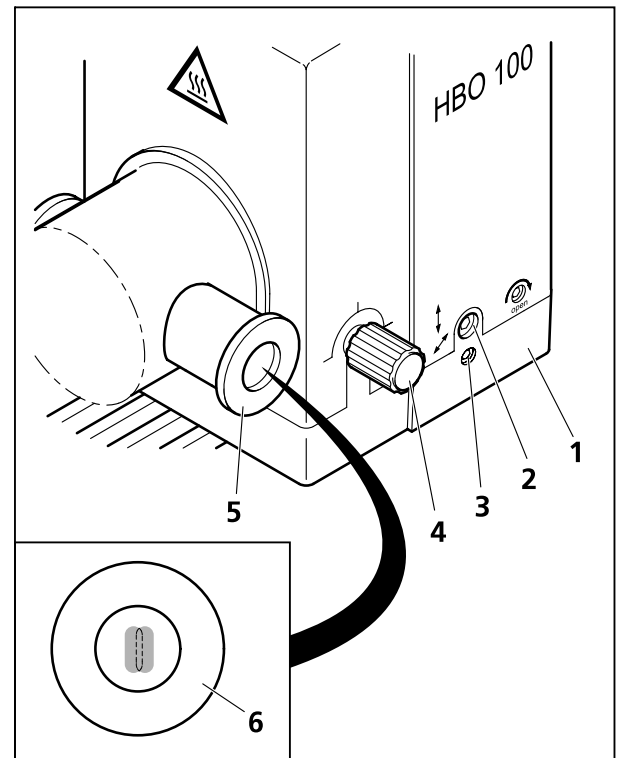


Fig. 3-41 Adjusting the HBO 100

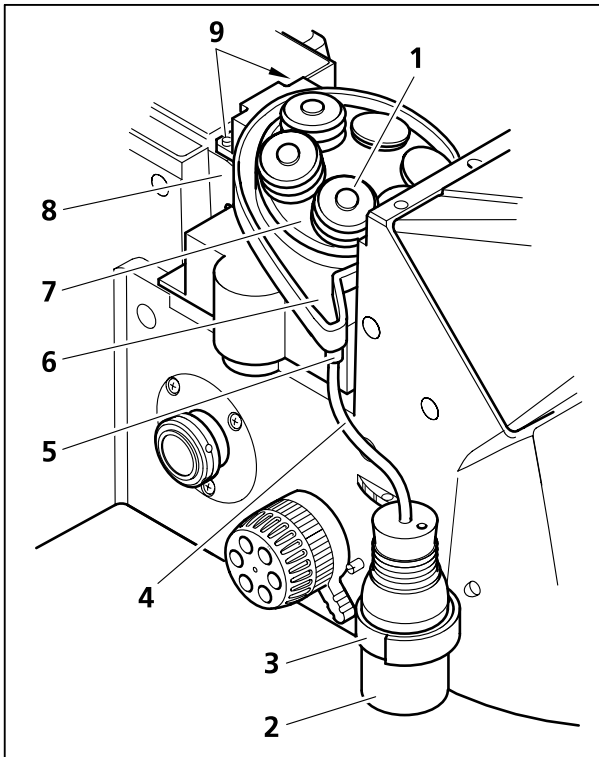


Fig. 3-42 Fitting the Aqua Stop II

3.15 Fitting the Aqua Stop II

The Aqua Stop II should be used to protect the objective and nosepiece when working with liquid specimens.


- Detach the microscope stage and screw out the objectives.
- Place the collecting trough (3-42/6) onto the nosepiece mount (3-42/8) and screw in place using two screws (3-42/9).
- Place the cover disk (3-42/7) onto the nosepiece.
- Load the nosepiece with the required objectives.
- Pull a suitably sized objective hood (3-42/1) over each objective.



Ensure that each objective hood is pulled up to the cover disk.


Two types of objective hood are available - size 1 (small) and size 2 (large).

- Objectives with a front diameter of **16 - 22.5 mm** should be protected using the **DMR small objective hood** (431716-0160-000).
- Objectives with a front diameter of **27.5 - 34 mm** should be protected using the **DMR large objective hood** (431716-0170-000).

 When attaching the objective hoods please take care that the upper edge does not form a collecting trough.


Unused nosepiece openings should be sealed using the caps supplied.

- Attach the drainage tube (3-42/4) to the drainage connector (3-42/5). Insert the other end of the tube through the bung on the collecting bottle (3-42/2) so that it projects 3 to 4 mm through the bung.

 The length of the tube must be such that the outlet of the collecting receptacle does not become bent during focusing.

- Insert the bung firmly into the collecting bottle.
- Attach the enclosed Velcro fastener (3-42/3) to the stand. Attach the collecting bottle to the stand using the Velcro fastener.
- Reattach the microscope stage.

In the event of any kind of accident involving liquids, the microscope stage should be removed and any drops of liquid soaked up using a lint free cloth. In particular, the front lens of the objective must be cleaned in order to continue to obtain optimum performance from the objective.

 For cleaning instructions please see the brochure "The Clean Microscope".

3.16 Attaching the mounting adaptor for Laserport to the stand

The Laserport adaptor can be used to couple external laser equipment to the microscope.



The connection of laser equipment to the Axio Observer stand is carried out at the customer's own risk.



The customer must ensure that all relevant regulations regarding the use of laser radiation for the relevant laser class are observed.

- Unscrew the four screws (3-43/3) from the plastic cover (3-43/4) and remove the cover from the stand by pulling to the right.
- Attach the mounting adaptor (3-43/2) and block (3-43/1) to the stand and screw into place using the six screws.
- Attach the laser in accordance with the manufacturer's instructions.

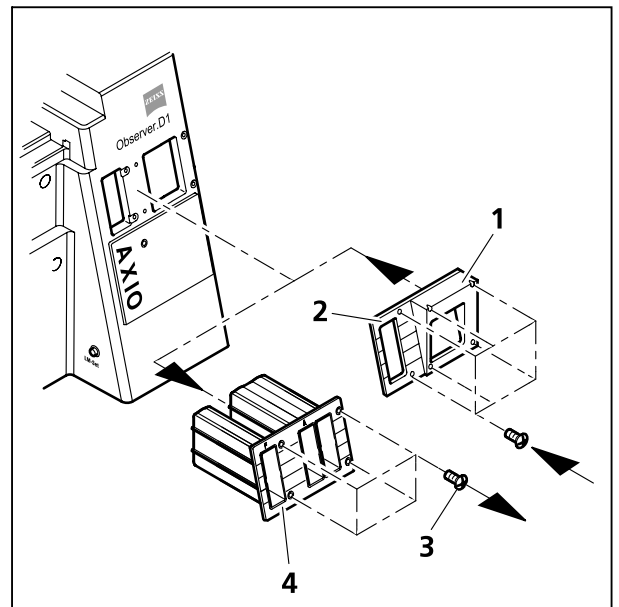


Fig. 3-43 Fitting the Laserport adaptor

4 OPERATION

The Axio Observer microscope is available with three different stand versions.

- Axio Observer.A1 (manual version)
- Axio Observer.D1 (coded / semi-motorized version)
- Axio Observer.Z1 (fully motorized version, including motorized Z-drive and TFT display)

The "Operation" section describes the configuration and operation of all three stand versions (see sections 4.1 and 4.2). Many of the operating functions are, however, identical.

The Axio Observer.D1 stand is equipped with a 4-line LCD display

The TFT display can only be used with the Axio Observer.Z1 stand. Operation of the microscope using the touchscreen TFT display is described separately (see section 4.6).

This manual does not describe operation of the motorized Axio Observer.Z1 using a PC.

The Axio Observer microscopes have been designed for use with incubators and micromanipulators. For information on connection and operation of these units, please see the relevant operating manual.

4.1 Overview of components and controls

4.1.1 Axio Observer.A1 (manual)

Key to figure 4-1:

- 1 On / Off switch
- 2 Left Sideport
- 3 Coarse / fine focus drive (left side) with fine drive, flat
- 4 Light path switching control (leftSideport / vis)
- 5 Objective nosepiece
- 6 Vertical adjustment knob for condenser
- 7 Condenser centering screw
- 8 Condenser
- 9 Microscope stage
- 10 3-position filter slider slot (diameter 25 mm)
- 11 Slot for iris stop slider as reflected light aperture stop or FL attenuator
- 12 Slot for iris stop slider as reflected light luminous-field stop
- 13 Drive knobs for controlling XY positioning of the mechanical stage
- 14 Reflector turret
- 15 Focus drive coarse / fine (right side)
- 16 TL button for switching the transmitted light halogen illuminator on and off or for opening and closing the transmitted light shutter
- 17 RL button for switching the reflected light shutter (fluorescence) on and off
- 18 Halogen illumination intensity control
- 19 Binocular tube
- 20 Binocular section of the binocular tube
- 21 Eyepiece
- 22 Eyepiece adjustment ring
- 23 Polarizer D with 2-position filter changer or 3-position filter changer
- 24 Luminous-field stop control

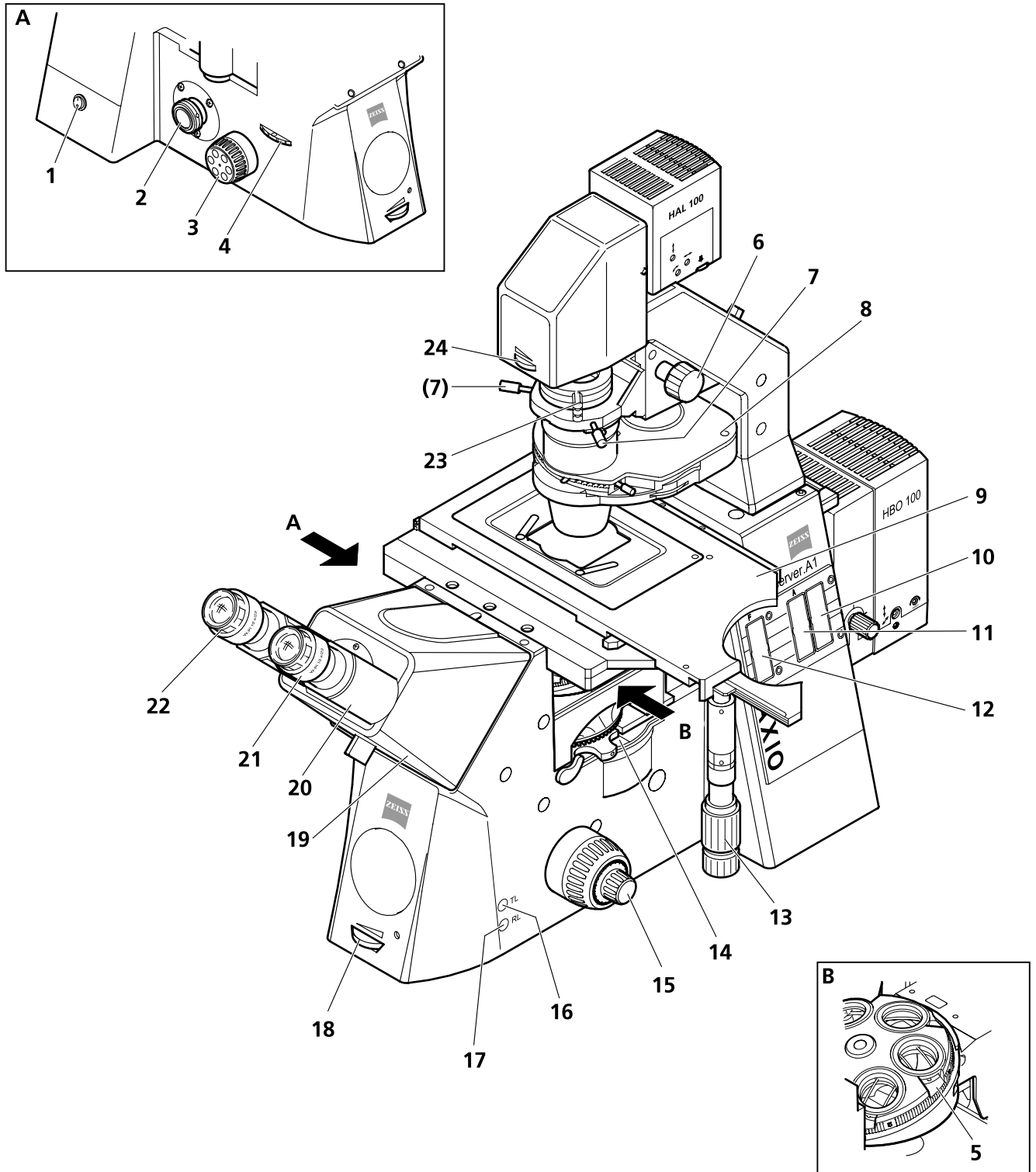


Fig. 4-1 Axio Observer.A1 components and controls (manual)

4.1.2 Axio Observer.D1 (coded, semi-motorized)

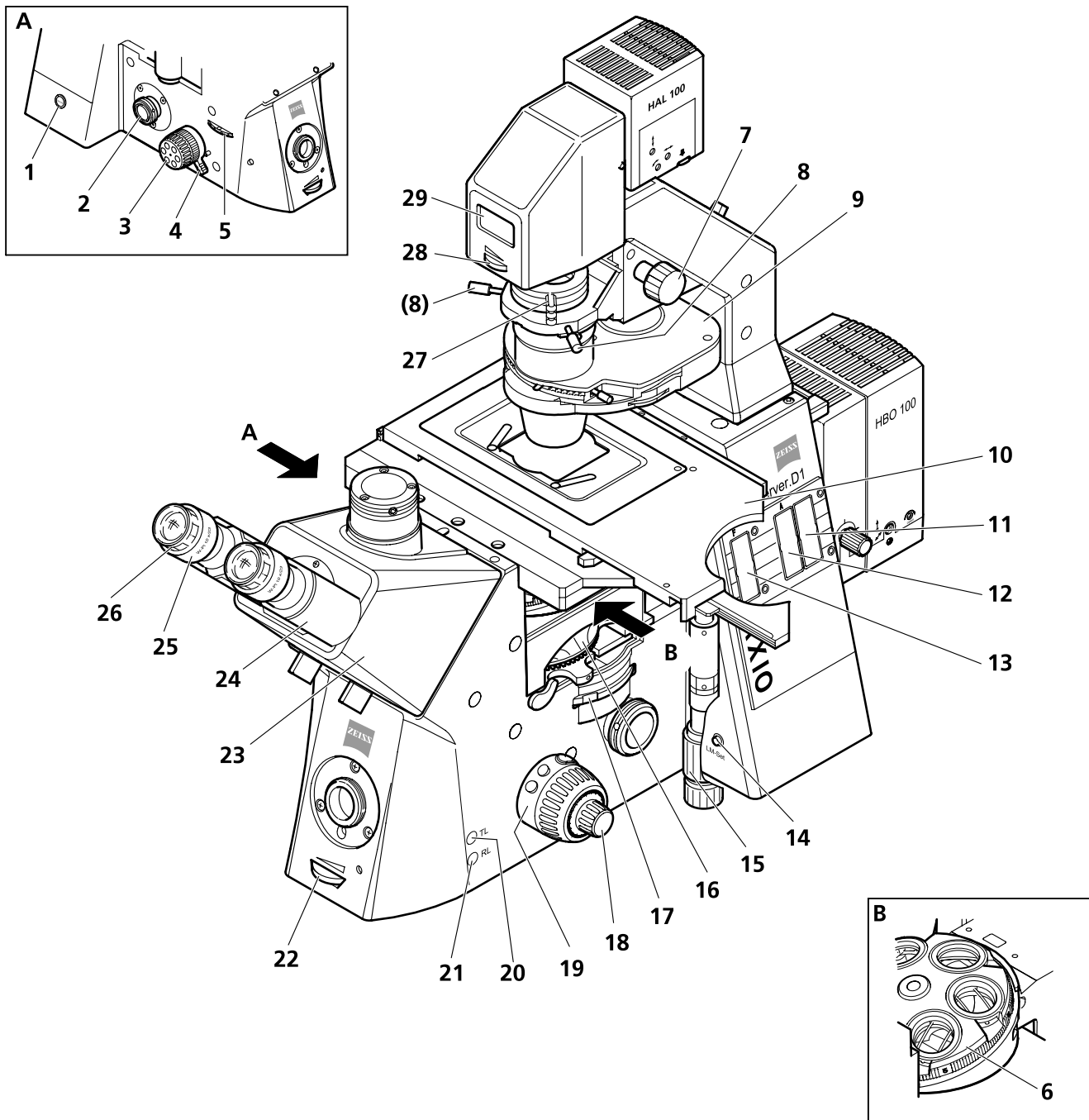


Fig. 4-2 Axio Observer.D1 components and controls (coded, semi-motorized)

Key to figure 4-2:

- 1 On / off button
- 2 Left Sideport
- 3 Coarse / fine focus drive (left side) with fine drive, flat
- 4 Vertical stop for focus drive
- 5 Light path switching control (left / right Sideport / vis)
- 6 Objective nosepiece
- 7 Vertical adjustment knob for condenser
- 8 Condenser centering screw
- 9 Condenser (manual or motorized)
- 10 Microscope stage
- 11 3-position filter slider slot (diameter 25 mm)
- 12 Slot for iris stop slider as reflected light aperture stop (manual or motorized) or FL attenuator (manual or motorized)
- 13 Slot for iris stop slider as reflected light luminous-field stop (manual or motorized)
- 14 LM set button
- 15 Drive knobs for controlling XY positioning of the mechanical stage
- 16 Reflector turret (coded or motorized)
- 17 Optovar turret control wheel (max. 3 positions)
- 18 Focus drive coarse / fine (right side)
- 19 Control ring, right
- 20 TL button for switching the transmitted light halogen illuminator on and off or for opening and closing the transmitted light shutter
- 21 RL button for switching the reflected light shutter (fluorescence) on and off
- 22 Halogen illumination intensity control
- 23 Binocular tube
- 24 Binocular section of the binocular tube
- 25 Eyepiece
- 26 Eyepiece adjustment ring
- 27 Polarizer D with 2-position filter changer or 3-position filter changer
- 28 Luminous-field stop control
- 29 LCD display

4.1.3 Axio Observer.Z1 (motorized)

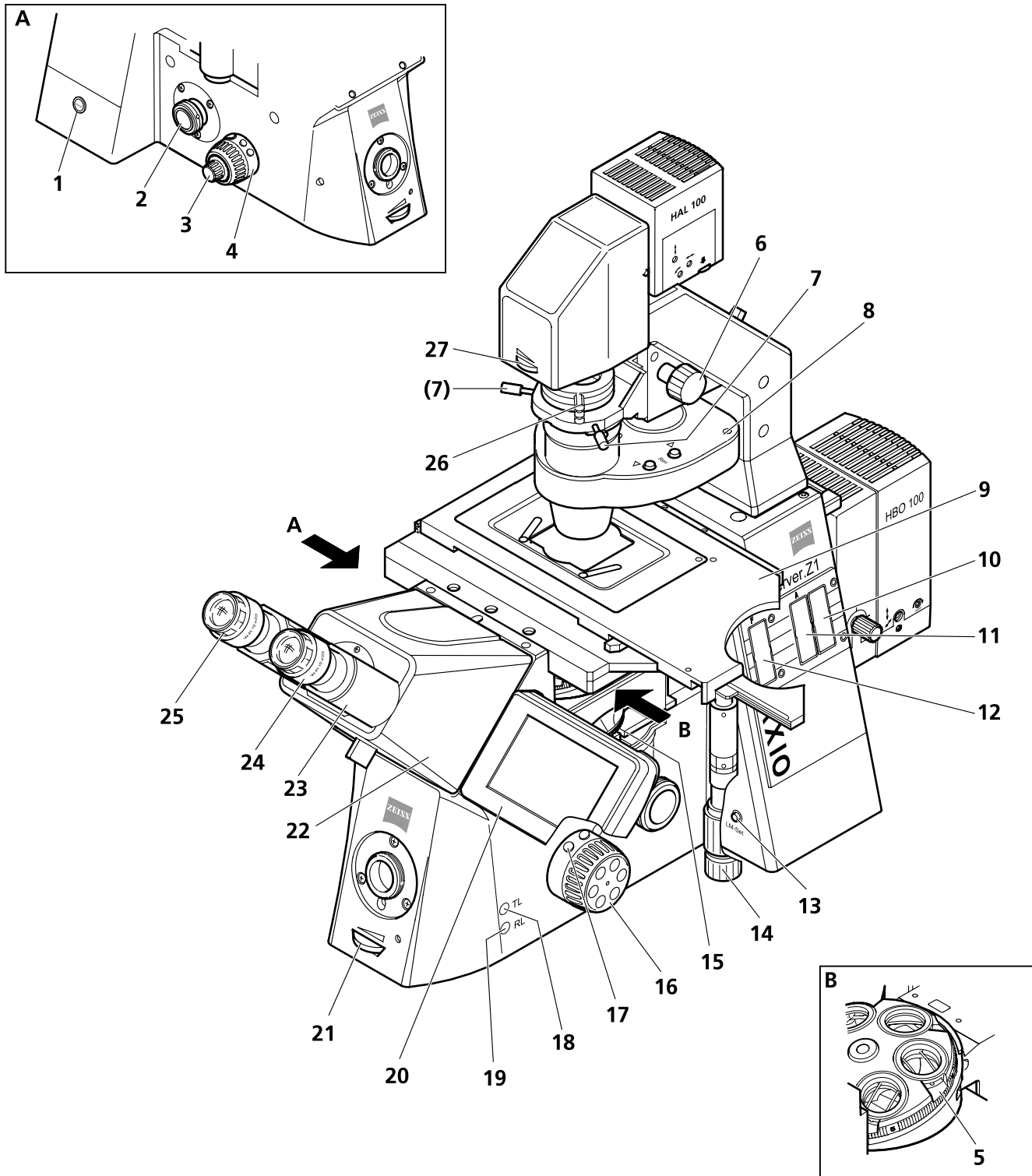


Fig. 4-3 Axio Observer.Z1 components and controls (motorized)

Key to figure 4-3:

- 1 Standby button
- 2 Left Sideport
- 3 Focus drive coarse / fine (left side)
- 4 Control ring, left
- 5 Objective nosepiece
- 6 Vertical adjustment knob for condenser
- 7 Condenser centering screw
- 8 Condenser (manual or motorized)
- 9 Microscope stage
- 10 3-position filter slider slot (diameter 25 mm)
- 11 Slot for iris stop slider as reflected light aperture stop (motorized) or FL attenuator (motorized)
- 12 Slot for iris stop slider as reflected light luminous-field stop (motorized)
- 13 LM set button
- 14 Drive knobs for controlling XY positioning of the mechanical stage
- 15 Reflector turret (coded or motorized)
- 16 Coarse / fine focus drive (motorized) with fine drive, flat (right side)
- 17 Control ring, right
- 18 TL button for switching the transmitted light halogen illuminator on and off or for opening and closing the transmitted light shutter
- 19 RL button for switching the reflected light shutter (fluorescence) on and off
- 20 TFT display
- 21 Halogen illumination intensity control
- 22 Binocular tube
- 23 Binocular section of the binocular tube
- 24 Eyepiece
- 25 Eyepiece adjustment ring
- 26 Polarizer D with 2-position filter changer or 3-position filter changer
- 27 Luminous-field stop control

4.2 Description of components and controls

On / off switch (4-1/1), on / off button (4-2/1), standby button (4-3/1)

- If the microscope is on, the power LED on the front of the stand will light up (see also section 3.12).

Left Sideport (4-1/2), (4-2/2), (4-3/2)



- Port for connecting documentation equipment.
- Various splitting ratios for right Sideport and visual observation (vis), depending on instrument configuration.




Manual or motorized focus drive, left side (4-1/3), (4-2/3), (4-3/3)




- Range of travel: approx. 10 mm
- Coarse adjustment (large knob):
Coarse focusing knob: 1 revolution = 2 mm
- Fine adjustment (small knob):
Fine adjustment: 1 revolution = 0.2 mm
- Optional: fine drive, flat




Light path switching control (left / right Sideport / vis) (4-1/4), (4-2/5)

- Selects beam splitting for right Sideport (doc), left Sideport (doc) and visual (vis) observation (A1 stand, left Sideport / vis only).
- 2 or 3 switch positions with various beam splitting ratios.
- Where instrument is configured with left Sideport 60N, 2 switching positions:

100 % vis : 0 % doc;	
20 % vis : 80 % doc left;	
- Where instrument is configured with left Sideport 60N, 3 switching positions:

100 % vis : 0 % doc;	
0 % vis : 100 % doc left;	
50 % vis : 50 % doc left	
- Where instrument is configured with right Sideport 60N, 3 switching positions:

100 % vis : 0 % doc;	
0 % vis : 100 % doc right;	
50 % vis : 50 % doc right	
- Where instrument is configured with left and right Sideport 60N, 3 switching positions:

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

Nosepiece with objectives (4-1/5), (4-2/6), (4-3/5)

- 6-position nosepiece H, DIC (4-4/1) with DIC slider slot (4-4/2) in all objective positions in manual, coded or motorized versions (A1 stand, three objective positions only with DIC slider slot).
- Manual and coded nosepieces: Rapid objective change by rotating the nosepiece using the adjustment ring (4-4/3).
- Motorized nosepiece: Rapid objective change by, for example, operating the pair of buttons on the right control ring (4-16/1). The nosepiece can also be operated manually.



If the heating stage or the gliding stage is used, the nosepiece must be lowered fully using the focus drive before changing objectives - otherwise the objective may collide with the stage.

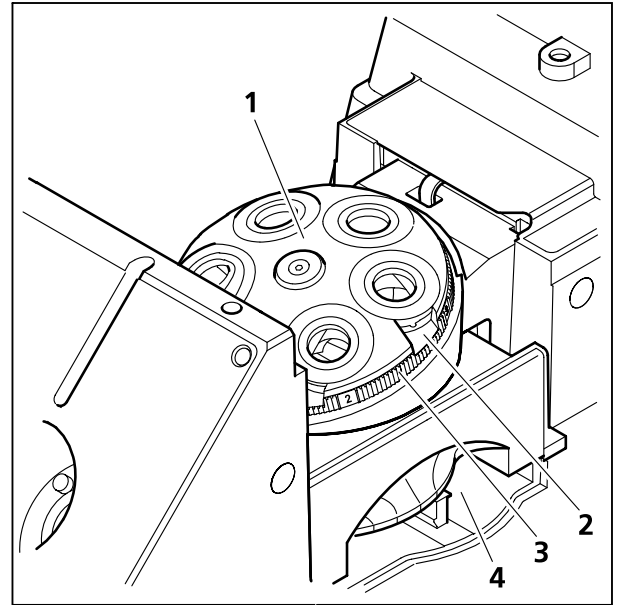


Fig. 4-4 Nosepiece with slot for DIC slider and PlasDIC slider; analyzer slider slot

Analyzer slider slot (4-4/4)

- For fixed analyzer sliders with two 32 mm filter positions, or $\pm 30^\circ$ analyzer sliders for DIC (available for all stands).

Vertical adjustment knob for condenser (4-1/6), (4-2/7), (4-3/6)

- Adjustment knob on the transmitted light illuminator carrier for raising and lowering the condenser to set KÖHLER illumination.

Condenser centering screw (4-1/7), (4-2/8), (4-3/7)

- Centering screws on both sides of the transmitted light illuminator carrier for centering the condenser.

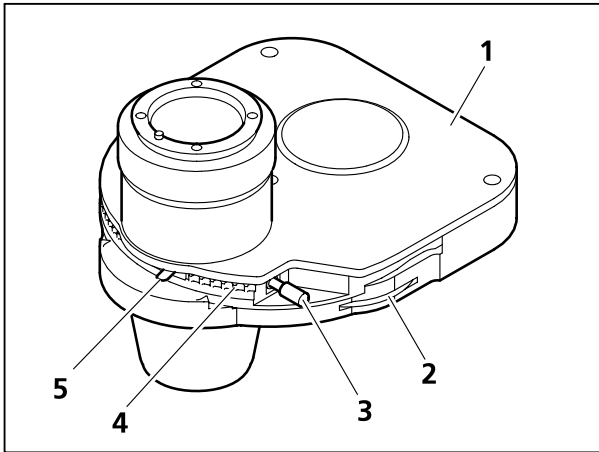


Fig. 4-5 LD condenser 0.55, 6-position
H Ph 1, Ph 2, Ph 3, Var 1/2

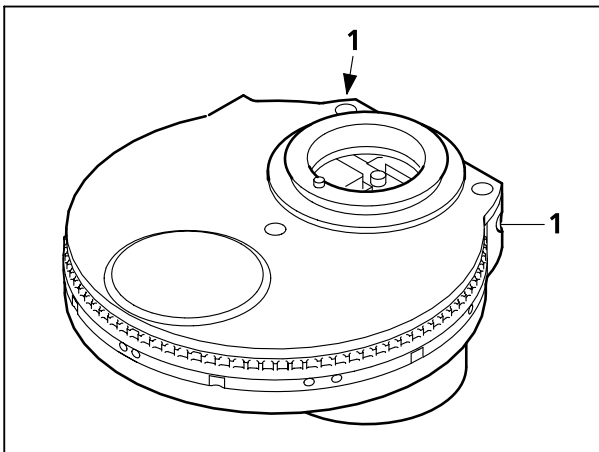



Fig. 4-6 LD condenser 0.35; 6-position
H, Ph 0, Ph 1, Ph 2, DIC, DIC

Manual condensers (4-1/8), (4-2/9)

Depending on the version, condensers (4-5/1) are equipped with the following:

- 5 or 6-position turret for:
 - bright field: H
 - Phase contrast: Ph 0, Ph 1, Ph 2, Ph 3 with centerable stops
 - Interference contrast: DIC
 - Varel contrast: Var 1, Var 2
 - PlasDIC: with 3.5 mm slit aperture for PlasDIC
- Aperture stop (iris stop). The aperture stop is opened and closed using the aperture control (4-5/2).
- Rotate the turret wheel (4-5/4) to swivel the bright field insert or contrast stops into the optical path.
- The short designation for the turret position (e.g. **H**) is displayed on the front of the turret, facing the user.
- Condensers for Varel contrast are equipped with a control (4-5/3) for adjusting the position of the Varel stop (when the Varel turret position is selected). The lever (4-5/5) is used to switch between Var 1 and Var 2.
- For condensers 0.35 and 0.55 for phase contrast, a 1.5 mm Allen key (inserted at 4-6/1) is used to center the phase stops.

 The condenser turrets feature a so-called automatic stop mechanism. The aperture stop (iris stop) is opened fully when a phase stop turret position is selected. The aperture stop opening is automatically reset to the previous setting when another turret position is selected.

Motorized condensers (4-3/8)

- The turret is moved by pressing the **Rev** \triangle ∇ (forwards and backwards, 4-7/1) keys on the right side of the condenser.
- Motorized aperture stop operation using the **A** key (open and close, 4-7/2) on the left side of the condenser. If there is a phase stop in the optical path, the aperture stop is always opened fully automatically (NA = 0.55).

Manual or motorized microscope stage with specimen holder (4-1/9), (4-2/10), (4-3/9)

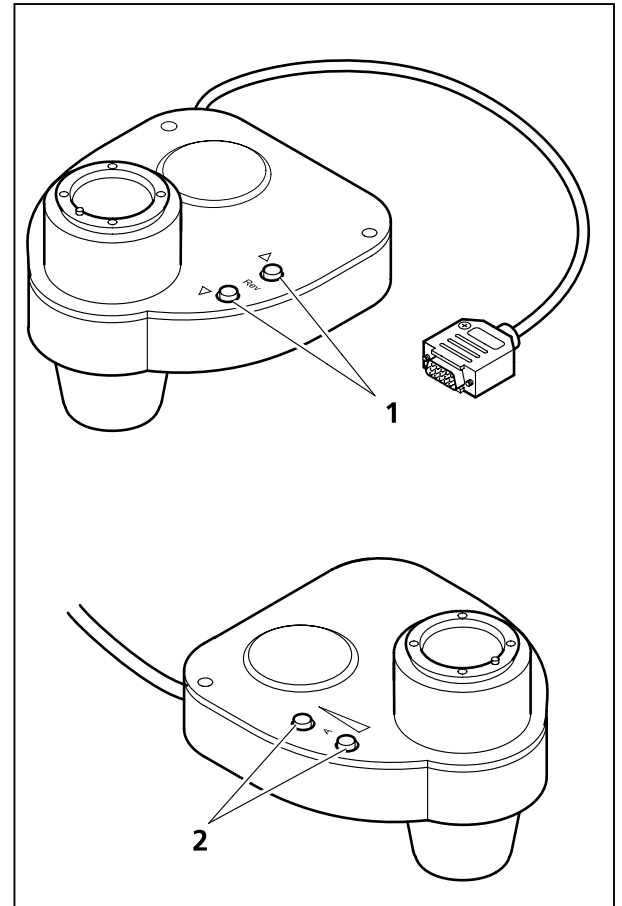
- Specimens are held, positioned and fixed by the specimen holder.
- Depending on instrument configuration, includes: specimen stage 250x230 with object guide 130x85 and mounting frame M for object guide;
mechanical stage 130x85,
mechanical stage 130x85 with short coaxial drive,
scanning stage 120x100 STEP,
scanning stage 130x85 mot. CAN,
Mounting frame K for mechanical stages and scanning stages;
Gliding stage Z

3-position filter slider slot (4-1/10), (4-2/11), (4-3/10)

- For 3-position, 25 mm diameter filter slider
- Insert the filter slider with the labeling visible from the front to the required stop position.

Slot for reflected light iris stop slider or FL attenuator (4-1/11 and 12), (4-2/12 and 13), (4-3/11 and 12)

- Slot for iris stop slider (4-8/1) as aperture / luminous field stop for setting KÖHLER illumination.
- The FL attenuator should only be used in the aperture stop plane (slot **A**).
- Insert the slider into the slot until it clicks into place. The aperture opening symbol (wedge) should be facing towards the user.



**Fig. 4-7 Condenser 0.55, 6-position
H, Ph 1, Ph 2, Ph 3, DIC, DIC**

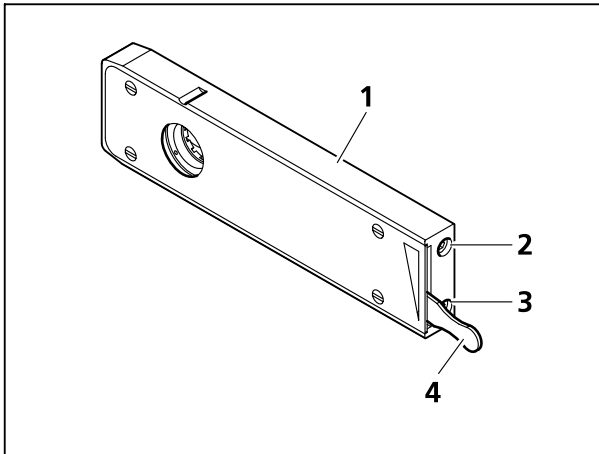


Fig. 4-8 Iris stop slider, manual

Manual iris stop slider for reflected light and manual FL attenuator

- The lever (4-8/4) on the slider is used to open and close (lower position) the iris stop.
- The two centering 3 mm Allen screws (4-8/2 and 3) can be used to center the stop in the optical path.
- The FL attenuator is used to attenuate the light in the fluorescence path when using the HBO 100 (slot **A**).
- The FL attenuator has six labeled positions which are selected by turning the control wheel.

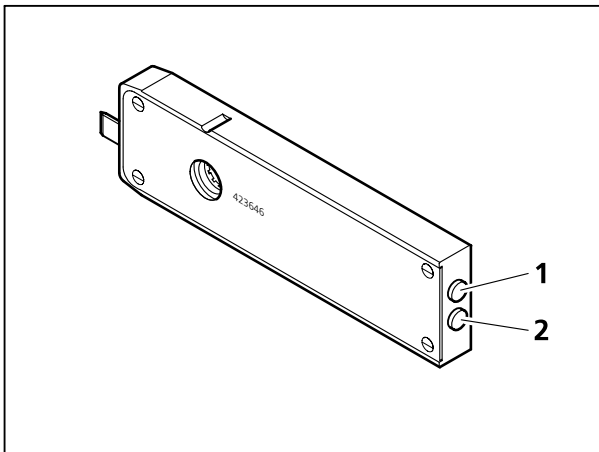


Fig. 4-9 FL attenuator, motorized

Motorized iris stop slider for reflected light and motorized FL attenuator

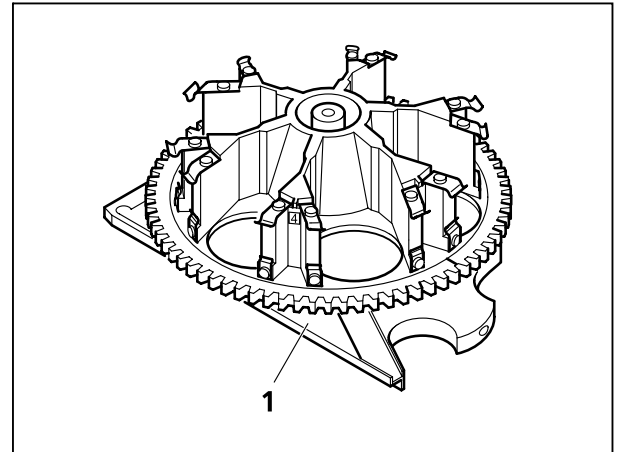
- The motorized slider and motorized FL attenuator are not compatible with the A1 stand.
- The stop is opened or closed by pressing the appropriate button on the slider.
- The motorized (or manual) FL attenuator is used to attenuate the light in the fluorescence path when using the HBO 100 (slot **A**).
- The FL attenuator has six positions which can be selected in forward or reverse order by pressing buttons (4-9/1) and (4-9/2).

Drive knobs for controlling XY positioning of the mechanical stage (4-1/13), (4-2/15), (4-3/14) or object guide (if the specimen stage 250x230 with object guide is fitted)

- Upper drive knob: movement in the Y-direction.
- Lower drive knob: movement in the X-direction.

Manual, coded or motorized 6-position reflector turret (4-1/14), (4-2/16), (4-3/15) / (4-10/1)

- Can be loaded with a maximum of six reflector modules for epifluorescence.
- Manual, coded reflector turret: Rapid reflector change by rotating the adjustment ring. The activated reflector is marked by a line on the right of the reflector turret.
- Motorized reflector turret: Rapid reflector change by, for example, operating the pair of buttons on the right control ring (4-16/1).

**Fig. 4-10 6-position reflector turret****Manual or motorized focus drive, right side** (4-1/15); (4-2/18), (4-3/16)

- Range of travel: approx. 10 mm
- Coarse adjustment (large knob):
Coarse focusing knob: 1 revolution = 2 mm,
- Fine adjustment (small knob):
Fine adjustment: 1 revolution = 0.2 mm
- Optional: fine drive, flat

TL button (4-1/16), (4-2/20), (4-3/18)

- Pressing briefly switches the halogen illuminator on or off. If a transmitted light shutter is built into the transmitted light illuminator carrier, it will be opened or closed.
- Pressing and holding down for > 1 s sets the brightness to the 3200 K value for color photography.

RL button (4-1/17) (4-2/21), (4-3/19)

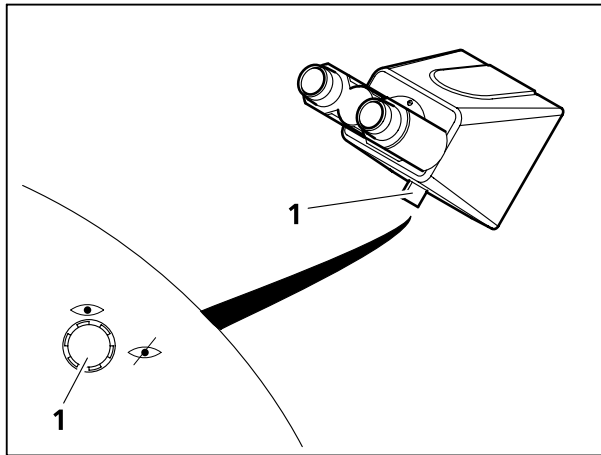
- Pressing briefly (< 1 s) activates or deactivates the external or internal fluorescence shutter (reflected light).



Halogen illuminator intensity control (4-1/18), (4-2/22), (4-3/21)

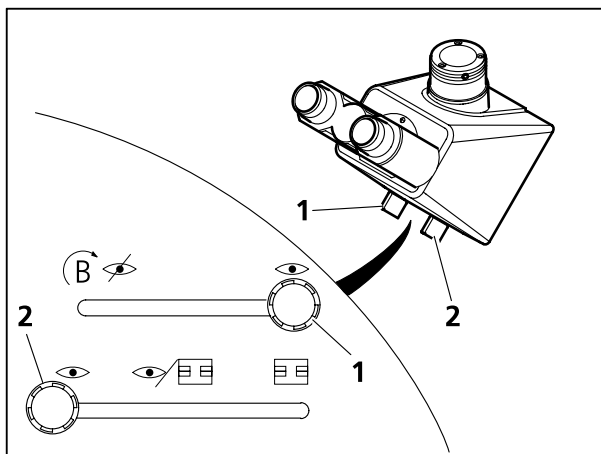
- Controls the brightness of the halogen illuminator.
- Voltage range from 0 to 12 V. On the A1 stand, the maximum is limited by a stop. On the D1 and Z1 stands, an acoustic signal sounds when the maximum brightness is reached; the control is not limited by a stop.






Binocular tubes (4-1/19), (4-2/23), (4-3/22)


- The binocular tubes provided allow the interpupillary distance and the viewing height to be individually adjusted within defined limits.

**Fig. 4-11 Binocular tube 45°/23****Binocular tube 45°/23 with manual shutter vis (4-11)**

- Shutter is opened and closed by turning the knob (4-11/1):
100 % vis 
0 % vis (light blocked) 

**Fig. 4-12 Binocular phototube 45°/23****Binocular phototube 45°/23 with sliding prism for vis / doc, Bertrand lens and manual shutter vis (4-12)**

- Shutter is switched on and off by moving the knob / slide (4-12/1):
100 % vis 
0 % vis (light blocked) 
Bertrand lens
- The Bertrand lens is focused by rotating the knob / slide (with the Bertrand lens in position)
- Light path switching (sliding prism vis / doc) using slide (4-12/2):
0 % vis : 100 % doc 
50 % vis : 50 % doc 
100 % vis : 0 % doc 

 The camera port of the binocular phototube is recommended up to an 18 mm camera field of view (referred to the intermediate image). That means, e.g., for AxioCam with camera adapter 0.63x.

Binocular section of binocular tubes (4-1/20)
(4-2/24), (4-3/23)

- The eyepieces are adjusted for the interpupillary distance by swiveling the two eyepiece sockets towards or away from each other (4-13/A and 4-13/B).
- Two different height settings are available by rotating the binocular section through 180°.

Eyepiece / eyepiece adjustment ring (4-1/21
and 22), (4-2/25 and 26), (4-3/24 and 25)

Both eyepiece models allow for correction of ametropia and can be fitted with eyepiece reticles (see also section 3.2.2).

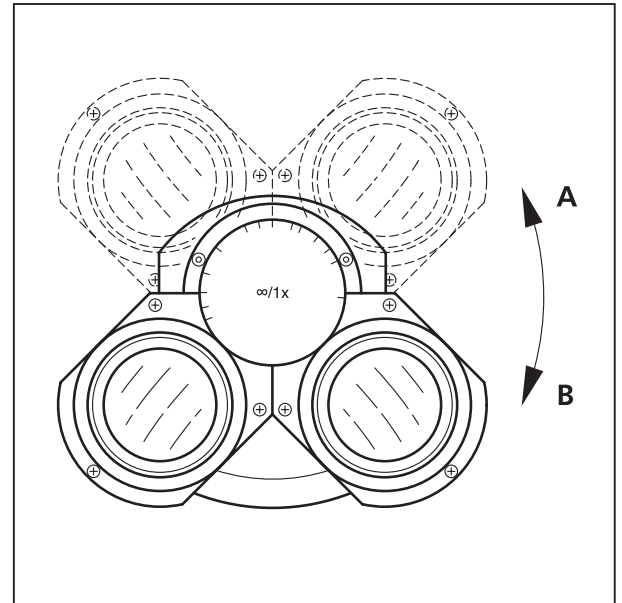


Fig. 4-13 Setting the binocular tube eyepiece distance

Polarizer D with 2-position filter changer or 3-position filter changer (4-1/23), (4-2/27), (4-3/26)

- The polarizer and filter positions can be swiveled into and out of position separately. There are stops for the swiveled in positions.

Luminous-field stop (transmitted light) control (4-1/24), (4-2/28), (4-3/27)

- Control on the transmitted light illuminator / condenser carrier for opening and closing the transmitted light luminous-field stop to set KÖHLER illumination.
- Rotating the control to the right closes the stop
- Rotating the control to the left opens the stop

LM set button (4-2/14), (4-3/13)

- On D1 and Z1 stands only.
- Pressing briefly (< 1 s) saves the Light Manager value
- Pressing and holding down for > 2 s activates configuration mode (D1 stand only)

Optovar turret control wheel (4-2/17)

- On D1 stand only, if the 1-position tube lens holder has not been configured permanently.
- Maximum 3 positions with:
 - Tube lens 1x (always existing);
 - Optovar 1.25x;
 - Optovar 1.6x;
 - Optova 2.5x
 A maximum of two Optovars can be configured.
- If fewer than 3 optics are to be used, the empty positions are blocked.

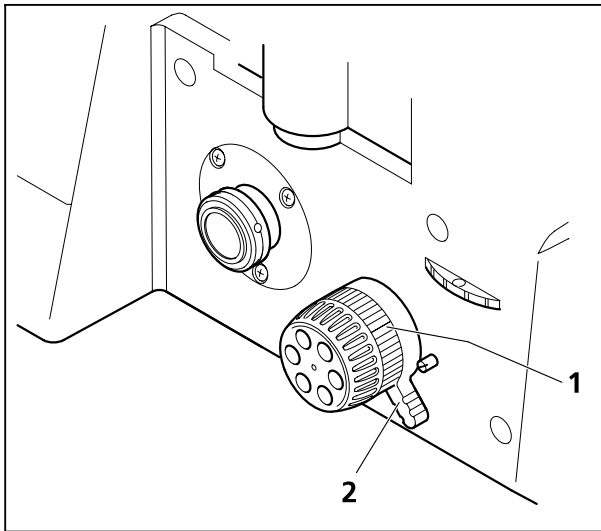


Fig. 4-14 Vertical stop for focus drive

Vertical limit stop for z focus drive (4-2/4)

- On D1 stand only.
- For limiting the upper position of the focus drive (to protect the specimen). The operating principle of the stop is explained by a graphic on the stand.
- Rotate the locking lever of the stop (4-14/2) upwards to the stop pin. Move the stage to the required position using the focus drive. Rotate the knurled wheel (4-14/1) clockwise as far as it will go. Press the locking lever downwards to lock the stop position.

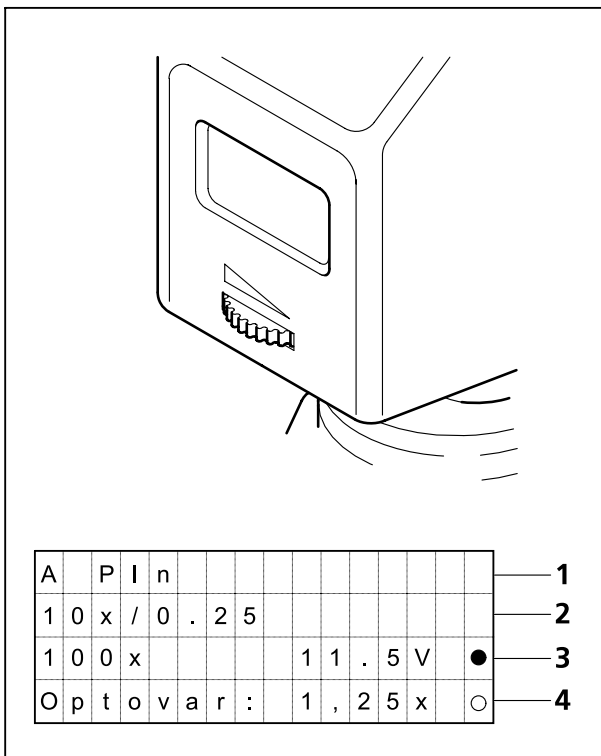


Fig. 4-15 LCD display

LCD display (4-2/29)

- On D1 stand only.
- LCD display with four lines of 16 characters each, located on the transmitted light illuminator housing.

Main information displayed:

- Line 1 (4-15/1):
Name of the objective
- Line 2 (4-15/2):
Objective magnification and contrast techniques
- Line 3 (4-15/3):
Total magnification, lamp and TL shutter details, condenser parameters
- Line 4 (4-15/4):
Reflected light information. If not available, Optovar magnification

TFT display (4-3/20)

- On Z1 stand only.
- For control and configuration of the microscope using the touchscreen, see section 4.6.

Control ring, right (4-2/19), (4-3/17)

- On D1 and Z1 stands only.
- Two pairs of buttons (4-16/1 and 3) and one single button (4-16/2) for operating motorized components or illumination settings.

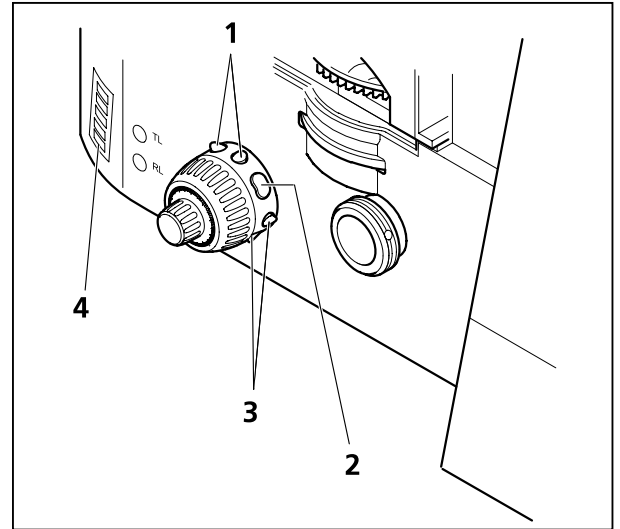
The factory set default button configuration is shown on the adhesive label (4-16/4) affixed to the stand.

D1 stand:

- If a motorized condenser is used, pressing the middle button will display the current condenser settings on the LCD display.
- The default button configuration can only be changed by using a PC connected to the microscope and running the **MTB2004 Configuration** program. If the button configuration is changed, the adhesive label affixed to the stand should be updated using the additional adhesive labels supplied.

Z1 stand:

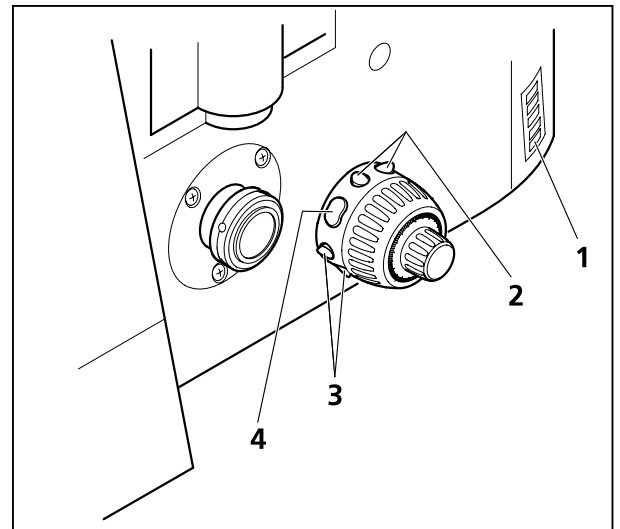
- The default button configuration can be changed by using the TFT display. If the button configuration is changed, the adhesive label affixed to the stand should be updated using the additional adhesive labels supplied.

**Fig. 4-16 Control ring, right (view from rear)****Control ring, left (4-3/4)**

- On Z1 stand only.
- Two pairs of buttons (4-17/2 and 3) and one single button (4-17/4) for operating motorized components or illumination settings.

The factory set default button configuration is shown on the adhesive label (4-17/1) affixed to the stand.

The default button configuration can be changed by using the TFT display. If the button configuration is changed, the adhesive label affixed to the stand should be updated using the additional adhesive labels supplied.

**Fig. 4-17 Control ring, left (view from rear)**

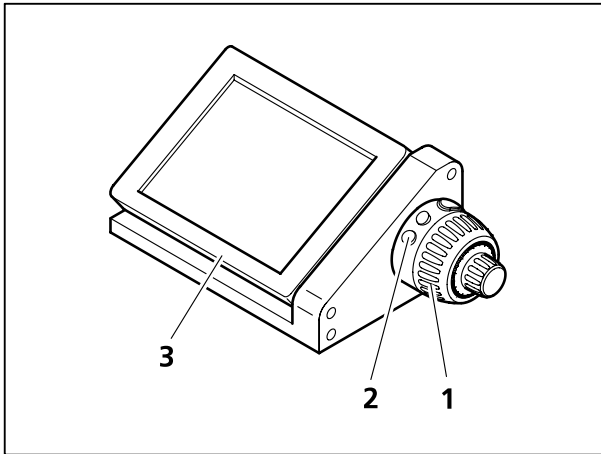



Fig. 4-18 Docking station with TFT display, control ring and focus drive

Docking station

- For use with the Z1 stand only.
- If it is awkward to operate the microscope stand from the right side, the functions of the touchscreen TFT display (4-18/3 on the docking station), the right control ring (corresponds to 4-18/2 on the docking station) and the focus drive on the right (corresponds to 4-18/1 on the docking station) can be performed using the docking station detached from the stand.
- The default button configuration can be changed by using the TFT display.
- The angle of the TFT display can be adjusted using the two control wheels (see also fig. 3-27/4) on the rear of the docking station.

 When using the incubator XL S1, mount the TFT display onto the docking station. It is not possible to mount the TFT display on the stand in this case.

Automatic component recognition (ACR) on the D1 and Z1 stands

Automatic component recognition (ACR) means automatic recognition of objectives and reflector modules on the Axio Observer.

When an objective (D1 and Z1) or reflector module is replaced, the new component is registered by the system. This offers important ease of use and safety benefits by preventing operator error and avoiding time-consuming programming.

The differences between automatic component recognition for the nosepiece and for the reflector turret are explained below.

- Nosepiece (optional, Z1 stand only)
Automatic component recognition for the nosepiece is initiated by pressing the appropriate button on the *Settings/Components/Objectives* page on the TFT display (see 4.6.5.1). The stand must be equipped with the ACR nosepiece and correctly configured beforehand.
- Reflector turret (optional, D1 and Z1 stands)
Automatic component recognition for the reflector turret is initiated automatically as soon as the ACR reflector turret cover is closed.

4.3 Basic settings for the D1 stand

After switching on, the instrument is initialized. The instrument status is shown on the LCD display.

LCD display while D stand is booting up:

While the instrument is booting up, "Axio Observer D1", the Zeiss logo and the status "Loading" are displayed.

4.3.1 Configuring the D1 stand

Pressing and holding down the LM set button for > 2 s will activate configuration mode with the following menu options:

- Set Objective (nosepiece),
- Set ReflModule (reflector turret),
- Set RL Slider (A) (slider for the reflected light illuminator aperture),
- Set Lamp Output,
- Set Ext. Shutter,
- Set Lightmanager,
- Set Dazzle Protection.

Two beeps indicates that the system has switched to configuration mode.

The selected menu is displayed on the **first** line of the LCD display. If a component (e.g. the reflector turret) is not available (cannot be configured), the relevant menu (e.g. Set ReflModule) will not be displayed. The current component position is shown on the right next to the menu designation (position number of the nosepiece or reflector turret).

Press the LM set button briefly (< 1 s, no beep) to switch to the next menu (cyclical).

The **second** line shows any available submenus.

The submenus can be selected using the TL and RL buttons. TL - next submenu, RL - previous submenu.

The **third** line shows the selected menu / submenu item. The **fourth** line shows the next item on the menu / submenu. For objectives, the fourth line shows the magnification, aperture and contrast technique of the selected objective.

The illumination intensity control can be used to scroll through the menu. Rotating the control to the right scrolls forwards, to the left backwards, through the menu. The currently selected item is indicated with an arrow (>).

During configuration, when a position (nosepiece and reflector turret) or a setting is changed, the settings are saved temporarily in RAM. Changes are only saved permanently when the user exits configuration mode. Some changes require the system to be reset before taking effect.

To exit configuration mode, press and hold the LM set button for at least 2 s. Two beeps indicates that the system is exiting configuration mode. The LCD display returns to status display mode.

4.3.2 Options during operation (when the status display is active)


The brightness of the LCD display can be adjusted by holding the RL button and rotating the illumination intensity control.

Pressing the RL button for at least 1 s turns the LCD display background lighting on or off. A low lighting level or no LCD background lighting can be useful for difficult fluorescence experiments with low emission intensities.

Pressing and holding the TL button for at least 1 s automatically sets the lamp voltage to the value for 3200 K (ideal for assessing colored specimens).

If a motorized condenser is connected, pressing the single button on the control ring (focus drive, right side) and pressing either of the buttons on the condenser will display the condenser status.

To save the current illumination intensity of the halogen illuminator in Light Manager, press the LM set button briefly. The message "Saving Light Manager Values" will be displayed on the monitor and an acoustic signal will be emitted. Precondition: The Light Manager CLASSIC or SMART has been activated (see the following chapter).

 If pressing first the TL button and then additionally the RL button briefly, the device will set automatically all components required (halogen lamp, all shutters, condenser, and reflector turret) in such a way that a bright field image will be seen when looking into the eyepieces. That is very useful if currently there is no image or light and one does not know which of the components is not correctly adjusted.

4.4 Light Manager on the Axio Observer.D1 and .Z1

 Light Manager is not available on the Axio Observer.A1.

The purpose of Light Manager is to generate specimen-dependent optimum illumination settings for the various contrast techniques and magnifications used, and to save these temporarily or permanently so that the user is able to reproduce these settings.

Light Manager has three operating modes: OFF, CLASSIC and SMART. The precise function of each mode depends on certain optional stand components. Use of Light Manager on both D1 and Z1 stand types requires that a coded or motorized nosepiece is used, so that the stand electronics can detect when the nosepiece is rotated into a new position.

Light Manager is available for transmitted light contrast techniques (bright field, phase contrast, DIC) and for epifluorescent contrast techniques. When operating with reflected light, the motorized FL attenuator (where used) will be considered by Light Manager.

Depending on configuration, Light Manager is activated (CLASSIC or SMART) or deactivated (OFF) when the microscope is switched on.

For transmitted light, the following parameters are saved for each nosepiece position (a motorized condenser is necessary):

- three light intensities (brightness values) for bright field, phase and DIC contrast techniques (different phase stops and DIC prisms are not registered) and
- for bright field and DIC, the aperture stop

For reflected light on bio / med stands, Light Manager operates in SMART mode in precisely the same way as in CLASSIC mode. In reflected light, Light Manager can only be used with the mot. FL attenuator (not with the iris stop slider).

The current FL attenuator settings are saved for each reflector position. Once the reflector turret has been moved through all positions, the parameters can be permanently saved by pressing the LM set button. Temporary changes can also be made.



Important:

Changes will only be correctly saved to the temporary memory and subsequently successfully saved permanently if the relevant shutter is open.

Light Manager only works if all the components involved are correctly configured.

The temporary memory is cleared when the microscope is switched off.

4.4.1 Light Manager mode: OFF

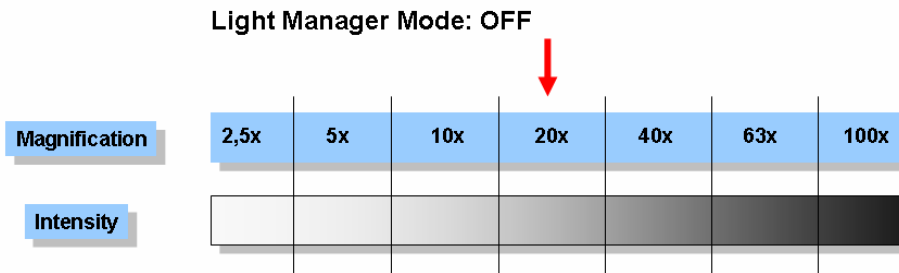


Fig. 4-19 Illumination intensity looking into the eyepieces, changing only the objective (starting from 20x).

If Light Manager is switched off, the microscope operates like a classical light microscope.

Starting from a selected magnification and an appropriate lamp voltage, the user must readjust the voltage manually to obtain a comparable brightness with a higher or lower magnification.

4.4.2 Light Manager mode: CLASSIC

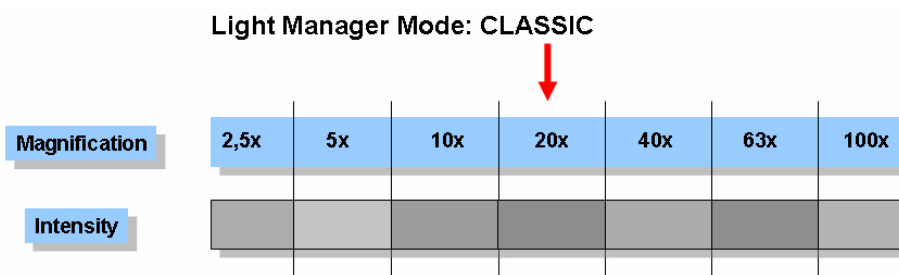


Fig. 4-20 Illumination intensity looking into the eyepieces, changing only the objective (starting from 20x). Light Manager settings for each objective have been saved previously.

If Light Manager is being used in CLASSIC mode, the user can select his or her own optimum illumination settings for each magnification (each combination of objective and Optovar).

The illumination must be set for all contrast techniques used (H, PH, DIC) for each objective.

The relevant values are automatically stored in Light Manager's temporary memory when the objective is changed.

To save the settings permanently (to be able to access the settings after switching off the microscope), press the LM set button on the right of the stand before switching off the microscope. A signal tone will be emitted to confirm that the settings are being saved and all available values will be permanently saved. This will be followed approximately 3 seconds later by a second signal tone. It is now safe to switch off the microscope.

Permanently saved settings can be changed temporarily during a session. If these settings are not saved permanently by pressing the LM set button, they will be discarded when the microscope is switched off. When the microscope is switched back on, the permanent settings will once more be used.

4.4.3 Light Manager mode: SMART

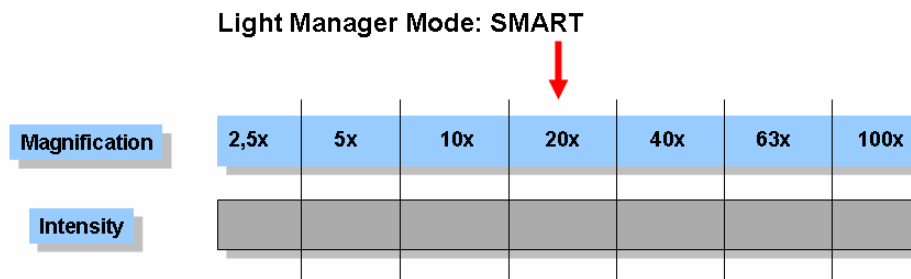


Fig. 4-21 Illumination intensity looking into the eyepieces, changing only the objective (starting from 20x). Settings for one objective have been saved previously.

In SMART mode, Light Manager automatically calculates the optimum brightness for all objectives configured using the LCD or TFT display (or MTB 2004) for a given contrast technique.

For a given contrast technique, when the illumination intensity is changed for one objective, the correct illumination intensity will be calculated for all other objectives based on the magnification and adjusted when the objective is changed.

The Optovar turret, if used, will also be considered in the brightness calculation.

As in CLASSIC mode, the illumination values can be permanently saved by pressing the LM set button on the right side of the stand. Permanently saved settings can be changed temporarily during a session.

4.4.4 Selecting Light Manager mode on the D1 stand

On the D1 stand, the Light Manager mode is selected using the LCD display.

- Select configuration mode by pressing and holding the LM set button for > 2 s. Two beeps indicates that the system has switched to configuration mode.
- Select the **Set Light Manager** menu, to select the Light Manager mode, by pressing the LM set button briefly (< 1 s, no beep).

```
Set Lightmanager
>Off
Classic
```

Fig. 4-22 Selecting Light Manager mode

The current menu is displayed on the first line of the LCD display (Fig. 4-22).

The third line shows the current setting marked with an arrow (>), the fourth line the next selectable setting.

- Rotate the illumination intensity control wheel until the required mode is displayed on the line marked with an arrow.
- Exit configuration mode by pressing and holding the LM set button for > 2 s. Two beeps indicates that the system is exiting configuration mode.

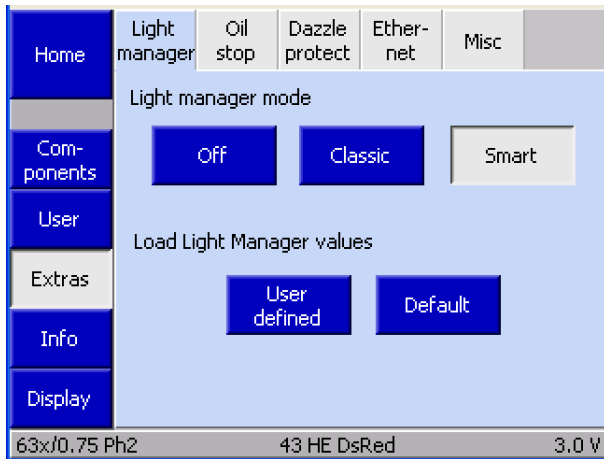


Fig. 4-23 Selecting and configuring Light Manager mode

4.4.5 Selecting and configuring Light Manager mode on the Z1 stand

- Light Manager can be deactivated or activated in the selected mode by pressing either **Off**, **Classic** or **Smart**.
- To discard any temporary settings and reset Light Manager to the previous settings saved using the LM-Set button, press **User defined**. The temporary settings are discarded and the permanently saved settings are set as active.
- To use the manufacturer's default settings, press **Default**. The default values will be loaded, written to the temporary memory and set as active. To overwrite the permanent settings with the default settings, press the LM set button. It is not possible to overwrite the manufacturer's default settings.

4.5 Contrast Manager on the Axio Observer.Z1

Contrast Manager is only available on the Z1 stand.

It is used for rapid switching between contrast techniques and for configuring mixed contrasts. This facilitates the detection of contrasted cells and the location of fluorescence signals in particular positions in the cell (see also section 4.6.1.3 (4)).

Necessary are mot. condenser, mot reflector turret and reflected light shutter.

When a new objective is moved into the optical path, all necessary settings for the contrast technique used will be applied. This affects both shutter settings and the position of the condenser turret.

If, for example, the user is working with phase contrast, the condenser turret position with the phase stop for the current objective will automatically be moved into the optical path. The shutter positions will be retained.

Using Contrast Manager's FL, BF, PH and DIC buttons, it is possible to combine contrast techniques as required, e.g. bright field, phase contrast or DIC with fluorescence.

4.6 TFT display touchscreen on the Axio Observer.Z1

4.6.1 Screen layout

On the motorized Axio Observer, the user can operate and configure the microscope and utilize optional functions using the TFT display. The TFT display is designed as a touch-sensitive screen.

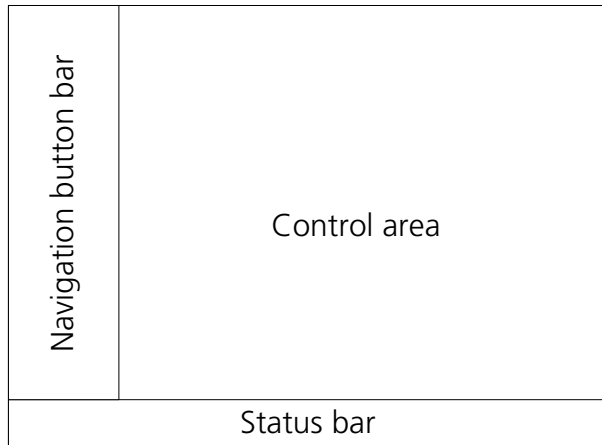


Fig. 4-24 Main areas of the TFT display

The controls and information displays are laid out on a series of tabs. The pages on the TFT display are generally divided into the following main areas (see Fig. 4-24).

4.6.1.1 Navigation bar

The navigation bar, on the left side of the screen, contains buttons for navigating between pages. The buttons displayed depend on the current page. The following buttons are available on all pages:

- **Home** Switches to the Home page
- **Display** Switches to the display page

4.6.1.2 Status bar

The status bar at the bottom of the screen displays information on the current settings.

4.6.1.3 Control area

The controls area is further subdivided into (see Fig. 4-25):

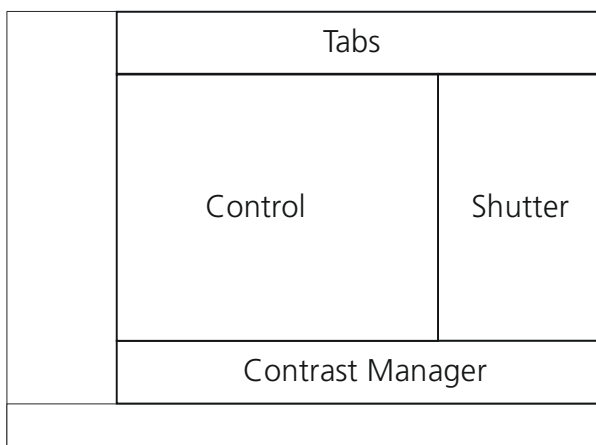


Fig. 4-25 Controls area of the TFT display

(1) Tabs

Tabs are used to select subsidiary functions. These are displayed in the *Control* area. A maximum of six tabs are available per page.

(2) Illumination

The RL shutter button for reflected light and TL shutter button for transmitted light are displayed on the right side of the control area. For transmitted light, the shutter is switched according to configuration. The **Off** and **On** buttons function as switches, i.e. the shutter in the optical path of the microscope is either open or closed.

(3) Control

This area contains controls relevant to the option selected on the navigation bar and the selected tab.

(4) Contrast Manager

At the bottom of the control area, there is a bar on which are displayed buttons for selecting the contrast technique. The contrast techniques available depend on the current microscope configuration. The following contrast techniques may be available:

Abbr.	Technique
FL	Fluorescence
BF	Bright field
PH	Phase contrast
DIC	Differential interference contrast



The contrast techniques arise from the interaction between the condenser, reflector turret, shutter positions and other parameters. The current contrast technique is displayed on the TFT display. No contrast technique is displayed for manual settings (e.g. empty reflector turret position with open RL shutter).

(5) Popup windows

Popup windows are displayed in order to:

- prompt the operator for additional entries. The user must make a selection (e.g. adjust the configuration after initialization, enter values, etc.)
- display error messages or special information. Some messages require the user to acknowledge the message by pressing **Close**
- display the operating status (waiting time). These windows close automatically



When a popup window is open, it is not possible to operate the page underneath.

4.6.2 Menu overview

The menu overview shown below may differ from the available menus for your microscope configuration. It represents all possible menu options including optional components and menu items which are accessible only if the user has administrator privileges. (Any user not logged in as an administrator has read privileges only).

Different tabs will be displayed on the **Microscope control** page depending on the stand type selected under **Settings, User, Stand type (Bio / Med or MAT)**. The menu overview below shows both versions.

Navigation bar 1 st level	Navigation bar 2 nd level	Tabs in control area 3 rd level						
Home		Magnification	Contrast	Optovar	Light path			MAT
Microscope	Control	Objectives	Reflector	Optovar	Light path	F/A	FluoArc	BIOMED
	Automatic	Soft keys	Settings	Scripts				
	XY Z	Position	Measure					
	Incubation	Incubation	Therm. Switch					
Settings	Components	Objectives	Reflector	Focus	Camera ports	Stage	Misc	
	User	Mode	Buttons left	Buttons right	Stand type	Language		
	Extras	Light manager	Oil stop	Dazzle protect	Ethernet	Misc		
	Info	Firmware						
Display								

Fig. 4-26 Menu overview


The *first level* buttons shown on the very left are displayed on the navigation bar (Fig. 4-26). Pressing **Microscope**, **Settings** or **Display** causes the buttons displayed on the navigation bar to change.

The buttons on the *second level* of the navigation bar cause the corresponding tabs to be displayed. Touching the tabs causes the corresponding buttons to be displayed on the controls area.

Controls are displayed in the Control area or in a Popup windows. They are not displayed on the status bar.

4.6.3 Home page

After switching on, the microscope is initialized. This process takes a few seconds. The **Home page** (Fig. 4-27) will then be displayed.




 If any coded or motorized microscope components have been changed or removed while the microscope was switched off, the new components will require configuration after switching on (see also section 4.6.5).

All menu pages can be accessed using the buttons on the navigation bar on the left.

The middle section of the controls area displays the configuration. All coded and motorized configurable elements detected during initialization are displayed on the status bar. If no elements are detected, the character "-" will be displayed. The elements are arranged in order of importance from top to bottom.

The following controls are displayed on the right side:

- **Load position** button
Pressing **Load position** causes the nosepiece to move to the loading position. Nosepiece movement can be interrupted by pressing **Stop** (Fig. 4-28). When the nosepiece has reached the load position, the **Load position** window is displayed. This window contains the following controls:

-  Moves the nosepiece back to the working position.
-  Moves the nosepiece upwards towards the working position until the button is released.
-  Moves the nosepiece downwards until the button is released (up to nosepiece limit stop).

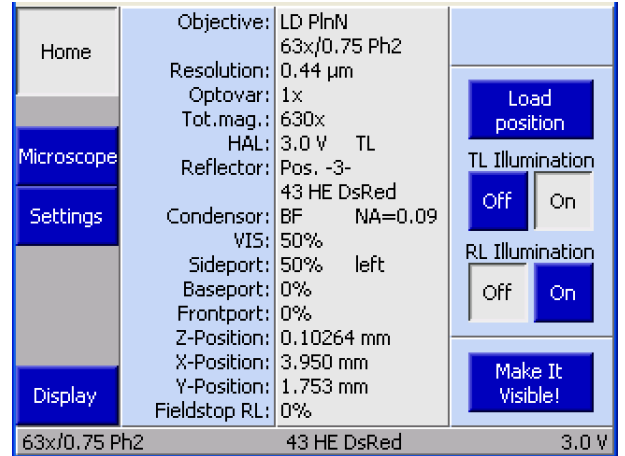


Fig. 4-27 Home page

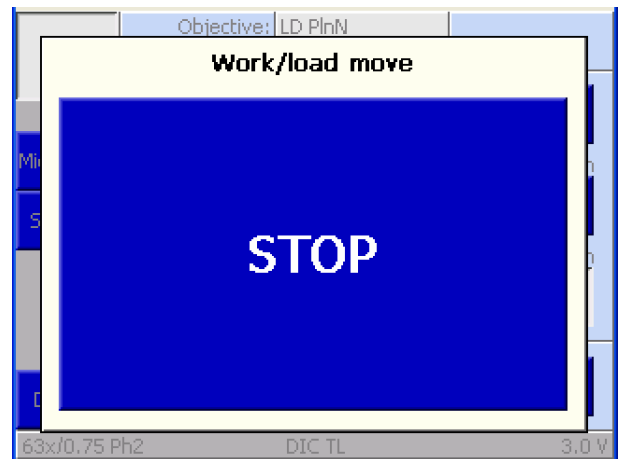


Fig. 4-28 STOP button

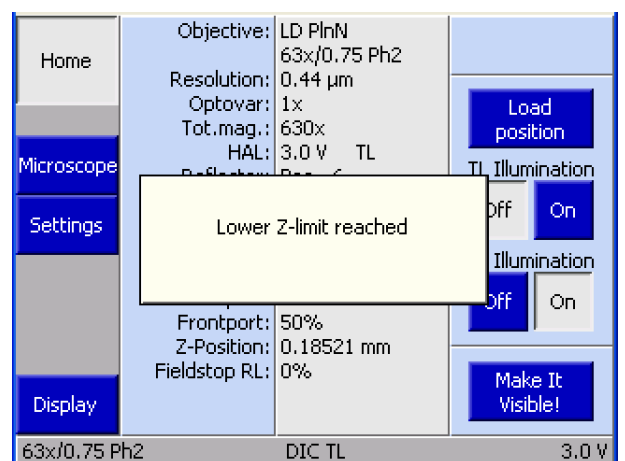


Fig. 4-29 Motorized focus drive limit reached

– **TL Illumination / RL Illumination** buttons

The **Off** and **On** buttons close and open the reflected light (RL) and transmitted light (TL) shutters or switch the lamp on and off.

– **Make it visible!** button

If the microscope is mis-adjusted such that no image of the specimen is visible, this button can be used to reset the microscope to a standard state in which the specimen is visible.

transmitted light illuminator is adjusted to medium intensity (3 V)

aperture stop is opened

TL shutter is opened, RL shutter is closed

mot. condenser is switched to bright field

reflector turret is rotated to the nearest bright field position

light path switching is set to 100% vis

The nosepiece position (or, if entered, the objective) will be shown on the left of the status bar, the halogen illuminator voltage on the right.

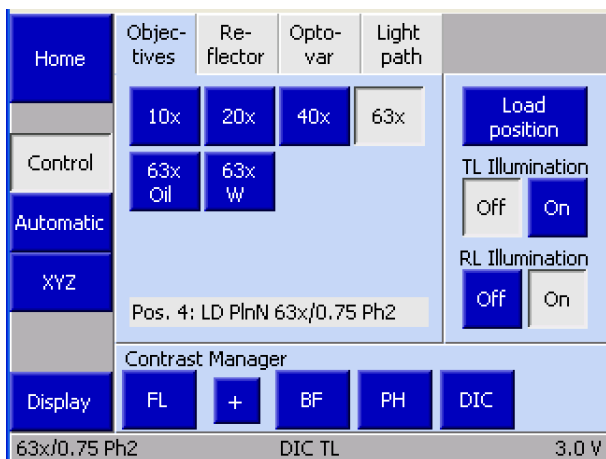


Fig. 4-30 Page Microscope -> Control -> Objectives

4.6.4 Microscope

Pressing **Microscope** on the navigation bar of the **Home** page brings up the **Microscope** page.

The **Control**, **Automatic** and **XYZ** pages are available from the **Microscope** page.

Different tabs will be displayed on the **Microscope** page depending on the stand type selected under **Settings, User, Stand type (Bio / Med or MAT)**.

4.6.4.1 Control Bio / Med

Depending on the optionally configurable motorized components, the **Control** page will contain up to six tabs:

- Objectives
- Reflector
- Optovar
- Light path
- FluoArc
- F/A

(1) Objectives

For objective positions which have already been configured, the magnification and, where applicable, the following additional information is displayed:

Oil	Oil immersion objective
W	Water immersion objective
Imm	Immersion

- To move an objective into the optical path, touch the button for that objective.



- If Light Manager is active, the brightness will be adjusted automatically when the objective is changed.
- If a contrast technique is set in Contrast Manager, when the objective is changed Contrast Manager will attempt to adjust the technique to the new objective (i.e. the condenser and reflector turret positions may be changed). If this contrast technique is not available for the new objective, the system will switch to bright field.

(2) Reflector



This tab will not be displayed if there is no motorized reflector turret installed. Instead the active reflector module will be displayed on the status page (Fig. 4-27).

Depending on the reflector turret installed, six controls for reflector positions 1 to 6 will be displayed. Reflector modules which have already been configured are identified by the description on the button.

- Touch the button for the reflector module required to move it into the optical path.

(3) Optovar



This tab will not be displayed if there is no motorized Optovar turret installed.

If a loaded optovar turret has been installed, the available lens magnifications will be displayed.

- Touch the button for the Optovar required to move it into the optical path.

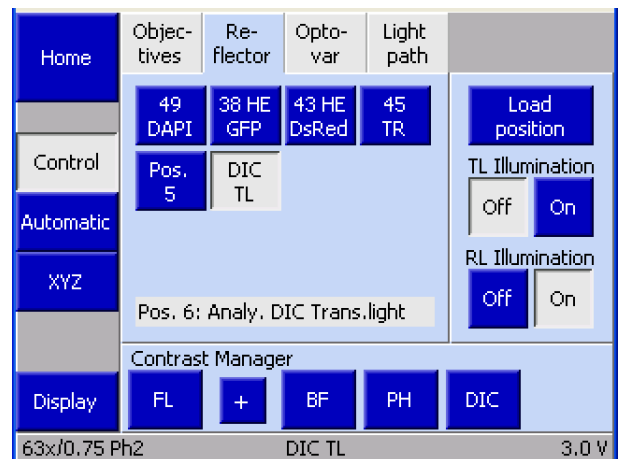


Fig. 4-31 Page Microscope -> Control -> Reflector

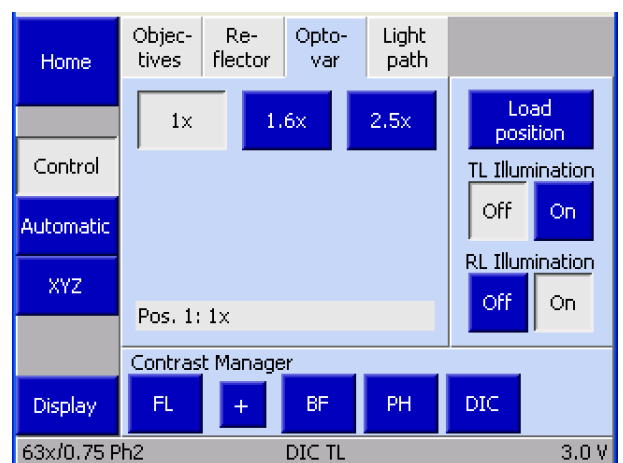


Fig. 4-32 Page Microscope -> Control -> Optovar

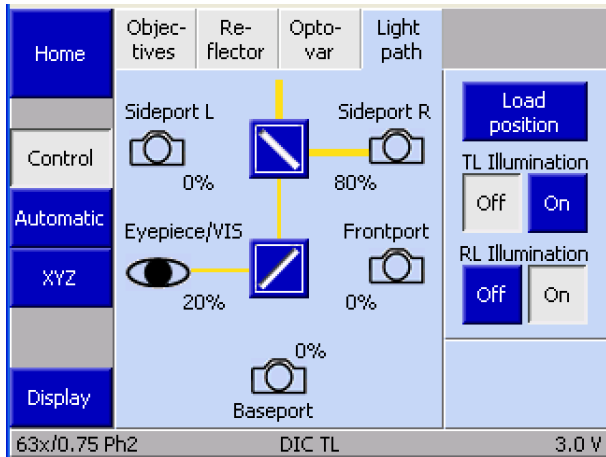


Fig. 4-33 Page Microscope -> Control -> Light path

(4) Light path

The light path is displayed schematically on the **Light path** tab.

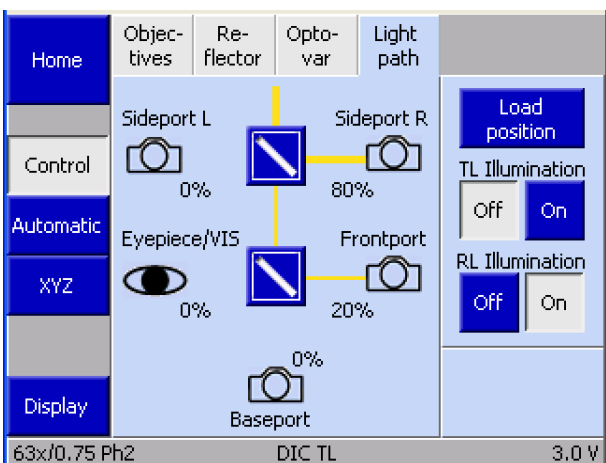
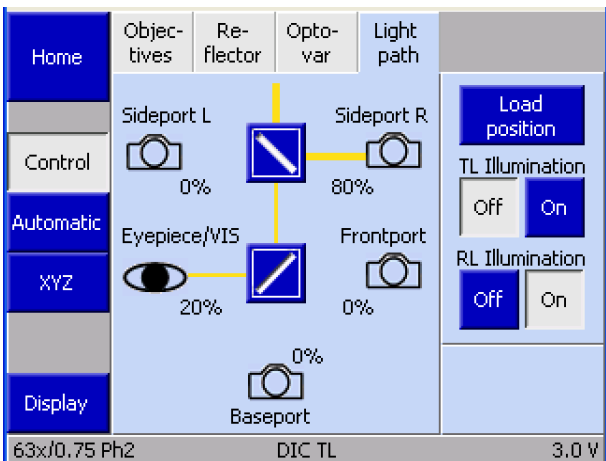
The following controls are available:



Active beam splitter: touching this button toggles through the available splitting ratios.

One of the following diagrams or a similar diagram representing the light path will be displayed in the controls area. The configuration is determined during initialization of the microscope.

- Two beam splitters (Sideport and Frontport / Baseport) are active.
- The active light paths are shown in yellow. The width of the active light paths is a function of the amount of light transmitted.
- Touching one of the icons (e.g. **Eyepiece/VIS**) will cause the maximum possible amount of light to be transmitted to this item.



(5) FluoArc

The **FluoArc** tab allows different brightnesses of the HBO 100 illuminator to be set via the variable intensity power supply unit FluoArc.

Two illumination intensities (level 1 and 2) can be preset and chosen as required (by pressing the **Level 1** or **Level 2** button). Presettings are made using the arrow buttons.

In the lower part of the register card the reached operating time of the mercury vapor short arc lamp of the HBO 100 is displayed. After replacing the mercury vapor short arc lamp, the lamp life counter has to be set to zero by pressing the **Reset** button.

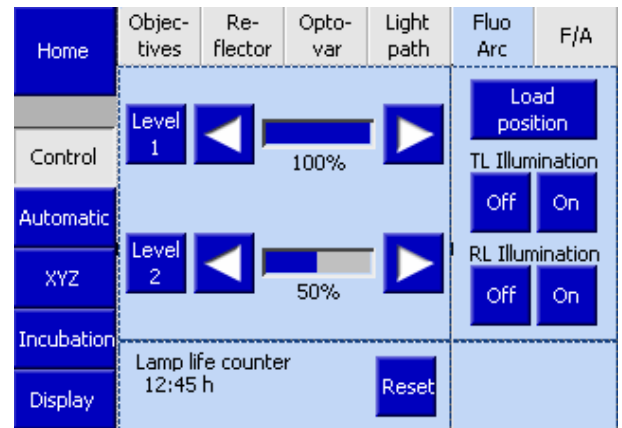


Fig. 4-34 Page Microscope -> Control -> FluoArc

(6) F/A

The **F/A** tab is used to configure the motorized iris stop slider for luminous-field stop (field stop) and the aperture stop.

The aperture is controlled using the arrow buttons. The current value is shown below the bar display.

The **Prev.** and **Max.** buttons reset the stops to the previous and maximum values respectively.

An existing motorized FL attenuator cannot be set via the TFT display. The lower aperture stop display does not exist in this case.



Fig. 4-35 Page Microscope -> Control -> F/A

4.6.4.2 Automatic

(1) Softkeys

Not implemented at present.

(2) Settings


Not implemented at present.


(3) Scripts

This tab is used to access scripts which have been generated, named and made available using the AxioVision program.

These scripts are activated by touching the relevant button on the TFT screen.

4.6.4.3 XYZ

-  The **XYZ** page is not available for all microscope stages.
 - Motorized stages (only CAN bus stages directly connected to stands .Z1)
 - Manual stage:
 - Z-drive settings only (XY controls are not available), **Measure** tab is not available
 - Manual stage / manual Z-drive:
 - XYZ** page is not available

-  The system detects whether a motorized stage is installed during microscope initialization. The stage should therefore only be changed when the microscope is switched off.

The **Microscope XYZ** page includes the tabs **Position** and **Measure**.

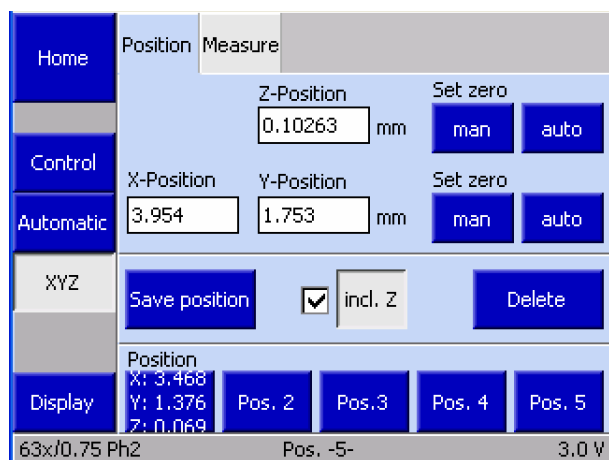



Fig. 4-36 Page Microscope -> XYZ -> Position

(1) Position

The controls area of the **Position** tab is divided into three blocks.

-  If you are not using a motorized stage, the XY controls are replaced by a **Start** button (see (2) *Measure*).

(a) Current position display / Set zero

The current Z, X and Y positions are displayed in millimeters (mm).



The Z position is the nosepiece position regarding height, the X and Y positions are the stage position.

The two **Set zero** buttons function as follows:

man

Manually sets the zero point. The current position is defined as the zero point and the display is set to zero.

auto

Automatically sets the zero point. The stage / nosepiece moves to the end position which has been defined as the zero point. The display is then set to zero.

(b) Save position

The **Save position** button is used to define coordinate positions for the five position buttons. To define a position proceed as follows:

- Move the stage and nosepiece to the required X, Y and Z positions.
- If you wish to save the Z value, activate the **incl. Z** checkbox.
- Press **Save position**. The **Current position save as** window will be displayed.

The window contains five buttons, Pos. 1 to Pos. 5. If coordinates have already been assigned to a button, the XYZ values will be displayed, otherwise the position number will be displayed.

- Save the current position by touching one of the position buttons. If coordinates have already been assigned to this button, you will be asked to confirm that you wish to overwrite the saved coordinates.
- Close the window by pressing **Exit**.
- To delete a set of coordinates, press **Delete**, select the position button and confirm by pressing **Yes**.

(c) Moving to a saved position

There are five buttons in the bottom, position area of the screen. Touching one of these buttons will cause the microscope to move to the saved coordinates. For details of how to save coordinates, see (b) *Save Position* above.

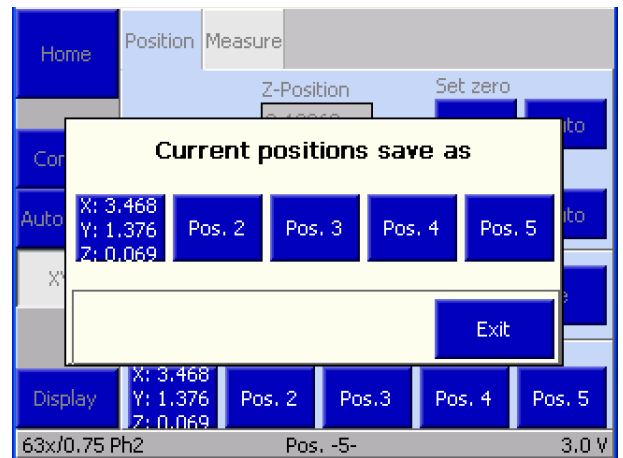


Fig. 4-37 Page Microscope -> XYZ -> Save position

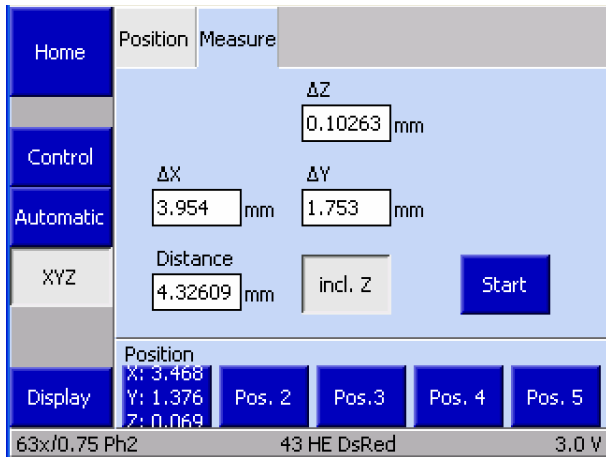


Fig. 4-38 Page Microscope -> XYZ -> Measure

(2) Measure



This tab is only accessible if a motorized (CAN bus) stage is used. Where no motorized stage is used, a **Start** button and the Z-distance ΔZ are displayed on the **Position** tab.

The **Measure** tab can be used to perform simple distance measurements in millimeters (mm). There are three ways of performing these measurements:

- Distance between two manually set positions
- Distance between a manually set position and a defined position
- Distance between two defined positions



If the Z distance is to be measured, activate the **incl. Z** button.

- Move the stage to the initial position.
- Press **Start**. The ΔX , ΔY and ΔZ fields will be set to zero.

All stage movements are displayed in the ΔX , ΔY and ΔZ fields.

The function of the position buttons is described under (1) *Position* above.

4.6.4.4 Incubation

The **Microscope/Incubation** page includes the tabs **Incubation** and **Y-Module**.

These tabs are used to control incubation components and thermostats connected to the microscope.

(3) Incubation

The **Incubation** tab lists all connected incubation components. Each button shows the target (bottom) and actual (top) temperatures.

Touching the button activates the **Incubation channel** window. This window is used to switch temperature control on or off, set the target temperature and save different target temperatures as parameter sets.

(4) Y-Module

The **Y-Module** tab lists the thermostats connected to the microscope. Each button shows the target (bottom) and actual (top) temperatures.

In the same way as for incubation components, thermostats can be switched on or off and target temperatures can be set from the popup thermostat window.

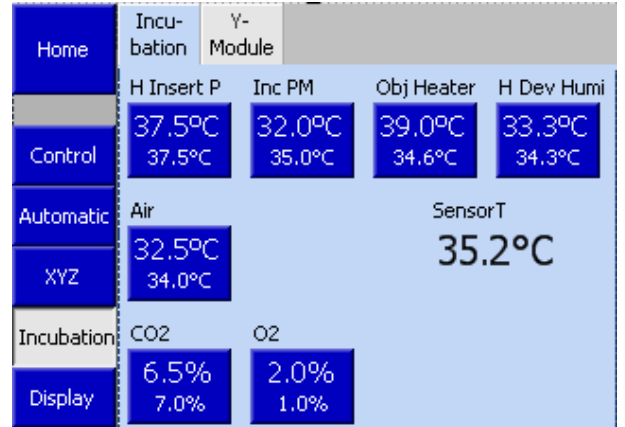


Fig. 4-39 Page Microscope -> Incubation -> Incubation

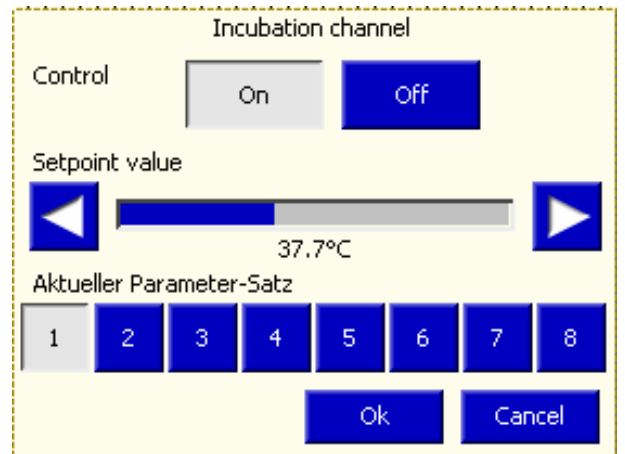


Fig. 4-40 Incubation channel window

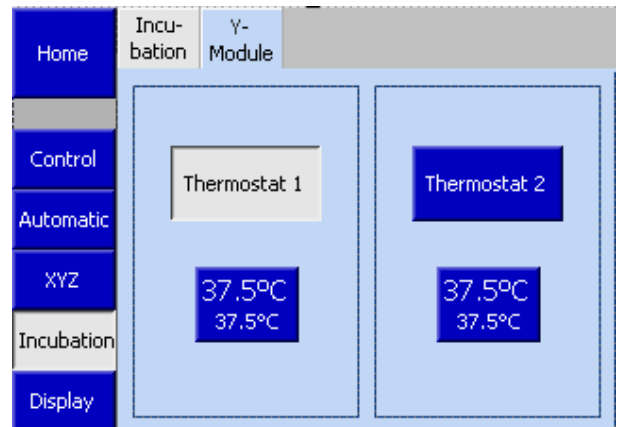


Fig. 4-41 Page Microscope -> Incubation -> Y-Module

4.6.5 Settings

Pressing **Settings** on the navigation bar of the **Home** page brings up the **Settings** page.

The **Settings** page provides access to the **Components**, **User**, **Extras** and **Info** pages.

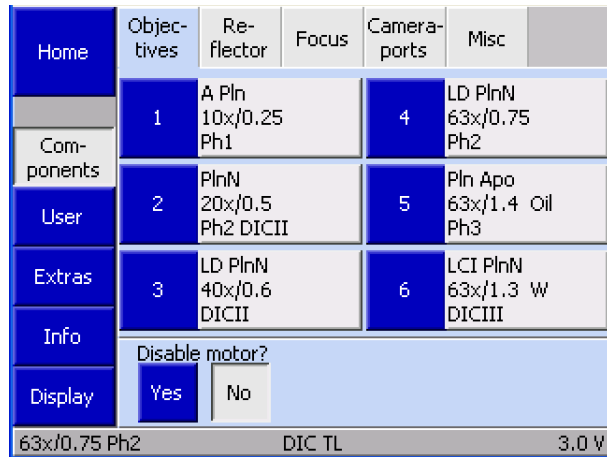


Fig. 4-42 Page Microscope -> Settings -> Components -> Objectives

4.6.5.1 Components

The **Settings/Components** page includes six tabs; **Objectives**, **Reflector**, **Focus**, **Cameraports** and **Misc**.

(1) Objectives

This tab is used to configure the nosepiece loading.

Up to six buttons are displayed. If no objectives have been configured, the buttons are labeled with the numbers of the nosepiece positions.

Once an objective has been assigned to a nosepiece position, the following information is displayed: objective name, magnification, numerical aperture (NA), immersion

Once a new objective has been assigned, the corresponding objective button on the **Microscope control** page will be labeled with the magnification and immersion type.

Press **Yes** in the **Disable motor?** area to deactivate the motor if an objective heater or a piezo focus drive is installed beneath an objective.

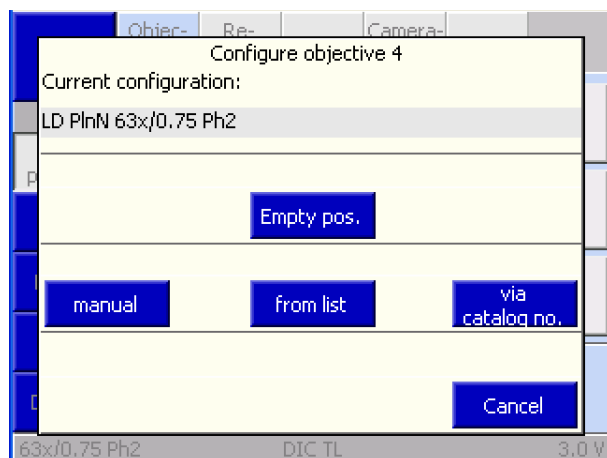



Fig. 4-43 Page Microscope -> Settings -> Components -> Objectives


- To configure a nosepiece position, touch the relevant objective button.

The following options can be selected from the **Configure Objective #** window:

- manual**
The user must enter the magnification, NA and immersion type himself.
- from list**
The user selects the magnification from the **Preselect Magnification** list and selects a suitable objective from the **Objective list**.
- via catalog no.**
To select the objective the user enters the Zeiss catalog number (XXXXXX-XXXX-XXX).

 Pressing **Empty pos.** clears the current objective selection. Select the relevant nosepiece position and confirm by pressing **Yes**.

- Press **Save** to save the objective configuration for the selected nosepiece position or press **Cancel** to close the window without saving.
- If you are overwriting an existing objective configuration, confirm by pressing **Yes**.

 If entering the 13-digit Zeiss catalog number, the leading six or trailing seven zeros do not need to be entered (enter a hyphen (-) after 123456 or enter 1234-567 and press **OK**). The missing zeros will be added automatically.

(2) Reflector

This tab is used to configure the reflector turret.

Up to six buttons are displayed, depending on the actual number of turret positions. The system detects the number of turret positions during initialization (and when the **Settings/Components** page is opened). If no reflectors have been configured, the buttons are labeled with the numbers of the turret positions only.

Once a reflector has been assigned to a reflector position the following data is displayed: designation (Type), reflected light module (RL), transmitted light position / module (TL)

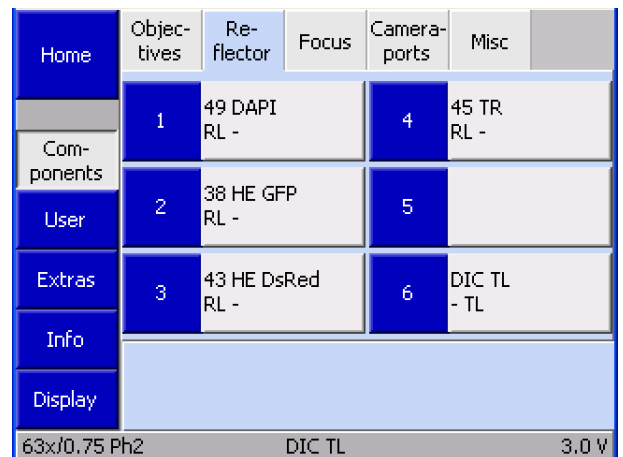



Fig. 4-44 Page Microscope -> Settings -> Components -> Reflector

 Once a reflector has been assigned to a position, the corresponding reflector button on the **Microscope control** page will be labeled accordingly.

- To configure a turret position, touch the relevant reflector button.
- Select the appropriate reflector from the list in the **Configure reflector position # in reflector turret** window. The current selection is shown in the **Resulting configuration** line.
- Select **RL** and / or **TL**.
- Press **Save**. If the turret position was already configured, you will be asked to confirm the new settings.

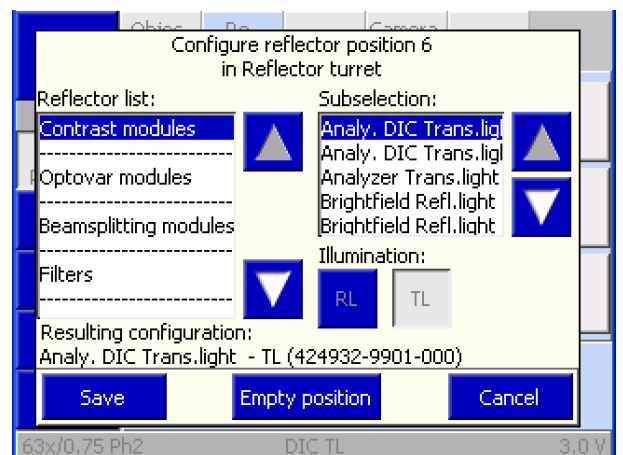


Fig. 4-45 Page Microscope -> Settings -> Components -> Reflector

(3) Focus

This tab is used to enter the firmware settings for the focus drive. The speed of the focus drive can be set individually for each objective.

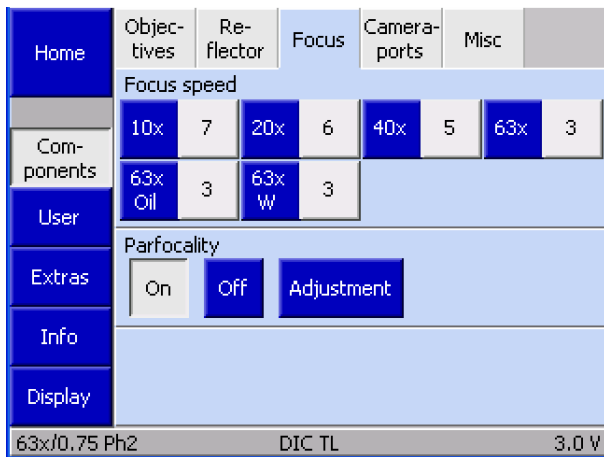


Fig. 4-46 Page Microscope -> Settings -> Components -> Focus

a) Focus speed

Up to six buttons are displayed, depending on the actual number of nosepiece positions. The system detects the number of nosepiece positions during initialization (and when the **Settings/Components** page is opened). If no objectives have been configured, the buttons are labeled with the numbers of the nosepiece positions.

If an objective has been assigned, the magnification is displayed on the blue part of the button on the left. The gray part of the button on the right shows the focus speed.

- To change the focus speed for an objective, press the gray part of the button.

- The **Focus Speed for Objective #** window will be displayed. Set the required speed (from 1 to 10) using the ◀▶ buttons. The higher the number, the higher the focus speed for the selected magnification.

The following focus drive speed factors in dependence on the objective magnification are recommended:

Objective magnification	Focus drive speed factor
1x	10
1.25x	8
2.5x	8
5x	7
10x	7

Objective magnification	Focus drive speed factor
20x	6
40x	5
50x	4
63x	3
100x	3

- Press **Save**.

b) Parfocality

The parfocality function is activated or deactivated using the **On** and **Off** buttons.

To configure the parfocality function press **Adjustment**. This will activate a wizard which will guide you through the configuration procedure.

All the objectives must be focused in sequence, starting with the dry objectives, from the highest magnification to the lowest, followed by the immersion objectives also from highest magnification to lowest. Press **Next Objective**, to move the nosepiece to the next objective. When all the objectives have been focused, press **End**.

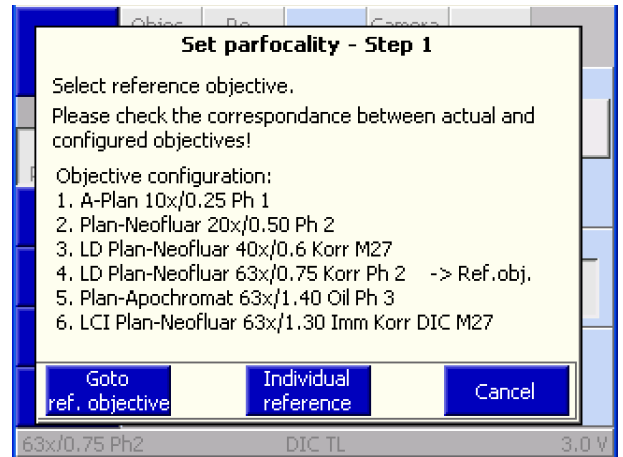


Fig. 4-47 Page Microscope -> Settings -> Components -> Focus

(4) Cameraports

This tab is used to configure the adapters for the camera ports (Frontport / Baseport / Sideport / Phototube).

Adapter

Up to five buttons are displayed depending on the configuration of the left camera splitter and the tube used. The system detects the status of the ports during initialization (and when the **Settings/Components** page is opened).

- To assign an adapter to a port, press the gray button. The **Select Camera Adapter** list will be displayed.
- Select the appropriate adapter from the list using the ▲ ▼ buttons.
- Press **Save** to assign the selected adapter to the port. Press **Cancel** to close the window without selecting an adapter.

The magnification is now shown on the button. Assign adapters to the other ports in the same way.

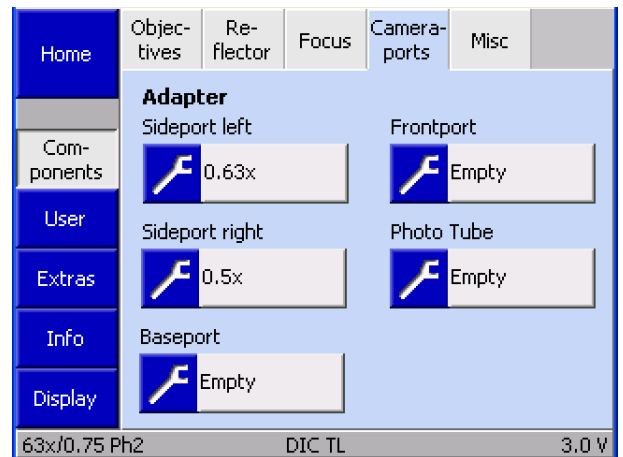


Fig. 4-48 Page Microscope -> Settings -> Components -> Cameraports

(5) Stage

This tab will be displayed if the scanning stage 130x85 mot. CAN is used. This tab is used to adjust the XY movement of the stage to the objective magnification.

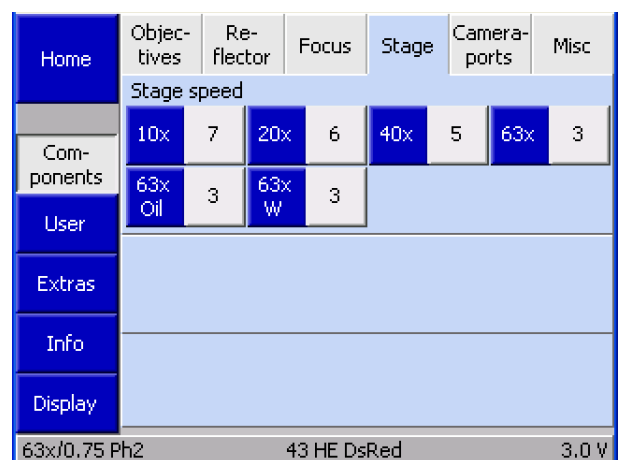


Fig. 4-49 Page Microscope -> Settings -> Components -> Stage

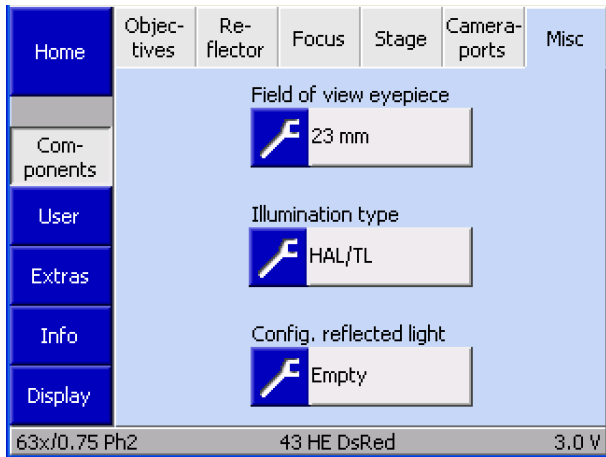


Fig. 4-50 Page Microscope -> Settings -> Components -> Misc

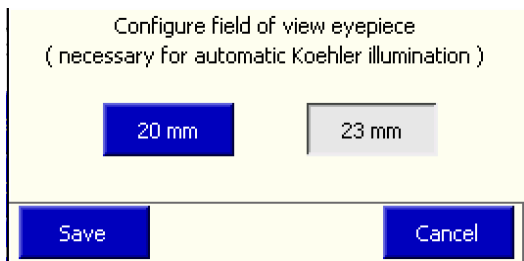


Fig. 4-51 Field of view eyepiece

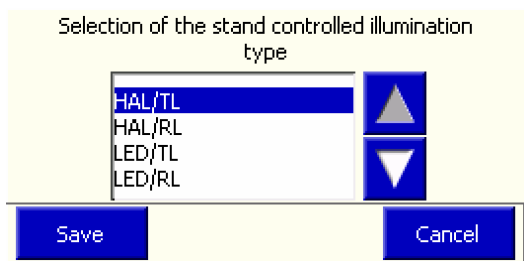


Fig. 4-52 Illumination type

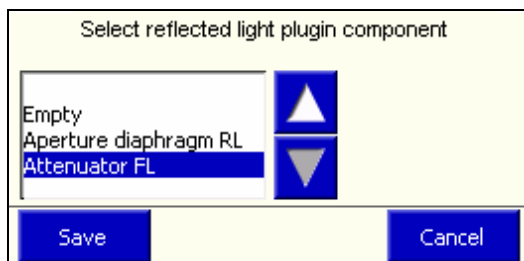


Fig. 4-53 Config. reflected light

(6) Misc

The Misc tab is used to configure additional, optional microscope components.



The number of buttons displayed depends on the components detected during initialization or when the **Settings/Components** page is opened.

- Field of view eyepiece

This is used to enter the eyepiece field of view. This is required because Light Manager automatically adapts the luminous-field stop to the eyepiece field of view, but cannot detect the field of view automatically.

- Illumination type

This is used to enter whether you are working with a halogen illuminator or the LED illuminator.

- Config. reflected light

If a motorized stop slider or an FL attenuator are used in reflected light, these components must be specified in this window. The settings will be applied after the microscope has been restarted.

4.6.5.2 User

Pressing **User** on the navigation bar opens the **User** page. The **User** page includes five tabs; **Mode**, **Buttons left**, **Buttons right**, **Stand type** and **Language**.

(1) Mode

This tab is used to select between **Standard** and **Personal settings** modes.

In **Standard** mode, all default functions (factory set) are active. In **Personal settings** mode, administrator defined settings are active for the following controls:

- five buttons on the Z focus drive, right / left

An administrator password must be entered before the button configuration can be changed.



You should ensure that access to the administrator password is controlled.

The factory-set password is "12345".

(2) Buttons left



An administrator password must be entered before the button configuration can be changed. Users who do not have administrator privileges will be able to view the button configuration, but will not be able to edit it.

This tab is used to configure the buttons on the left control ring. The controls are shown symbolically. The two upper buttons and two lower buttons are configured as pairs.

- Press the gray button to open a drop-down list.
- Select the required function from the list using the ▲ ▼ buttons. Only functions which are actually available with the current microscope configuration are listed.
- Press **Save** to assign the required function. Press **Cancel** to close the window without selecting a function.

The other buttons are configured in the same way.

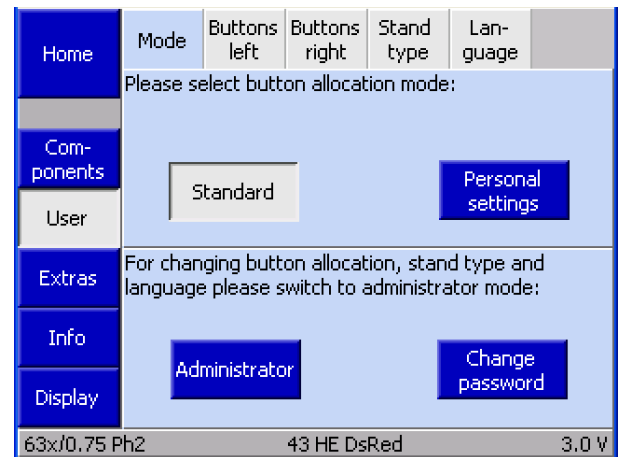


Fig. 4-54 Page Microscope -> Settings -> User -> Mode

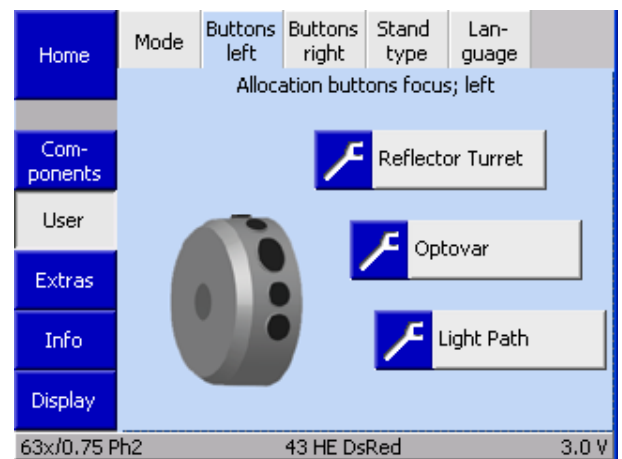


Fig. 4-55 Page Microscope -> Settings -> User -> Buttons left

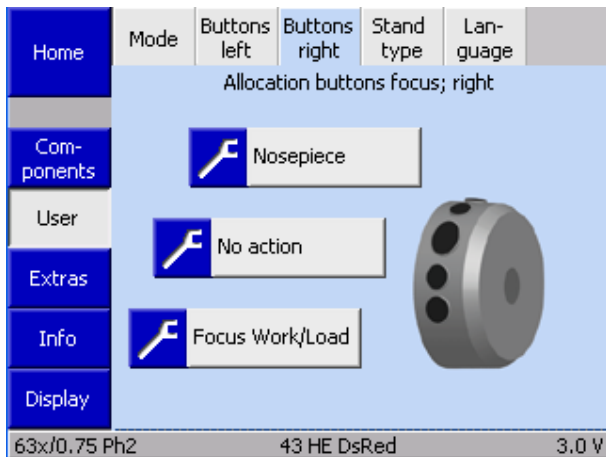


Fig. 4-56 Page Microscope -> Settings -> User -> Mode

(3) Buttons right



An administrator password must be entered before the button configuration can be changed. Users who do not have administrator privileges will be able to view the button configuration, but will not be able to edit it.

To set the button configuration for the right control ring, see (2) *Buttons left*.

(4) Buttons docking station

If the docking station is used, this can, if required, be configured in the same way.

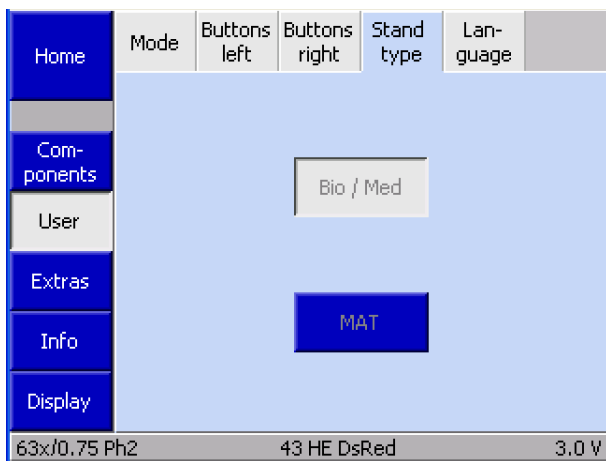


Fig. 4-57 Page Microscope -> Settings -> User -> Stand type

(5) Stand type

This tab is used to select whether the Axio Observer should be configured as a biological/medical microscope or a materials microscope (MAT). Changes to the basic settings will take effect after the microscope has restarted automatically.

(6) Language

This tab is used to select the language used on the TFT display. At present, the available languages are English and German. Changes to this setting will take effect after the microscope has restarted automatically.

4.6.5.3 Extras

The **Settings/Extras** page includes **Light manager**, **Oil stop**, **Dazzle protect**, **Ethernet** and **Misc** tabs.

(1) Light manager

This tab is used to activate or deactivate Light Manager or to change the Light manager mode. Light manager is used for automatic brightness adjustment (see section 4.4).

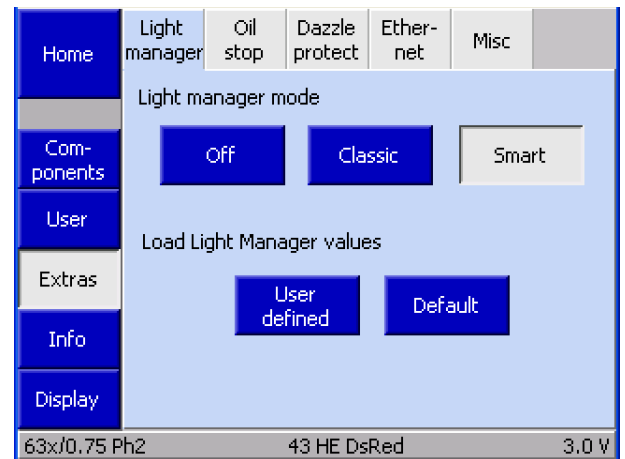


Fig. 4-58 Page Microscope -> Settings -> Extras -> Light manager

(2) Oil stop

The oil stop tab is used to activate or deactivate the oil stop function. The oil stop function prevents a dry objective from being moved into the immersion fluid. The nosepiece is always lowered when switching between a dry and an immersion objective.

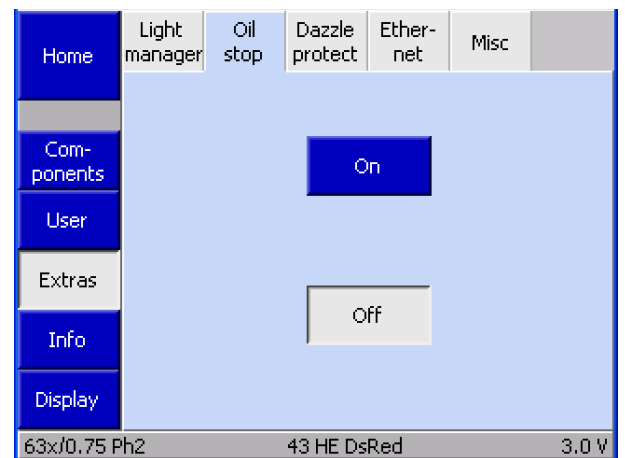


Fig. 4-59 Page Microscope -> Settings -> Extras -> Oil stop

(3) Dazzle protect

Note:

If the dazzle protection function is deactivated globally, all other fields on this tab will be grayed out.

If one of the components described is not installed, the corresponding buttons will not be displayed.

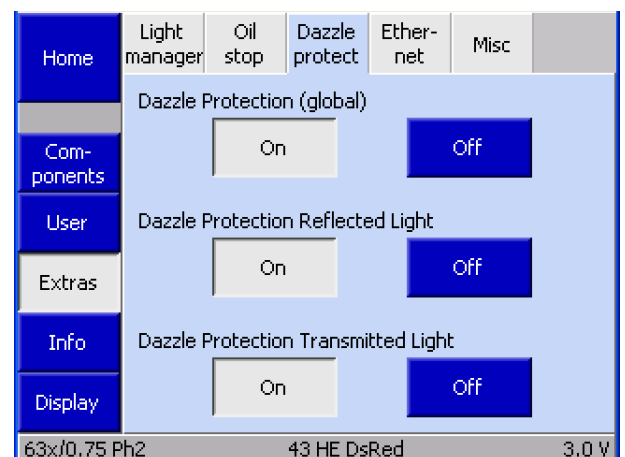


Fig. 4-60 Page Microscope -> Settings -> Extras -> Dazzle protect

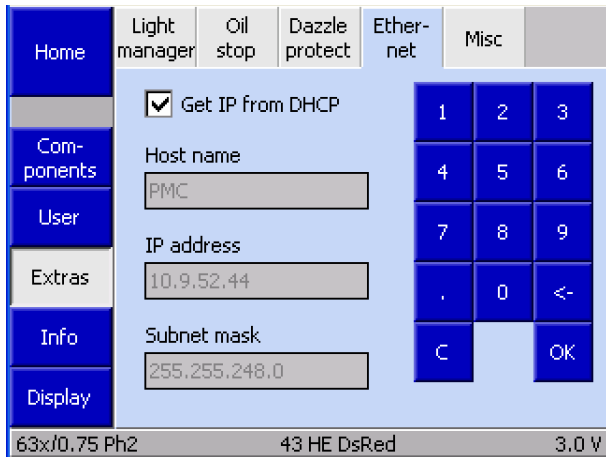


Fig. 4-61 Page Microscope -> Settings -> Extras -> Ethernet

(4) Ethernet

This tab is used to configure the Axio Observer's Ethernet connection.



Fig. 4-62 Page Microscope -> Settings -> Extras -> Misc

(5) Misc

The Misc tab is used to calibrate the touchscreen display.

After pressing the **TFT Calibration** button, several crosses will appear at different positions. By pressing a blunt pencil exactly on the cross centers the touch matrix is made to coincide with the image presentation.

4.6.5.4 Info

The **Settings/Info** page contains the **Firmware** tab only.

The Firmware tab shows the firmware version.

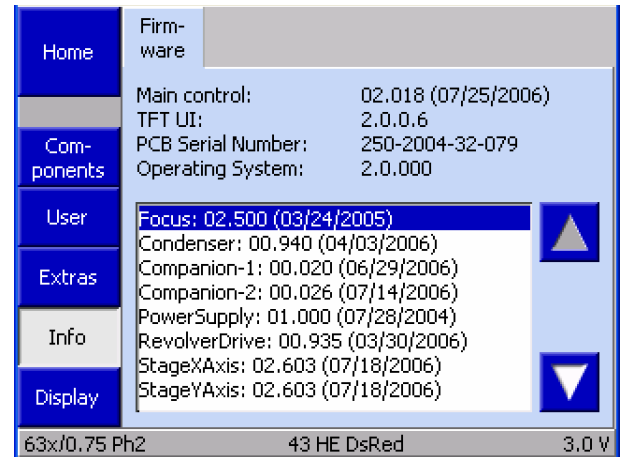


Fig. 4-63 Page Microscope -> Settings -> Info -> Firmware

4.6.6 Display

Pressing **Display** on the navigation bar of the **Home** page brings up the **Display** page.

The **Display** page enables the user to adjust the brightness of the TFT display using the ◀▶ buttons.

Press and hold **Display** on the navigation bar for at least one second to dim the TFT display. To turn the display back on, touch anywhere on the TFT screen.

Pressing **Display off** switches the TFT display off. Pressing it again switches the TFT display back on.

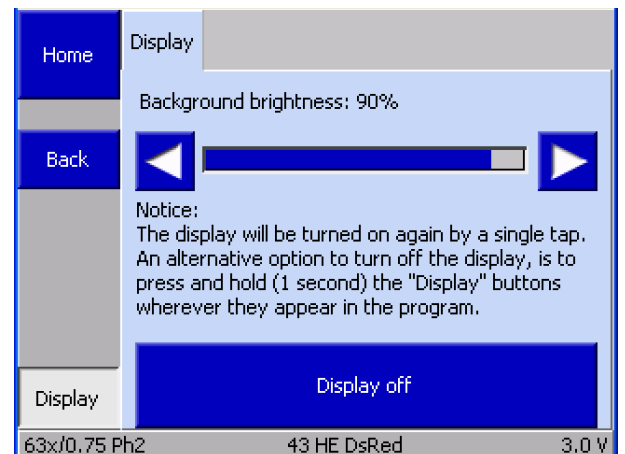


Fig. 4-64 Page Home -> Display



When the TFT display is switched off, it returns to the page from which the **Display** page was activated. This page will be displayed when the display is switched back on.

4.7 Illumination and contrast techniques

4.7.1 Setting transmitted light bright field KÖHLER illumination

4.7.1.1 General operating principle

Transmitted light bright field microscopy is the simplest of all optical microscopy techniques, since it allows high contrast and stained specimens (e.g. blood smears) to be viewed quickly and easily.

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

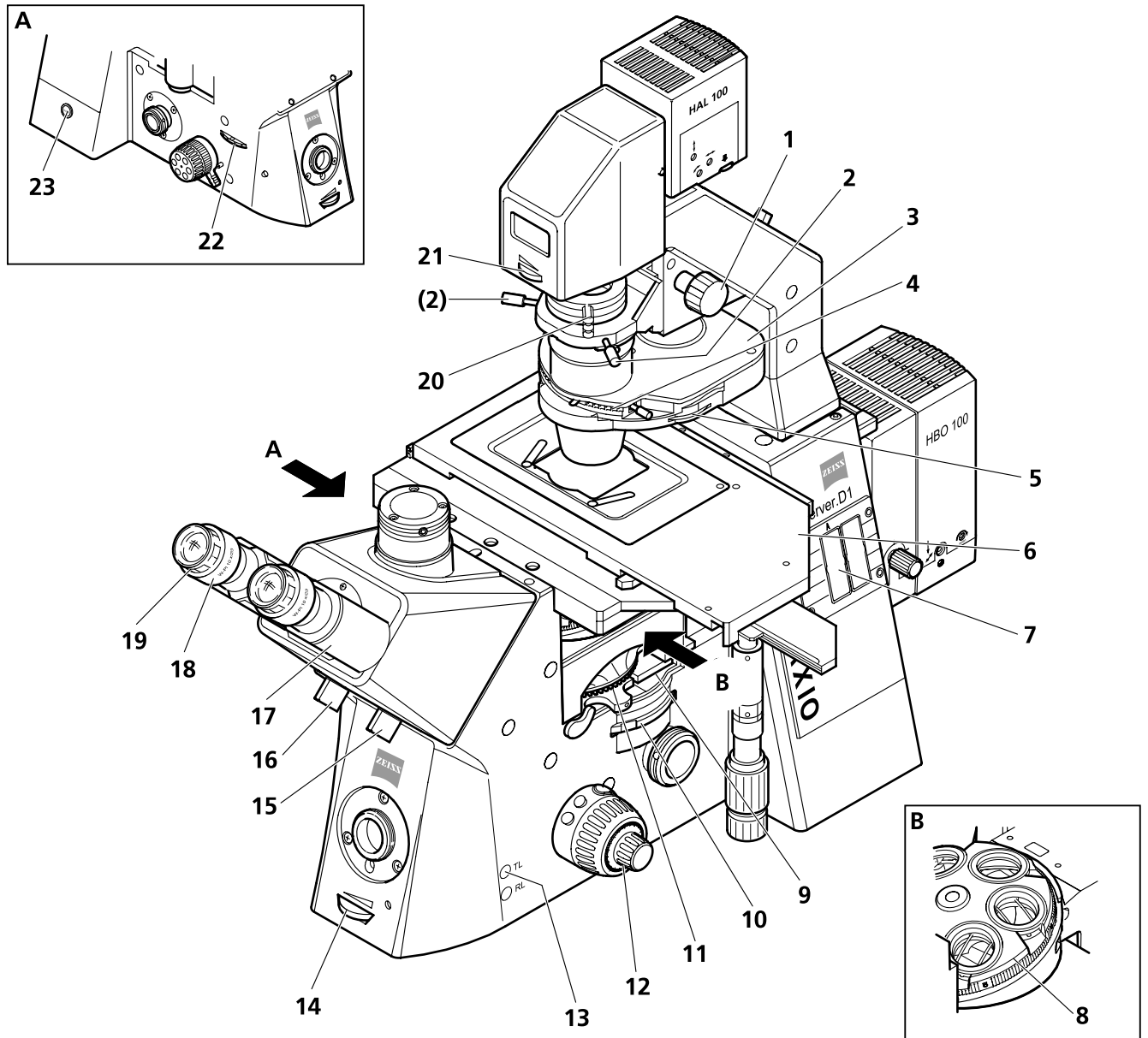
To make use of the microscope's full optical performance, and in particular that of the objective, the condenser, luminous-field stop and aperture stop should be adjusted in line with the requirements for KÖHLER illumination. This basic rule of microscopy is described in detail in section 4.7.1.3.

4.7.1.2 Equipment for transmitted light bright field

Every Axio Observer microscope is equipped with the components required to carry out transmitted light bright field microscopy.

4.7.1.3 Setting up transmitted light bright field KÖHLER illumination

- The microscope should be set up as described in section 3.
- The microscope has been switched on.
- Select the objective with the lowest magnification (e.g. 10x objective, yellow ring) on the nosepiece (4-65/8). Ensure that it clicks into position correctly.
- Using the Optovar turret control wheel (4-65/10), set the factor to 1x. Ensure that it clicks into position correctly.
- Open the luminous-field stop completely by turning the luminous-field stop control (4-65/21) on the transmitted light illuminator carrier to the left as far as it will go.
- Open the aperture stop completely by rotating the aperture stop control (4-65/5) on the condenser forwards as far as it will go.
- Turn the condenser turret adjustment ring (4-65/4) to move the condenser turret to the **H** position for bright field (if H is not available, to the **DIC** position).
- Turn the reflector turret adjustment ring to move the reflector turret (4-65/11, where used) to a position which contains no filter combination. Ensure that it clicks into position correctly.
- If necessary remove the analyzer slider from the slot (4-65/9) or move to the open position. Ensure that it clicks into position correctly.
- Turn the optical path switching control for the Sideport (4-65/23) to the 100 % vis (visual) position.
- Set the beam splitting ratio to 100 % vis (4-65/16) on the binocular (photo)tube. Remove the Bertrand lens from the optical path (where used) by moving the combined knob / slide (4-65/15) to the 100 % vis position.



- | | | | |
|----|--|----|---|
| 1 | Vertical adjustment knob for condenser | 14 | Illumination intensity control |
| 2 | Condenser centering screw | 15 | Knob / slide for vis / doc beam splitting |
| 3 | Condenser | 16 | Knob / slide for Bertrand lens and manual shutter |
| 4 | Condenser turret disk | 17 | Binocular section of the binocular tube |
| 5 | Condenser aperture stop control | 18 | Eyepiece |
| 6 | Microscope stage | 19 | Eyepiece adjustment ring |
| 7 | Aperture stop slider slot | 20 | Polarizer D with 2-position filter changer or 3-position filter changer |
| 8 | Objective nosepiece | 21 | Luminous-field stop control |
| 9 | Analyzer slider slot | 22 | Sideport control |
| 10 | Optovar turret control wheel | 23 | On / off button (D1), on / off switch (A1), Standby button (Z1) |
| 11 | Reflector turret | | |
| 12 | Focus drive coarse / fine | | |
| 13 | TL button for switching halogen illuminator on / off | | |

Fig. 4-65 Axio Observer.D1

- Swivel the 3-position filter changer (4-65/20) out of the optical path.
- Place a high-contrast specimen on the microscope stage (4-65/6).
- Adjust the eyepieces to the correct interpupillary distance by moving the binocular section (4-65/17) of the binocular tube apart or together.
- Set the ametropia correction zero point using the adjustment ring (4-65/19) of the eyepieces (4-65/18)
 - with no eyepiece reticle: set to the white point,
 - with eyepiece reticle: set to the red point,
- To correct for ametropia, bring the selected detail of the specimen into optimum focus using the relevant eyepiece adjustment ring.
- Use the coarse / fine focus drive (4-65/12) to bring the selected detail of the specimen into sharp focus. If no light is visible in the eyepieces, check that light is being emitted from the housing of the halogen illuminator. If no light is being emitted, switch on the halogen illuminator by pressing the TL button (4-65/13).
- Set the light intensity to a comfortable brightness using the illumination control (4-65/14).

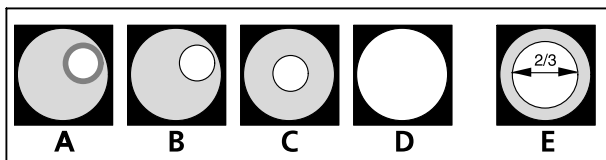



Fig. 4-66 Setting the stops for transmitted light bright field KÖHLER illumination

- Close the luminous-field stop (4-65/21) until it becomes visible in the field of view (not necessarily in focus - 4-66/A).
- Bring the edge of the luminous-field stop into focus (4-66/B) by adjusting the height of the condenser (4-65/1).
- Center the luminous-field stop using the centering screws (4-65/2 - 4-66/C) and open until the edge of the stop just disappears from the field of view (4-66/D).
- To set the aperture stop, remove one eyepiece from the eyepiece tube and adjust the aperture stop (4-65/5) to approximately 2/3 the diameter of the exit pupil of the eyepiece (4-66/E).
The settings required for optimum contrast depend on the specimen.
- Reinsert the eyepiece and where necessary refocus on the specimen using the fine focusing drive.
- Adjust the light intensity using the rocker switch.

 The field size and objective aperture change each time the objective is changed, so that for optimal results the luminous-field stop and aperture stop should be readjusted whenever the objective is changed.

4.7.2 Setting up transmitted light phase contrast

4.7.2.1 General operating principle

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

The phase contrast technique uses "phase stop and phase plate" 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.

The high-intensity, direct light components are attenuated and given a constant phase shift by the annular channel defined optically by the "phase stop and phase plate". By contrast, the indirect light components, diffracted by various cell components, bypass this optical channel and their phase is determined by differences in refractive index and thickness of the specimen.

In the intermediate image plane these two beams interfere and are enhanced or attenuated depending on their phase position. This interference results in images with intensity and color differences which can be perceived by the human eye.

The use of objectives with two phase plates enables the user to switch rapidly between positive and negative contrast by changing the aperture using the condenser turret. Positive phase contrast is useful for thin cell structures (e.g. filopodia), negative contrast for thicker parts of the cell, as fine structures are also better visualized.

4.7.2.2 Instrument configuration

- Phase contrast objectives with phase plates Ph 0, Ph 1, Ph 2 or Ph 3 for various mid-range numerical apertures. They can also be used for bright field techniques without any limitations.
- Condenser with turret containing centerable phase stops Ph 0, Ph 1, Ph 2 and Ph 3 for various mid-range numerical apertures.
- The active condenser phase stop must match the designation on the objective, e.g. Ph 1.

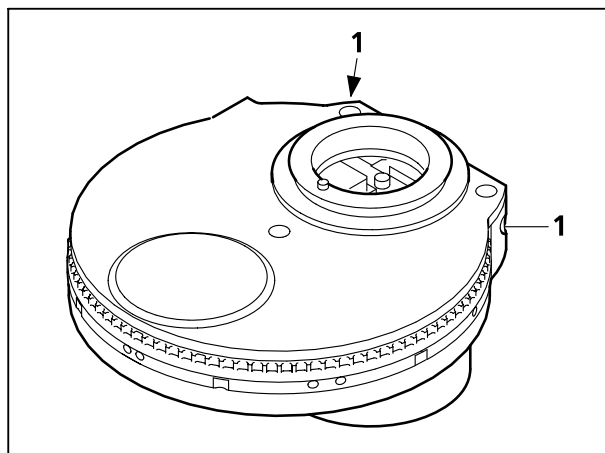


Fig. 4-67 Centering the condenser phase stop

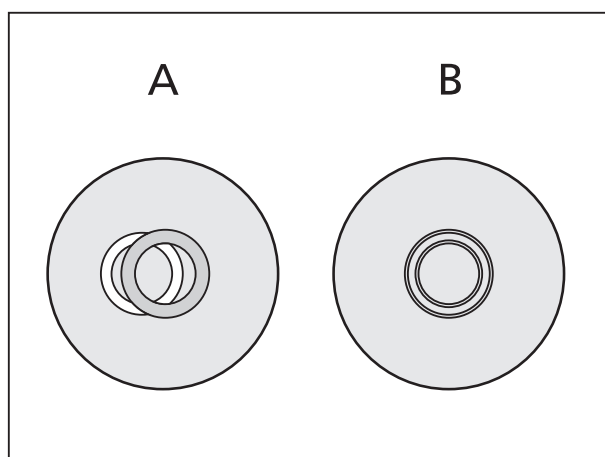


Fig. 4-68 Centering the phase stop (bright, in the condenser) with the phase plate (dark, in the objective)

4.7.2.3 Setting up transmitted light phase contrast


- Swivel the phase contrast objective, e.g. Ph 1, into the optical path.
- On the condenser turret select the phase stop with the same designation as the phase contrast objective (e.g. Ph 1).
- To check the centering and the congruence of the bright phase stop (in the condenser) with the dark phase plate (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 plate in the exit pupil of the objective.

If the phototube is used, the Bertrand lens can also be inserted in order to observe the exit pupil of the objective. Where the Bertrand lens is used, the Optovar turret adjustment wheel must be set to factor 1x.

- If the congruence is not perfect (4-68/A), the bright stop must be centered using two 1.5 mm Allen keys (4-67/1) until it is completely congruent with the dark phase plate (4-68/B).
- Finally, remove the centering telescope from the tube and replace it with an eyepiece / remove the Bertrand lens from the optical path.

It is not usually necessary to center the phase stops, as they are factory centered.

To enhance image contrast, a wide-band interference filter, green 32 x 4, can be inserted in the filter changer. 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 (4-68/B).

 All phase contrast objectives used require adjustment of the phase plates. When examining liquid objects in small vessels, the optical path must be aligned to the center of the vessel, as liquids at the edge of a vessel act as a lens and adversely affect the microscope image.

4.7.3 Setting up differential interference contrast (DIC) for transmitted light

4.7.3.1 General operating principle

The transmitted light DIC technique is used to produce high-contrast, 3D images of transparent specimen details.

Light which has been linearly polarized by a polarizer is split into two beams in a birefringent prism. These two beams pass through adjoining regions of the specimen a small distance apart and experience different path differences as a result of different refractive indices and specimen thicknesses. The two beams are then recombined by a second birefringent prism and after passing through the analyzer have the same direction of vibration. This allows the two beams to interfere in the intermediate image, with the path differences being transformed into differences in intensity (gray scale).

4.7.3.2 Instrument configuration

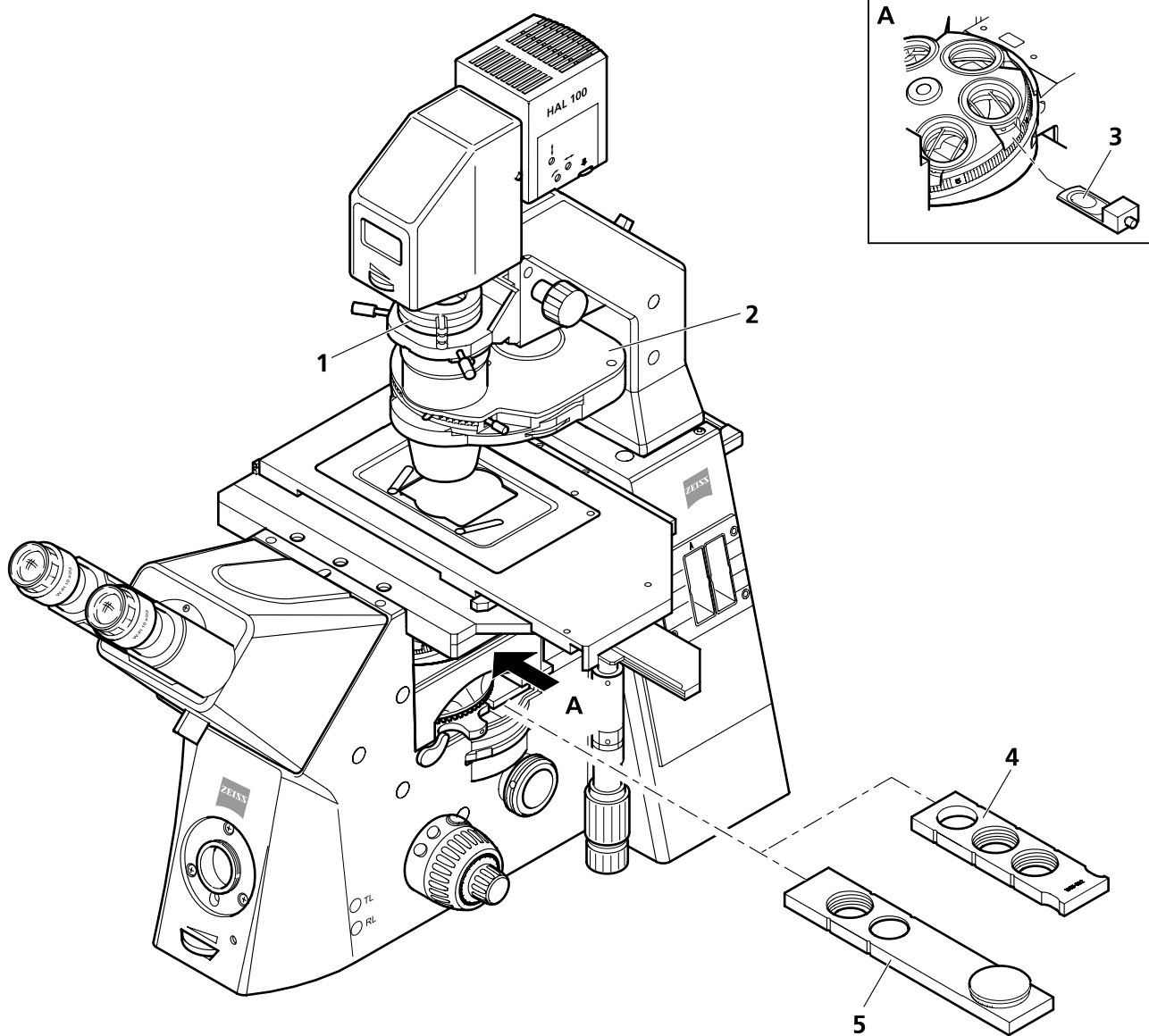
- Objectives suitable for DIC use, e.g. Plan-Neofluar
- DIC slider suitable for the objectives used
- Condenser turret equipped with DIC prisms (DIC I, DIC II, DIC III)
- Polarizer, e.g. polarizer D with 2-position filter changer (only required where the condenser used does not have a DIC prism with integrated polarizer)
- Analyzer module D in the reflector turret or analyzer, e.g. fixed analyzer slider or $\pm 30^\circ$ analyzer slider (de Sénarmont)

4.7.3.3 Setting up transmitted light DIC

- Select an objective suitable for DIC on the nosepiece. Insert the corresponding DIC slider (4-69/3) into the slot on the nosepiece. Ensure that the DIC slider clicks into position.
- Select the appropriate DIC prism I, II or III on the condenser turret.
- Insert the analyzer slider (4-69/4) into the stand. Ensure that it clicks into position correctly.

(1) Transmitted light DIC with fixed analyzer slider

- Move the polarizer (4-69/1) on the transmitted light illuminator carrier into position. Ensure that it clicks into position correctly.
- Place the specimen onto the stage.
- Configure the luminous-field stop and aperture stop on the condenser (4-69/2) for KÖHLER illumination.
- Set the optimum contrast using the knurled screw on the DIC slider. Symmetrical adjustment of the DIC slider around its center position allows specimen details to be viewed in 3D as if raised or lowered.



- 1 Polarizer D (fixed, optionally rotatable)
- 2 Condenser
- 3 DIC slider
- 4 Analyzer slider, fixed
- 5 $\pm 30^\circ$ analyzer slider


Fig. 4-69 Components for transmitted light DIC on the Axio Observer

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

 This technique can only be applied using the 0.35 H/DIC condenser.

If the $\pm 30^\circ$ analyzer slider is used, the DIC slider must first be centered.

- Move the polarizer into position and adjust the $\pm 30^\circ$ analyzers (4-69/5) to the 0° (dark) position (polarizer and analyzer are perpendicular).
- Deselect the DIC prism on the condenser turret (e.g. use bright field or phase contrast).
- Remove one eyepiece and replace with the centering telescope (or swivel the Bertrand lens into position on the phototube).
- If the field is viewed using the centering telescope (or Bertrand lens), a diagonal black line on the DIC slider (left top to bottom right) will be visible.
- Move the diagonal black line to the center of the field of view by adjusting the knurled screw on the DIC slider.
- Remove the centering telescope and reinsert the eyepiece (or move the Bertrand lens out of the optical path).
- Select the DIC position on the condenser.
- Place the specimen onto the stage.
- Using the analyzer control wheel, rotate the analyzer away from the 0° position until optimum contrast is obtained.

 Because the DIC technique uses polarized light, it will be disrupted if birefringent objects, e.g. foils occasionally used with histological sections, are positioned between the polarizer and analyzer. This problem may also arise with Plexiglas culture chambers if the chamber bottom is made of plastic. In such cases, it is advisable to use chambers with glass bottoms to avoid impairment of the optical performance.

4.7.4 Setting up transmitted light PlasDIC contrast

4.7.4.1 General operating principle

PlasDIC provides a relief-like image and is particularly useful for thicker specimens. The contrast can be varied. Microtiter plate wells can be contrasted right up to the edge. Glass-bottomed culture vessels are not required.

4.7.4.2 Instrument configuration

PlasDIC contrast requires a microscope stand with the following components:

- 3.5 mm slit aperture for PlasDIC in the condenser turret,
- PlasDIC slider, depending on the objective,
- D P&C analyzer module in the reflector turret or analyzer slider.

4.7.4.3 Setting up PlasDIC

- Open the condenser aperture stop (4-69/2) fully.
- Place the specimen onto the stage.
- Select the 3.5 mm slit aperture for PlasDIC on the condenser. When switching from bright field to PlasDIC, the brightness should be increased.
- Select the D P&C analyzer module on the reflector turret or insert the analyzer slider (4-69/4 or 5) into the optical path beneath the reflector turret.
- Select a PlasDIC objective. Suitable objectives include the A-Plan 10x, LD A-Plan 20x, 32x or 40x, LD Plan-Neofluar corr 20x, 40x or 63x.
- Insert the PlasDIC slider into the DIC slot of the selected objective.
- Adjust the contrast using the knurled screw on the PlasDIC slider. Structures can be visualized in relief or pseudo-dark field. Relief offers the best contrast.

4.7.5 Setting up transmitted light VAREL contrast

4.7.5.1 General operating principle

VAREL contrast provides a relief-like image of specimens and can be used as an alternative to phase contrast. VAREL contrast can also be used on curved surfaces, such as 96-well microtiter plates, for which no contrast can be achieved using the phase contrast technique (ring congruence is not possible!).

4.7.5.2 Instrument configuration

- Condenser with turret equipped with VAREL stops.
- Objectives suitable for VAREL contrast, e.g. A-Plan Ph 1 Var 1 or LD A-Plan Ph 2 Var 2.

4.7.5.3 Setting up VAREL contrast

- Open the aperture stop (4-70/1) fully.
- Select the VAREL stop on the condenser turret (**Var** position).
- Select the required VAREL objective.
- Adjust the Varel aperture using the adjustment screw (4-70/2) until optimum VAREL contrast has been achieved (relief-like image).

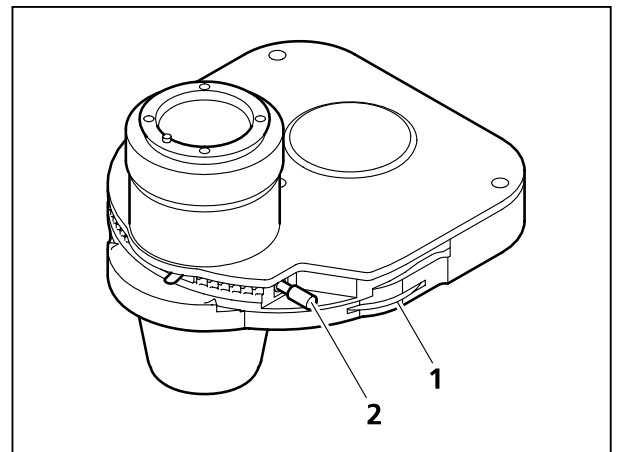


Fig. 4-70 Setting up VAREL contrast



Microtiter plates:

Select the VAREL stop ring opposite to the well edge for illumination on the edge of a well; the right or left VAREL rings can be used in the middle of the well.

The specimen field and associated image of the VAREL stop is shown in fig. 4-71. The stop appears rotated by 180°.

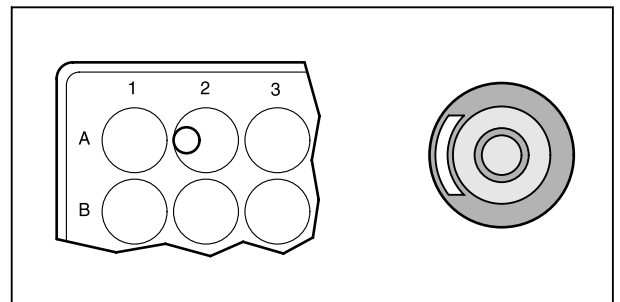



Fig. 4-71 VAREL contrast for microtiter plates

-  - Shifting the VAREL illumination to outside the pupil is equivalent to unilateral dark field illumination.
- Shifting the VAREL illumination to between the Ph and VAREL rings of the objective is equivalent to oblique bright field illumination.

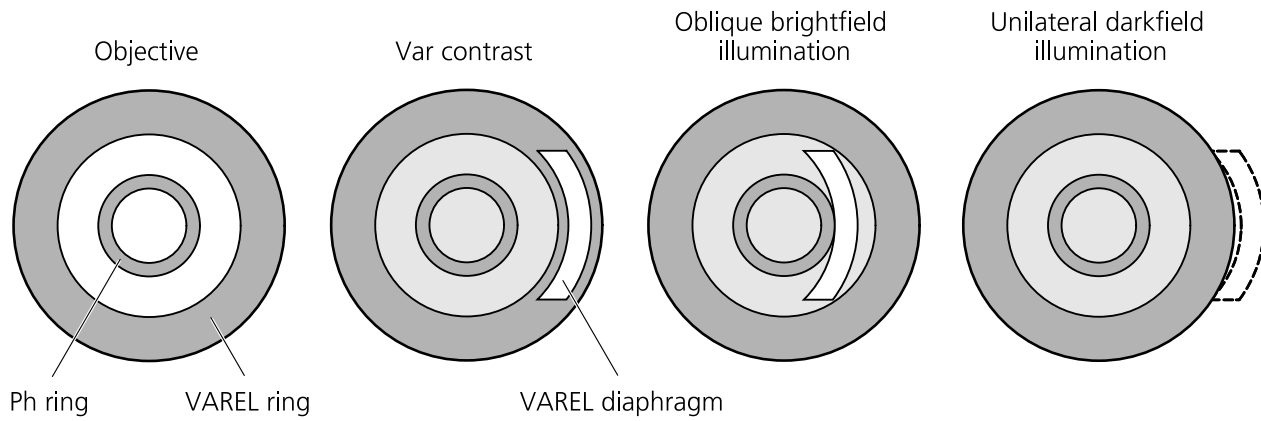


Fig. 4-72 Pupil images with VAREL contrast

4.7.6 Setting up epifluorescence contrast

4.7.6.1 General operating principle

The epifluorescence technique allows high-contrast images of fluorescent substances in typical fluorescence colors to be obtained. In the epifluorescence microscope, the light generated by a high-performance illuminator arrives at an excitation filter via a heat filter. The filtered, short-wavelength excitation beam is reflected by a dichroic beam splitter and is focused on the specimen via the objective. The specimen absorbs the short-wavelength beam and emits longer-wavelength fluorescent light (Stoke's law), which is gathered by the objective and transmitted by the dichroic beam splitter. The light then passes through a barrier filter which only transmits the long-wavelength light emitted by the specimen.

The excitation and barrier filters, which are positioned, together with an associated dichroic beam splitter, in the FL reflector module, must have very precisely matched spectra.

4.7.6.2 Instrument configuration

- Recommended objectives: bright field objectives
- FL reflector module in the reflector turret
- Optional: HBO 100, HBO 50 fluorescence illuminators
- HAL 100 halogen illuminator for transmitted light illumination

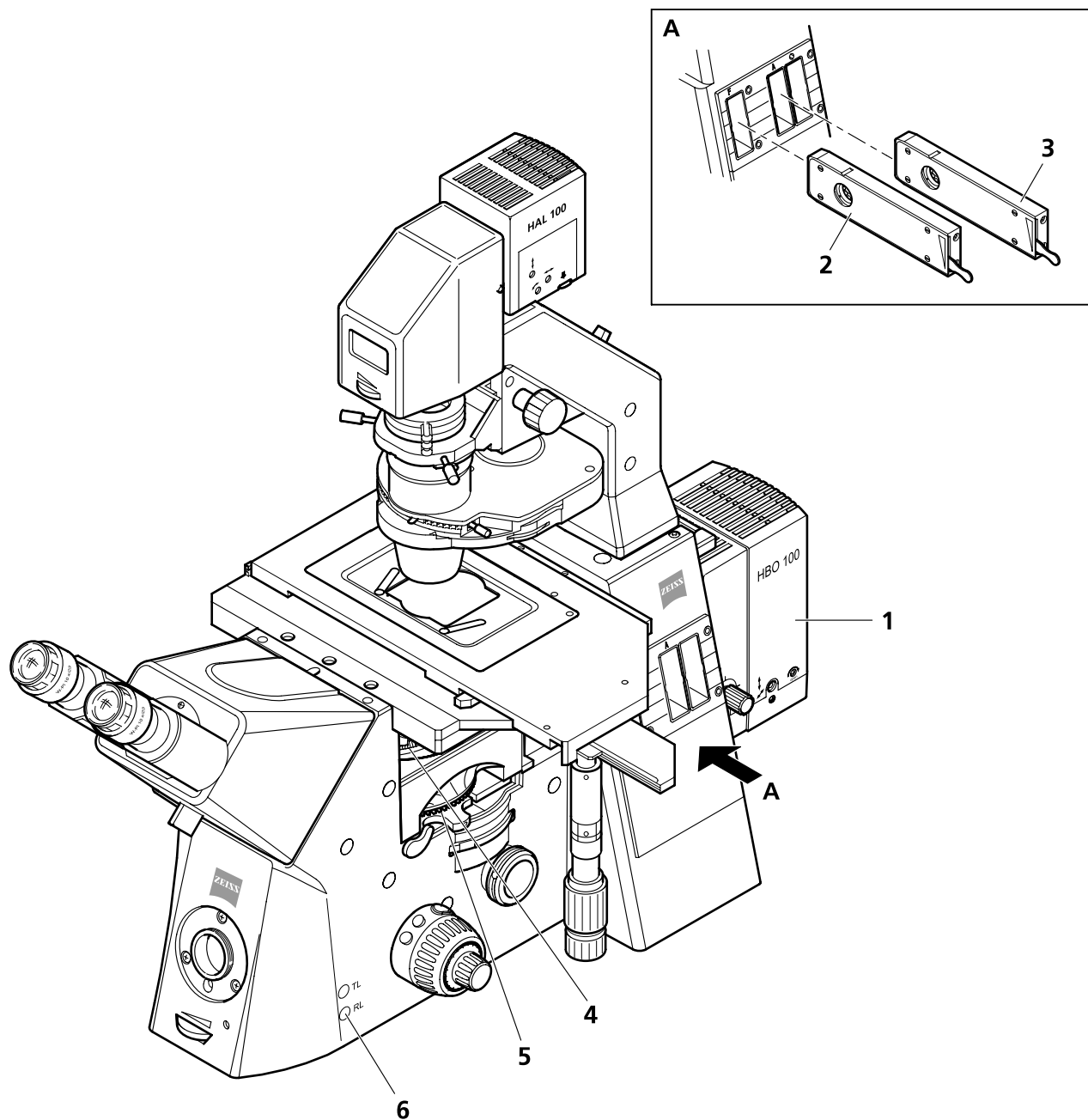


The mercury vapor short-arc lamp should always be adjusted using the adjusting aid as described in section 3.14.3 before utilizing the epifluorescence technique. It may be necessary to readjust the lamp after extended use. The self-adjusting HBO 100 can also be used.

4.7.6.3 Configuring epifluorescence

The first step in setting up epifluorescence is considerably easier if, e.g. the 20x/0.50 Plan-Neofluar objective and a strongly fluorescing specimen are used. Demonstration specimens may also be used.

- Switch on the HAL 100 halogen illuminator.
- Select a suitable objective, e.g. 20x/0.50 Plan-Neofluar, on the nosepiece (4-73/4).
- Select position **H** (transmitted light bright field, or alternatively phase contrast) on the condenser turret, and select an appropriate region of the specimen.
- Bring the specimen into focus.
- Block the reflected light path with the fluorescence shutter by pressing the RL button (4-73/6).



- 1 HBO 100 fluorescence illuminator
- 2 Iris stop slider (luminous-field stop)
- 3 Iris stop slider (aperture stop)
- 4 Nosepiece
- 5 Reflector turret
- 6 RL button

Fig. 4-73 Components for epifluorescence on the Axio Observer

- Switch on the HBO 100 fluorescence illuminator (4-73/1) at the power supply and leave to warm up to operating temperature for approx. 15 minutes.

- Select the FL reflector module with the required fluorescence filter combination (depending on the type of excitation) on the reflector turret (4-73/5).
- Open the fluorescence shutter by pressing the RL button (4-73/6).

For epifluorescence, two iris stop sliders are used as aperture and luminous-field stops. However, since the aperture stop is not visible during the epifluorescence procedure, the aperture stop slider must first be centered in the luminous-field stop slot and then inserted in the aperture stop slot.

The aperture stop and luminous-field stop are centered in the same way:

- Insert an iris stop slider into the luminous-field stop (F) slot (4-73/2) until it clicks into place.
- Using the lever, close the stop until it becomes visible in the field of view.
- Center the stop using the two adjusting screws on the slider (using a 3 mm ball-end Allen key). Finally, open the stop until the entire field is clear.
- Insert the centered slider into the slot (A) for the aperture stop (4-73/3) until it clicks into place.
- Insert a further iris stop slider into the luminous-field stop slot (4-73/2).
- Close the luminous-field stop until it becomes visible in the field of view.
- Center the luminous-field stop to the edge of the field of view using the two centering screws.
- Either open the luminous-field stop until it just disappears from the edge of the field of view or, if there is a risk of bleaching the specimen, close it until it is visible in the field of view.
- Finally, refocus on the specimen and optimize the fluorescence illuminator collector position by using the control on the fluorescence illuminator to adjust the collector such that illumination of the field of view by the short-wave excitation reflector module is as homogeneous as possible. Correction of the collector position is not required for modules with long-wavelength excitation.

4.8 Documentation

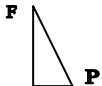
Axio Observer microscopes are fitted with up to five cameraports which can be used for record-keeping purposes:

- Frontport for connecting an SLR, video or digital camera (e.g. Zeiss AxioCam) via a special video or camera adapter.
- Sideport (right or left) for connecting record-keeping equipment via an interface 60N.
- Baseport (bottom) for connecting record-keeping equipment via an interface 60N.
- Binocular phototube with an interface 60N.

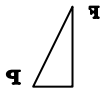
4.8.1 Image orientation for camera outputs

The following table gives a detailed overview of image orientation at the Axio Observer camera outputs.

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

Original object:  viewed without using the microscope

The object is placed on the microscope stage with the **readable side**, as shown above, **towards the objective** and then appears, as viewed from above by the microscope user, as follows:

 **This view is the reference view for the description which follows.**



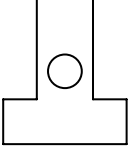
Likewise viewed from above, a movement of the specimen stage in the Y direction backwards (thick arrow) and in the X direction to the right (thin arrow) looks like this:



The table below provides, for a given viewing angle for the relevant stand component, the following information:

- the intermediate image of the object / monitor image, as captured by the camera, and
- the direction of motion of the object when the stage is moved.

Order no. / Description	Viewed with / Switch position	Intermediate image / monitor	Direction of motion	Viewing angle, schematic
425537-0000-000 Binocular tube	Eyepieces (100% vis)			
425536-0000-000 Binocular phototube	100% vis : 0% doc 50% vis : 50% doc 0% vis : 100% doc	 	 	
425535-0000-000 Binocular ergotube	Eyepieces (100% vis)			
425150-0000-000 Sideport 60, left, 2 switch positions	20% vis : 80% doc			
425152-0000-000 Sideport 60 links, 3 switch positions	50% vis : 50% L 0% vis : 100% L			
425153-0000-000 Sideport 60, right, 3 switch positions	50% vis : 50% R 0% vis : 100% R			
425154-0000-000 Sideport 60, left and right, 3 switch positions	0% vis : 100% L 20% vis : 80% R	 	 	
000000-1069-228 Beam path switching	100% Frontport			
000000-1069-229 Beam path switching mot.	100% Frontport			

Order no. / Description	Viewed with / Switch position	Intermediate image / monitor	Direction of motion	Viewing angle, schematic
425126-0000-000 Baseport	100% doc			

Use of camera adapters which work without using intermediate imaging does not affect image orientation. The same applies to the Frontport with adapters V200 T2 2.5x for SLR 000000-1279-493 and video adapter V200 C 2/3" 0.63x on the Frontport 000000-1071-171.

The use of Optovars (e.g. 1.25x or 1.6x) does not affect image orientation.

The above description applies equally to all other specimens.

5 CARE, MAINTENANCE, TROUBLESHOOTING AND SERVICE

5.1 Care

Care of the microscope is limited to the following procedures:

- Cover the instrument with the dust cover after each use.
- Do not install the instrument in damp areas.
- Cover open tubes with dust protection caps.
- Remove dust and loose dirt from visible optical surfaces using a brush, airblower, cotton wool bud, optical cleaning paper or a 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 Cleaning Mixture L.

This cleaning mixture is manufactured from 90 % vol benzine and 10 % vol isopropanol (IPA). The ingredients are also known under the following synonyms:

Benzine: petroleum ether
Isopropanol: 2-propanol, dimethyl carbinol, 2-hydroxypropane



Before cleaning, switch off the microscope and disconnect it from the mains power supply.

Clean the optical surface using a circular motion working from the middle outwards. A light pressure should be exerted on the optical surface.

The following instructions should be followed where the Axio Observer is used in warm, humid climates:

- Keep the microscope in a bright, dry, well-ventilated room with a humidity of less than 65%. Sensitive components and accessories, such as objectives and eyepieces, should be stored in a dry cabinet.
- If the microscope is to be stored in closed containers for a prolonged period, fungicide-soaked cloths should be placed in the containers to prevent mould.



The following conditions always present a risk of mould growth on fine opto-mechanical instruments:

- exposure to a relative humidity > 75 % and a temperature of +15 to +35 °C for a period longer than three days.
- installation in poorly-ventilated, dark rooms and
- dust deposits and fingerprints on optical surfaces.

The microscopes are not equipped with special protective devices for handling corrosive, potentially infective, toxic, radioactive or other specimens which are dangerous to health. If handling such specimens, all legal requirements especially national rules for accident prevention have to be regarded.

- Eliminate any contamination of the microscope according to the rules of accident prevention.
- Switch off the device and put dust cover (protection against dust and humidity) on it after use.

5.2 Maintenance

5.2.1 Checking the instrument

The following checks should usually be performed every six months.

General

- Check the power cable and plugs for damage.
- If any damage is visible, switch off the instrument and prevent its use. Any damage should be repaired by the Zeiss service team.
- Check that the maximum operating times of the halogen and HBO illuminators have not been exceeded (weekly).

Illumination

- Check that the halogen and HBO illuminators are correctly configured.
- Check the electrical contacts of the illuminators.

Optics

- Visual inspection of the cleanliness of objectives and eyepieces

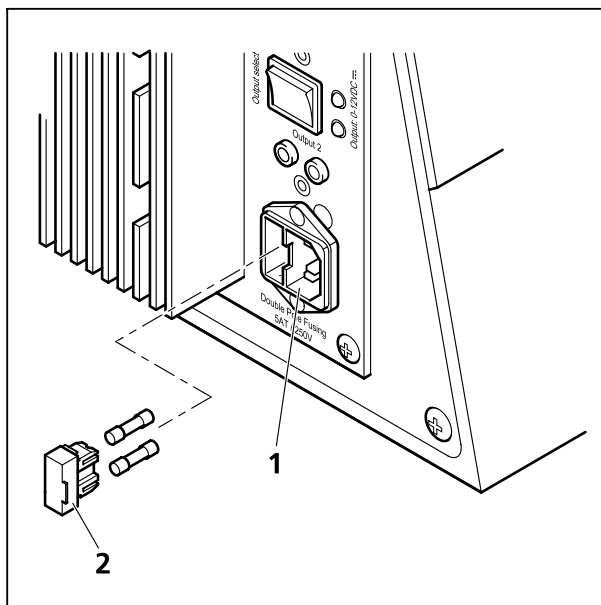


Fig. 5-1 Replacing the fuse

5.2.2 Replacing the microscope fuses

The main instrument fuses are located at the rear of the Axio Observer.A1 and .D1 stand. The fuse compartment is integrated into the power socket and contains two **T 5.0 A/H 250 V 5 x 20 mm** fuses.

If one of the fuses blows, the cause should be identified and any technical defect remedied before replacing the fuse.



The instrument must be disconnected from the mains power supply before replacing a fuse.

- Disconnect the instrument from the mains power supply. Remove the fuse carrier (5-1/2) by pulling it towards you.
- Remove the fuses from the fuse carrier and insert new fuses.
- Reinsert the fuse carrier into the fuse compartment (5-1/1) until it clicks into place. Plug in the power cable.

The Axio Observer.Z1 is supplied with power from the VP231 power supply. The fuses (2x type **T 6.3 A/H / 250 V**) of the power supply are located in a box on its backside. The procedure to exchange a fuse is identical with the procedure for the manual stands.

5.2.3 Replacing the fuses in the power supply for the HBO 100

The fuse carrier for the F1 and F2 fuses is located on the rear of the power supply. It is integrated into the power socket and contains two **T 2.0 A/H 250 V** fuses.

If one of the fuses blows, the cause should be identified and any technical defect remedied before replacing the fuse.



The instrument must be disconnected from the mains power supply before replacing a fuse.

- Disconnect the instrument from the mains power supply.
- Remove the fuse carrier (5-2/1) from the fuse compartment (5-2/2) by pulling it towards you.
- Replace the defective fuse.
- Reinsert the fuse carrier into the fuse compartment until it clicks into place.
- Plug in the power cable.

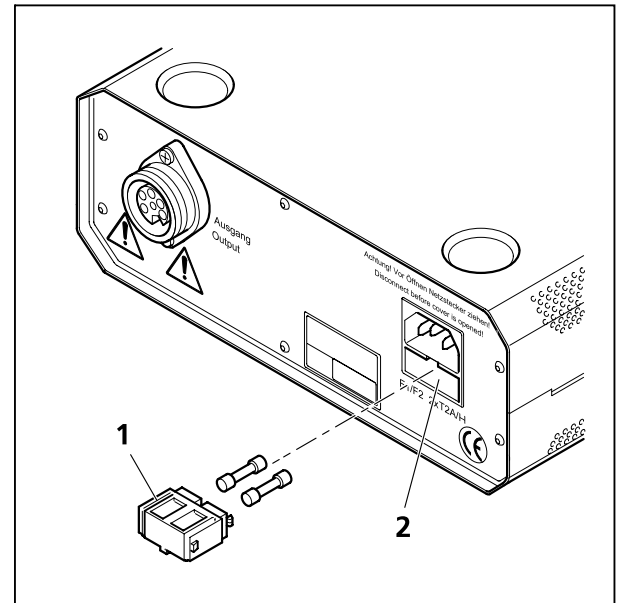


Fig. 5-2 Replacing the power supply fuses

5.3 Servicing

Any repairs to optical components or moving parts inside the instrument or any work on the power supply may only be carried out by service technicians or specially **authorized** personnel.

If servicing is required, please contact your local representative or

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6 ANNEX**6.1 List of abbreviations**

a	Free working distance
AC	Alternating current
ACR	Automatic component recognition
A-Plan	Achromatic objectives featuring improved image flatness (ICS line)
BF	Bright field
Br.	Suitable for spectacle wearers
CAN bus	Controller Area Network Communication Bus
Cod.	Coded
CSA	Canadian Standards Association
D	Transmitted light / cover slip thickness
d	Diameter
DC	Direct current
DIC	Differential interference contrast
DIN	Deutsches Institut für Normung (German standards institution)
doc	Documentation
EC	European Community
EN	Euronorm (European standard)
EMC	Electromagnetic compatibility
EEC	European Economic Community
foc.	focusable
H	Bright field
HAL	Halogen illuminator
HBO	Mercury vapor short-arc lamp
HD	Bright / dark field
HF	Bright field
ICS	Infinity color corrected system
IEC	International Electrotechnical Commission
IP	International Protection (protection class)
ISO	International Organization for Standardization
LCD	Liquid Crystal Display
LED	Light Emitting Diode
LD	Long Distance

M, mot.	motorized
Man	Manual
N. A.	Numerical aperture
Ph, PH	Phase contrast
PL	Plan
PlasDIC	Plastic differential interference contrast
RL	Reflected light
SLR	Single lens reflex
SW	width across flats
T	Slow-blow (fuse type)
TFT	Thin film transistor
TL	Transmitted light
TV	Television
UL	Underwriter Laboratories (American testing institute)
USB	Universal serial bus
VIS	Visual
XBO	Xenon short-arc lamp

6.2 Index

	Page
3	
3200 K	69
A	
ACR	74
Ametropia	31
Analyzer	65
Aperture stop	117
Aqua Stop II	56
Automatic component recognition	74
B	
Basic settings	75
Beam splitter	43
Binocular section	71
Binocular tube	26, 30
Binocular tubes	70
Bright field	104
C	
Care	121
Checks	122
Color photography	69
Compatibility	16
Components and controls	58, 64
Condenser	65
Condenser, manual	66
Condenser, motorized	67
Condensers	25, 38, 39
Configuration	75
Contrast Manager	81
Contrast techniques	104
Control ring	73
D	
Description	14
Designation	14
DIC	109
DIC prisms	40
Differential interference contrast	109
Diffuser	52
Docking station	45, 74
Documentation	118
E	
Equipment	16
Eyepiece reticle	31
Eyepieces	25, 30, 71

F

Filter changer.....	71
Filter set.....	42
Filter slider	67
FL attenuator.....	68
FL P&C reflector module	42
Fluorescence contrast	115
Fluorescence illuminator	116
Focus drive	64, 69

G

Gliding stage Z	38
-----------------------	----

H

Halogen bulb.....	53
Halogen illuminator	50
HBO 100 illuminator	54, 55
Heating stage	37

I

Illumination intensity.....	69
Image orientation	118
Installation.....	29
Intended use.....	14
Iris stop slider.....	68

K

KÖHLER.....	65, 67, 71, 104
-------------	-----------------

L

Laserport	57
LCD display.....	72
Left Sideport.....	64
Light Manager	77
LM set	71
Luminous-field stop	71, 117

M

Main features	15
Maintenance	122
Menu overview.....	84
Microscope stage.....	34, 67
Mounting frame	36

N

Notes on warranty	12
-------------------------	----

O

Object guide.....	36
Objective nosepiece	33, 65
Objectives.....	23, 33
On / off switch.....	64

Operation	58
Optovarrevolver	71
P	
Phase contrast	107
PlasDIC contrast	112
Polarizer	71
Power connection	46
R	
Reflected light	115
Reflector turret	41, 69
Replacing the fuses	122, 123
RL button	69
S	
Safety notes	8
Scanning stage	35
Servicing	123
Setting up the instrument	29
Sideport	64
Sockets	47
Specimen holder	67
Specimen stage	26, 36
Stand types	13
Switching on / off	49
System overview	19
T	
Technical specifications	27
TFT display	45, 72, 82
TL button	69
Touchscreen	82
Transmitted light	104, 107, 109, 112, 113
Transmitted light illuminator	32
U	
Unpacking	29
V	
VAREL contrast	113
Vertical adjustment of the condenser	65
Vertical stop for focus drive	72
X	
XY positioning	68

6.3 Patent rights

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