

Axioplan 2 imaging and Axiophot 2 Universal Microscopes

Operating Manual



Knowledge of this manual is required for the operation of the instrument. Please therefore familiarize yourself with its contents and pay special regard to the sections dealing with the safe handling of the instrument.

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Notes on the safe operation of the instrument

The Axioplan 2 imaging Universal Microscope has been designed and tested in compliance with EN 61010, part 1 (DIN VDE 0411) and IEC 1010-1 and left the factory in a perfect state with regard to its safety facilities. To ensure that this remains the case and to guarantee the safe operation of the equipment, the instructions and warnings given in this manual must be observed.

The instrument meets the requirements of the EC directive 89/336/EEC and the EMC legislation of November 9th 1992. It has been allocated the protection degree IP 20 and is categorized as Class 1 Equipment. The power plug must be inserted in a socket featuring a grounding (earth) contact. The grounding effect must not be nullified by an extension cable which does not have a protective ground wire.

For your own safety and for the protection of the instrument against damage

Axioplan 2 imaging features special protective devices such as attenuation filters to protect the eyes against intense radiation, and stops to protect specimens and objectives against knocks and mechanical damage. These protective devices must be used and must not be removed. You must familiarize yourself with the protective devices provided by Axioplan 2 imaging under all circumstances.



Danger of crushing!

In stands with a motorized focusing drive, there is a danger of crushing your hand between the stage carrier and stand base when the stage is lowered. For this reason, do not place your hand under stage carrier when the stage is being lowered.



Gas discharge lamps!

In unfavorable circumstances and with improper use, gas discharge lamps can explode, flinging splinters of glass through the air and causing possible injury. Therefore, it is imperative that the safety and operating instructions of the manufacturer of the gas discharge lamp be followed (→ chapter on *Care, Maintenance*), Gas discharge lamps emit ultraviolet radiation which can cause burns on the eyes and skin. Never look directly into the light of these lamps and avoid direct, unprotected incidence of their light on your skin. When using the microscope, always use the protective devices belonging to the instrument, e.g. special attenuation filters. Gas discharge lamps are contained, for example, in our microscope illuminators HBO 50, HBO 100 and XBO 75.



WARNING!

Specimens hazardous to health!

The Axioplan 2 imaging is not equipped with any special devices for protection against corrosive, toxic, radioactive or other substances hazardous to health. All legal requirements, especially national accident regulations, must be observed when handling such specimens.



WARNING!

Hot surfaces!

Do not touch the hot lamp housing. Always disconnect the power plug before changing a lamp and allow the unit to cool down for approx. 15 minutes.



WARNING!

Thermally sensitive fluorescence filters!

Fluorescence filters are sensitive to the thermal radiation of the microscope lamp and their performance can be permanently impaired by it. Therefore, never remove the heat-reflecting filter on the microscope illuminators when you are working with fluorescence filters.



WARNING!

Heat build-up!

Placing objects against or covering ventilation slots on the microscope or its components can lead to a build-up of heat which will damage the instrument and, in extreme cases, cause a fire. Therefore, always keep the ventilation slots clear (minimum distance 15 cm). Always check whether the microscope is switched off before placing the dust cover over it.



WARNING!

Dust und dirt!

Dust and dirt can impair the performance of the Axioplan 2 imaging. Therefore, protect the microscope as far as possible against these influences. Always use the dust cover if you do not intend to use the Axioplan 2 imaging for longer periods of time (more than 6 hours).



WARNING!

Operation!

The instrument must be operated by trained staff only. They must be instructed in the hazards involved in microscopy and the respective field of application. This includes an awareness of the risk of eye injury due to intensive irradiation by light.

General

Note on exchangeable components

Perfect functioning of the instrument requires that you use spare parts and components which are marketed and approved by us. In the event of doubt, please contact our service staff. The use of parts from other manufacturers may impair the performance of the Axioplan 2 imaging or indeed damage the instrument. The use of such parts is the sole responsibility of the user.

No other care, maintenance or repair work must be carried out apart from the activities specified in → *Care, Maintenance*.

More extensive repairs may only be performed by our customer service experts or by specially authorized persons. Damage to the instrument may otherwise result.

Note: We would like to expressly emphasize here that any adjustments not described in the *Microscope Components* chapter must only be performed by persons expressly authorized by us to do so.

The Axioplan 2 imaging is a precise optical instrument which may be impaired in its function or even damaged by inexperienced handling.

Note on power unit integrated in stand

The integrated power unit is used to supply voltage to the microscope illumination, the Axiophot 2 Photo module and the coded motorized components. The power unit must not be used to supply voltage to other external power consuming devices. This can lead to overloading and destruction of the power unit.

General

The Axioplan 2 imaging is a universal microscope which is suitable for all applications relevant to light microscopy. Its modular design, numerous components and wide range of accessories allow the Axioplan 2 imaging to be adapted and extended to perform a large number of special applications.

There is practically no field of application in light microscopy for which the Axioplan 2 imaging cannot be used. However, users wishing to utilize the motorized and automatic functions of Axioplan 2 imaging must take this into account when choosing the basic stand configuration. You will already have made this decision before starting to read this manual. But we would nevertheless like to point out that the configuration for which you have opted must not necessarily be the definitive one. If you wish to change your basic configuration at a later date, the possibility most certainly exists. Our specialist staff will be pleased to provide you with any advice you may need.

The aim of this manual is to describe the many possible functions of Axioplan 2 imaging. However, the sheer number of functions offered may sometimes lead to a certain amount of confusion. Therefore, if you have any doubts concerning the capabilities of your Axioplan 2 imaging, please contact our subsidiary or ourselves direct. Our address and telephone number are given on the inside cover of this manual.

In the chapter entitled *Stand* you will find the various basic versions of the Axioplan 2 imaging stands. Starting with the manual version, this chapter deals with all stand functions available, up to and including the stand featuring all motorizable functions and the light manager.

The chapter *Microscope Components* describes the operating functions with which you yourself can equip or extend the Axioplan 2 imaging. Not all parts described there must be present on the Axioplan 2 imaging. However, if you are considering adding some of the components to your configuration, the corresponding catalog numbers are listed.

The chapter *Microscopy Techniques* provides you with information on the basic settings of the microscope, e.g. KÖHLER illumination adjustment, and contains instructions concerning the operation of the microscope for specific applications.

Abbreviations

achr.	achromatic
AL	incident light
apl.	aplanatic
Br	suitable for eyeglass wearers (eyeglasses can be placed directly against eyepieces)
C	camera
C-Mount	camera mount
D	diameter or coverglass thickness
D	darkfield
DIC	Differential interference contrast
DL	transmitted light
E-PI	flat-field eyepieces with aspheric correction
WD	working distance
Fl	fluorescence
foc	focusable diopter compensation on eyepiece
phot.	photographic
FT	chromatic beam splitter
HAL	halogen illuminator or lamp
HBO	mercury pressure short arc lamp
HD	bright/darkfield
H	brightfield
ICS	Infinity Colour-corrected System
Korr	correction mount
LD	long working distance
LFB	luminous field diaphragm
MC	microscope camera
MPM	microscope photometer
N.A.	numerical aperture
P	photometry
Ph 1, 2, 3	phase contrast; the numbers refer to the diameter of the ring stop used; with an objective with the designation Ph 2, you use the ring stop with the corresponding designation Ph 2.
Pol	polarization
SFZ	field of view number
SI	system integration
SLR	single lens reflex
Stemi	stereomicroscope
SW	wrench size
UD	universal rotary stage
Var	VAREL contrast
vis.	visual
W-PI	wide angle flatfield
XBO	xenon short arc lamp
ZBE	intermediate image plane

Purpose

The Axioplan 2 imaging has been designed as a universal microscope and can be used for all areas of light microscopy, provided it is configured and equipped appropriately. Depending on its equipment, it is used in the following fields:

- transmitted light microscopy
- incident light microscopy
- fluorescence microscopy
- photomicrography
- videomicroscopy.

The Axioplan 2 imaging also performs the contrasting methods in transmitted light and incident light:

- darkfield
- phase contrast
- polarization contrast
- differential interference contrast.

Motorizable functions and the recognition of coded microscope components used permits the performance of applications in such fields as process automation and telepathology. Here, the microscope is controlled by a software which activates the programmable CAN-BUS and RS 232 interfaces. Here, the microscope is controlled by a built-in microprocessor and a control software which activates the standard interfaces CAN-BUS and RS 232 C in personal computers.

Installation conditions

- Dust-free environment
- Maximum relative air humidity 85 %
- Vibration-free worktop

Specifications concerning power supply, storage temperature and other technical details are contained in the chapter entitled *Technical Data*.

Overview and Connections

The stand shown in the illustration is equipped with a wide variety of functional units. It may therefore differ from the stand you have purchased. Should you therefore see any components in the drawing which are not present on your own microscope, this does not mean that you have an incorrect Axioplan 2 imaging configuration. Setting and adjustment of certain components and modules of the Axioplan 2 imaging sometimes require special tools which are included in the delivery package of the microscope. This particularly concerns the SW 3 ball-headed screwdriver and the SW 1.5 screwdriver for Allen head screws.

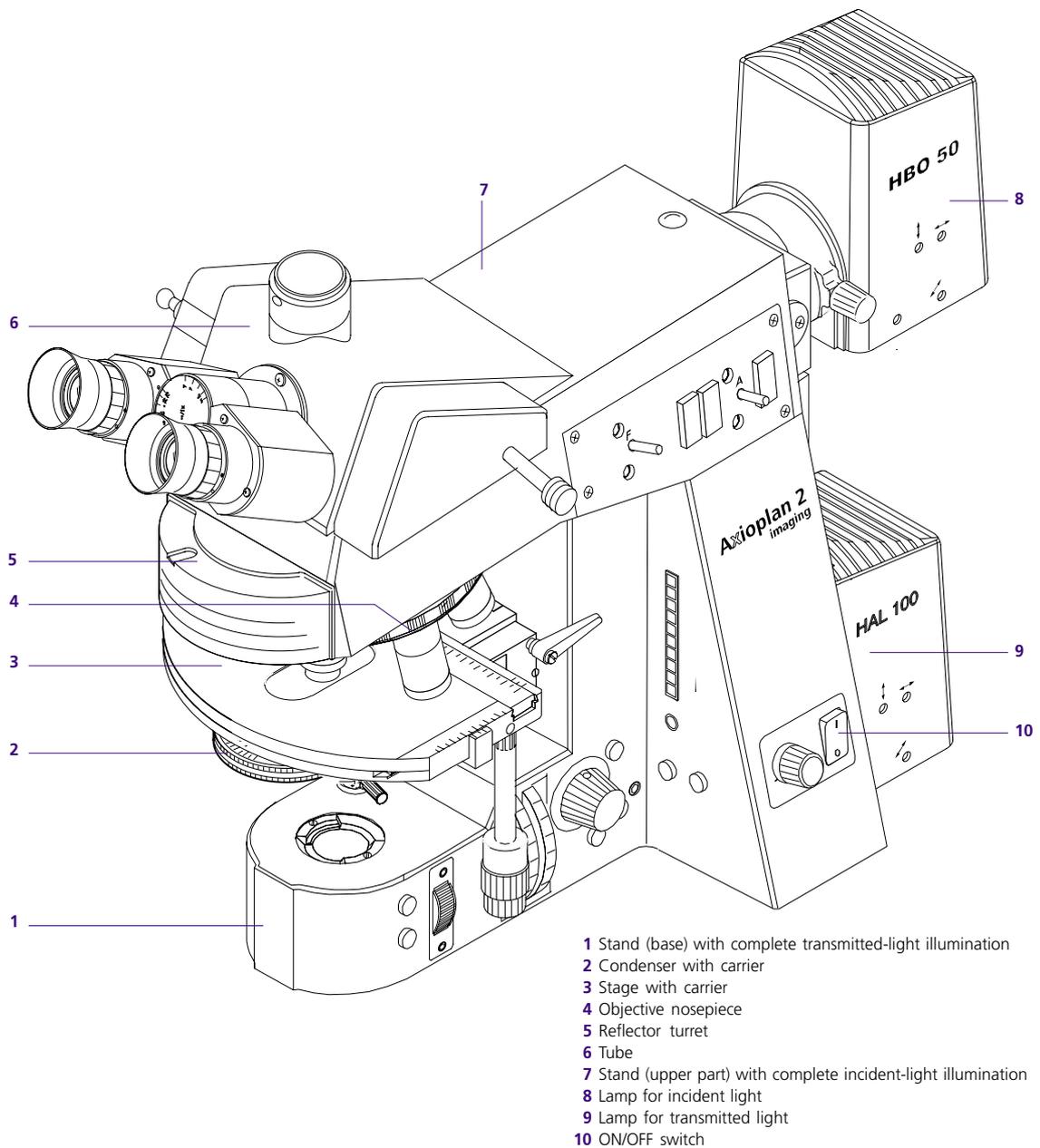


Fig. 1 Axioplan 2 imaging (motorized)

Putting into Operation

Unpacking and installing the microscope

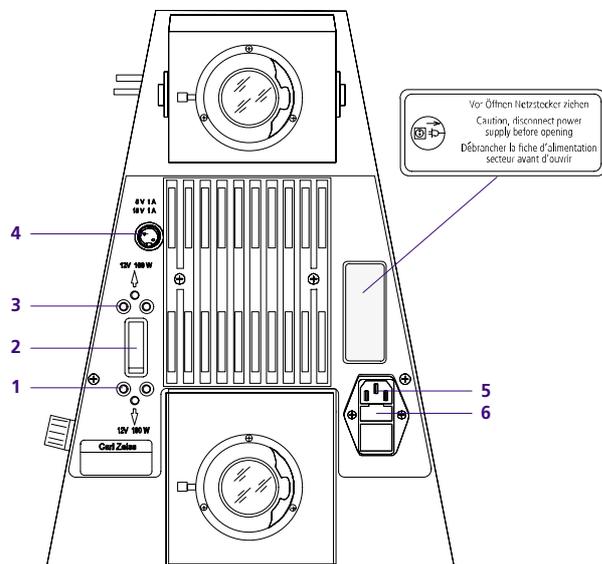
Normally, your microscope is supplied completely assembled in a specially designed container. The Axiophot 2 Photo module and the notebook required for its control, plus any required intermediate tubes, are packed separately. The transport containers include instructions for unpacking the instrument.

Please make sure that you observe the instructions contained therein.

Due to the complexity of the Axioplan 2 imaging and the necessity to ensure its perfect functioning, it will generally be installed and set up on site by our service personnel.

The Axioplan 2 imaging is handed over to you in a state which will enable the users to attach all the items listed in the *Microscope Components* chapter themselves. The procedures required are described.

Putting into Operation



- 1 Sockets of the integrated power unit for the transmitted-light illumination
- 2 Toggle switch to change between transmitted and incident light
- 3 Sockets of the integrated power unit for the incident-light illumination
- 4 Socket for Axiophot 2 Photo module
- 5 Socket for instrument plug (line voltage)
- 6 Compartment for instrument fuses

Fig. 2 Axioplan 2 imaging (instrument back)

Setting up the microscope

The Axioplan 2 imaging microscope features an integrated wide-range power unit and can be connected to line voltages ranging from 100...240 V AC, 50 ... 60 Hz. The wide-range power unit sets itself automatically to the appropriate line voltage.

- Check that all connected components have been mounted correctly and are sitting properly.
- Set ON/OFF switch (Fig. 1/10) to position **O**.
- Connect power unit first to the instrument and then to the line.
- Make the other electrical connections, such as microscope illuminators, connecting cable to TV camera, Axiophot 2, notebook, etc.

Note: All the electrical and electronic connections must be made before switching on the instrument so that they can be recognized by the initialization routines.

- Set ON/OFF switch to position **I**.

This last setting switches on the microscope and makes it ready to use after approx. 10 s. When the instrument is equipped with the light manager, this last position is set.

- Switch on notebook or PC (if connected).

Switching off the instrument

- Set ON/OFF switch to position **O**.

Stand

All the stand configurations described in the following are usually tailored to the customers special requirements. For example, it is possible that the stand configuration delivered to you includes only incident-light or fluorescence equipment and that transmitted-light components are therefore not available.

The same applies to the objective nosepiece, of which seven models are available.

The following stand types exist for the Axioplan 2 imaging:

- Manual type
This stand can only be operated manually. Motorized and codable functions cannot be easily retrofitted.
- Type E
Unlike the manual type, this stand is equipped with an electronic system permitting all the appropriately prepared microscope components (except the z-focusing drive) to be motorized. These functions can also be easily retrofitted.
- Motorfocus type (MOT)
Like type E, plus motorized focusing equipment

Aside from these basic types we also use the following abbreviations in this manual:

- LM/E type
Like type E, plus additional light manager
- LM/MOT type
Like Motorfocus type, plus additional light manager

Upgrading to include motorized functions is possible, but requires the stand type E or higher.

Should you intend to upgrade your microscope to include motorized functions, please get in touch with the Zeiss service department. Retrofitting is normally possible without difficulty.

The components of the upper stand part are available in the following versions:

- reflector turret: manual or motorized
- nosepiece: manual,
coded or motorized
- reflected-light shutter: motorized

The components of the lower stand part are available in the following versions:

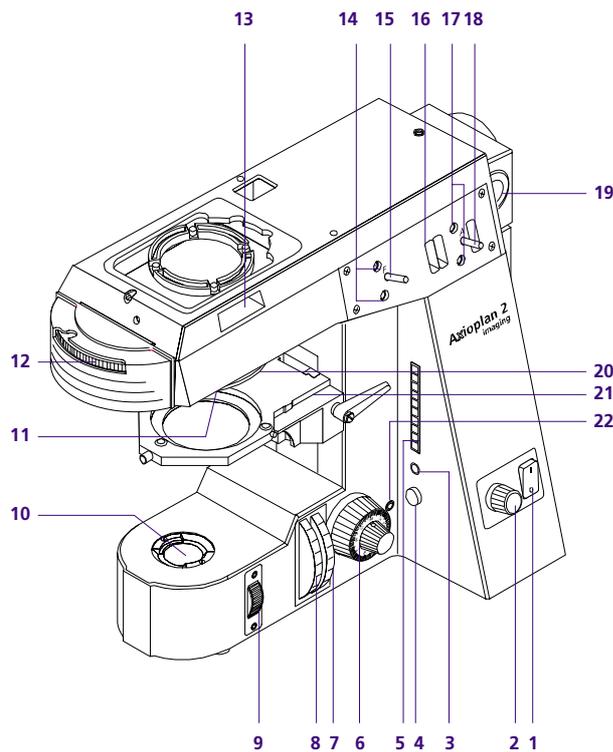
- condenser turret: manual or motorized
- luminous-field diaphragm: manual or motorized
- filter wheels: manual,
coded or motorized
- z-focusing drive: manual or motorized

Features and components common to reflected- and transmitted-light stand types:

- lamp connection
- light exit
- power units for HAL 100 microscope illuminator and electronic control system
- switches, adjusting and display components
- mechanical parts for stage focusing

The following description of stands starts with the manual model. On this basis, only the differences and additional functions are described for the other stand models.

Stand



- 1 ON/OFF switch
- 2 Light intensity control
- 3 On/Off display of color temperature key
- 4 Color temperature key
- 5 LED line lamp voltage
- 6 Focusing drive
- 7 Filter wheel 2
- 8 Filter wheel 1
- 9 Luminous-field diaphragm (transmitted light)
- 10 Light exit of transmitted-light equipment
- 11 Compartment for compensators
- 12 Reflector turret
- 13 Analyzer compartment
- 14 Centering screws for luminous-field diaphragm (incident light)
- 15 Pushrod for luminous-field diaphragm (incident light)
- 16 Slot for filter slider
- 17 Centering screws for aperture diaphragm (incident light)
- 18 Pushrod for aperture diaphragm (incident light)
- 19 Adjustment aid for lamp setting (incident light-option)
- 20 Objective nosepiece
- 21 Lowerable stage carrier
- 22 Swinging in/out of diffusion disk (transmitted light)

Fig. 3 Manual stand

Manual stand

The stand shown here may differ from your stand model. Most differences will be present in the upper part of the stand, i.e. they concern incident-light and polarizing microscopy. If the configuration illustrated is different from your own Axioplan 2 imaging, this does not necessarily mean that an error has been made, but is probably due to the stand equipment you have chosen for your respective application.

Controls

(The numbers refer to Fig. 3.)

1 ON/OFF switch

Position **O** = instrument switched off

Position **I** = instrument switched on

For optical status checking, the switch lights up in green in position I (for defects see *chapter Care, Maintenance*).

When the Axioplan 2 imaging is switched on, not only the HAL 100 microscope lamp is supplied, but also the Axiophot 2 Photo module, if mounted, and the motorized internal components.

2 Light intensity control

Knob to adjust the light intensity of connected 100 HAL microscope illuminators.

The power unit integrated in the stand is highly stabilized against voltage fluctuations and supplies adjustable d.c. voltage in the range from 3 to 12 V. A yellow LED line displays the set voltage range and the light intensity.

Note: If the light intensity cannot be adjusted, please check whether the color temperature key (4) is switched off.

3 On/Off display of color temperature key

Lights up when the color temperature of 3200 K has been switched on.

4 Color temperature key

Knob to set the 3200 K color temperature for photomicrography using color films (artificial light). When switched on, a green LED lights above the knob.

A constant color temperature of 3200 K is required for color photography. This is achieved if a fixed d.c. voltage of 10.5 V is supplied to the HAL 100 microscope illuminator. Correct functioning can not be guaranteed if other lamps than the 12 V/100 W lamps supplied by Carl Zeiss are used.

Stand

7, 8 Filter wheels for transmitted light

Two rotatable filter wheels (filter magazine) with 4 positions each are equipped with different filters; two different models are available. The wheels, which feature click stops, are rotated into position. The set filters are marked on the wheels.

Filter magazine D 452155-0000-000

Filter magazine for general use.

The positions of the two wheels can be combined as required.

Filter wheel 2 (7) 100 open position
25 neutral-density filter 0.25
6 neutral-density filter 0.06
1.5 neutral-density filter 0.015

Filter wheel 1 (8) 100 open position
6 neutral-density filter 0.06
G wide-band interference filter
green
CB conversion filter 3200 K ... 5500 K

Filter magazine D PHOTO 452157-0000-000

Filter magazine for the brightness control at a constant color temperature of 3200 K. The 3200 K color temperature must be selected. Green LED above the key lights constantly. The positions of the two filter wheels can be combined as required.

A high current and a correspondingly bright lamp are needed to achieve the color temperature of 3200 K. Filter wheel 2 can be used to reduce the brightness of the lamp to a "normal degree" more beneficial to the eye.

The following brightness steps can be set:

Filter wheel 2 (7) coarse steps

100 maximum brightness at
3200 K 100 %
6 normal brightness at 3200 K 6 %
0.4 low brightness at 3200 K 0.4 %
0 transmitted light blocked

Filter wheel 1 (8) fine steps

100 brightness 100 % of coarse step
50 brightness 50 % of coarse step
25 brightness 25 % of coarse step
12 brightness 12 % of coarse step

Conversion or green filters are additionally inserted into the filter mount or laid onto the light exit of the transmitted-light illumination.

9 Luminous-field diaphragm

Wheel for the continuous setting of the aperture of the luminous-field diaphragm (transmitted light).

10 Light exit of transmitted-light equipment

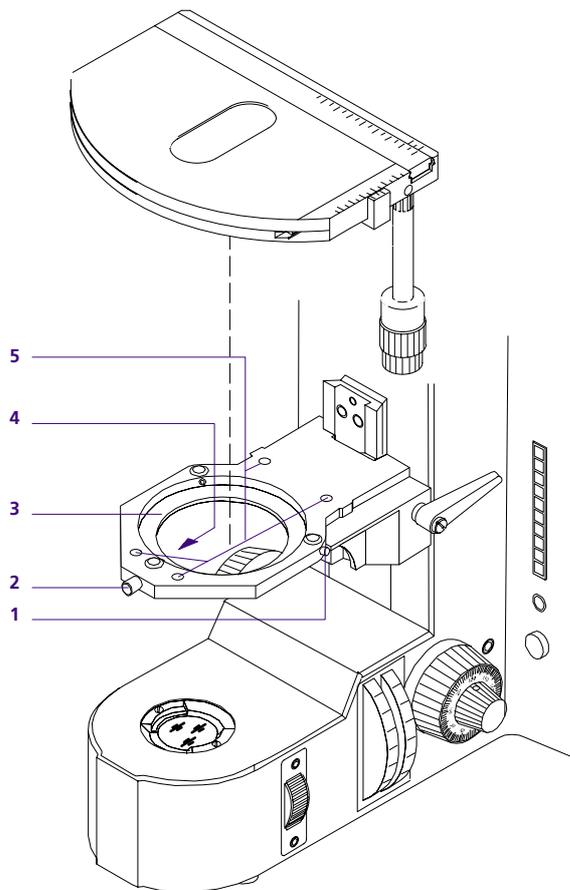
Light filters with dia. 32 mm can be placed on the plane surface of the centering ring supplied.

Note: Additional filters can be inserted in the color glass carrier of the condenser mount.

14 Centering screws

To center the luminous-field diaphragms (incident light) using SW 3 screws.

Stand



- 1 Stage centering screw for stage centration (on both sides)
- 2 Screw-on cap for spring pin of stage clamp
- 3 Angular guidance for attachment of dovetail for specimen stages
- 4 Spring pin of stage clamp
- 5 Drilled holes to screw on fixed stages and scanning stages

Fig. 4 Stage mounting

15 Pushrod

For the continuous diameter setting of the luminous-field diaphragm (incident light)
Pushed in = open diaphragm
Pulled out = closed diaphragm.

6 Manual focusing drive

The universal microscope Axioplan 2 imaging is focused via coaxial drives on both sides of the stand.

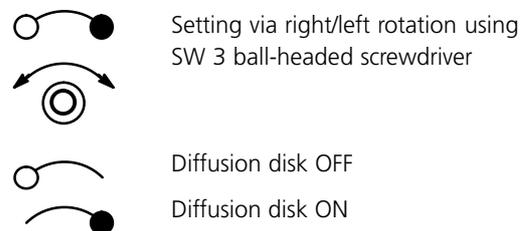
Coarse drive (large knob on the inside):

1 revolution = approx. 2 mm, 1 increment = approx. 2 μ m.

Fine drive (small knob at the outside):

Gear ratio 1:10 (1 revolution approx. 0.2 mm)

22 Diffusion disk (transmitted light)



The diffusion disk improves the homogeneous illumination of the object plane. Normally, it is swung into position. It can be swung out using the SW 3 ball-headed screwdriver to increase the visibility of the lamp filaments during adjustment of the microscope illuminator.

21 Stage carrier

The stage carrier is used to mount the stage and the condenser carrier to which the condenser is attached. The stage carrier is either fixed to the stand or removable. The non-rotating mechanical stage and the scanning stage are fixed to the carrier using screws.

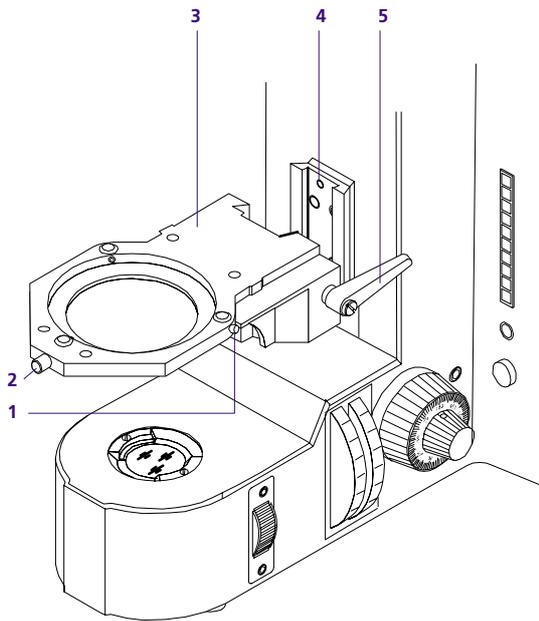
Attaching/removing rotary mechanical stages

(Fig. 4)

- Loosen screw-on cap (2) (3 ... 4 mm).
- Attach notch of stage dovetail to the spring pin (4) at the front.
- Push stage against spring pin and lower into the stage carrier in the back, then let go.
- Tighten screw-on cap (2).

The stage can now be rotated to the right and left.

Stand



- 1 Stage centering screw for stage centration (on both sides)
- 2 Screw-on cap for spring pin of stage clamp
- 3 Stage carrier
- 4 Dovetail guiding device
- 5 Clamping lever

Fig. 5 Height adjustment of the stage carrier

Stage centering

All stages are factory-precentered, i.e. when the stage is rotated, a set specimen feature will remain in the image center. Should a set feature move away from the image center when the stage is rotated, the stage must be centered again.

- Loosen stage clamping screw (Fig. 4/6).
- Correct the drift of the image part by simultaneously rotating the two stage centering screws (1).
- When the stage is centered, tighten the screw-on cap.

Note: When highly magnifying objectives are used the centration is only exact for one chosen objective.

Height adjustment of the removable stage carrier

(Fig. 5)

The height of the removable stage carrier is adjustable, which is very useful, for example, in the case of very high specimens (max. specimen height = 49 mm).

- Hold stage carrier (3) with your left hand. Loosen clamping lever (5).
- Change height of stage carrier, tighten clamping lever (5). Do not press too hard.

Note: To remove the stage carrier, loosen the clamping lever and take the carrier out of the dovetail guiding device (2) by moving it to the left. Orientation of the clamping lever can be selected as required by pressing against the spring and rotation in to the required position.

Condenser carrier (Fig. 6)

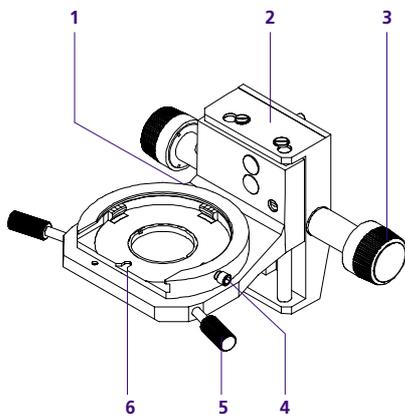
The condenser carrier is screwed to the stage carrier. All the condensers available for the Axioplan 2 imaging are attached to the condenser carrier. The height of the carrier can be adjusted on both sides and permits the centering of the inserted condensers.

Note: For the setting of KÖHLER illumination, the image of the luminous-field diaphragm must be centered in the field of view.

Setting the height stop (Required KÖHLER illumination setting must have been performed):

- Loosen clamping screw (1) for height stop using SW 3.
- Set the specimen.
- Image the luminous-field diaphragm sharply (by adjusting the height of the condenser).
- Move up the condenser by approx. half a rotation of the control.
- Tighten clamping screw for height stop (1).

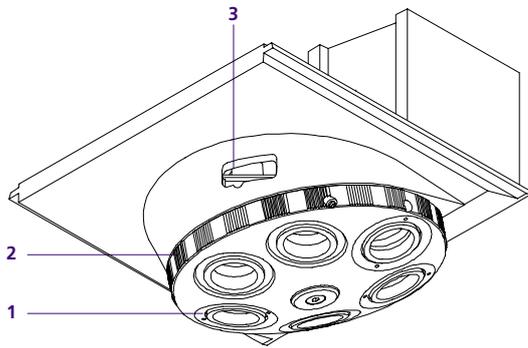
Note: Depending on which stage carrier is mounted (fixed or removable), the appropriate special condenser carrier is screwed on. Its difference mainly lies in its height adjustment, and is of interest only for incident-light microscopy and where high specimens in the range of up to 49 mm are used. If you intend to change the condenser carrier, please get in touch with our service department.



- 1 Clamping screw for height stop, prevents the specimen from being pressed out inadvertently from below by the condenser
- 2 Carrier guidance
- 3 Height adjustment controls on both sides
- 4 Clamping screw to fix the condenser
- 5 Centering screws on both sides
- 6 Orientation groove for the condenser

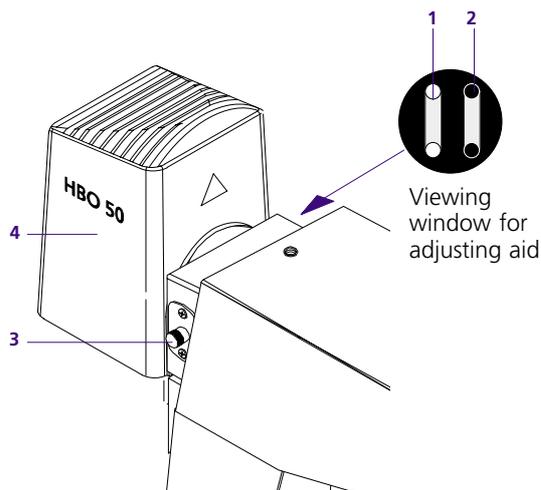
Fig. 6 Condenser carrier

Stand



- 1 Thread (normally W0.8", M27 for incident light darkfield) to screw in the objectives
- 2 Knurled ring for nosepiece rotation
- 3 Compartment for compensators

Fig. 7 Objective nosepiece



- 1 Image of the light arc
- 2 Mirror image of the light arc of the lamp reflector
- 3 Slide-in knob for adjusting aid activation
- 4 HBO 50 lamp housing

Fig. 8 Adjusting aid

20 Objective nosepiece

The objective nosepiece is used for mounting the objectives and changing them quickly. Depending on the application, the customer can choose from nine different nosepieces.

Note: Nosepiece positions which are not being used must be covered with dust caps.

The nosepieces for polarization is equipped with centering screws (SW 1.5) for the centration of the objectives.

Nosepieces for DIC feature one compartment for insertion of a DIC slider in each objective mount.

Note: Please do not try to remove the nosepiece from the stand, since this will result in the loss of the centration; this can only be reset by our service staff.

11 Compartment for the insertion of auxiliary objects and compensators

Fig. 7/3 and Fig. 3/11
(→ *Microscope Components*)

12 Reflector turret

The reflector turret (Fig. 3/12) consists of a filter wheel with 5 click stops to which the required reflector modules are attached. Attachment and change of modules can be performed by the user (→ *Microscope Components, Reflector Modules*).

13 Analyzer Compartment

Fig. 3/13
(→ *Microscope Components*)

14 Adjusting aid for HBO/XBO incident microscope illuminators

(Fig. 8)

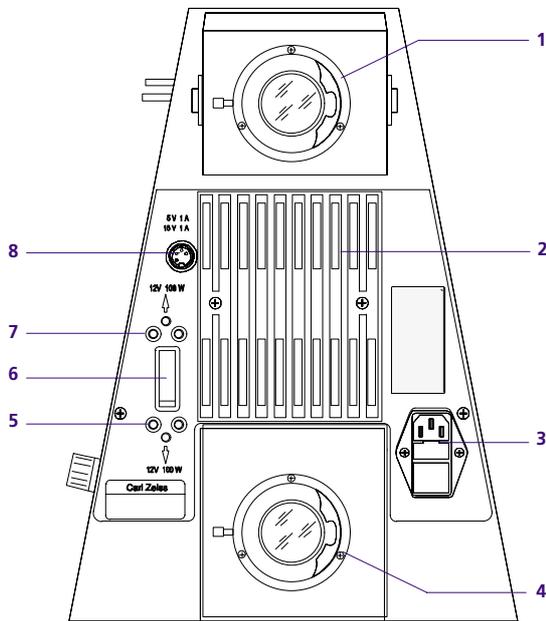
The adjusting aid simplifies optimum setting of the HBO 50/100 and XBO 75 lamps in fluorescence microscopy. A mirror, directs the image of the light arc to a round window visible from the outside (matt black filter). Here, the position change of the focal point and its mirror image, performed by adjusting the screws on the microscope illuminator, can be viewed.

The lamp is set optimally if the image and the reflected image of the light arc are shown in the window centrally and in focus.

Stand

Back of manual stand

Key to Fig. 9 (back of manual stand)



- 1 Connecting tube for microscope lamps-incident light with locking screw for dovetails to fix the lamps
- 2 Ventilation slots (always keep clear; minimum distance: 15 cm)
- 3 Sockets for instrument plug with integrated compartment for instrument fuses (change of fuses → chapter *Care, Maintenance*)
- 4 Connecting tube for microscope lamps-transmitted light, with locking screw for dovetails to fix the lamps
- 5 Sockets of the integrated power unit for HAL 100 / transmitted light microscope lamps
- 6 Toggle switch to change between incident and transmitted light.
The short delay after you push the switch helps prevent you from hurting your eyes in the unexpected bright light.
- 7 Sockets of the integrated power unit for HAL 100 incident light microscope lamps
- 8 Socket for Axiophot 2 Photo module

- 1 Connecting tube for microscope lamps-incident light
- 2 Ventilation slots
- 3 Socket for instrument plug
- 4 Connecting tube for microscope lamps-transmitted light
- 5 Sockets for microscope lamps-transmitted light
- 6 Toggle switch transmitted light/incident light
- 7 Sockets for microscope lamps-incident light
- 8 Socket for Axiophot 2 Photo module

Fig. 9 Back of manual stand, also see Fig. 2

Stand E/Stand mot

Specialities

- Integrated electronic system in the stand base
- Motor focusing (only MOT stand)

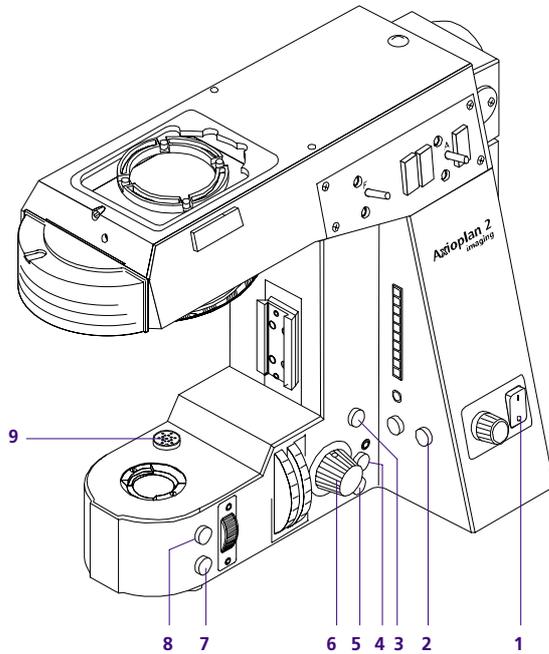
Manual operation of these stands differs only slightly from the operation of the manual stand. The following therefore describes only those stand functions which the instructions for the operation of the manual stand do not cover.

The stand described here is equipped with all the available motorized and coded functions and may therefore differ from your stand configuration.

Note: Microscope Software

Some of the motorized functions of the stands can only (or also) be operated from the PC via the software. For a description of this function, please see → the chapter *Microscope Software*.

The following numbers refer to Fig. 10 (motorized stand).



- 1 ON/OFF switch
- 2 Shutter switch incident light
- 3 Gear switchover to motor focusing
- 4 Turning to the right of the connected motorized components
- 5 Turning to the left of the connected motorized components
- 6 Coarse/fine drive of focusing
- 7 Rapid stage lowering CHANGE (on both sides of the stand)
- 8 Rapid stage lowering VIEW (on both sides of the stand)
- 9 Socket for connection of motorized condensers

Fig. 10 Motorized stand

1 ON/OFF switch

- O** Instrument switched off
- I** Instrument switched on

For checking the operating status, the switch lights up green in position I (for malfunctions see → chapter *Care, Maintenance*).

When the Axioplan 2 imaging is switched on, not only the HAL 100 microscope lamp is supplied with power, but also the Axiophot 2 Photo module, (if it is attached), and the motorized and coded components.

Note: Motorized focusing and all the other motorized or codable functions are only available when the instrument is switched on.

Stand

2 Shutter switch incident light

In incident-light microscopy or when using an externally supplied gas discharge lamp, it may be necessary to block the light path without switching off the lamp. A motorized shutter is used for this, which interrupts the light path by swinging in a diaphragm.

Normally, the shutter is opened and the lamp for transmitted light therefore switched off.

A short press of switch (2) blocks the incident light and the transmitted-light illuminator is switched on.

If you want to combine transmitted-light fluorescence and epi-fluorescence, press switch (2 - shutter) for more than 2 s. The incident-light or transmitted-light beam path can then be used. Pressing switch (2) again returns to the change mode between transmitted and reflected light.

Note: If you see no light in the transmitted or incident light technique, check by pressing switch (2) whether the shutter is blocking the light path.

3 Gear switchover to motor focusing

Pressing the switch changes from coarse to fine drive, and vice versa (Fig. 10/3).

6 Motorized focusing drive

Focusing is manual by means of a control knob (on both sides) that acts on an electronic encoder.

Difference: To change between coarse and fine drive, the gear switchover (Fig. 10/3) must be operated.

4, 5 Turning to the left or right of the connected motorized components (Fig. 10)

Using the keys (4) and (5) attached to the right and left of the stand you can operate two of the following motorized components:

1. Condenser turret
2. Reflector turret
3. Nosepiece

Key (4) turns the appropriate control element of the component to the right, key (5) to the left.

Each time the microscope is switched on, the existence of motorized components is "enquired" and recorded. The switching function for the first two components available is then assigned to keys (4) and (5) in the above sequence.

The first component found is always assigned to the keys on the right of the stand. For example, if your instrument is equipped with the motorized components 1 to 4, keys (4) and (5) on the right will switch the condenser turret and the keys on the left switch the reflector turret.

7, 8 Rapid stage lowering CHANGE/VIEW

Knobs on both sides of the stand for rapid stage lowering and moving it up again in the previous position.

Stage lowering

- Press CHANGE knob (7).
Stage will be lowered. The current focus setting is stored. When the stage is lowered, motorized focusing by focusing drive is switched off.

Moving stage up

- Press VIEW knob (8).
Stage moves up and the stored focus position is precisely reset.
The user-friendly design of this function enables you to operate the CHANGE/VIEW keys with either your right or your left hand.



CAUTION!

Risk of injury and instrument damage

Please do not insert your hand or any objects between the stage and the objective when the stage is moved upwards. The same applies to the downward motion: in that case, the space between condenser and stand base is reduced.

9 Socket for motorized condensers

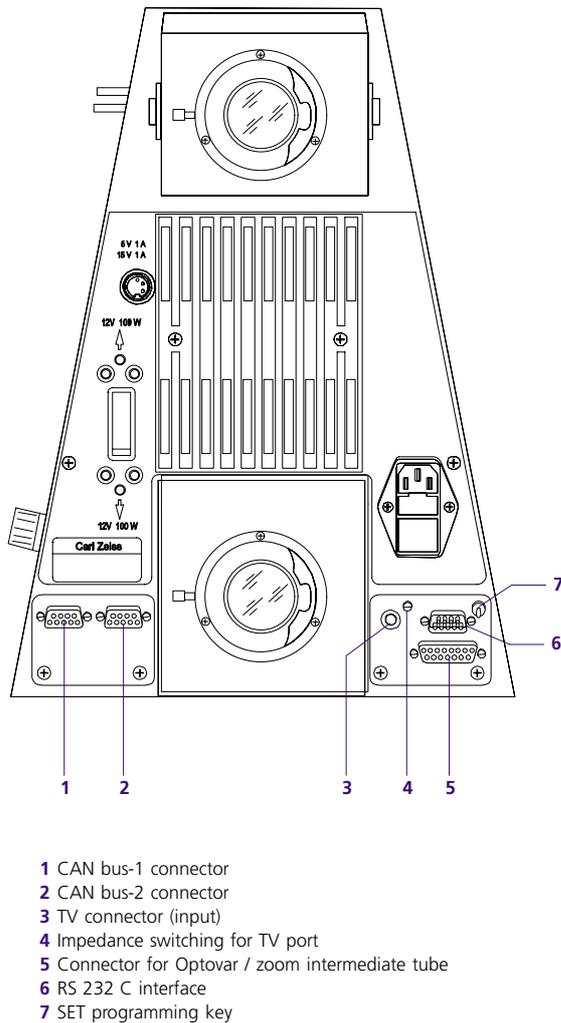
Both the voltage supply and the control of motorized condensers is made via this socket.

- Switch off Axioplan 2 imaging
- Attach condenser to condenser carrier
- Connect plug of the condenser cable with socket (9) on stand base.

Note: When the Axioplan 2 imaging is switched on, instrument initialization is performed to allow recognition of the connected modules. This means that the system cannot recognize any modules which are connected **after** the Axioplan 2 imaging has been switched on.

If the condenser is to be activated via the Microscope control, it must be activated via the Setup program (→ *microscope control software*).

Stand



- 1 CAN bus-1 connector
- 2 CAN bus-2 connector
- 3 TV connector (input)
- 4 Impedance switching for TV port
- 5 Connector for Optovar / zoom intermediate tube
- 6 RS 232 C interface
- 7 SET programming key

Fig. 11 Back of motorized stand

Back of stand E/MOT

1 CAN bus-1 connector

For connection of the Axiophot 2 photo module.

2 CAN bus-2 connector

These interfaces permit activation of the Axioplan 2 imaging via a CAN bus. Normally, the signals from a PC - which must be equipped with an appropriate driver board (CAN bus interface) - are transferred to the Axioplan 2 imaging and read off from there. The second connector also allows cascading. This enables several series-switched instruments to be activated by a PC.

The control commands for programming languages under WINDOWS™ are provided by DLLs (Dynamic Link Libraries).

For details, please see → the chapter *Interface Description* or get in touch with our service department.

5 Connector for Optovar/zoom intermediate tube

The intermediate tube (coded or motorized) installed is connected to the microscope via this connector using the cable supplied.

Stand

6 RS 232 C interface

The intermediate tube (coded or motorized) installed is connected to the microscope via this connector using the cable supplied.

7 Programming key (SET)

This key only functions in conjunction with the light manager described later.

- A short press of the SET programming key (< 3 s) stores the current settings of the following microscope parts, provided they are adjustable by motor or electronically readable:
 - aperture diaphragm for transmitted light
 - luminous field diaphragm for transmitted light
 - incident-light aperture diaphragm
 - filter wheels
 - condenser front optics
(only with use of achromatic motorized dual condenser 0.5, 445341-0000-000, the so-called pathology condenser mot.)

One separate storage is possible for each position of the nose-piece.

The stored settings are automatically activated when the respective positions of the nosepiece are adopted.

The stored values are retained after you switch off the instrument.

If you have changed the settings of the current position and now wish to call the stored settings, all you have to do is turn the nosepiece further and then return it to the previous position.

Note: Optovar and zoom intermediate tube

If you have inserted an Optovar intermediate tube on your Axioplan 2 imaging, its Light Managers settings (field stop and filter wheels) are also stored depending on the position of the motorized objective nosepiece.

The microscope software can be used to store several settings for each mount of the nosepiece. The settings are stored by the program in parameter files and are called with these again.

The microscope software can be used to store several settings for each mount of the nosepiece. The settings are stored by the program in parameter files and are called with these again.

Coded microscope parts

Various modules of the Axioplan 2 imaging and some additional microscope components can be coded.

Codings is beneficial when the microscope software is used. The software can recognize coded parts of the microscope and display their position or status, even if the parts involved are not motorized. For example, filter wheels or intermediate tubes can be coded without being motorized. In this case, the software would record and display a manual change of the filter or optovar/zoom position. However, the microscope software must be appropriately configured if it is to recognize the coded positions.

The configuration is performed with the aid of a setup program delivered with the instrument. Normally, the setup program has already been implemented in the factory prior to delivery of Axioplan 2 imaging.

Note: Equipping the nosepiece

The microscope software must be started via the PC before the nosepiece is fitted with objectives (normally, the nosepiece is fitted with objectives in the factory as ordered). Fit the nosepiece mounts with the objectives displayed for these mounts by the program. If the objective to be used is not displayed by the program, the setup program must be called and this objective input for a mount on the nosepiece.

Always check whether the objective position and the appropriate display correspond in the program. The same applies for the positions of the reflector turret.

Stand

Light manager

Light manager means the microscope's ability to adjust the KÖHLER illumination setting automatically. At the same time the image illumination is adjusted at a constant color temperature.

Configured settings are stored and are reproduced automatically whenever you change the position of the objective nosepiece.

It is not necessary to connect the microscope to a computer to perform this function.

Essential requirements for the light manager, however, are the motorized or coded nosepiece and the possibility of motorized adjustment for the following components:

- luminous field diaphragm for transmitted light
- aperture diaphragm for transmitted light
- filter wheels
- condenser front optics
(only with use of achromatic motorized dual condenser 0.5, 445341-0000-000)
- aperture diaphragm for incident light (only for incident-light techniques)

The KÖHLER illumination setting procedure should already have been performed prior to the initial use of your Axioplan 2 imaging (→ page 74). You should also make an occasional check to ensure that this setting has not changed.

The factory-programmed setting of the light manager guarantees a practical starting situation, from which you can make your own settings and then store them with the SET key.

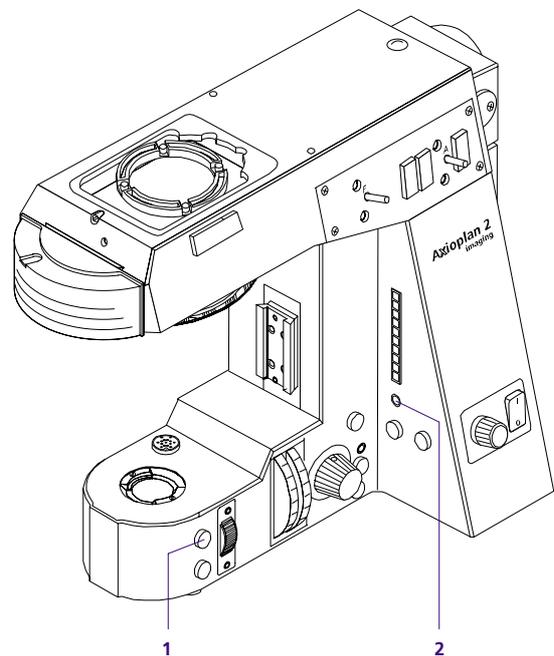
- Give SET key (in Fig. 11/7) a short press to store the setting.

Automatic balancing of focusing speed and focus position (parfocality) of different objectives

The lower part of the MOT stand is equipped with a Harmonic Drive™ of high precision which converts the hand movement in the focusing process into different sensitive vertical stage movements (proportionally to the objective's magnification). Motor focusing also compensates minor focus differences which always occur between the objectives. First, however, the following learning process needs to be activated:

- Focus dry objective exactly with the highest magnification/aperture.

- Press and hold (> 3 s) both upper switches for fast stage lowering (Fig. 12/1 left and right) at the same time until the orange LED display (Fig. 12/2) lights up briefly. This activates the so-called "learning mode" with the dry objective mentioned above as reference system.
- If you want to adjust the focusing speed in the fine drive, press both left switches for fast lowering. The upper switch causes a coarse focus adjustment, while the lower switch causes a fine focus adjustment. Holding the switch slowly changes the focusing speed in the fine drive. You can now choose among approximately 40 different focusing speed parameters.
- Focus again exactly on the specimen and press briefly the upper right switch. This stores focusing speed and focus position for the reference objective currently in use.
- Now switch to the objective with the next lower magnification/aperture and adjust focusing speed and focus position accordingly. You may store the parameters for this objective by pressing briefly the upper right switch for fast lowering.
- Proceed with the other objectives accordingly. Oil immersion objectives should be adjusted last.
- Finally press and hold (> 3 s) the upper right switch for fast lowering until the orange LED display lights up briefly. This switches the "learn mode" off and you may now check the objectives' parfocality visually.



1 Fast stage lowering VIEW
2 LED

Fig. 12 Motorized stand

Microscope control software AxioVision Control

What does AxioVision Control offer?

AxioVision Control permits the remote control of the following Carl Zeiss microscopes from your PC:

- Axioplan 2 imaging / Axiophot 2
- Axiovert 100 M
- Axioskop 2 MOT

Remote control with AxioVision Control provides the following benefits:

- convenient remote control function with visual navigation aid
- flexible storage of microscope settings and activation via function keys
- AxioMouse permits microscope operation via PC-mouse
- convenient photomicrography with Axiophot 2 and MC 200
- management of different user profiles

System requirements

Recommended hardware and software:

- Pentium II processor 300 MHz
- 128 MB RAM
- 4 GB hard disk
- video adapter 1024 x 768 x 24 bits, 4 MB for true color
- CD-ROM drive
- 17" monitor
- Windows 98 / Windows NT 4.0 Service Pack 3

Handling

Minimum hardware and software requirements:

- Pentium II processor 233 MHz
- 32 MB RAM
- 4 GB hard disk
- video adapter 1024 x 768 x 24 bits, 4 MB for true color
- CD-ROM drive
- 17" monitor
- Windows 95

Note: Detailed information on the microscope control software is contained in the AxioVision Control manual B 40-623 (Cat. No. 000000-1068-563)

Microscope control software AxioVision Control

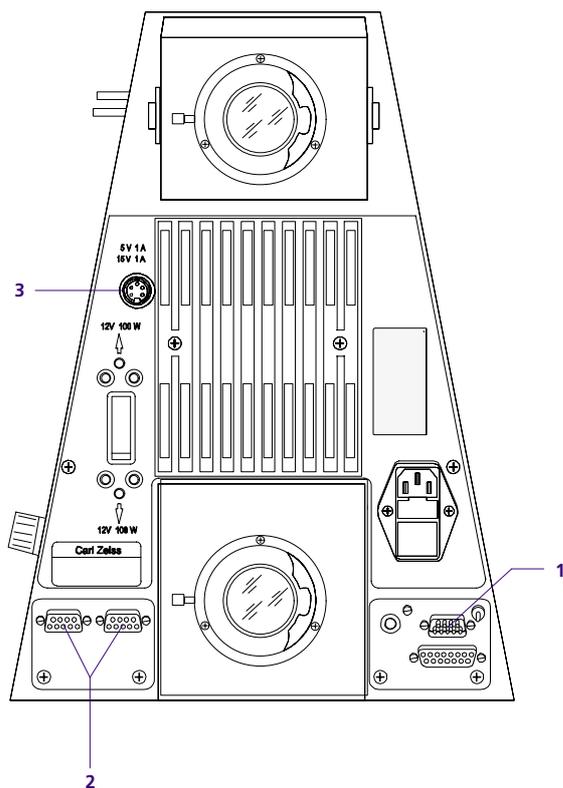


Fig. 13 Motorized stand (back)

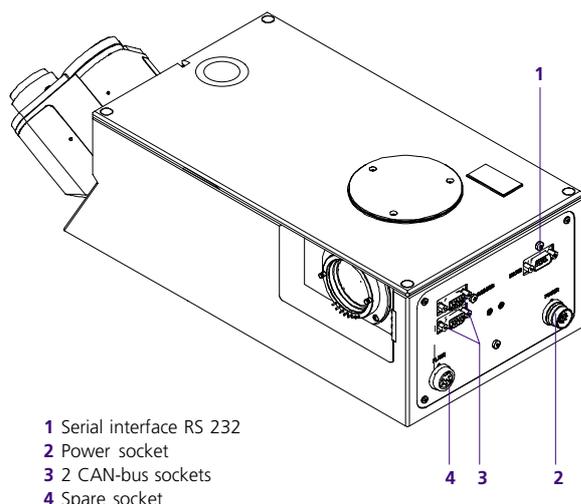


Fig. 14 Electrical connections of the Axiophot 2

Electrical connections

Microscope

The coupling of the Axioplan 2 with the PC or the notebook is performed via the RS 232 C serial interface (Fig. 13/1) using a connecting cable.

Axiophot 2 photo module

The electrical connections are on the back of the Axiophot 2 photo module:

- CAN-bus socket for cable connection to the stand and other microscope components (Fig. 14/3)
- RS 232 serial interface for the connection of a PC or notebook (Fig. 14/1)
- Spare socket (Fig. 14/4)
- Power socket (Fig. 14/2)

Connection to the manual stand

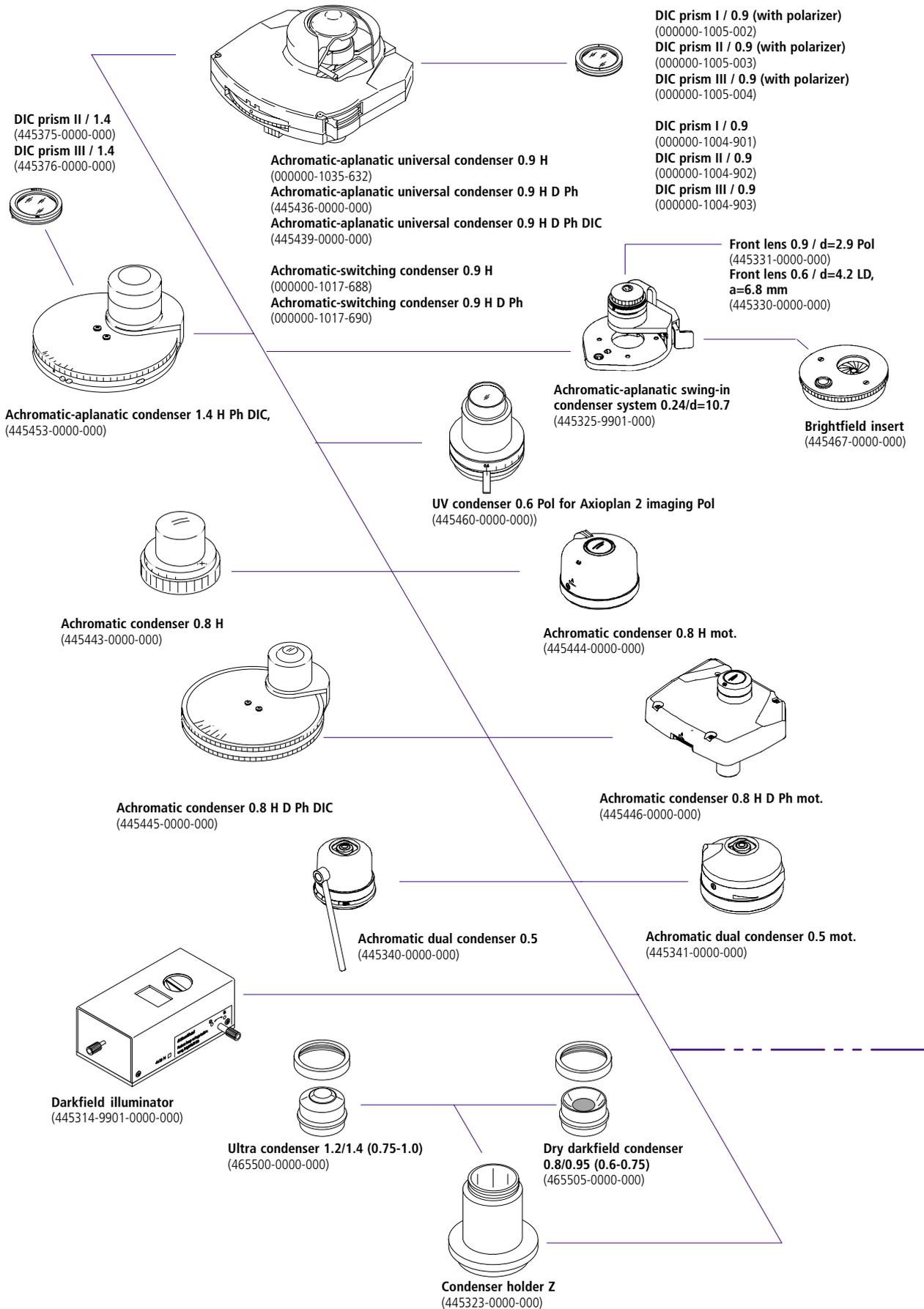
- Connect the supplied RS 232 C connection cable to the serial interface of the photo module (Fig. 14/1) and to the serial interface of your notebook/PC.
- Connect the internal voltage supply cable supplied to the POWER socket (Fig. 14/2) of the photo module and to socket (Fig. 13/3) at the back of the stand.
- Tighten all the safety screws on the plugs.

Connection to the stand E/MOT

- Use the RS 232 C cable supplied to connect the serial interface of the photo module (Fig. 14/1) or the stand (Fig. 13/1) to the serial interface of your notebook/PC.
- Connect the internal voltage supply cable supplied to the POWER socket (Fig. 14/2) of the photo module and to socket (Fig. 13/3) at the back of the stand.
- Connect photo module and stand by connecting the CAN bus cable to the appropriate CAN bus sockets (Fig. 14/3 and Fig. 13/2).
- Tighten all the safety screws on the plugs.

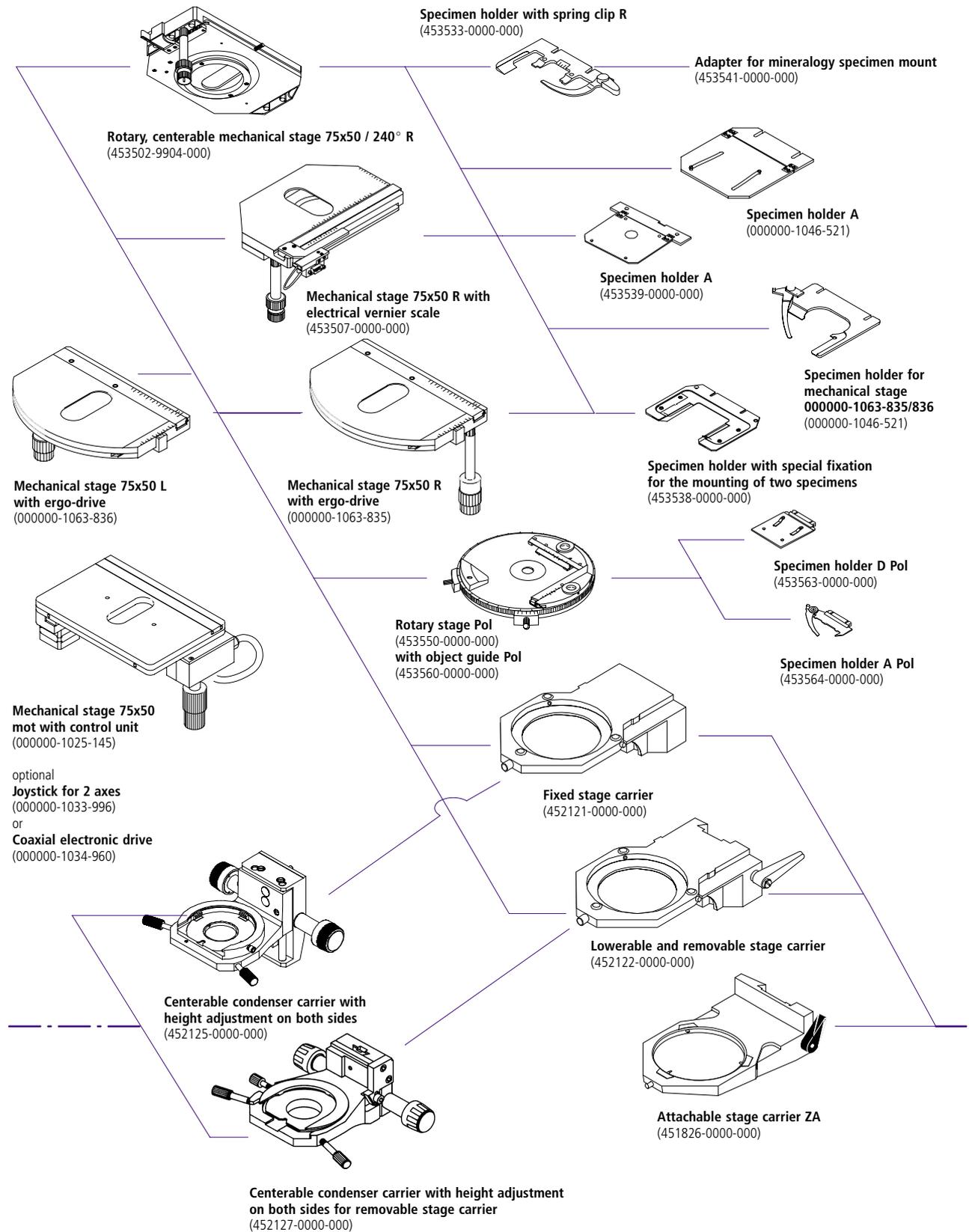
System Overview

Condensers



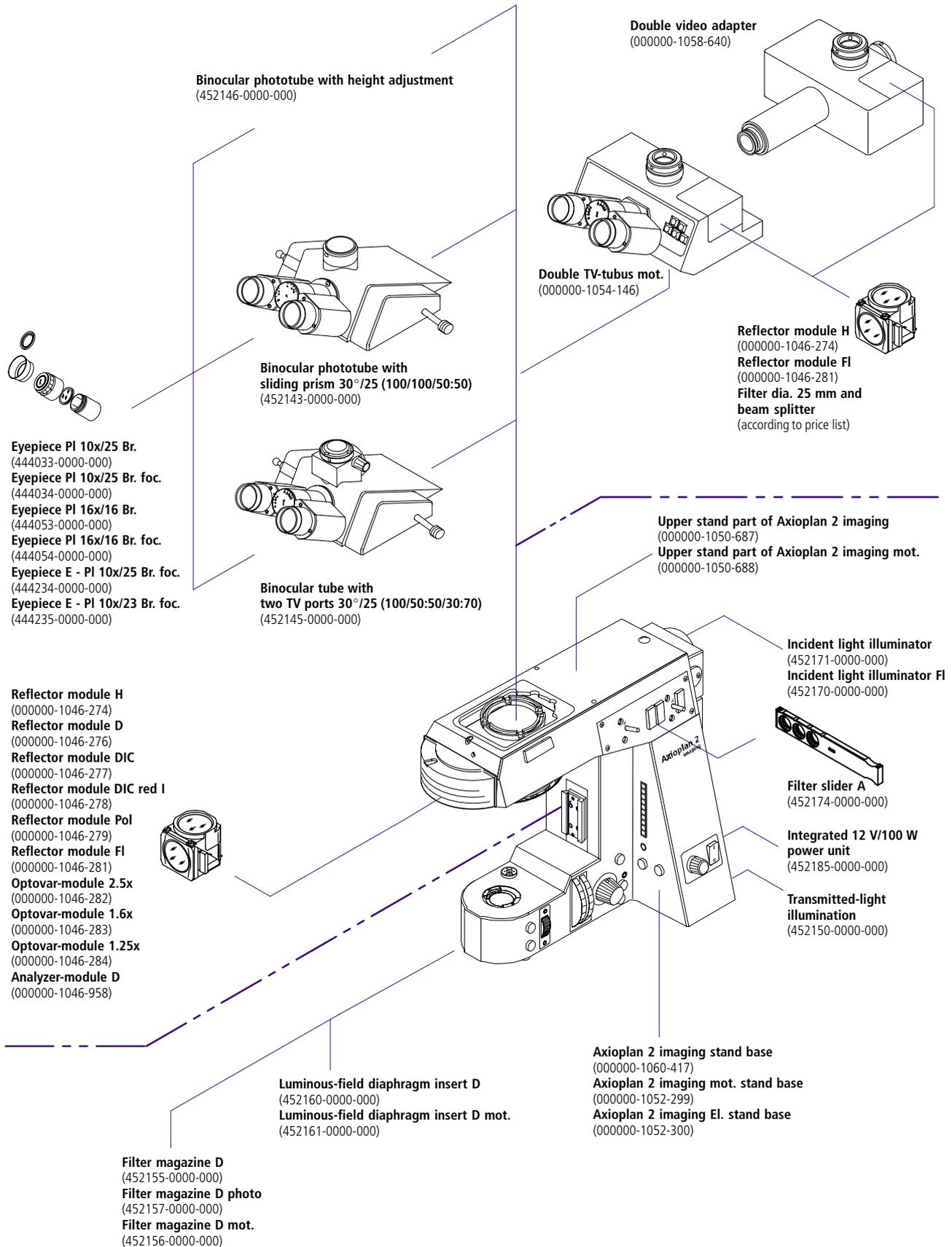
System Overview

Stage carriers, Stages, Condenser carriers



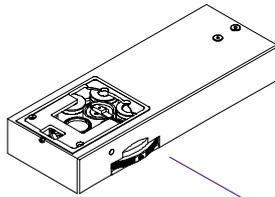
System Overview

Stand, Tubes, Eyepieces, Filters, Reflectors

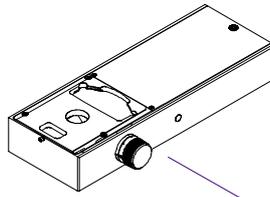


System Overview

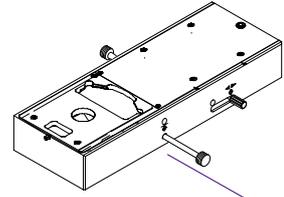
Intermediate tubes, Nosepieces, Compensators



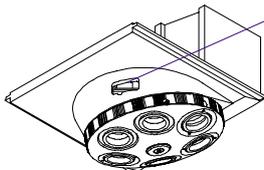
Optovar intermediate tube
1.0x/1.25x/1.6x/2.0x/2.5x cod.
(452175-0000-000)



Intermediate zoom tube 1.0x ... 2,5x cod.
(452180-0000-000)



Intermediate tube Pol
(with quartz depolarizer)
(452184-0000-000)



- 7x nosepiece H W 0.8
(452130-0000-000)
- 6x nosepiece H DIC W 0.8
(452131-0000-000)
- 6x nosepiece H D DIC M27 cod.
(452136-0000-000)
- 7x nosepiece H W 0.8 mot.
(452137-0000-000)
- 6x nosepiece H D DIC M27 mot.
(452138-0000-000)
- 6x nosepiece H DIC W 0.8 mot.
(000000-1067-351)
- 6x nosepiece Pol W 0.8 cod.
(452139-0000-000)



DIC sliders
entire line according to price list



Objectives
entire line of ICS objectives
according to price list



Mirau interference equipment for LD 20
for objects with thread W 0.8
(444942-0000-000)



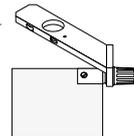
Antiflex cap for Epiplan-Neofluar 2.5 HD
(444920-0000-000)

Antiflex cap for Epiplan-Neofluar 1,25x
(444921-0000-000)

Antiflex cap for Epiplan-Neofluar 2.5x
(444922-0000-000)

Intermediate ring M27 on W 0.8 H = 0.94
(444911-0000-000)

Intermediate ring H "O" M 27 on W 0.8
(444910-0000-000)



Fluorescence protection screen
(452163-0000-000)



Compensator lambda 6x20
(473704-0000-000)



Compensator lambda / 4 6x20
(473714-0000-000)

Wedge compensator 0 - 4 lambda 6x20
(473724-9902-000)

Sénarmont compensator 546:4, 6x20
(473718-0000-000)

Brace-Koehler rotary compensator $\lambda/8$
(453716-0000-000)

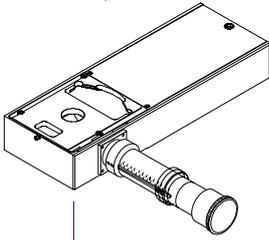
Ehringhaus tilting compensator 0 - 6 λ
(453720-0000-000)

Ehringhaus tilting compensator 0 - 130 λ
(453722-0000-000)

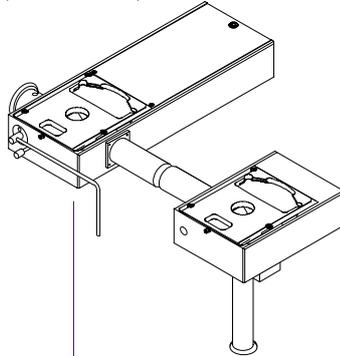
System Overview

Intermediate tubes, Illumination, Analyzers

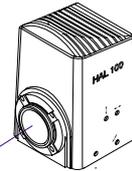
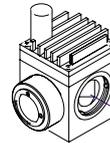
Intermediate tube for image projection
(452181-0000-000)



Intermediate assistant's tube 2x
(452179-0000-000)



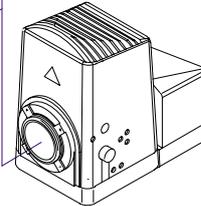
path-deflecting mirror for 2 illuminators (mot)
(447131-9901-000)



HAL 100 lamp housing with collector, lamp mount and heat-reflecting filter
(447219-0000-000)

HBO 50 lamp housing with lamp mount
(447220-0000-000)
Collector HBO 50/SF 25
(447270-0000-000)

Power supply unit for HBO 50
(392642-0000-000)



N HBO 103 lamp housing
(000000-1007-980)
N XBO 75 lamp housing
(000000-1007-981)
Collector N HBO 103/XBO 75
(000000-1007-976)
Quartz collector N HBO 103/XBO 75
(000000-1007-977)
HBO 103 W/2 mercury short arc lamp
(380301-9350-000)
XBO 75 W/2 xenon short arc lamp
(380079-9190-000)

HBO 100 W ebq 100 dc power unit
(000000-1003-928)
ebx 75 isolated power unit
(000000-1003-924)



HAL 100 lamp housing with collector, lamp mount and heat-reflecting filter
(447219-0000-000)

Bertrand lens slider Ph
(453671)



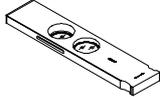
Adjusting aid HBO/XBO
(452169)



Path-deflecting mirror for two illuminators
(447230-9901)



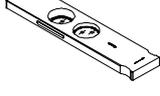
Fixed analyzer slider
(453657)



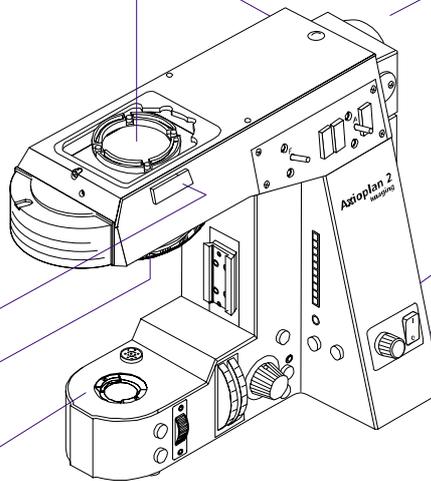
Slider with analyzer with rotary lambda plate
(453663) (in preparation)



Slider for quartz depolarizer
(453659)



Quartz depolarizer d=32 mm
(453653)



Filter magazine D
(452155)

Filter magazine D photo
(452157)

Filter magazine D mot.
(452156)

Luminous-field diaphragm insert D
(452160)

Luminous-field diaphragm insert D mot.
(452161)

Notebook with German keyboard

(000000-1056-852)

Notebook with US keyboard

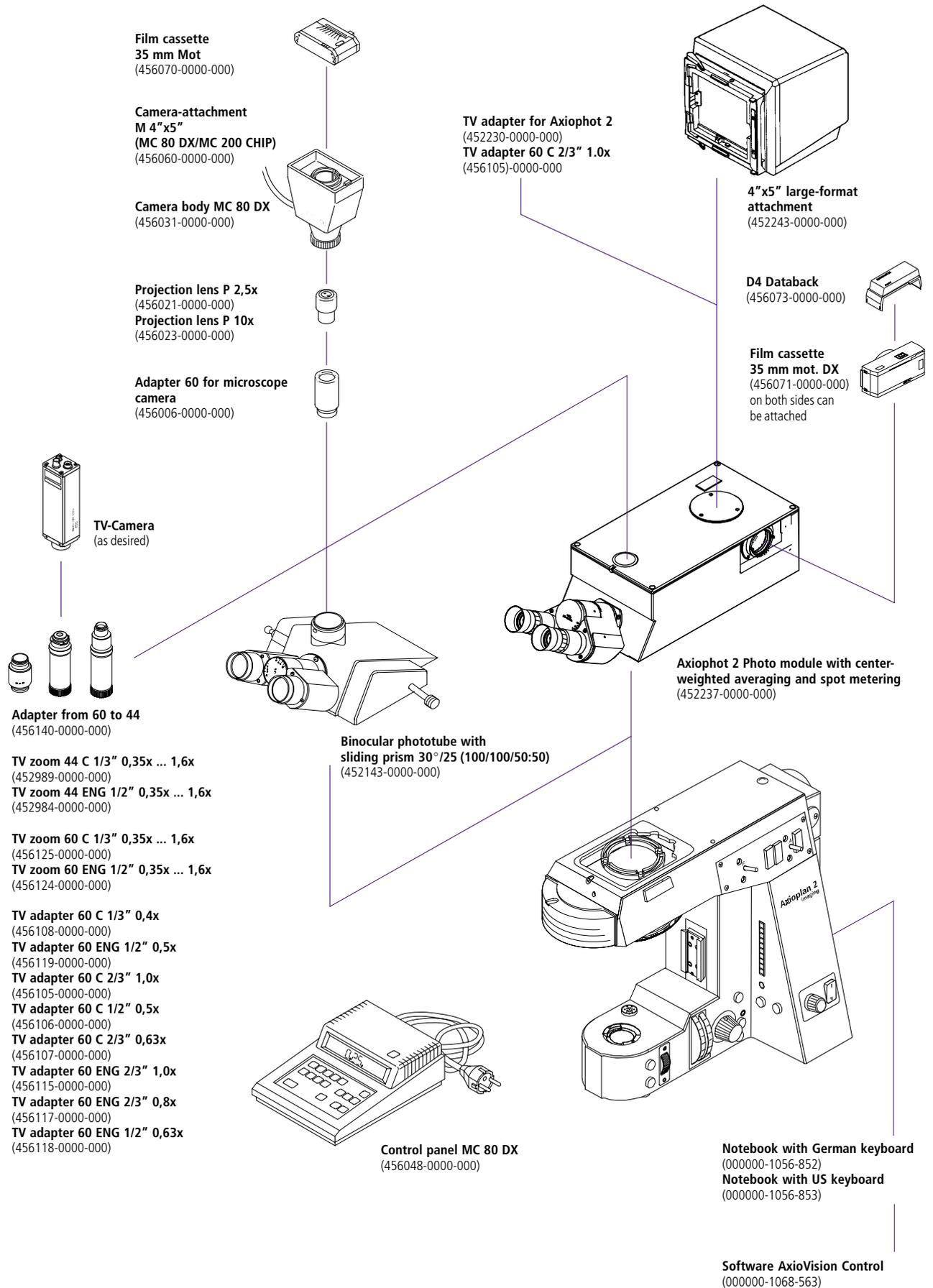
(000000-1056-853)

Software AxioVision Control

(000000-1068-563)

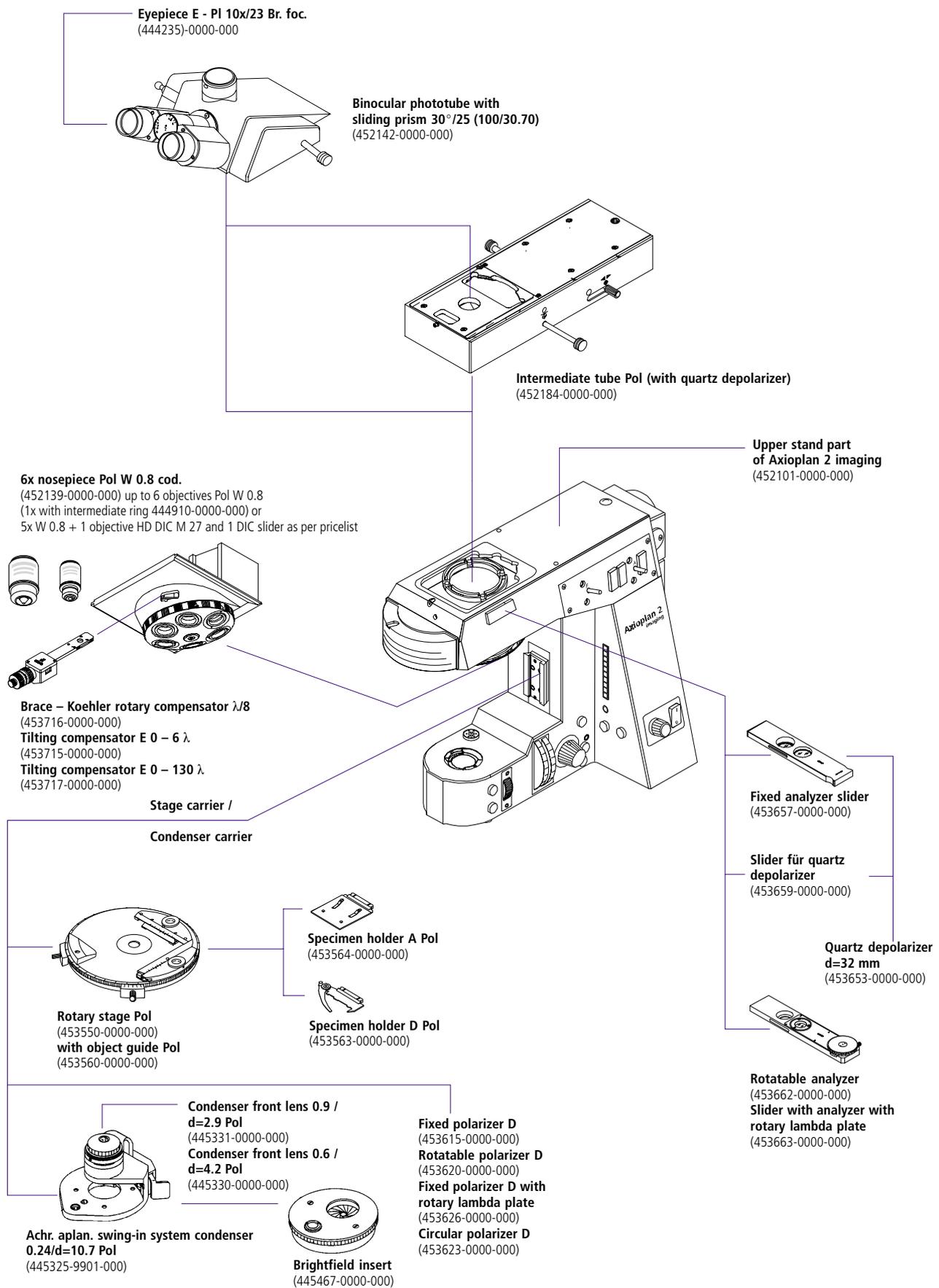
System Overview

Equipment for Documentation



System Overview

Equipment for polarization



Microscope Components - Overview, Description, Instructions for Use

The Axioplan 2 imaging incorporates the System Integration (SI) principle. This SI design allows the Axioplan 2 imaging to be configured or converted to meet various requirements.

The following part of the manual describes all of the components with which the stands of the Axioplan 2 imaging can be combined. Please note that some components require a specific stand model. For example, the use of parts controllable by motor is not possible with the purely manual version of the stand, but requires at least a type E stand.

If the retrofitting of a motorized function is not possible with the equipment which you have purchased, please contact Zeiss Customer Service. It is possible that the retrofitting procedure can be performed without difficulty.

If components can only be used with the Axioplan 2 imaging or only under certain conditions, this is indicated in the overview given for the components in question.

Microscope illuminators 32

Tubes 36

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Reflector modules 43

Objectives 46

Stages 48

Condensers 52

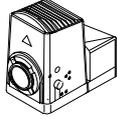
**Analyzers, compensators,
auxiliary objects and
DIC sliders 57**

**Axiophot 2
Photo Module 59**

Microscope Components

Microscope illuminators

Overview

Designation	Application	Component parts	Cat. no.
HAL 100 halogen illuminator 	Incident light and transmitted light All stands	Lamp housing (with collector and heat-reflecting filter, power unit integrated in Axioplan 2 imaging) 12 V/100 W halogen lamp	447219-0000-000 380079-9540-000
N HBO 103 gas discharge illuminator Mercury External power unit required! 	Epi-fluorescence All stands	N HBO 103 lamp housing N HBO 103/XBO 75 collector N HBO 103/XBO 75 quartz collector HBO 103 W/2 mercury short arc lamp Transformer HBO 103 W ebq 100 dc	000000-1050-373 000000-1007-976 000000-1007-977 380301-9350-000 000000-1003-928
HBO 50 gas discharge illuminator Mercury External power unit required! 	Epi-fluorescence All stands	HBO/XBO lamp housing HBO 50/SF 25 collector HBO 50 lamp Power supply unit for HBO 50	447220-0000-000 447270-0000-000 392642-0000-000 392642-0000-000
XBO 75 gas discharge illuminator Xenon External power unit required! 	Epi-fluorescence and for high light intensities in incident light All stands	N XBO 75 lamp housing N HBO 103/XBO 75 collector N HBO 103/XBO 75 quartz collector XBO 75 W/2 xenon short arc lamp ebx 75 isolated transformer	000000-1007-981 000000-1007-976 000000-1007-977 380079-9190-000 000000-1003-924

Note: For the procedure for the assembly and adjustment of the HBO 50 and HBO 103/XBO 75 illuminators, please see the separating operating manuals G 42-160 (Microscope Illuminator with HBO 50) and G 42-165 (Microscope Illuminator with HBO 100/XBO 75).

Microscope Components

General notes



WARNING!

Thermally sensitive fluorescence filters!

Fluorescence filters are sensitive to the thermal radiation of the microscope lamp.

Therefore, never remove the heat-reflecting filter from the illuminator tube.



WARNING!

Risk of damage to instrument!

Before you drape the dust cover over the microscope, switch off the microscope and the external power supply of the microscope illuminator.

Notes on the handling of lamps



CAUTION!

Risk of injury!

- Operate the lamps in the closed housing only.
- Gas discharge lamps emit intensive UV light. With longer periods of direct exposure, this can lead to skin burns and, in the long term, to skin cancer.
Blindness or injury to the conjunctiva may result from looking directly into the lamp.
- Change lamps in cold state only: there is a risk of explosion due to the high internal pressure of warm lamps. Xenon lamps are also under pressure when cold.
Maximum safety: protective visor and leather gloves with long cuffs.
- Always switch off the power unit and microscope prior to lamp change.
- Never touch the glass bulbs of the lamps to be changed, but use the protective caps delivered with the new lamps. Do not forget to remove the protective caps after the new lamps have been inserted.



WARNING!

Heat build-up!

Placing objects against or covering ventilation slots will lead to a build-up of heat which can destroy the instrument or cause a fire. For this reason, always keep the ventilation slots clear.



CAUTION!

Risk of burning!

Do not touch the lamp housing when using the microscope for long periods! The area of the upper ventilation slots in particular is subject to pronounced heating, resulting in a risk of burning.

Microscope Components

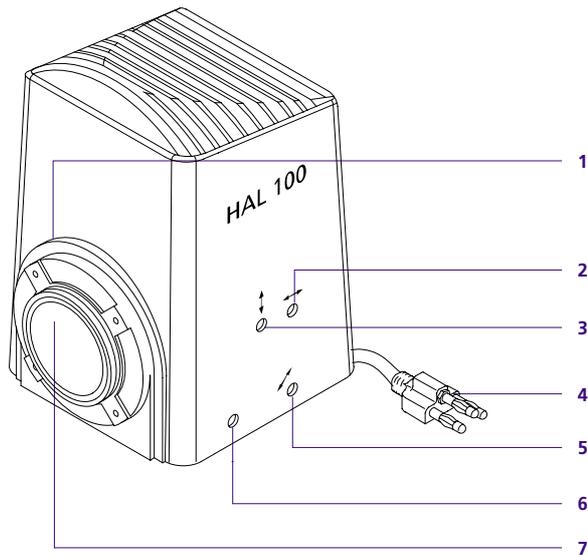
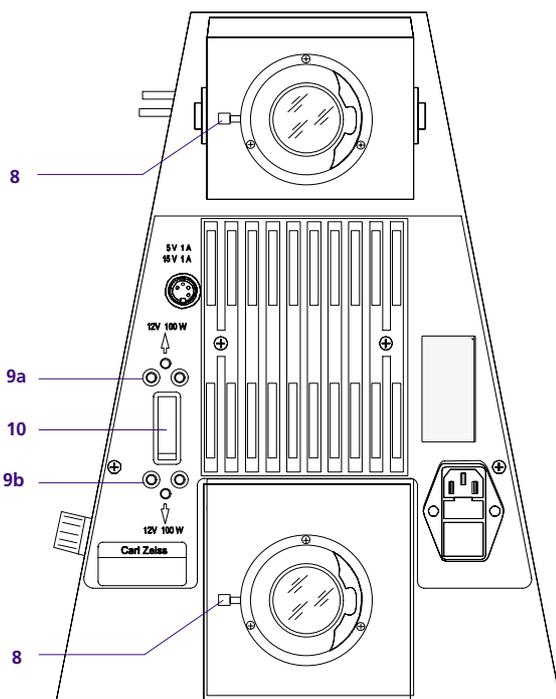


Fig. 15 HAL microscope illuminator



- 1 Dovetail ring
- 2 Focus adjustment of lamp filament
- 3 Vertical adjustment of lamp filament
- 4 Lateral adjustment of lamp filament
- 5 Securing screw for lamp housing
- 6 Plug for 12 V DC voltage supply
- 7 Light exit
- 8 Clamping screw for securing illuminator
- 9a Socket, illuminator voltage for incident light
- 9b Socket, illuminator voltage for transmitted light
- 10 Toggle switch for switching between incident light/transmitted light

Fig. 16 Connection of microscope illuminators

HAL - halogen illuminator

(Fig. 15; Fig. 16)

The HAL microscope illuminator is suitable for transmitted light and incident light microscopy and is part of the standard equipment of the Axioplan 2 imaging.

It consists of a reflector, collector, heat-reflecting filter, lamp mount and a 12 V/100 W halogen lamp.

The illuminator is attached via a clamp system with dovetail ring (1) and clamping screw (8).

Note: A second heat-reflecting filter (467828) is required for Pol applications. The filter is installed in illumination tube using a retaining ring.

Electrical supply

The microscope Axioplan 2 imaging features a wide-range power unit and can be connected to line voltages ranging from 100 ... 240 V AC, 50 ... 60 Hz. The wide-range power unit sets itself automatically to the required line voltage.

- Connect illuminator to 3-pin plug (4) at socket (9a, reflected light) or (9b, transmitted light).

Note: If the stand is not equipped with 3-pin connectors, the third pin of the lamp connector must be unscrewed first.

- Connect the instrument to the line and switch it on via the ON/OFF switch (Fig. 18/13).
- Set toggle switch (10) in the appropriate position (transmitted light/reflected light).
- Set illumination intensity via the voltage regulator (Fig. 18/14).

After switching on and setting (10), the lamp may take a few seconds to light.

Note: An adjustable d.c. voltage of 3...12 volts stabilized against power fluctuations is present at sockets (9a) and (9b). Only the HAL illuminator may be connected. Other illuminators are supplied by external power units which are adapted to the powers of the respective lamps.

Microscope Components

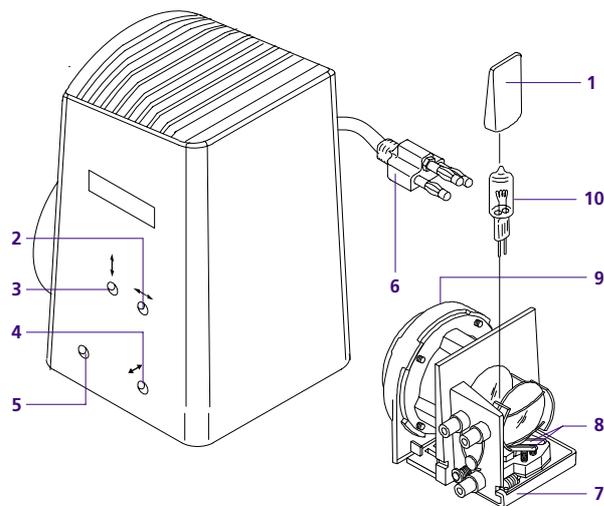
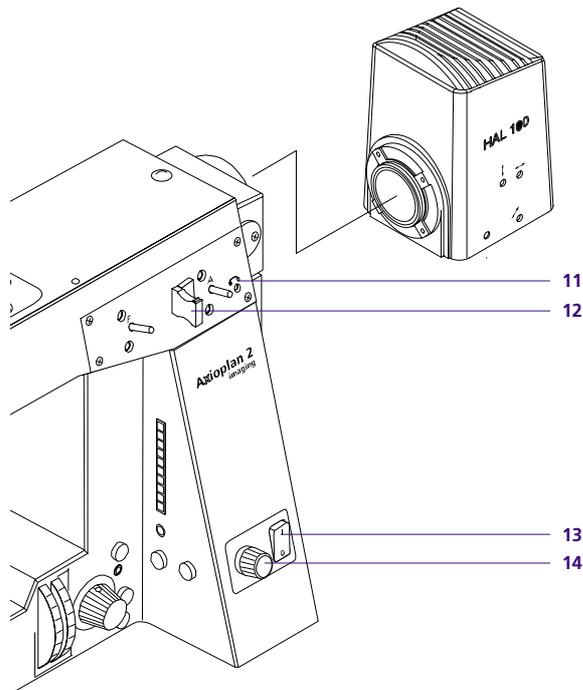


Fig. 17 Lamp housing and lamp mount



- 1 Protective cap
- 2 Focus adjustment of lamp filament
- 3 Vertical adjustment of lamp filament
- 4 Lateral adjustment of lamp filament
- 5 Securing screw for lamp housing
- 6 Plug for 12 V DC voltage supply
- 7 Lamp mount
- 8 Spring
- 9 Internal tube
- 10 Halogen lamp
- 11 On/Off switch for diffusing screen (incident light)
- 12 Filter, incident light
- 13 On/Off switch (I = on, 0 = off)
- 14 Voltage control for setting the illumination intensity

Fig. 18 Connection for incident light

Adjusting the halogen lamp

(Fig. 17; Fig. 18)

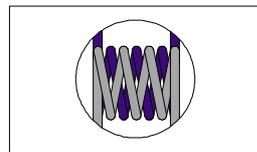
The SW 3 mm screwdriver delivered with the equipment is required to set screws (2), (3) and (4).

Coarse adjustment

- Remove microscope illuminator.
- Switch on Axioplan 2 imaging.
- Direct light ray towards a projection surface (wall, paper) at least 3 m away.
- Adjust screw (2) until the lamp filament is imaged sharply on the projection surface.
- Adjust screws (3) and (4) until the image of the lamp filament fills the gaps in the reflector image (→ below).

Fine adjustment

- Switch off diffusing screen and any filters present using switches (11) (incident light) or Fig. 3/22 (transmitted light).
- Attach microscope illuminator.
- Focus on specimen with $\leq 40x$ objective and look for a free object area.
- Insert Bertrand lens or remove eyepieces so that you can observe pupil image with lamp filament and its mirror image.
- Adjust screws (3) and (4) until both images are centered.



- Reactivate diffusing screen and filters used.
- Optimize homogeneous illumination of pupil image with screw (2).

Changing the halogen lamp

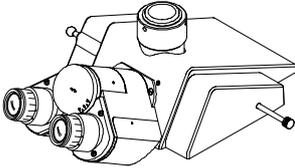
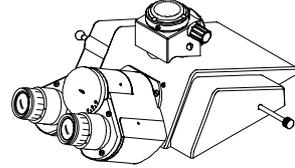
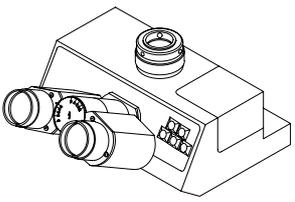
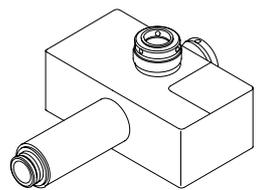
- Remove plug (6) from sockets (Fig. 16/10a) or (10b).
- Remove microscope illuminator.
- Loosen screw (5) and pull out lamp housing in upward direction.
- The lamp mount is now accessible.
- Turn lamp housing upside down and remove old lamp by pressing spring (8).
- After removing protection cap (1), insert new lamp in socket with the springs pressed.
- Give spring short press downward to center the lamp.
- Re-attach lamp housing.

Note: Hold new halogen lamps by the protective caps (1) only. Even the tiniest traces of grease on the glass bulb of the lamp can impair the performance and service life of the lamp.

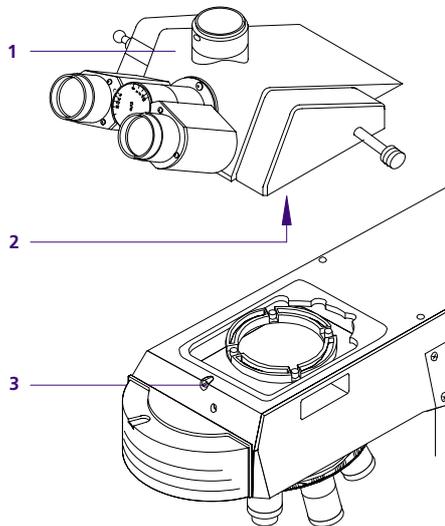
Microscope Components

Tubes

Overview

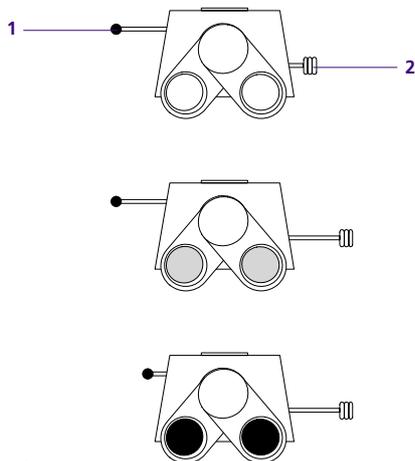
Specification	SFZ	Splitting	Application/Combination	Cat. no.
Binocular phototube with sliding prism 30°/25 	25	100 : 0 50 : 50 0 : 100	3 switch positions 1 eyepiece shutter as light shutter with 50:50 ratio	452143-0000-000
Binocular phototube with two ports 30°/25 	25	100 : 0 50 : 50 30 : 70	3 switch positions and switching mirror in the phototube for two ports as an alternative for photo/TV 1 eyepiece shutter as light shutter with 50:50 ratio	452145-0000-000
Binocular phototube 25 with height adjustment (5 ... 30°)	25	100 : 0 20 : 80		452146-0000-000
Double TV tube mot. 	25	100 : 0 50 : 50 0 : 100	Motorized light path switching; 2 switching levels with: – 3 switching positions for beam splitting – 2 switching positions for the two camera/TV ports (100 % to the top, 100 % to the back, or splitting freely assignable with reflector module H or FL and dia. 25 mm filter)	000000-1054-146 available from 4/2000
Double video adapter 	25		Upgrading of one camera/TV port to 2 switchable camera/TV ports (100 % to the top, 100 % to the back, or splitting freely assignable with reflector module H or FL and dia. 25 mm filter)	000000-1058-640 available from 4/2000

Microscope Components



- 1 Tube
- 2 Tube lens
- 3 Securing screw

Fig. 19 Tube mounting



- 1 Upper pushrod
- 2 Lower pushrod

Fig. 20 Light splitting

General

All binocular tubes have a viewing angle of 30°. The interpupillary distance can be set between 55 and 75 mm by pressing the two halves of the tube together or pulling them apart.

Note: If intermediate tubes are used, the tube lens (Fig. 19/2) of the binocular tubes must be removed. It can be stored in the container supplied. The cover of the container, which includes two pins, can be used as a tool for the removal.

Attaching tubes

(Fig. 19)

To attach the tubes, you need the SW 3 Allen key.

- Loosen securing screw (3) on the stand.
- Place tube (1) onto the upper part of the stand and align it.
- Tighten securing screw (3).

Light splitting

(Fig. 20)

The binocular tubes are equipped with pushrods fitted to the sides of the tubes. These pushrods move a sliding prism which directs part or all of the light to the photo/TV ports.

In principle, the position of the pushrods is the same for all tubes.

Upper pushrod pulled out and lower pushrod inserted: 100 % light to eyepieces.

Upper and lower pushrods (1) and (2) pulled out: 50% light to the camera/TV ports, 50% to the eyepieces.

In tubes with 3 switching positions (452143-0000-000, 452145-0000-000), the center position of the pushrod (2) splits the beam in 50% for camera/TV and 50% for the eyepieces (452146-0000-000: 0% to camera/TV and 20% to eyepieces)

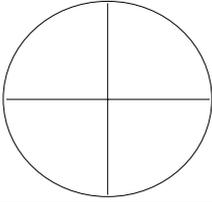
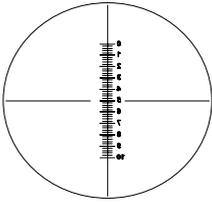
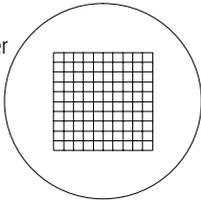
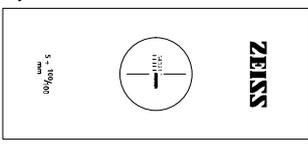
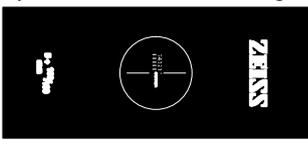
Upper pushrod (1) pushed in: eyepiece blocked.

In tubes with more than one camera/TV port, an additional knob is included on the respective port for blocking or deflecting the light.

Microscope Components

Eyepieces

Overview

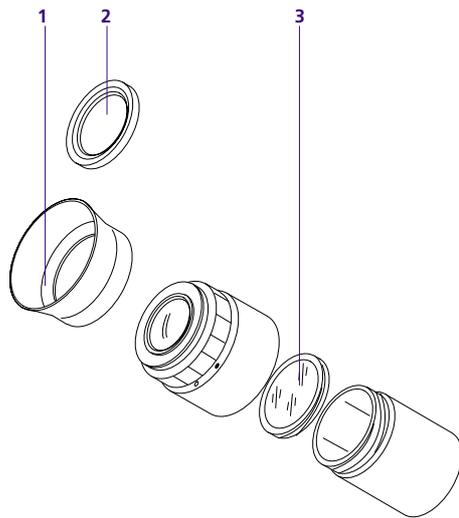
Specification	Application/Combination	Cat. no.
Eyepieces		
Eyeiece E-PI 10x/25 Br. foc.	Standard eyepiece of research category for large field of view 25 mm	444234-0000-000
Eyeiece E-PI 10x/23 Br.foc.	High-performance aspheric eyepiece for Epiplan objectives or for use of intermediate tube Pol in field of view	444235-0000-000
Eyeiece PI 16x/16 Br.foc.	These highly magnifying special eyepieces are of help when you are working constantly with high additional magnifications	444054-0000-000
Photo eyepiece S-PI 8x/16	For the attachment of the MC 200 CHIP microscope camera	444029-0000-000
Photo eyepiece S-PI 10x/20	For the attachment of microscope cameras	444049-9902-000
Photo eyepiece S-PI 12,5x/16	For the attachment of microscope cameras	
Reticles for 10x eyepieces (Measuring and counting)		
Eyeiece crossline disk 	$d = 26 \text{ mm}$	
Eyeiece crossline micrometer 	$14 : 140/d = 26 \text{ mm}$	
Eyeiece grid micrometer 	$12,5x \ 12,5/5; \ 10/d = 26 \text{ mm}$	
Object micrometers (for calibrations)		
Object micrometer 	positive 5+ 100/100 μm , $d = 0,17 \text{ mm}$	
Object micrometer for incident light 	negative 5+ 100/100 μm , $d = 0$	
Other aids on request.		

Microscope Components

General

(Fig. 21)

The Zeiss microscopes are generally supplied with two focusing eyepieces. Eyeglass protective rings (2) are attached to the eyepieces to prevent lens scratching. Folding eyecups (1) can be used alternatively. Folding eyecups (1) can be helpful here.



- 1 Folding eyecup
- 2 Rubber ring for spectacle wearers (instead of 1)
- 3 Reticle

Fig. 21 Eyepiece

Designations/Markings

Br.

The designation **Br.** on the eyepieces means that eyeglass-wearers can use their eyeglasses for microscopy and place them directly against the eyepieces. Users who do not wear glasses should keep a distance to the eyepieces allowing them to view the entire field.

fof.

The designation **fof.** on the eyepieces means that they are focusable. This allows you to compensate for differences in the visual performance of your two eyes. For focusing, turn the eyepiece for the eye with better vision to the zero position and focus on the specimen using the fine drive. Then turn the focusing ring of the other eyepiece until you see the specimen in focus.

If you choose to wear your correctly fitted glasses when using the microscope, this adjustment is not necessary, since the compensation is performed by the glasses. Both eyepieces are set to zero. Visual defects, such as astigmatism, are not corrected. Eyeglasses or contact lenses should be worn.

If one of the eyepieces contains a reticle (3), you first have to focus the eyepiece on the reticle.

- To do this, remove the eyepiece from the tube and turn the upper part of the eyepiece until stop. Hold the eyepiece against a bright surface and turn the upper part in again until the reticle is visible in focus.
- Insert the eyepiece into the tube again and focus both eyepieces on the specimen (as described above).

White dot

This is the zero position of the eyepiece if no reticles are used.

Red dot

This is the zero position if a reticle is used in the eyepiece.

Note: Before looking through the microscope, check whether the eyepiece is in a zero position and set it to diopter power, if required.

Use of reticles

Eyepieces featuring a red dot allow the use of reticles.

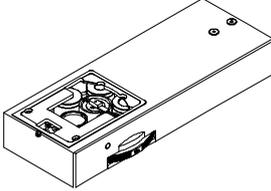
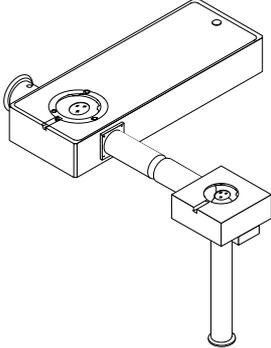
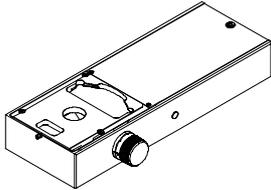
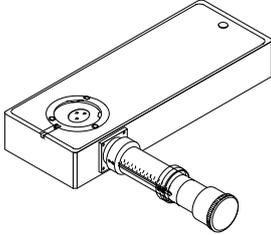
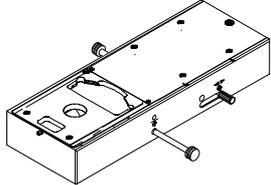
Make sure that the reticle always faces the field stop.

Reticles should be inserted by the Zeiss servicing staff in dust-free conditions.

Microscope Components

Intermediate tubes

Overview

Specification		Application/Combination	Cat. no.
Optovar 1.0x/1.25x/1.6x/2.0x/2.5x coded		Additional magnification, in steps; for correctly dimensioned scale projection (Databack) with Axiophot 2 photo module (can also be used for microscopes without Axiophot 2 and without data transfer)	452175-0000-000
Coobservation equipment with light pointer		For connection of a second binocular tube as a discussion device	452179-0000-000
Zoom intermediate tube coded		Additional magnification, stepless, coded;	452180-0000-000
Intermediate tube for data projection switchable		Projection of data into intermediate image	452181-0000-000
Intermediate tube Pol		Crossline or iris stop can be inserted in field of view, focusing of Bertrand optics for axial image observation (conoscopy) Removal of individual object details from field of view using iris diaphragm, for Axioplan 2 imaging Pol / Axiophot 2 Pol	452184-0000-000

Microscope Components

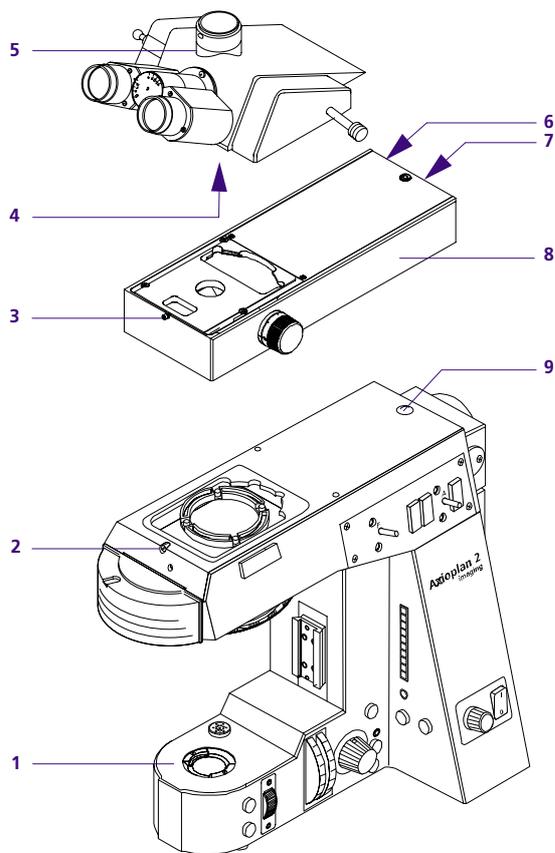


Fig. 22 Mounting of intermediate tube

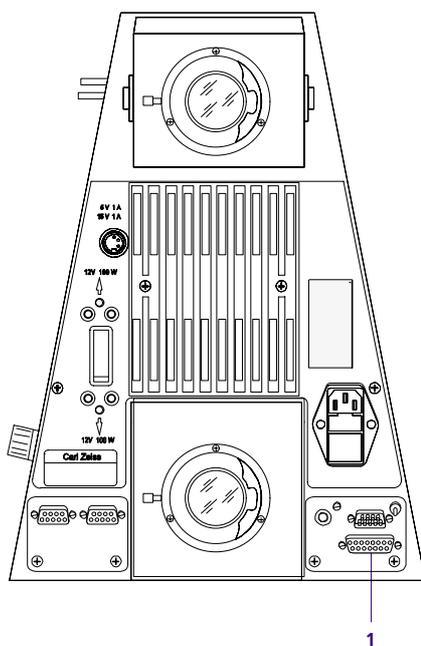


Fig. 23 Back of stand

General

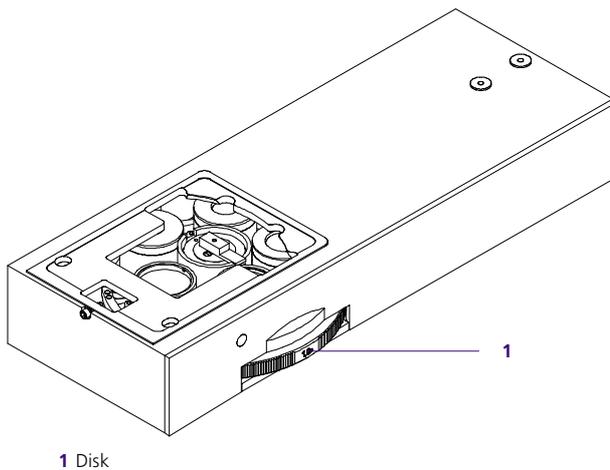
Intermediate tubes are always attached to the upper part of the stand. To do this, the binocular tube must first be removed and its tube lens removed.

Note: Use the cover of the tube lens container to remove the tube lens (4).

Mounting of an intermediate tube

- Switch off your instrument.
- Loosen clamping screw (2) on stand (1) and remove the tube (5) upwards.
- Attach the intermediate tube (8) to the stand. Centration is made using the dovetail.
- Tighten clamping screw (2) on stand only slightly at first.
- Insert the supplied clamping screw with washer (7) into the drilled hole provided and screw it into the threaded hole (9) of the stand.
- Align the intermediate tube with the edges of the intermediate tube and screw tight clamping screws (2) and (7).
- Remove the tube lens (4) from the tube because a tube lens is integrated in the intermediate tube.
- Attach the tube (5) to the intermediate tube (8) and center via the dovetail.
- Align the tube with the edges of the intermediate tube and tighten the clamping screw (3).
- For coded or motorized intermediate tubes, connect the serial interface of the intermediate tube (Fig. 22/6) with that of the stand (Fig. 23/1) via the RS 232 C serial cable supplied.

Microscope Components



1 Disk

Fig. 24 Optovar intermediate tube

Optovar intermediate tube

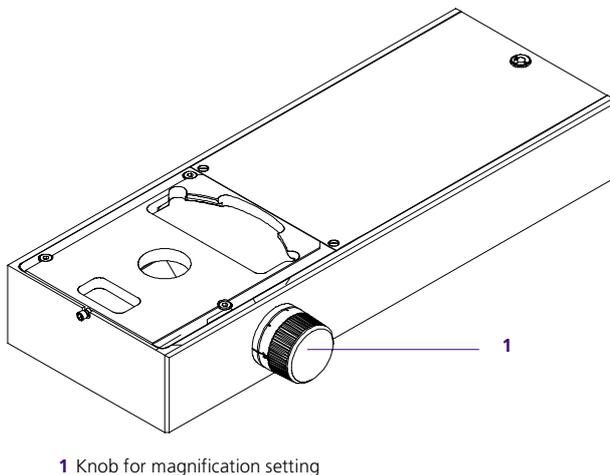
(Fig. 24)

The Optovar intermediate tube additionally allows a convenient magnification change in the steps 1.0x, 1.25x, 1.6x, 2.0x and 2.5x. You can read the magnification factors at the projecting disk (1) on the right-hand side.

To set a different magnification factor, turn the disk to the appropriate click stop position.

Note: E-PI eyepieces should be used instead of the PI models because of their better imaging quality. Optovar in position 1x should only be used with eyepieces E-PI 10x/25 Br.

If, when determining the microscope magnification, you do not want to work with these factors, then combine the magnification of Optovar and the 10x eyepiece and you practically have the eyepiece powers 10x, 12.5x, 16x, 20x and 25x.



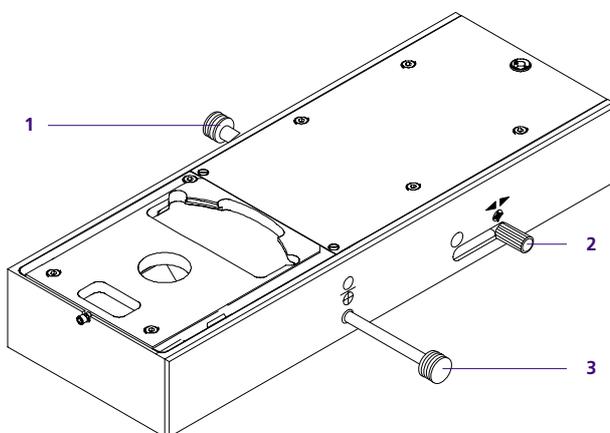
1 Knob for magnification setting

Fig. 25 Zoom intermediate tube

Zoom intermediate tube 1,0x ... 2,5x

(Fig. 25)

The intermediate tube with zoom extends the magnification range of the microscope continuously from 1x ... 2.5x. The required magnification can be set with knob (1) and the zoom factors can be read off at the scale.



1 Pushrod for field diaphragm
2 Rotary and slider knob for Bertrand lens
3 Pushrod for reticle

Fig. 26 Intermediate tube Pol

Intermediate tube Pol

(Fig. 26)

The intermediate tube Pol is equipped with a Bertrand lens, a quartz depolarizer, a reticle which can be switched on/off and a field diaphragm (iris).

The combined rotary and slider knob (2) is used to swing in/out and to focus the Bertrand lens. In its rear position (symbol \oplus), the Bertrand lens is in the beam path (conoscopy).

Pushrod (1) is used to adjust the field diaphragm:

- pushrod in – diaphragm open
- pushrod out – diaphragm closed

Pushrod (3) is used to project a reticle into the beam path:

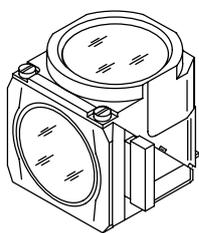
- pushrod in – reticle out of beam path
- pushrod out – reticle in beam path

Microscope Components

Reflector modules

Overview

Specification	Application/combination	Cat. no.
Reflector module H	Brightfield, incident light	000000-1046-274
Reflector module D	Darkfield, incident light	000000-1046-276
Reflector module DIC	Differential Interference contrast, incident light	000000-1046-277
Reflector module DIC Red I	Differential interference contrast, incident light, The integrated λ -plate converts optical path differences into colors.	000000-1046-278
Reflector module Pol	Brightfield, incident light, polarization	000000-1046-279
Reflector module FL	Fluorescence, usable filter sets	000000-1046-281
Optovar module 2.5x	Focusing aid for photography for additional magnification at a weak magnification, can be used only in transmitted light	000000-1046-282
Optovar module 1.6x	For additional magnification in transmitted light	000000-1046-283
Optovar module 1.25x	For additional magnification in transmitted light	000000-1046-284
Analyzer module D	Differential Interference Contrast, polarization	000000-1046-958



Microscope Components

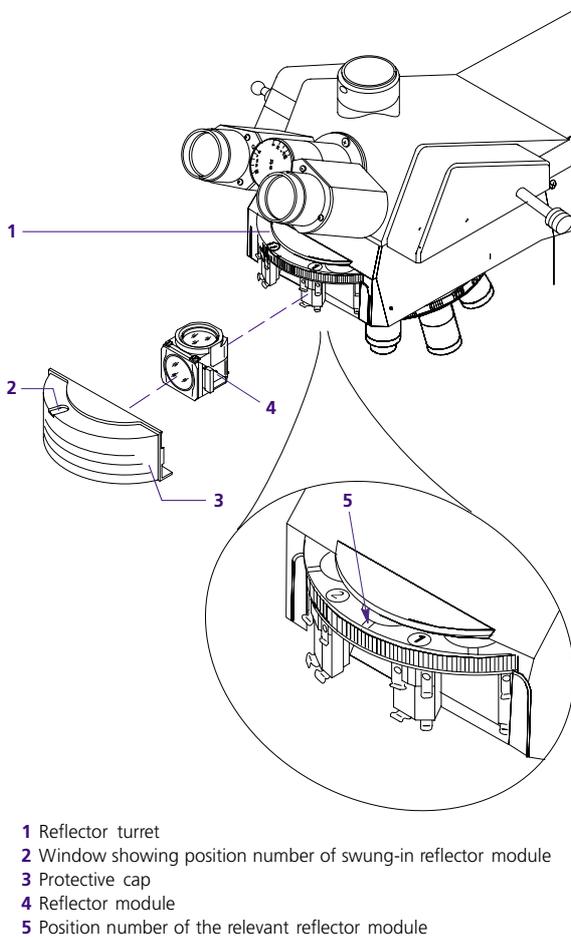


Fig. 27 Attachment of reflector modules

Attachment of reflector modules

Usually, the reflector turret is correctly equipped in the factory. The reflector turret features eight reflector positions and can be equipped as required. To retrofit or change the equipment, proceed as follows (see Fig. 27):

- Swing the required opening of the reflector turret (1) into the beam path (see window 2). Then turn the reflector turret clockwise by four clickstops (manually or via microscope control software). The required opening is now accessible on the front of the reflector turret. For checking purposes, the position number (2) is engraved in the reflector turret (see smaller digit at (5))
- Grasp protection cap (3) at the top and bottom and remove it in forward direction.
- Remove the non-required reflector module (if available) from the reflector turret opening by pulling it out of the lower spring elements first and then from the upper spring elements.
- Insert the new module (4) into the upper spring clamps on the reflector turret from below via the holding elements on the right and left. Then press on the module at the bottom until it engages also in the lower spring elements of the reflector turret.

The reflector module requires no further adjusting procedures.

Reflector module FL

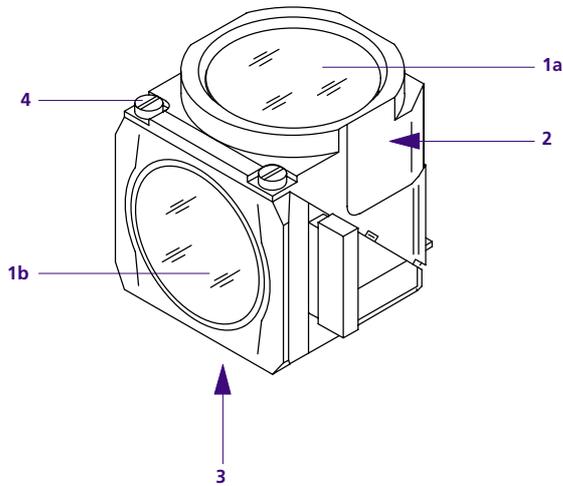
You can configure the reflector module FL (000000-1046-281) for fluorescence microscopy with filters and beam splitters to meet your own personal requirements. You can order the filter and beam splitter combination separately from us. However, you can also purchase completely assembled reflector modules from us.

Mounting the filter inserts

- First, remove the reflector module from the reflector turret in accordance with the above instructions.
- Use the mounting tool plate (contained in tool set) to loosen the ring screw for the filter to be changed and screw it out completely.
- Remove the filter by turning it out onto the surface of your hand.
- Insert the new filter into the opening, place the ring screw in position and re-tighten.

Hinweis: The reflecting surfaces of the filters must always be oriented in the direction of the illumination.

Microscope Components



- 1a Barrier filter
- 1b Exciter filter
- 2 Surface for sticker attachment
- 3 Reflecting surface of beam splitter
- 4 Attachment screws for module halves

Fig. 28 Mounting of beam splitter

Mounting the beam splitter

To enable the mounting of the beam splitter, the reflector module is divided diagonally into two halves which are connected via two slotted screws.

- Loosen the two slotted screws using a screwdriver of a suitable size.
- Fold apart the two reflector halves and carefully remove the beam splitter lying between the two halves
- Place the new beam splitter on the spring frame intended for it in one of the reflector halves (reflecting surface showing downwards, 3)

Note: You can recognize the reflecting surface of the beam splitter by carefully viewing the edge: the reflection coating does not reach up to the edge.

- Fold over the other half and screw both halves together using the two slotted screws.
- After completion of the mounting procedure, please attach the supplied sticker indicating the filter combination to the side of the reflector module intended for it (2).

Note: Please be extremely careful when performing the above steps and make sure to avoid dirt on the filter inserts and the beam splitter or damage to these units.

Ideally, the filters and the beam splitter should be assembled by Carl Zeiss service staff.

Microscope Components

Objectives

General Information

All the objectives of the Axioplan 2 imaging incorporate the principle of ICS optics, i.e. they project the image to infinity. Only the tube lens produces the intermediate image which can be viewed via the eyepieces

Abbreviations

ICS	Infinity Color-Corrected System
H	brightfield
D	darkfield
HD	brightfield/darkfield
Ph 1, 2, 3	phase contrast; the numbers refer to the diameter of the annular diaphragm to be used: for an objective described Ph 2, use the annular diaphragm Ph 2.
DIC	Differential interference contrast
AA	working distance: distance between objective and sample surface or cover slip surface
Pol	polarization
Fl	fluorescence
N.A.	numerical aperture
Imm	immersion medium
W	water
Oil	oil
Glyc	glycerin
Korr	adjustable correction of cover slip thickness
LD	Long working distance
∞	infinity

Attachment of objectives

- Remove dust caps from the openings of the objective nosepiece.
- Screw in objectives (in the order of increasing magnifications).

Note: When using a coded nosepiece, please make sure to insert the objectives in accordance with the positions displayed in the Microscope Software.

Check the positions by comparing the objectives displayed in the program to the objectives actually inserted.

Nosepiece positions which are not required must always be covered with dust caps.

Microscope Components

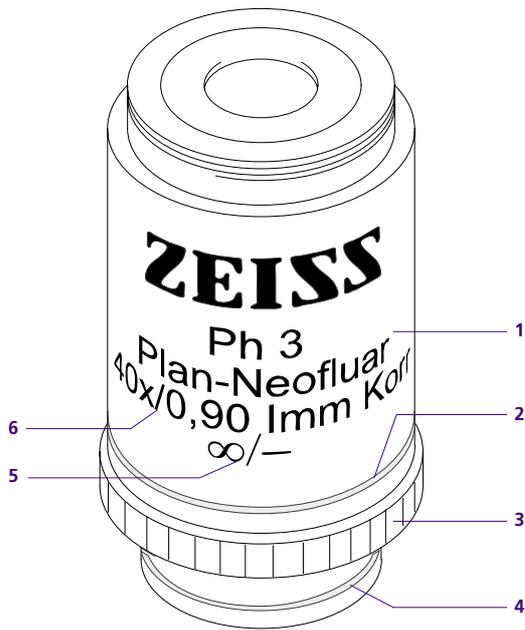


Fig. 29 Labelling of objectives

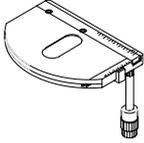
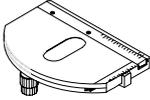
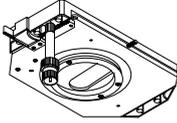
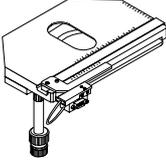
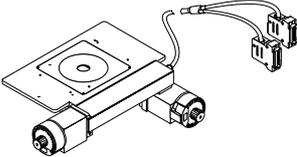
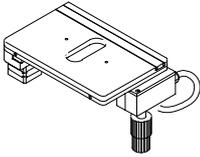
Labelling of objectives

Number	Explanation	Color	Meaning
1	The color of the labelling marks the contrasting technique intended for this objective	black	Standard
		red	Pol/DIC
		green	Ph 1, 2, 3
2	Color coding of the magnification	black	1.25x
		brown	2.5x
		red	4x; 5x
		orange	6.3x
		yellow	10x
		green	16x; 20x; 25x; 32x
		light blue	40x; 50x
		dark blue	63x
		white	100x; 150x;
		3	Mechanical setting ring (only for special objectives). For optical correction when using different immersion media or in the case of different thickness of cover slips/chamber bottoms. Also available with objectives with adjustable aperture iris
4	Color coding for immersion liquid to be used	black	oil
		white	water
		orange	glycerin
		red	variable
5	Tube length/cover slip thickness; tube length ∞ marks ICS optics	like 1	
6	Magnification/numerical aperture	like 1	

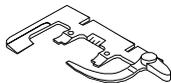
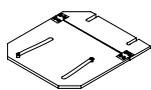
Microscope Components

Stages

Overview

Specification		Comments	Cat. no.
Stages			
Mechanical stage 75x50 R with ergo-drive		stage focusing on right; abrasion-proof coating; height adjustment and adjustment of smoothness of drive controls	000000-1063-835
Mechanical stage 75x50 L with ergo-drive		stage focusing on left; abrasion-proof coating; height adjustment and adjustment of smoothness of drive controls	000000-1063-836
Rotary, centerable mechanical stage 75 x 50, 240° R		rotatable about 240°; abrasion-proof coating	453502-9905-000
Mechanical stage 75 x 50 R electronic		with electronic vernier; abrasion-proof coating	453507-0000-000
Pol rotary stage with object guide		for polarizing microscopy	453550-0000-000 and 453560-0000-000
Scanning stage 100 x 100			453585-0000-000
Mechanical stage 75x50 mot with control unit		options: with joystick for 2 axes (000000-1033-996) or with coaxial electronic drive (000000-1034-960)	000000-1025-145

Microscope Components

Specification		Comments	Cat. no.
Specimen holders			
Specimen holder with spring clip R			453533-0000-000
Special specimen holder		with special fixing device, for mounting 2 specimens	453538-0000-000
Specimen holder for mechanical stages 000000-1063-835/836			000000-1046-521
Specimen holder A			000000-1070-588
Specimen holder A			453539-0000-000
D Pol object guide		combination with 453560-0000-000	453563-0000-000
A Pol object guide		combination with 453560-0000-000	453564-0000-000

Microscope Components

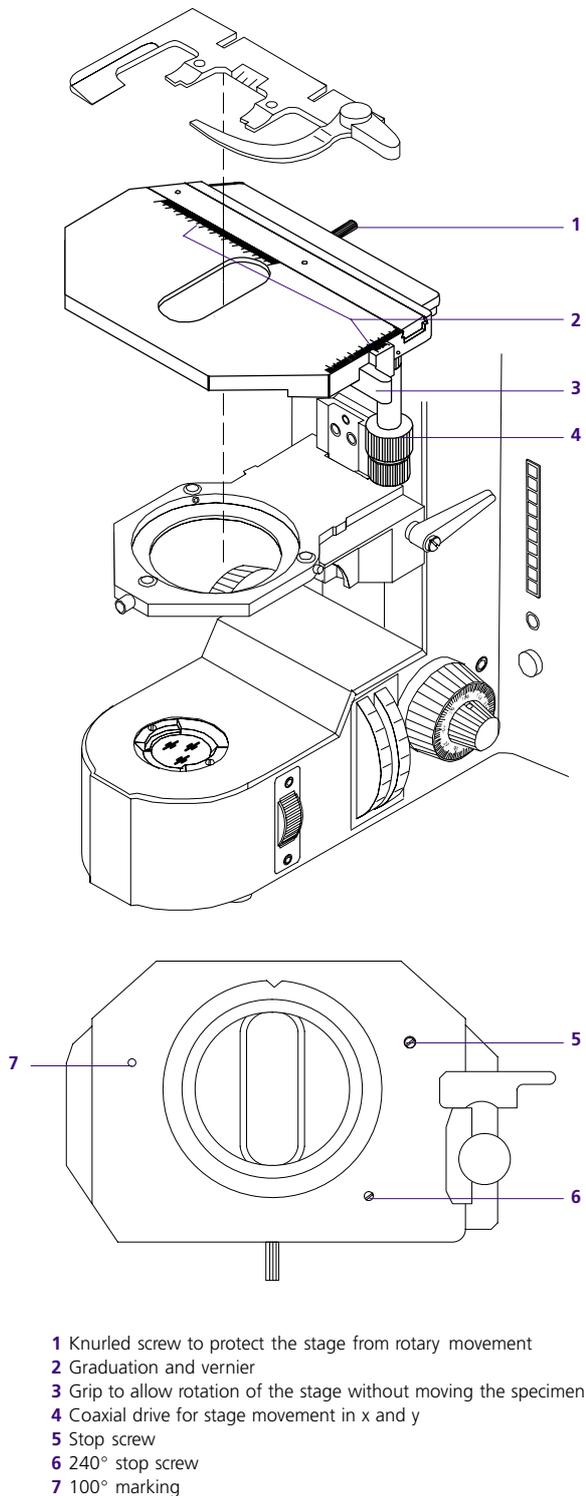


Fig. 30 Mechanical stage

General Information

(Fig. 30)

Mechanical stages can be rotated and centered. They can be moved in x (25 mm) and y (50 mm). There is a stage drive on the right which also moves when the stage is rotated.

Attachment/removal and centration of stages

(→ see *Stage carriers* in the chapter *Manual stand*)

Height adjustment of drive controls

(000000-1063-835/836, 000000-1067-325)

- Remove the sleeves of the drive controls by unscrewing both screws.
- Set the height of the drive rods as required.
- Set the smoothness of the drives.
- Attach sleeves again and fix them in the required height.

Conversion of the rotary mechanical stage

The rotary and centerable mechanical stage (453502-9905-000) is factory-aligned to a y-range of 28 mm and a rotation range of 240°.

The combination of the achromatic dual condenser 0.5 mot (445341-0000-000, see page 24) with the rotary mechanical stage limits the turning range and the Y movement of the stage.

In the focus position of the condenser, the stage can only be turned through 100° (standard 240°). The Y movement is limited to the length of the microscope slide.

Note: If you do not need a rotary mechanical stage (e.g. mechanical stage 000000-1063-835), two microscope slides can be screened without limitation.

Specimen holder 453538-0000-000 is recommended for this.

Extending the y-range to 50 mm requires conversion of the stage. The rotation range is then reduced to 100°.

- Remove mechanical stage (→ see *Stage carriers* in the chapter *Manual stand*).
- Remove stop screw (5).
- Remove stop screw from the 240° marking (6) to the 100° marking (7).
- Insert mechanical stage so that the spring catches the groove of the dovetail.
- Loosen knurled screw (1).

Microscope Components

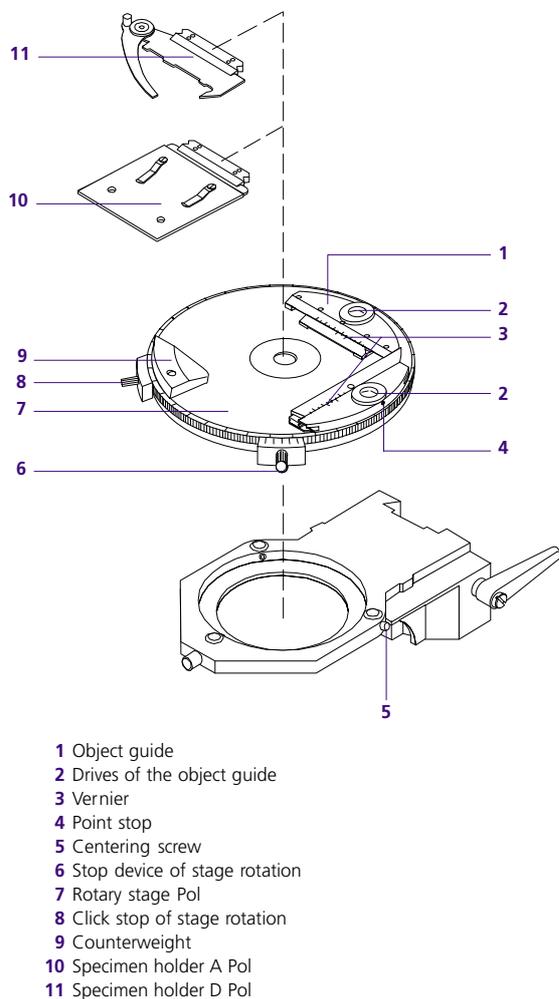


Fig. 31 Pol rotary stage

Rotary stage Pol

The rotary stage Pol (7), used with polarizing microscopes, is a rotatable and centerable stage with an object guide (1) for a travel range of 45 mm in x and 25 mm in y.

Object coordinates can be determined with an accuracy of 0.1 mm using graduations and verniers.

Either the specimen holder D Pol (11) or A Pol (10) for incident-light specimens can be attached to the object guide. Together with a reticle in the intermediate tube, a 360° graduation (3) and one vernier each to read off 1/10° are used for angle measurement.

The stage rotation can be locked by screw (6). Tightening screw (8) provides a click stop every 45°.

Stage centering is required to make sure that an object feature in the center of the field of view does not migrate when the stage is turned. Checking the centering before each examination is a matter of routine for the user of a polarizing microscope and is performed as follows:

- The nosepiece Pol of your microscope features 5 centerable threaded mounts and one fixed mount containing an objective. Swing in the objective and focus on a high-contrast specimen.
- Turn the stage. Almost all object features move on circles; the center of all these circles is the center of rotation of the stage (→ Fig. 32).
- With the small Allen wrench inserted at (5), bring this object spot to the point of intersection of the eyepiece cross lines, i.e. the optical axis of the 10x objective. This centering procedure may have to be repeated.

The point counter on the controls of the object guide (1) provides a click stop after every 0.5 mm of specimen movement (the point counter with 0.2 mm steps (453570) is optional). The point counter simplifies systematic screening of a specimen, and it can also be used for volume and quantitative analysis. The click stops are set with the supplied Allen wrench on screw (4) (unscrew (4) to activate the click stop, screw it in to deactivate it). To prevent defocusing, clip plastic rings on the drive controls (2) and move the controls from the side.

The attachable mechanical stage is always supplied with counterweight (9) which can be screwed on to the stage using the SW3 Allen wrench. It is used for symmetrical weight distribution and prevents defocusing when the stage is rotated. It should be used at high magnifications in particular. If it is not factory-mounted, please refer to the leaflet supplied with the device.

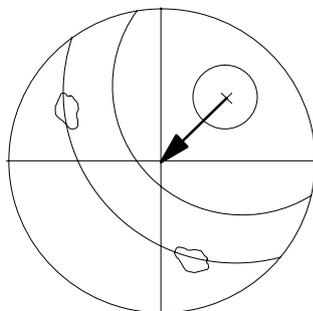


Fig. 32 Centering of Pol rotary stage

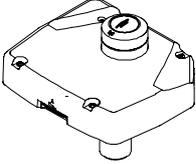
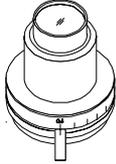
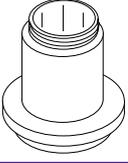
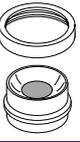
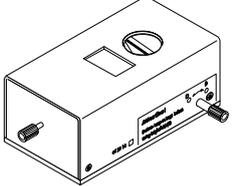
Microscope Components

Condensers for Transmitted Light

Overview

Specification	Comments	Cat.no.
Achromatic-aplanatic universal condenser 0.9	highest flexibility and resolution; front lens swung in: for objectives 10x...100x	000000-1035-632
Achromatic-aplanatic universal condenser 0.9 H D Ph	front lens swung out: for objectives 1.5x ...10x	445436-0000-000
Achromatic-aplanatic universal condenser 0.9 H D Ph DIC	brightfield insert or turret disk for contrasting elements with 5 or 7 positions	445439-0000-000
Achromatic-aplanatic sSystem condensor 0.24/d=10.7 swing-in Pol	highest flexibility and resolution; front lens 0.9 swung in: for objectives 10x ... 100x front lens swung out: for objectives 2.5x ... 10x front lens 0.6 swung in: for objectives 10x ... 40x	445325-9901-000
Achromatic switching condenser 0,9	For objectives A-Plan 5x ... 100x	000000-1017-688
Achromatic switching condenser 0,9 H D Ph	For objectives A-Plan 5x ... 100x	000000-1017-690
Achromatic-aplanatic condenser 1.4 H DIC	oil immersion on the illumination side for optimum resolution in transmitted light; fixed front lens; for brightfield and Differential Interference Contrast, for objectives 20x ... 100x	445453-0000-000 also for phase contrast and darkfield
Achromatic condenser 0.8 H	brightfield condenser, fixed front lens, for objectives 5x ... 100x with iris diaphragm for aperture adjustment	445443-0000-000
Achromatic condenser 0.8 H mot.	like 445443-0000-000, but motorized	445444-0000-000
Achromatic condenser 0.8 H D Ph DIC	for brightfield, darkfield and phase contrast with objectives Ph 1, Ph2 and Ph3 DIC III fixed front lens; for objectives 5x ... 100x; with iris diaphragm for aperture adjustment	445445-9901-000

Microscope Components

Specification		Comments	Cat. no.
Achromatic condenser 0.8 H D Ph mot.		like 445445-0000-000, but motorized	445446-0000-000
UD condensor 0.6		Primarily intended for use with UD stage; also suitable as LD condenser 0.4 for brightfield and polarization	445460-0000-000
Achromatic dual condenser 0.5		brightfield condenser for objectives 1.25x ... 40x; lever for rapid change of fixed front lens; with iris diaphragm for aperture adjustment for objectives $\geq 5x$	445340-0000-000
Achromatic dual condenser 0.5 mot.		like 445340-0000-000, but motorized	445341-0000-000
Condenser holder Z		to allow mounting of: ultracondenser 1.2/1.4 or dry darkfield condenser 0.8/0.95	445323-0000-000
Ultra condenser 1.2/1.4		special condenser for darkfield, high magnification and aperture suitable for objectives with aperture 0.75 ... 1.0	465500-0000-000 (replaces 445315-0000-000)
Dry darkfield con- denser 0.8/0.95		special condenser for darkfield, medium magnification and aperture special condenser for darkfield, medium magnification and aperture, suitable for objectives with aperture 0.6 ... 0.75	465505-0000-000
Darkfield illuminator		for unilateral darkfield illumination at low magnifications between 1.25x and 10x; switching to brightfield is possible	445314-0000-000
Condenser for photometry		insert suitable ICS objective in condenser carrier for photometry 452126-0000-000	in preparation

Microscope Components

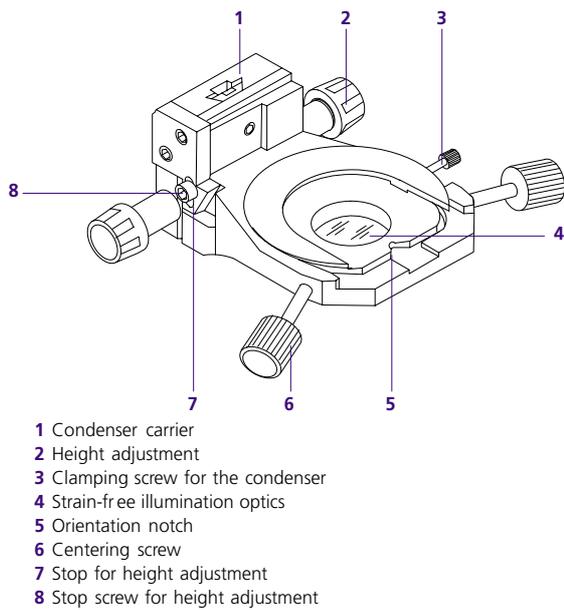


Fig. 33 Condenser carrier

Condenser carrier

(Fig. 33)

The condenser you selected is contained in the condenser carrier. These are its control elements:

- Height adjustment on both sides (max. 34 mm) (2). The ease of motion is factory-adjusted (to be changed only by the service staff).
- Clamping screw (3) for the condenser (used only for condenser exchange using SW 3 key).
- Two centering screws (6) for the condenser. These are used to center the luminous-field diaphragm image for the setting of KÖHLER illumination. To prevent the specimen from being pressed out of the object holder by mistake, the height movement of the condenser is limited by a stop screw.

To prevent the specimen from being pressed upward out of the object holder, the height movement of the condenser is limited by a stop screw.

The stop (7) is adjusted as follows:

- Loosen stop screw (8), pin will fall downwards.
- Adjust the specimen (use a thick specimen mount).
- Image the luminous-field diaphragm (close it until it becomes visible).
- Move the condenser slightly upwards (diaphragm image becomes unsharp).
- Press stop screw (8) upwards and - tighten it again.

Your specimen can now no longer be touched by the condenser.

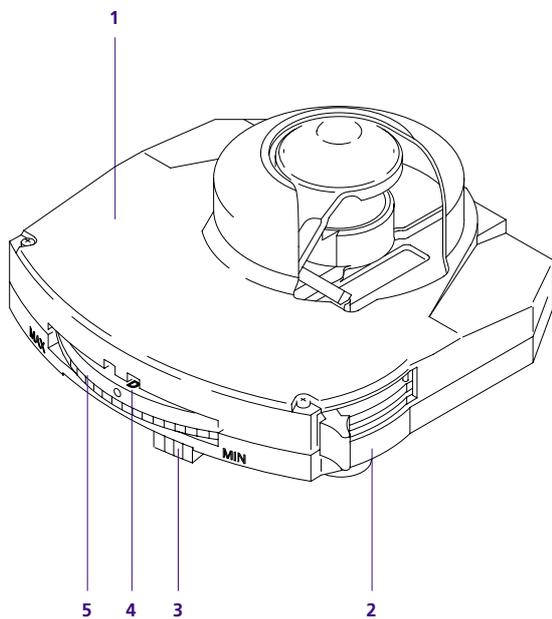


Fig. 34 Condenser modules

Achromatic-aplanatic condenser system

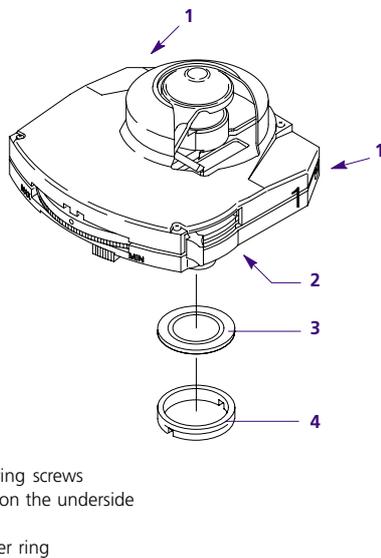
(Fig. 34)

Depending on the version, the achromatic-aplanatic universal condenser (1) is equipped with:

- Fold-out front lens
- Turret disk for:
 - brightfield – without or with DIC I, II, III
 - darkfield – D
 - phase contrast – Ph 1, Ph 2, Ph 3
 - interference contrast – DIC I, II, III
- aperture diaphragm (iris stop)

The front lens is folded in / out using lever (2). Turning the turret wheel (4) swings the brightfield insert and the contrast stops in the beam path. The abbreviation of the set turret position (e.g. D) is displayed in one of the windows (5). Slider (3) opens and closes the aperture diaphragm.

Microscope Components



- 1 Centering screws
- 2 Cover on the underside
- 3 Stop
- 4 Retainer ring

Fig. 35 Changing the phase stop

Changing the phase stops or darkfield stops in the universal condenser (if required)

(Fig. 35)

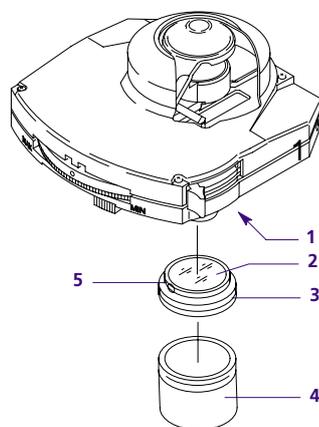
- Tighten both centering screws (1) until stop using SW 1.5 ball-headed screwdriver.
- To loosen the cover on the condenser underside (2), loosen both grub screws using SW 2 screwdriver and remove the cover. Position turret disk with stop to be changed in the exchange opening and hold it on the knurled ring, since no click-stop is effective in this position.
- Use mounting device contained in tool set to unscrew retainer ring (4) from the condenser underside (2) and allow phase stop (3) to slide out.
- To insert the new stop, turn condenser round, insert the new stop, and tighten retainer ring. The shiny glass underside of the stop must point downwards when the condenser is inserted.
- Loosen both centering screws and recenter stop using the centering telescope. Make sure that the correct label is visible on the knurled ring of the turret when the unit is swung into the beam path.

Note: Phase or darkfield stops may only be inserted in centerable openings, DIC prisms only in openings with an iris stop.

Changing the DIC prisms in the universal condenser

(Fig. 36)

The positions of the DIC prisms are marked with I, II, III.

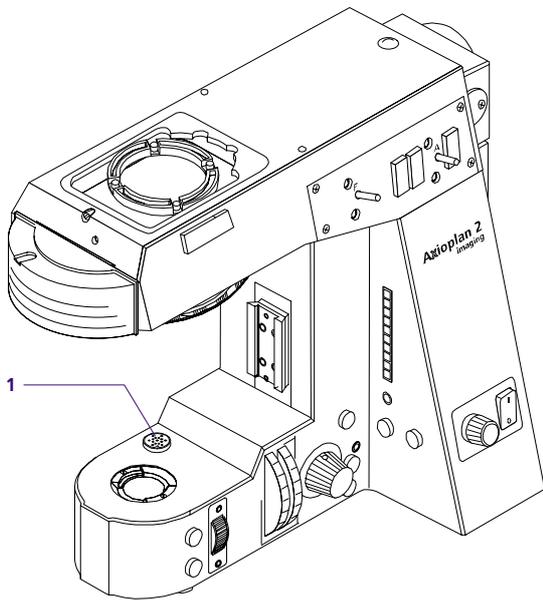


- 1 Cover on the underside
- 2 DIC prism
- 3 Prism mount
- 4 Double-function tool
- 5 Groove

Fig. 36 Changing the DIC prism

- To loosen the cover on the condenser underside (1), loosen both grub screws using SW 2 screwdriver and remove the cover. Position turret disk with DIC prism to be changed in the exchange opening and hold it on the knurled ring, since no click-stop is effective in this position.
- Unscrew retainer ring using the so-called double function tool (4).
- Screw threaded side (4) of the tool into the prism mount (3) and then pull out the DIC prism.
- Remove the DIC prism (2) from the tool and screw on the new, required prism.
- Installation of the DIC prism is made in reverse order. Make sure that the DIC prism is oriented correctly (groove (5) must engage in the pinion of the mount). Make sure that the knurled ring of the turret disk is labeled correctly.

Microscope Components



Achromatic-aplanatic condenser 1.4

The achromatic-aplanatic condenser 1.4 is equipped with fixed front lens and turret disk. The turret disk contains 6 positions and accepts max. 2 DIC prisms, 2 phase stops and 1 darkfield diaphragm. A position for brightfield with iris has been provided. The DIC prisms are inserted in the condenser 1.4 with the same orientation as in the achromatic-aplanatic system condenser. A retaining ring is used to secure them in position on the condenser 1.4.

Attachment of motorized condensers to the stand

The stand E or the motorized stand with light manager is required to enable the integration of motorized condensers. The motorized luminous-field diaphragm, mortised filter wheels and at least a coded nosepiece are available.

- Switch off instrument.
- Insert plug of the condenser into the socket on the left side of the stand base and secure it using the knurled ring.
- Insert ring dovetail of condenser into the condenser carrier and clamp it.
- Switch on instrument again. The instrument automatically recognizes the condenser type used.

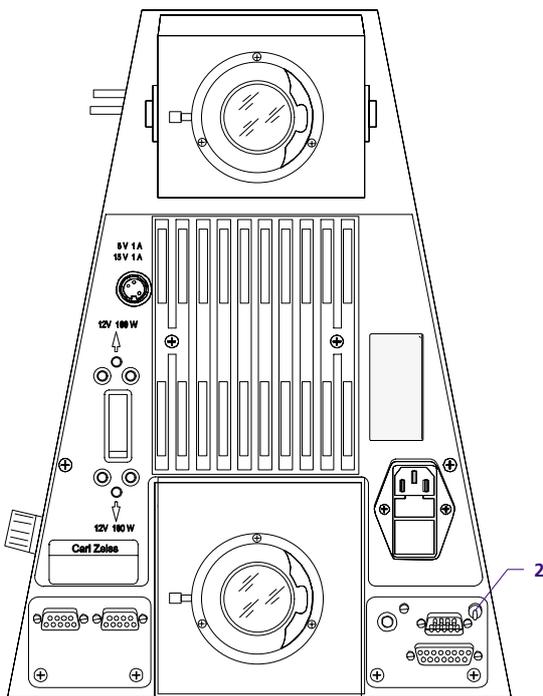
Normally, the microscope is equipped with objectives and condenser in the factory and the basic settings of the light manager (object field adjustment, aperture adjustment and, if required, brightness adjustment) are performed afterwards. The parameter settings of the light manager are made with reference to the objective in the beam path and stored accordingly. If certain parameters need to be changed, this can be performed manually on the condenser.

The SET key (2, on the instrument back) permits the new parameters to be stored. The light manager will then use these parameters.

Regardless of this, it is always possible to operate motorized condensers via PC, which is even recommended for reasons of higher operating convenience.

The achromatic condenser 0.8 H D Ph mot. (445446) also offers the possibility of motorized switching of the turret disk by pressing a key on the condenser. The aperture iris diaphragm is manually set using the knurled wheel.

Note: When an objective or the entire nosepiece equipment is changed, resetting of the light manager in accordance with the new instrument configuration is absolutely necessary.



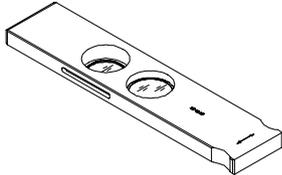
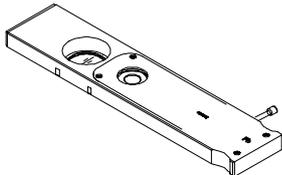
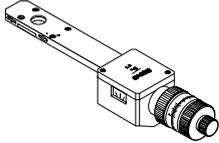
- 1 Connection socket for the plug of the motorized condenser
- 2 SET button

Fig. 37 Back of instrument

Microscope Components

Analyzers, Compensators, Auxiliary Objects and DIC Sliders

Overview

Specification		Cat. no.
Analyzers		
Fixed analyzer slider		453657-0000-000
Slider with analyzer and lambda plate, rotary		453663-0000-000 in preparation
Rotary analyzer		453662-0000-000
Slider for quartz depolarizer		453659-0000-000
Quartz depolarizer $d = 32$ mm (can also be used in 453657-0000-00)		453653-0000-000
Bertrand lens sliders		
Bertrand lens slider Ph		453671-0000-000
Compensators		
Compensator λ 6 x 20 mm		473704-0000-000
Compensator $\lambda/4$. 46 x 20 mm		473714-0000-000
Quartz wedge 0 ... 4 λ , 6 x 20 mm		473724-9902-000
Sénarmont compensator 546:4, 6 x 20 mm	available as customized product	473718-0000-000
Rotary Brace - KÖHLER compensator $\lambda/8$		473716-0000-000
Tilting compensator E 0 - 6 λ	available as customized product	473715-0000-000
DIC-sliders for ICS optics (only usable with DIC nosepiece or nosepiece Pol 1x)		according to price list
Polarizers		
Fixed polarizer D		453615-0000-000
Rotary polarizer D		453620-0000-000
Fixed polarizer D with rotary λ -plate		445226-0000-000

Microscope Components

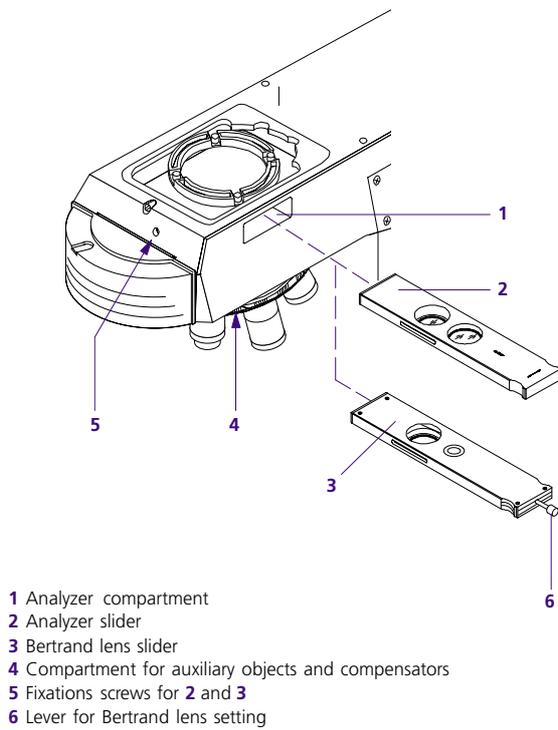


Fig. 38 Mounts for sliders, analyzers and auxiliary objects

General Information

(Fig. 38)

The components listed in the overview on page 57 are used to regulate the optical contrast in the microscope and to adjust the auxiliary devices necessary to regulate the contrast. Compensators are required for contrast enhancement and measurements.

In brightfield/phase contrast, the Bertrand lens slider (3) is inserted at (1), and the fixed or rotary analyzer slider (2) or the quartz depolarizer is included in the DIC equipment.

Compensators are inserted in compartment (4).

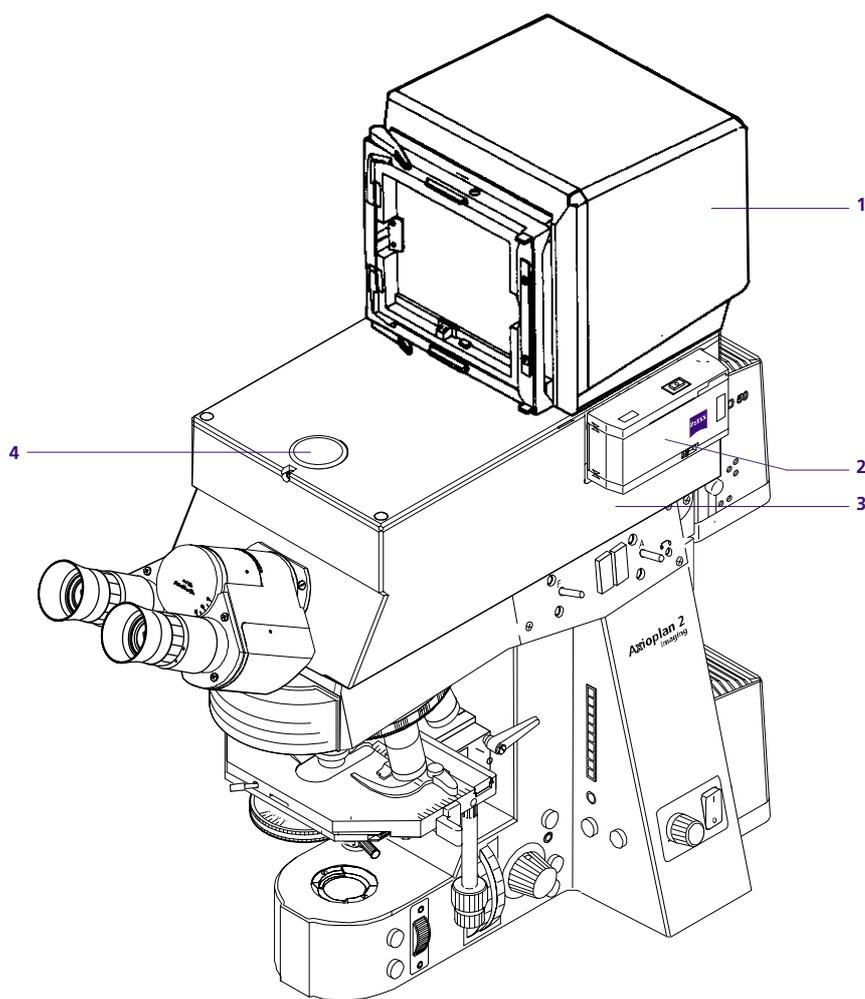
Note: To allow the use of DIC sliders, your microscope must be equipped with an objective nosepiece for DIC.

Please refer to page 67 for information on how to adjust the microscope with polarizers, analyzers and DIC prisms for differential interference contrast.

Microscope Components

Axiophot 2 Photo module

Overview



- 1 4x5" large-format camera
- 2 35 mm Mot film cassette (right)
- 3 Axiophot 2 Photo module
- 4 Port for TV camera

Fig. 39 Axiophot 2 Photo module

Microscope Components

General Information

The Axiophot 2 Photo module turns your microscope into a universal and completely new photomicroscope. Motorized control elements and operation via notebook (or PC) only makes work with the Axiophot 2 methodically richer and at the same time considerably easier.

If you opt for a microscope equipped with the Axiophot 2 Photo module, we will supply you with the completely assembled stand, including an intermediate tube, if required. Installation and setup of the entire instrument and retrofitting of the photo module to existing instruments (hardware and software) will be performed by our service personnel oder Außendienst.

Key features and technical data

- Two 35 mm cameras (35 mm Mot film cassette) and one 4x5" camera with automatic exposure control
- With 35 mm film cassette: automatic film advance, automatic advance to 1st picture when a new film is loaded, and motorized rewinding
- Decimal display of exposure time, countdown during exposure
- Automatic exposure time extension for exposures requiring long exposure times (reciprocity failure compensation) in 9 steps
- Optional spot metering or center-weighted averaging
- Possibility of fixing the automatic exposure time for comparison photos
- Multiple exposures
- Exposure corrections: 3 exposure values; maximum correction amount: 1/3 exposure value
- Automatic autobracketing with preselected correction (calibration series, etc.)
- Illuminated frame visible with both eyes, adjustable brightness
- Projection of data and reference scale
- Motorization of following settings:
 - switchover observation/documentation
 - switchover camera/video port
 - switchover light metering/film
 - switchover center-weighted averaging/spot metering
 - switchover of cameras

Standard 35 mm film cartridges 135 (visible in the window) are used for the 35 mm Mot DX film cassette; for the 4x5" back of the large-format camera, sheet-film, Polaroid sheet-film and pack-film cassettes (000000-0000-545 and 550) and roll-film cassettes on plate for international back are used.

Magnification on film for 35 mm film:

objective magnification x 2.5,

for 9 x 12cm/4x5" large formats:

objective magnification x 10.

35 mm Mot DX film cassette

Removing the cassette

- Use your two hands to grasp the film cassette on the right and left, press unlocking button (2) until stop using your thumb, and only then pull cassette off in upward direction.

Inserting a film

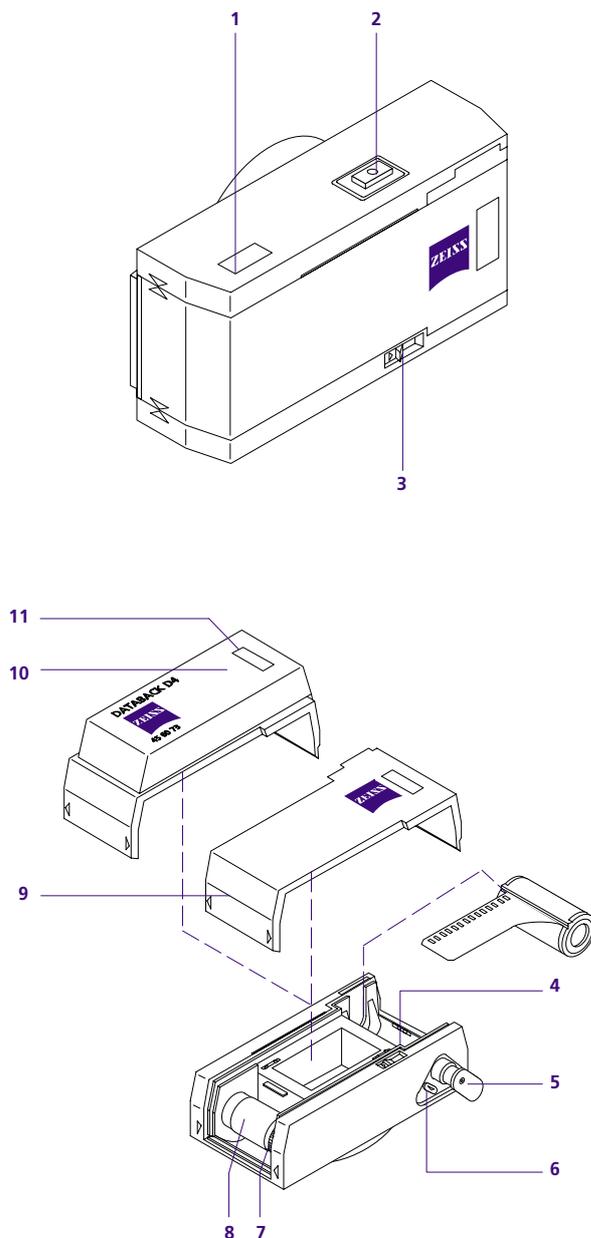
- Push locking slider (6) in arrow direction. This will cause the cartridge holding pivot (5) to pop out. Now the D4 databack (10) or plain cassette back (9) can be removed.
- Insert cartridge into cartridge compartment (4).
- When pulling the film out of the cartridge, make sure that it is flat; place the film leader on the rubber cladding of the take-up reel (8) in such a way that your fingers do not touch the rubber cladding. Press in the cartridge holding pivot (5).
- Insert plain back (9) or D4 databack (10) on left side (see arrows on the backs and on the cassette) and press right side of the back to snap it shut. Make sure again that the rubber cladding of the take-up reel is not touched. Mechanical frame counter (1) shows S (Start).

Note: For No. 135 (35 mm) film you may use film cartridges with or without DX coding. We advise you not to use bulk film (see page 79).

Carefully clean (remove remainders of lubrication) the rubber cladding of the take-up reel (8) after exposure of 60 ... 80 films or when problems arise with the film advance (motor is not switched off, mechanical counter (1) is not set to S (Start). Cleaning agents: Q-tips and water with a little washing-up liquid.

- Set rewind button (3) to R.
- Soak Q-tips in the water with washing-up liquid.
- Use Q-tips to clean rubber cladding of the take-up reel, move reel by turning its knurled ring (7). If required, repeat cleaning procedure until the cotton remains clean.
- Dry humid rubber cladding using a dry Q-tip.

Microscope Components



- 1 Mechanical counter
- 2 Eject key
- 3 Slider for film rewinding
- 4 Compartment for film cartridge
- 5 Cartridge holder
- 6 Lock for back of cassette
- 7 Knurled ring
- 8 Take-up reel
- 9 Back of cassette
- 10 Data back for film cassette
- 11 Control window

Fig. 40 35 mm Mot film cassette

Attaching the cassette

- Hold film cassette with both hands on the right and left of the camera body. The unlocking button (2) shows to the user and must not be pressed during attachment.
- Hold the film cassette parallel to the upper edge of the camera body and insert it in the basic body until the unlocking button (2) jumps out. The film leader is wound automatically until the mechanical counter (1) reads 1.

After exposure of the first frame (and, if a databack is used, exposure of the data), the film is automatically advanced to the next frame. The mechanical counter (1) of the film cassette displays the current frame number. When the end of film is reached, the film advance motor is shut down; the left-hand display window of the control console shows the message **FILM END**.

Rewinding the film

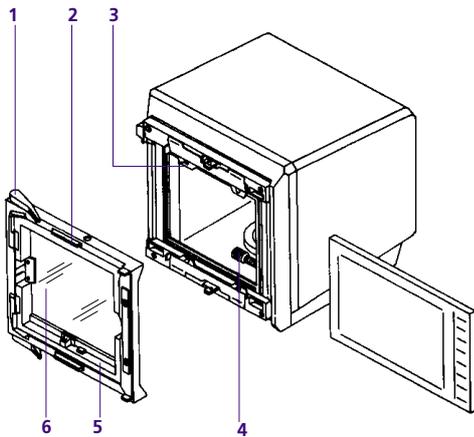
Operating slider "R" (3) automatically rewinds the film, the END display blinks.

After unloading the film, the slider "R" is automatically set to the normal film advance position when the camera back is attached.

Data back for 35 mm Mot DX film cassette

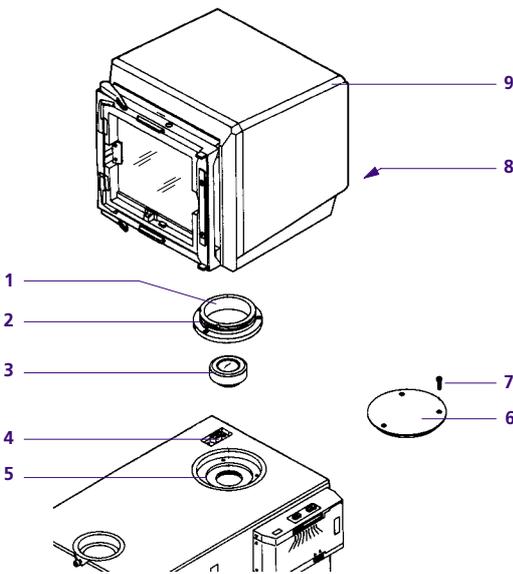
The data back (10) is attached to the Mot film cassette instead of the standard back (9). The film cassette with data back is then attached as usual.

Microscope Components



- 1 Lever
- 2 Key for unlocking
- 3 Lock
- 4 Knurled screw
- 5 Cassette mount
- 6 Groundglass

Fig. 41 4 x 5" large-format camera



- 1 Mounting ring
- 2 Notches
- 3 Large-format optical system
- 4 Socket
- 5 Holder
- 6 Cover
- 7 Screws
- 8 Clamping screw
- 9 4x5" large-format camera

Fig. 42 Mounting the 4x5" large-format camera

4x5" Large-Format Camera (Fig. 41)

Large-format groundglass and cassette mount (5). Cassettes for universal camera backs are slid behind the large-format groundglass which can be lifted with lever (1). To take off the groundglass:

- Press (2) and move it to the right; mount accordingly. The groundglass can be used for microprojection for small discussion groups, provided there is sufficient light: This requires that you perform the following steps in the **Photo** program module:

- Select 4x5" camera; T ; **START** opens the shutter for observation; pushing **START** closes it again.

Knurled screw (4) for the positioning of data on different film formats becomes visible at the lower right after removal of the groundglass (6). Control of positioning on the groundglass (ASA 12: it lights for approx 1 s).

Mounting the 4x5" large-format camera (Fig. 42)

Proceed as follows to mount the 4x5" camera to your Photo (9) module:

- Remove cover (6) by screwing out 3 screws (7) using hexagonal screwdriver.
- Screw supplied large-format optical system (3) into the mount.
- Insert mounting ring (1) for 4x5" camera into the opening in such a way that the notches (2) point towards the user and tighten it using the 3 screws (7).
- Loosen clamping screw (8) at 4x5" camera and move the camera to the red dot.
- Remove protection cap. Attach 4x5" camera and make sure that the optical system exactly fits the mount.
- Swing clamping screw (8) to the right and tighten it.

Second TV camera

Instead of the 4x5" camera, a second TV camera can be mounted as follows:

- Remove cover (6) by screwing out 3 screws (7).
- Screw on TV adapter (452230-0000-000) using the 3 screws.
- Only use TV adapter 1.0x (456105-0000-000) to connect TV camera C 2/3"; TV adapter 0.63x (456107-0000-000) to connect TV camera C 1/2" and TV adapter 0.50x (456106-0000-000) to connect TV camera C 1/3"
- A 3C-CTV camera cannot be connected to this port.
- The camera factor of the TV camera is obtained by multiplying the factor of the TV camera by 2.5.

Microscopy Techniques

Transmitted light brightfield

To set the incident light illumination in accordance with the KÖHLER principle, proceed as follows:

- On the rear of the instrument, switch on the transmitted light illumination using the toggle switch and then switch on the microscope at the ON/OFF switch (9).
- At voltage regulator (8) set approx. 3 ... 4 volts as the supply voltage for the illumination.
- First, place a high-contrast specimen on the stage (small, thin coverglass face up!).
- Swing in 10x objective (yellow ring) (1) on nosepiece into beam path.
- Check the 0 positions on the eyepiece scale (→ page 39).
- Raise the condenser - do not swing front optics to the side to the microscope slide.
- Set H (brightfield) on index of condenser turret and use knurled ring (3) of turret disk to close aperture diaphragm to approx. half its size.

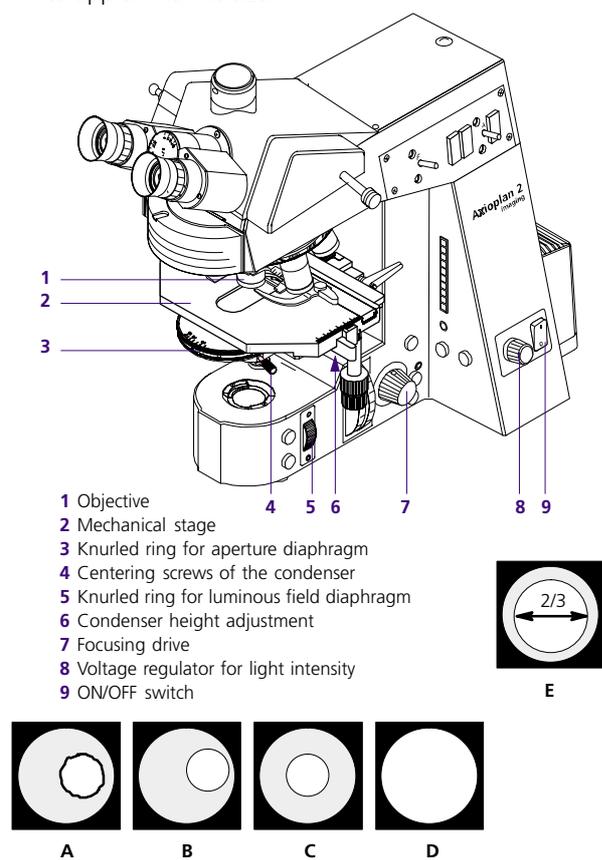


Fig. 43 Microscope setting for transmitted light brightfield

You should now see spots of light (the exit pupils) behind the eyepieces. If you are working with a binocular phototube, all of the light will be directed to the binocular tube if the push-rod is slid in all the way.

When you look into the tube you will see a bright circle (the eyepiece stop) with each eye.

- Merge the two circles into one by adjusting both eyepieces tubes to your PD.
- Focus the specimen using the focusing drive (7). The eyepiece setting for spectacle wearers should be "0" (if you are working without glasses (→ *Microscope components, Eyepieces, page 39*).
- Close luminous field diaphragm with knurled ring (5) moderately. It will then appear unsharp in the image (A).
- Focus the diaphragm image (B) by lowering the condenser slightly with (6).
- Use centering screws (4) of the condenser to move the diaphragm image to the center of the field of view (C).
- Open luminous field diaphragm on knurled ring (5) until it just disappears from the field of view (D).
- Now adjust the contrast with the aid of the aperture diaphragm (3) to suit the needs of the specimen being examined. The value of the aperture diaphragm can be read off on the scale of the condenser.

Note: If you are not certain how much to stop down, proceed as follows: for objects displaying moderate contrast, approx. 2/3 of the exit pupil of the objective should be illuminated (E) (eyepiece removed) using the aperture diaphragm (3).

Every objective change also changes the field of view and the objective aperture, i. e. the above procedure must be repeated.

- When a low-power objective images more than the condenser can illuminate, swing the condenser front optics out of position via the lever and lower it, if required.
- If your microscope features a light manager, the illumination is set automatically. You can store the microscope setting via the SET key (→ *Stand, Light Manager*).

If your instrument is motorized and you are using the microscope software via a notebook/PC, you can store a total of 16 reproducible microscope settings (→ *Microscope Software*).

Microscopy Techniques

Transmitted light darkfield

Use

- ❑ To examine small and extremely small specimens and specimen features such as treponemas, spirochaetae, flagella, bacteria, etc. or emulsions if the contrast supplied by phase contrast is insufficient.
- ❑ If the inherent colors of natural, i.e. unstained, specimens such as living organisms in water (algae, protozoa, lower order animals) are clearly visible.

Additional equipment

- ❑ Always required: a condenser with a central stop whose numerical aperture is higher than that of the objective used.
- ❑ Objectives with aperture 1.0 must feature an integrated iris aperture diaphragm.

Adjustments required

- ❑ Set the illumination as for brightfield; the luminous field diaphragm must be imaged and centered. If the height of the condenser has been correctly set, an almost sharp image of the luminous field diaphragm will be obtained.
- ❑ Check the objective pupil to ensure that it really is dark.

Note: With darkfield illumination, there may be a ring of light in the pupil which you should eliminate by focusing the condenser and (if an iris objective is available) by closing the iris diaphragm. The decisive criterion of a high-quality darkfield is, of course, a totally black background in the field of view.

Additional notes

Darkfield requires even cleaner specimens than other methods; films of grease (fingerprints) in particular will cause lightening of the background.

The somewhat difficult adjustment of the darkfield is simplified by initially precentering with a low-power objective. As the luminous field can only be seen in areas where particles light up and since large areas of the specimen ultimately examined may contain no such particles, a specimen displaying uniform detail distribution should be chosen, e.g. a blood smear.

Plan-Neofluar	Plan-Apochromat	Illumination
10x/0.30	10x/0.32	Ph stop $3 \geq 0.44$
20x/0.50 40x/0.75 D	20x/0.75	darkfield stop 0.76 - 0.90
100x/1.3 oil iris	40x/1.0 oil iris 100x/1.3 oil iris	dry darkfield condenser 0.8/0.95 ultra darkfield con- denser 1.2 - 1.4 oil

The table shows recommended combinations for some selected objectives

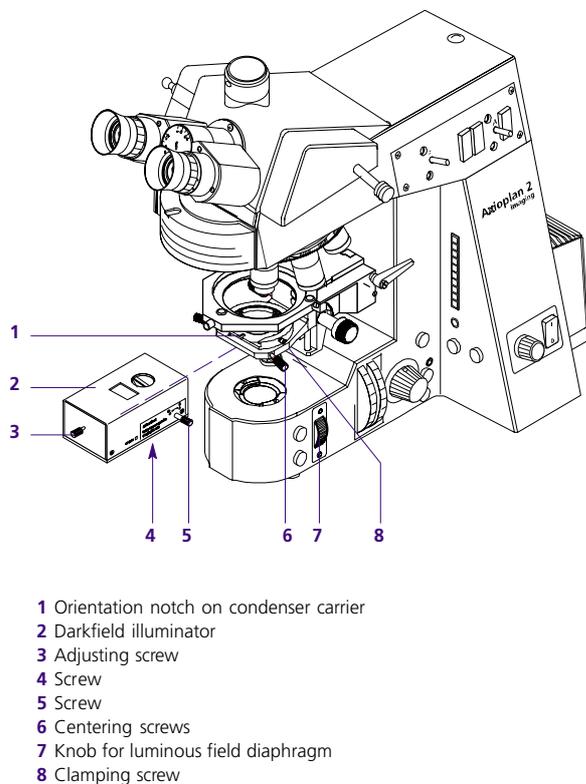


Fig. 44 Microscope configuration for darkfield

Darkfield illumination in low magnifications

Darkfield illuminator (2) 445314-9901
 (for low magnifications from 1.25x to 20x)



Glare hazard!

When switching from darkfield to brightfield, the lamp brightness must be reduced under all circumstances.

CAUTION!

Setting the optimum darkfield illumination

- Insert illuminator (2) in lowered condenser carrier, ensuring that screw (4) on ring dovetail engages with orientation notch (1). Clamp with screw (8).
- Use screw (5) to select darkfield setting (see symbol).
- Move illuminator under the stage carrier with aid of condenser drive.
- Select objective 1.25x.
- Place auxiliary, strongly scattering specimen (strip of paper) on the stage and focus.
- Close luminous field diaphragm (7). Use screw (3) to produce uniform vignetting on the upper and lower edges of the field of view. Produce right/left symmetry with the two centering screws (6) of the condenser carrier. For technical reasons, the vignetting remaining in the upper and lower areas of the field of view is inevitable with the 1.25x objective. However, the photo frame is uniformly illuminated.
- Place specimen in position; use large slides and large coverglasses, as the light-scattering edges then lie well outside the object field.
- If the image brightness is insufficient, the diffusion screen can be removed from the illumination ray path without causing any drawbacks. Opening of luminous field diaphragm (7) also directs more light to the specimen, but also more reflections outside the photo frame.
- When switching to next highest objective magnification (2.5x), turn screw (3) until the field of view is fully illuminated without reflections. Adjust the luminous field diaphragm until the image background is optimally dark. With higher objective magnifications up to 20x, adjust the luminous field only without changing the illuminator setting.
- This illuminator can also be used for brightfield by swinging out the deflection mirror via screw (5).

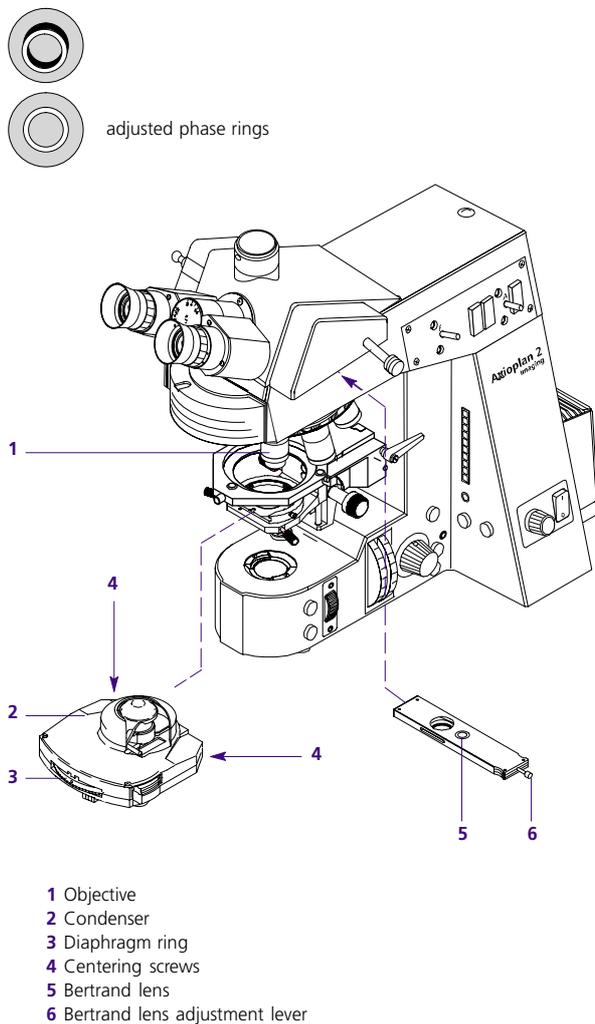


Fig. 45 Microscope configuration for phase contrast

Phase contrast

Use:

These techniques are used for unstained specimens in particular in order to enhance their contrast.

Additional equipment

- Objectives (1) designated Ph. These can also be used for brightfield.
- A condenser (2) featuring Ph positions.

Additional adjustment

The phase rings in the various objectives are of different sizes and marked Ph1, Ph2 and Ph3 on the objective (1). The turret bears the same designations, e.g. Ph 1.

- Combine the designation on the turret disk with the corresponding objectives.

Perfect phase contrast is only obtained if the (dark) ring in the objective and the (bright) ring in the condenser exactly coincide.

The Bertrand lens slider (5) provides more convenient viewing of the objective pupil, especially with phase stop centering.

- Loosen screw (Fig. 38/5) visible on the front of the stand using Allen key SW3 until the slider can be inserted and tighten it again until the stops become effective.
- When moved to the left, the Bertrand lens, focused via a lever (6), is effective.

(Checking without this attachment is also possible with the eyepiece removed, in the same way as for the condenser diaphragm).

If the centration is not perfect (the two rings must coincide as shown in Fig. 45) this can be corrected by using centering screws (4) and SW 1.5 key. This type of centration is suitable for all condensers designed for phase contrast.

The centration remains unchanged when the condenser disk is turned or changed, and even if the entire condenser is re-moved.

To enhance the contrast, a green filter can be moved into the ray path either via the filter wheel or placed on the light exit (transmitted light) or the color glass carrier.

Note: Meticulously clean glass-to-air specimen surfaces (fingerprints) are of greater importance in phase contrast than in brightfield. Diaphragm ring (3) of the condenser has no function, since the Ph openings do not contain iris diaphragms. The diaphragms in the Ph positions of the condenser are part of the front optics with which the condenser was supplied; if the front optics are changed, other diaphragms are required.

Microscopy Techniques

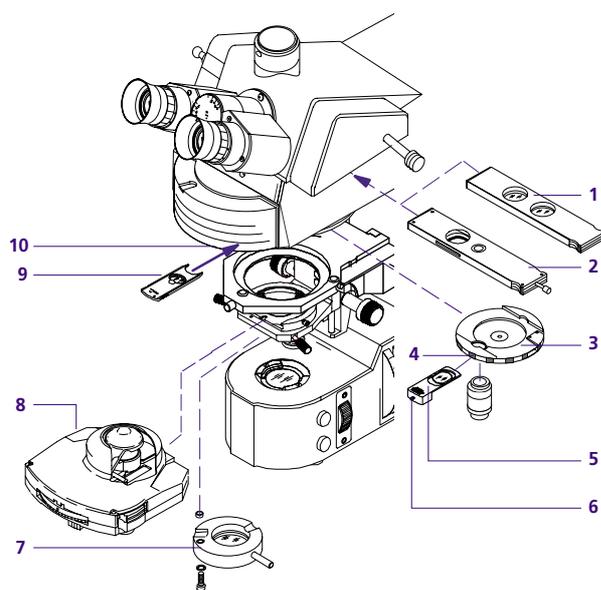
DIC - Differential interference contrast in transmitted light

Use:

- For unstained specimens which are too thick for phase contrast examination, with the result that layers of the specimen outside the focal plane impair the clarity of the image.
- If the halo typical of phase contrast impairs the observation of small details in the specimen.

Additional equipment

- normally Plan-Neofluar objectives,
- a special nosepiece (3) with slots (4) for mounting the DIC sliders,
- DIC slider (5) showing on its surface the magnification and aperture of the objective for which it is intended. Insert DIC slider in slot (4) until the click stop is reached.
- a condenser turret (9) with DIC positions,
- a polarizer (8) which is swung into position beneath the condenser and
- an analyzer slider (1) which is inserted on the right side of the upper stand part.



- 1 Analyzer slider
- 2 Bertrand lens slider
- 3 Nosepiece
- 4 Slot for DIC slider
- 5 DIC slider
- 6 Knurled screw
- 7 Polarizer
- 8 Condenser turret
- 9 Compensator
- 10 Compensator compartment

Fig. 46 Microscope configuration for Differential Interference Contrast

Additional adjustments

Like the 3 (or 2) Ph positions of the condenser, there are 3 (or 2) DIC positions, marked I, II, III, suitable for combination with the appropriately marked DIC sliders.

This permits the following combinations:

Objective	DIC position
10x/0.30	DIC I condenser setting
20x/0.50	DIC II condenser setting
40x/0.75	
usually $\geq 40x$	DIC III condenser setting

Unlike the Ph positions, the DIC positions are provided with an iris diaphragm (10). Open this completely at first. To enhance contrast, it can then be closed slightly, this generally being the last stage in the adjustment (see also "KÖHLER illumination", page 63).

Optimum contrast is set by using the knurled screw (7) of the DIC sliders (6) in the nosepiece.

- Swing in the DIC-suitable objective via the nosepiece. Insert the appropriate DIC slider into the nosepiece compartment until click-stop.
- Swing in the DIC analyzer module on the reflector.
- Swing in the suitable DIC prism I, II or III (number visible on the condenser turret disk). No further steps are required if a condenser with integrated polarization filter (e.g. 445439-0000-000) is used. Otherwise, a polarization filter must be inserted below the condenser.

Microscopy Techniques

DIC setting using the SENARMONT polarizer

The following must be taken into consideration when a SENARMONT polarizer, consisting of a carrier with $\lambda/4$ plate (above) and a rotary polarizer (below), is used:

- Insert SENARMONT polarizer below the condenser until click-stop.
- Set luminous-field diaphragm and aperture diaphragm in accordance with the rules of Köhler illumination.
- Set optimum contrast using the knurled screw on the DIC slider. When the DIC slider is moved symmetrically around its center position, specimen details can be displayed in 3D in such a way that they appear in different planes.
- In SENARMONT DIC, the optimum contrast is set by turning the polarizer using the rod in the lower part of the SENARMONT polarizer. The $\lambda/4$ plate above it must always be swung in to allow contrasting according to SENARMONT to function. If the DIC slider has been moved to the center position before, there will be darkness in the click-stop position of the SENARMONT polarizer, i.e. the path difference is 0 nm in this position.

Additional notes

In DIC, contrast is created by (pseudo) relief and, in the case of linear structures, is therefore dependent on their orientation: with orientation in the same direction as the "light shadow", contrast is low, while in the direction perpendicular to the shadow optimum contrast is obtained. The possibility of specimen rotation is therefore (almost) imperative for adjustment. Please bear in mind that the mechanical stage can be used as a rotary stage.

To ensure reflection-free illumination, the luminous field and aperture diaphragms should not be opened any wider than allowed for the KÖHLER principle.

DIC uses polarized light and is therefore impaired if "optically active" elements are located between the polarizer (7) and the analyzer (1), e.g. foils which are sometimes used for histological sections, or plexiglass culture dishes with plastic bases (dishes with glass bases are also available).

Color DIC is obtained if you use compensator λ (9) (473704-0000-000). This slider may be used only in transmitted light.

Use the reflector module DIC Red I (000000-1046-278) for color DIC in incident light.

The DIC prisms in the DIC positions of the condenser are part of the front optics supplied with the condenser on delivery.

Microscopy Techniques

Transmitted-light polarization - Detection of birefringence

Use

This technique is used for the examination of transparent, birefringent objects. It is a characteristic feature of birefringent objects that, with polarizer and analyzer in a crossed position, the otherwise dark field of view turns bright 4 times when you rotate the object stage through 360°. In the process, interference colors from only just visible gray (e.g. biological specimens), white, red, yellow blue etc. up to higher-order white can occur, regardless of birefringence, the thickness and the orientation of the object.

Adjustments

- Adjust your polarizing microscope for standard brightfield examination (see → page 63).
- Center the rotary stage Pol (see → page 51) to ensure that the optical axis of the objective in the non-centerable turret opening has been centered on the rotary axis of the stage. If this alignment has been performed correctly, an object feature in the center of the reticle will remain in position when the stage is turned.
- Tighten screw (2) locking the stage in position and swing the first objective in a centerable threaded mount into the beam path. If the threaded mount is not centered, the previously focused object feature will no longer be in the center of the reticle.
- Bring the displaced feature into the center of the reticle by adjusting the two centering screws of the objective using two small Allen wrenches. The centering screws are located on the left and the right of the objective position on the knurled ring of the nosepiece (4). Proceed in the same manner for the next centerable threaded mount of the nosepiece. After you have centered all objectives, loosen the screw locking the stage rotation.
- Re-check the position of each objective by turning the stage. An object feature located in the center of the reticle should now remain in the center.
To maintain this centration, we recommend using the knurled ring of the nosepiece to change the objectives instead of the objectives themselves.
- Swing the polarizer (3) into the beam path and adjust it to 0°, if your microscope is equipped with a rotary polarizer.

- Insert the analyzer (1) until it snaps in and the field of view is dark.
If you work with a measuring analyzer, adjust the measuring scale to 90° and secure it in position.
If you use a $\pm 10^\circ$ rotary analyzer with a rotary λ -plate, adjust the analyzer to the central clickstop.
The λ -plate will be ineffective if it is positioned above the analyzer, and effective if it is below the analyzer.
- Bring the object to be examined into the field of view and turn the stage including the object.
As described above, colorless or colored changes of the object indicate the presence of birefringence. Optically anisotropic materials can also remain dark, however, if an isotropic direction, e.g. of optically uniaxial or biaxial crystals, is parallel to the viewing direction.
In this case, you can establish whether the object is optically isotropic or anisotropic by using the conoscopic viewing method (see → page 72).

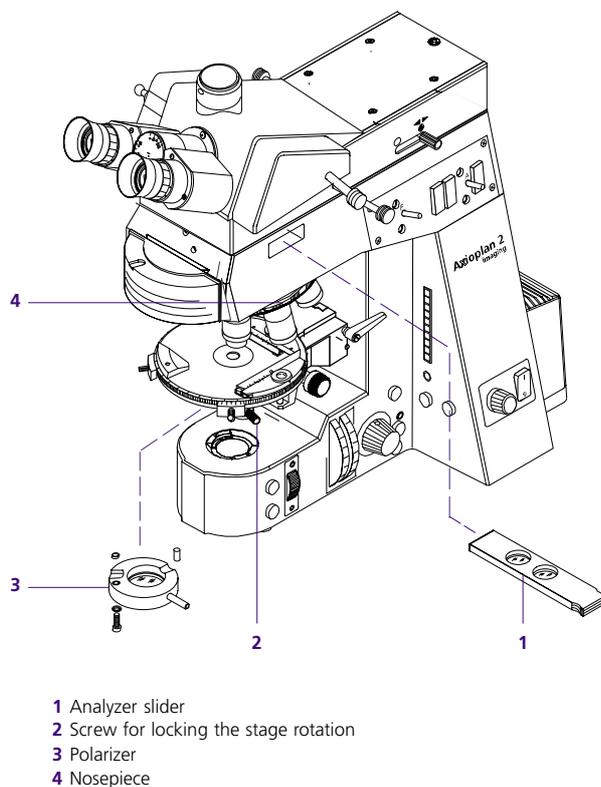


Fig. 47 Transmitted-light polarization

Microscopy Techniques

Transmitted-light polarization - Determining the n_{γ} direction of oscillation

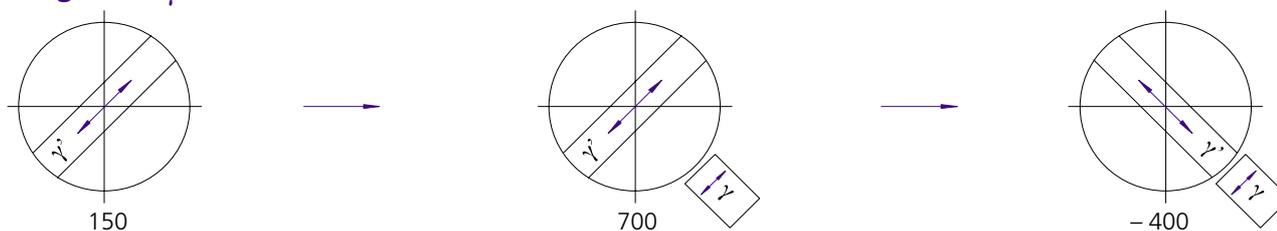


Fig. 48 Determining the n_{γ} direction of oscillation using a synthetic fiber as an example

Use

The position of the two directions with the greatest (n_{γ}) and the smallest (n_{α}) refractive index (both in relative terms) or with the greatest (n_{γ}) or with the smallest (n_{α}) refractive index (both in absolute terms) relative to the morphological directions of crystal surfaces, crystal needles or fibers is an important feature used, for example, in the diagnosis of biocrystals (gout, pseudogout).

Adjustments

- Perform the adjustments described under Adjustments on page 69. The fiber lights up in the field of view.
- Turn the stage to the first clickstop, deactivate the clickstop and turn the stage in such a way that the object displays maximum darkness.
- Activate the clickstop and turn the stage by 45° to the next clickstop so that the longitudinal axis of the fiber is oriented in the NE-SW direction (Fig. 48). Here, the object displays maximum brightness (diagonal position), e.g. gray-white.
- This color corresponds to a path difference of 150 nm in the Michel-Lévy color chart (Fig. 48 and Fig. 49).
- After you have inserted the compensator λ , the color turns to yellow-orange (path difference approx. 400 nm).
- When you turn the stage by 90° , the fiber appears green-blue (path difference approx. 700 nm).

Conclusions

The n_{γ} direction of the compensator λ is oriented in the NE-SW direction. The color of the surroundings of the fiber is a deep red of the 1st order (path difference is one λ ; approx. 550 nm). The fiber itself appears green-blue (path difference approx. 700 nm). The higher interference color (700 nm) can only be the result of the addition of the path differences of (approx. 150 nm) and compensator

The colors will be added if the n_{γ} of the compensator and the n_{γ} of the object are parallel. Hence, the n_{γ} of the object also lies in NE-SW direction at a higher interference color and is oriented in parallel to the longitudinal axis of the fiber.

Summary

Compare interference colors (path differences) in the two diagonal positions. The larger path difference will occur if both n_{γ} directions are parallel. Thus, the n_{γ} direction of the object has been determined.

Note:

Michel-Lévy color charts are available under Cat. No. 42-312.

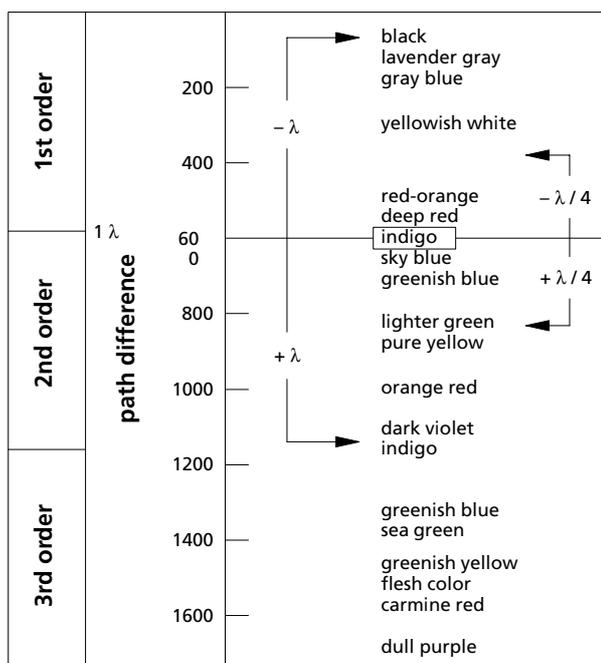


Fig. 49 Schematic illustration of the Michel-Lévy color chart

Microscopy Techniques

Transmitted-light polarization - Determining and measuring path differences

Use

The color chart only allows the rough estimation of path differences of transparent, anisotropic substances, such as minerals, synthetic crystals, plastics, strained glass, biocrystals or erythrocytes. For exact measurement, a compensator is needed. This compensator reduces the path difference caused by the object to zero (1st order black), i.e. it compensates for the path difference. Unlike determining the n_γ direction where the position of addition is of importance, the object must be in the position of subtraction relative to the compensator, i.e. the n_γ of the object must be turned against the n_γ direction of the compensator by 90° .

Selecting the correct compensator

We can provide the suitable compensator for every path difference measurement: rotary Brace-Köhler compensator $\lambda/8$ with a minimum measuring range from 0 to 72 nm and tilting Ehringhaus compensator E 0 - 6 λ with a maximum range from 0 to 3,276 nm. Path differences can only be measured if the path difference of the object lies within the measuring range of the compensator used. To obtain as high measuring accuracy as possible, the measuring range of the compensator used and the path difference should approximately be of the same magnitude. To find the correct compensator, insert your object in the dark field between the crossed polarizer and analyzer. Turn the stage and note the interference colors obtained (see → table below).

Measurement

- Focus on the specimen (objective 10x).
- Bring polarizer and analyzer into crossed position.
- Turn specimen
 - to extinction or standard position,

- activate the stage clickstops,
- set a diagonal position displaying maximum brightness at 455.

Insert the compensator selected, move it out of its zero position and watch whether:

- the interference colors become deeper, i.e. the path difference decreases. The n_γ direction of the compensator and the n_γ direction of the object are perpendicular to each other (position of subtraction). The specimen is correctly positioned for compensation.
- the interference colors become paler (position of addition), i.e. the path difference increases. The black of the 1st order never appears. In this case, turn the specimen by another 90° .
- All you have to do now is to adjust the compensator in such a way that the point of measurement is completely dark. Using the recorded angles, you can read off the path difference in nm from the relevant table or calculate the path difference when you work with the rotary Brace-Köhler compensator.

Sénarmont compensation

The Sénarmont technique allows the measurement of path differences up to 1λ . This technique differs in the following points from the above description:

- a Sénarmont compensator 546/4 is used which is a $\lambda/4$ plate with n_γ in the east-west orientation.
- a rotary analyzer is used for measurement.
- the path difference is calculated using the angle measured.

Note: It is advisable to use monochromatic light for measurement. To achieve this, use the green interference bandpass filter 546, d=32x3 (467807-0000-000).

Interferenc color	Using compensator λ you will obtain	Path difference	Compensator / Technique
Dark gray	colors (blue, yellow), 1st order gray present	$\lambda/10$ (approx. 50 nm)	rotary Brace-Köhler compensation $\lambda/8$
White	colors, 1st order white present	$\lambda/2$ (approx. 270 nm)	de Sénarmont technique or Ehringhaus tilting compensator 0 - 6 λ
More or less deep interference colors	colors or white, colors of 1st to 4th order are present	$\lambda/2$ to 5 λ (approx. 270 to 2700 nm)	Ehringhaus tilting compensator 0 - 6 λ

Microscopy Techniques

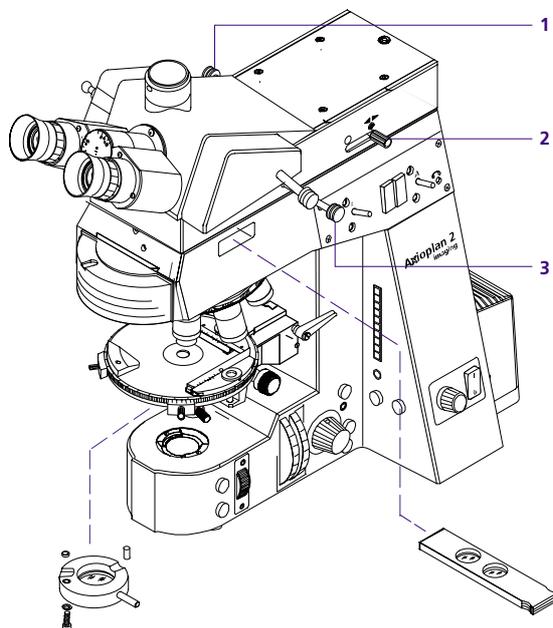
Transmitted-light polarization - Determining the optical characteristics of crystal

Use

Determining the optical characteristics of transparent and weakly absorbing crystals is part of the diagnosis of crystals. This technique is also termed conoscopic observation. Its main field of application is the classic microscopy of rocks. It is also possible, however, to identify and characterize synthetic crystals, industrial minerals and plastics (e.g. films).

Adjustments

- Adjust your polarizing microscope for standard brightfield examination (see → page 63) and polarization in transmitted light (see → page 69) eingestellt.



- 1 Pushrod for field diaphragm
- 2 Rotary and slider knob for Bertrand lens
- 3 Pushrod for reticle

Fig. 50 Transmitted-light polarization

- Bring the low-power objective (2.5x/0.075) into the beam path. The best orientation for conoscopic observation have those crystals (e.g. a thin rock section) whose brightness level is changed as little as possible in orthoscopic observation when you turn the stage. In this case, the optical axis of uniaxial crystals or one of the optical axes of a biaxial crystal is nearly parallel to the viewing direction.
- After selecting a crystal in this manner, position it in the center of reticle (3). Bring the dry objective providing the largest aperture (40x/0.85) into the beam path, swing in the condenser front lens and re-check that the condenser diaphragm is fully open. Lift the condenser until the image of the luminous field diaphragm is sharp (see → page 63).

- Close the field diaphragm (1) until the grain boundaries of the crystal selected are no longer visible. This ensures that the interference figures of neighboring crystals are not superimposed on the interference figures of the crystal to be examined. Thus, object features with as small a diameter as 10 mm can be eliminated from the field of view.

When you turn the stage, the object must remain in the center of the reticle, i.e. it must also remain in the visible area (re-center, if necessary).

- Swing in the Bertrand lens (symbol ⊕) with knob (2) on the intermediate tube Pol. Use the Bertrand lens to focus the pupil image (interference figure).

The conoscopic image obtained now shows you whether the crystal has one or two axes. Use the compensator λ to determine the optical characteristics of the crystal (optically positive or optically negative); the compensator $\lambda/4$ can also be used for this purpose.

Use the wedge compensator 0 - 4λ (quartz wedge) to determine the optical characteristics of absorptive crystals (e.g. augite, hornblende) or crystals with anomalous birefringence.

Uniaxial crystals

If the optical axis of a uniaxial crystal is oriented in parallel to the viewing direction, a dark cross becomes visible in conoscopic viewing which can be surrounded by concentric interference fringes (dependent on the birefringence and the thickness of the specimen). These interference fringes are also termed isochromats (Greek isos = equal, chroma = color).

The cross remains visible while the stage is turned. Focus your attention on the NE quadrant of the cross (1st quadrant; counting is counterclockwise).

You work with the compensator λ :

- After you have inserted the compensator I, the following appears in the 1st and 3rd quadrant near the center of the dark cross:
 - yellow = optically negative
 - blue = optically positive

You work with the compensator $\lambda/4$:

- After you have inserted the compensator $\lambda/4$, a dark spot appears near the center of the dark cross:
 - in 1st and 3rd quadrants = optically negative
 - in 2nd and 4th quadrants = optically positive

You work with the wedge compensator 0 - 4λ :

- After insertion of the wedge compensator 0 - 4λ , the isochromats in the 1st and 2nd quadrant are moving
 - outward = optically negative
 - inward = optically positive

Miroscopy Techniques

Biaxial crystals

If biaxial crystals display a cross in conoscopic observation which resolves into the two branches of a hyperbola, the acute bisectrix (1st center line) is oriented in parallel to the viewing direction. Turn stage until the dark branches of the hyperbola (isogyres) are in the 1st and 3rd quadrants.

You work with the compensator λ :

- After you have inserted the compensator λ , the following appears:
 - yellow on the outside (concave side of hyperbola) and
 - blue on the inside (convex side of hyperbola) = optically negative
 - blue on the outside and yellow on the inside = optically positive

You work with the compensator $\lambda/4$:

- After you have inserted the compensator $\lambda/4$, a dark spot appears:
 - on the outside of the dark isogyre = optically negative
 - on the inside of the dark isogyre = optically positive

You work with the wedge compensator $0 - 4\lambda$:

- After insertion of the wedge compensator $0 - 4\lambda$, the isochromats in the 1st and 3rd quadrants are moving
 - outward = optically negative
 - inward = optically positive

Note: If an optical axis of a biaxial crystal is parallel to the viewing direction, only one branch of the hyperbola will be visible in conoscopic viewing, with the vertex of this branch lying in the center of the field of view. If the stage is turned, the branch of the hyperbola will move about its vertex. Proceed in the same manner as described above to determine the optical characteristics of the crystal.

	Optically uniaxial		Optically biaxial		
	positive	negative	positive	negative	
λ plate (white \rightarrow blue \rightarrow yellow)					+ = blue - = yellow
Quartz wedge (direction of movement during insertion)					direction of movement
$\lambda/4$ plate (position of black spots)					

Microscopy Techniques

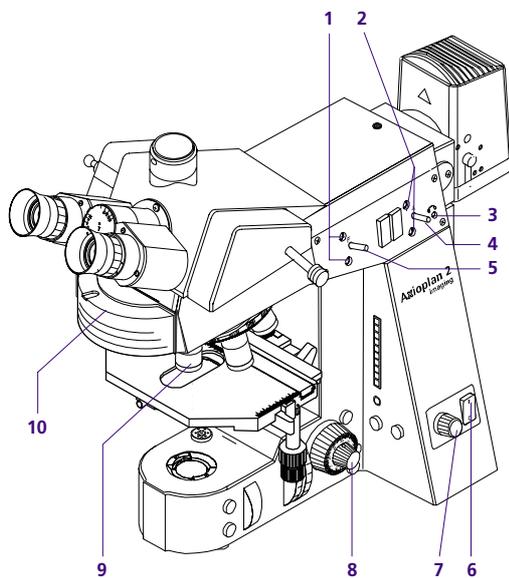
Incident light brightfield

(Fig. 51)

Note: In all incident-light techniques, the compensators 6 x 20 (see also → overview on page 57) must be removed from the beam path to prevent image quality from being impaired.

To set the incident light illumination in accordance with the KÖHLER principle, proceed as follows:

- On the rear of the instrument, switch on the incident light illumination using the toggle switch and then switch on the microscope at the ON/OFF switch (6).
- At voltage regulator (7) set approx. 3 ... 4 V as the supply voltage for the illumination.
- Place a polished specimen on the stage (align top surface parallel to support, e.g. with leveling press).



- 1 Centering screws for luminous field diaphragm
- 2 Centering screws for aperture diaphragm
- 3 Swing-in, swing-out facility for diffusing screen
- 4 Pushrod for aperture diaphragm
- 5 Pushrod for luminous field diaphragm
- 6 ON/OFF switch
- 7 Voltage regulator for light intensity
- 8 Focusing drive
- 9 Objective
- 10 Reflector turret

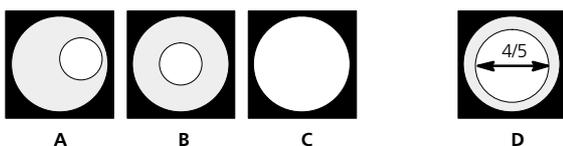


Fig. 51 Microscope setting for incident light brightfield

- Swing the 10x objective (yellow ring) (9) on nosepiece, and the reflector module for brightfield on reflector turret (10) into the beam path.
- Check the 0 positions on the eyepiece scale (→ page 39).

You should now see spots of light (the exit pupils) behind the eyepieces. If you are working with a binocular phototube, all of the light will be directed to the binocular tube if the pushrod is slid in all the way.

When you look into the tube you will see a bright circle (the eyepiece stop) with each eye.

- Merge the two circles into one by adjusting both eyepieces tubes to your PD.
- On the illumination device, swing in the diffusing screen by turning screw SW 3 (3) to the black dot, and make the luminous field diaphragm narrower by pulling out push rod (5).
- Focus the specimen with focusing drive (8). The image of the luminous field diaphragm (A) which lies exactly in the specimen plane may be helpful here.
- Move the diaphragm image into the center of your field of view (B) with the aid of centering screws (1). Use pushrod (5) to open this diaphragm until the field of view is just free and no more (C).
- Now adjust the contrast with aperture diaphragm (4) to suit the needs of the specimen being examined.

Note: If you are not certain how far you should stop down, a good rule of thumb is that approx. 4/5 of the exit pupil of the objective should be illuminated (D). [The exit pupil is visible at the bottom of the tube when the eyepieces are removed or when the Bertrand lens is swung in and focused (Bertrand lens slider)].

If necessary, the aperture diaphragm can be brought into the center of the pupil using centering screws (2) and then stopped down to 4/5 its size using pushrod (4).

Every objective change also changes the objective aperture, i.e. the aperture diaphragm must be readjusted.

If your microscope features a light manager (motorized aperture diaphragm, at least coded nosepiece), you can store the microscope setting via the SET key (→ *Stand, Light Manager*).

Microscopy Techniques

Incident light darkfield

Use

Additional equipment

Object features which heavily scatter light, such as scratches, cracks, pores or the surfaces of metal fractures light up bright in darkfield illumination. Ideal brightfield objects such as specular surfaces including features with different degrees of reflection remain completely dark, however.

- Nosepiece with connecting thread M 27
- HD Objectives
- reflector module D

Adjustments required

- Set illumination as for brightfield.
- Then switch to darkfield by swinging in reflector D on the reflector turret.
- Open luminous field diaphragm and aperture diaphragm fully, as this work in darkfield using the incident light technique requires maximum illumination intensity.

Note: When switching back to brightfield, do not forget to close the luminous field diaphragm and the aperture diaphragm to the normal values. There will otherwise be a risk of glare.

Miroscopy Techniques

DIC - Differential interference contrast in incident light

Use

This technique is used for the high-contrast display of reflecting surfaces featuring height differences of several wavelengths up to the $\lambda/20$ range.

Additional equipment

- Epiplan-Neofluar Pol, DIC or HD DIC objectives
- Special HD DIC W 0.8 or HD DIC M 27 nosepiece
- DIC sliders matched to the objectives used.
The magnification and the aperture of the suitable objective are given on the top surface of the sliders. Push DIC slider into the slot until hear it snap in.
- DIC slider+ for maximum contrast at reduced resolution provided by objective
- DIC or DIC red I reflector module

DIC enhancement

If contrast is unsatisfactory using the standard DIC equipment, use the sliders marked + which are available for the objectives listed below (overview). The increased contrast may result in decreased resolution.

Objective	DIC slider
5x / 0,15	5x / 0,15 Epi +
10x / 0,30	10x / 0,30 Epi +
20x / 0,50	20x / 0,50 Epi +
LD 20x / 0,40	LD 20x / 0,40 Epi +

Note: If you want to work with the reflector module Pol, sliders with or without a compensator λ must be used for the analyzer compartment (see → Fig. 46 on page 67).

Additional comments

In DIC, contrast is caused by surface relief. With linear structures, therefore, contrast is dependent on whether the orientation of these structures is in the "light - shadow" direction (very low contrast) or at right angles to it (maximum contrast). For this reason, it is advantageous to have the possibility of object rotation to obtain an image displaying the highest contrast. This can be achieved using either a rotary mechanical stage or a rotary polarization stage.

Colored DIC is obtained if you use the DIC red I reflector module or if you insert a reflector module Pol together with an analyzer slider and a λ plate, rotatable by $\pm 10^\circ$ (453662).

Microscopy Techniques

Incident-light polarization - Detection of bireflection and reflection pleochroism

Use

Polished sections of ore minerals, coals, ceramic products, specific metals and metal alloys display a different reflection behavior depending on the orientation of their crystals or object features. Thus, this technique is also another contrasting technique.

Adjustments

- Adjust your polarizing microscope for standard examinations in incident light (see → page 74).
- Bring the reflector module Pol into the beam path by turning reflector turret (6). The incident-light polarizer is oriented in the E-W direction.
- Insert analyzer (5) into the analyzer compartment. The analyzer is oriented in the N-S direction.

- Close the aperture diaphragm by 2/3 of its diameter by pulling out pushrod (4) as far as required.
- Your object will display bireflection if object features possess differences in brightness or color which change when the stage is turned.
- Pleochroism is present when color changes occur in the object during the turning of the stage (incident-light polarizer in beam path, analyzer swung out).

If the polarizing microscope you use for the examination of incident-light objects is equipped with an intermediate tube Pol, you must remove the Bertrand lens from the beam path using knob (2) and push in the pushrods for field diaphragm (1) and for reticle (3).

Note: When knob (2) is in its front position, the Bertrand lens is swung out of the beam path, in its rear position, the lens is in the beam path.

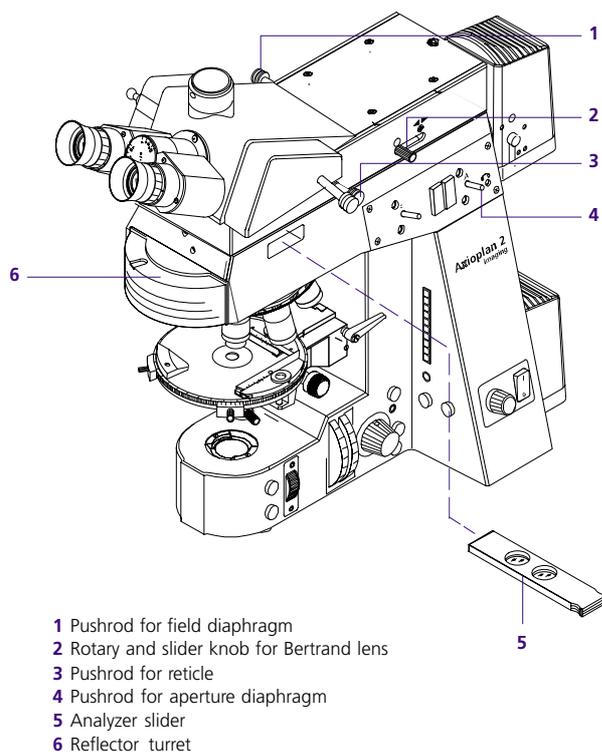


Fig. 52 Incident-light polarization

Microscopy Techniques

Epi-fluorescence

Additional equipment

- ❑ Recommended: Plan-Neofluar or Fluar objectives (UV excitation)
- ❑ FI reflector module (with the relevant filter combination) and shutter module in the reflector turret
- ❑ HBO 103 or HBO 50 mercury vapor short-arc lamp for reflected-light illumination
- ❑ HAL 100 halogen illuminator for transmitted-light illumination

Note: It is absolutely necessary that the mercury vapor short-arc lamp has been aligned before the reflected-light fluorescence technique is used. Realignment might also become necessary depending on the hours of use.



WARNING!

Explosion hazard

The HBO 50 W lamp must be exchanged after expiry of the average service life of 100 hrs. The average service life of the HBO 103 is 300 hrs. Their illuminance decreases in the course of many hours of use so that homogeneous illumination of the object field can no longer be guaranteed. Furthermore, there is a risk of explosion. The remaining service hours can be read off on the power supply unit.

Please refer to manual "N HBO 103 and N XBO 75 Microscope Illuminators" (B 40-065) for further details on how to change and align the lamps.

Setting of the illumination

The initial epi-fluorescence setting is made considerably easier if a Plan-Neofluar 20x/0.50 objective and a strongly fluorescent specimen is used. It is also possible to use demonstration specimens first.

Note: If the I-compensator has still remained in the compartment above the nosepiece after use of the DIC technique, it must be removed before epi-fluorescence can be set.

- Switch on the HAL 100 halogen illuminator
- Swing in the Plan-Neofluar objective 20x/0.50.
- Move condenser turret to position H, transmitted-light brightfield (or phase contrast), and search the specimen spot to be examined.
- Keep the light path in the reflected-light illuminator blocked with the shutter module A on the reflector turret or with the barrier position of the reflected-light filter slider (A).

- Switch on the HBO 103 or HBO 50 mercury vapor short-arc lamp and allow it to heat up to its operating temperature for approx. 15 minutes.
- Select and switch on the required fluorescence filter combination (depending on the type of excitation) on the reflector turret of the FI reflector module.
- When using the reflected-light filter slider, unblock the light path in the reflected-light illuminator.
- Remove one eyepiece from the tube and use your eye to set the aperture diaphragm. Open the aperture diaphragm until the entire objective exit pupil is free.
- Insert eyepiece in the tube again and close luminous field diaphragm until it becomes visible in the field of view.
- Use the two centering screws on the condenser carrier to center the luminous-field diaphragm in the edge of the field of view.
- Either open the luminous field diaphragm until it just disappears behind the edge of the field of view, or close it into the field of view if there is a risk of bleaching of the specimen.
- Finally, refocus on the specimen and optimize the collector position of the HBO 103, if required. Set the collector in such a way that the illumination of the field of view is as homogeneous as possible if the reflector module of short wavelength excitation is used. Modules with an excitation of a longer wavelength do not require the correction of the collector position.

Note: To avoid eye injury, please use the fluorescence protection screen (452163-0000-000 → *System Overview*).

Microscopy Techniques

Photomicrography with the Axiophot 2

Note: If your polarizing microscope (Axioiplan 2 imaging Pol, Axiophot 2 Pol) is equipped with an intermediate tube Pol, you must remove the wire reticle (see → Fig. 26/3) from the beam path if you do not want it recorded in the photo.

How to proceed for photography

- Carefully set your Axiophot 2 Photo module for observation.
Set the beam splitter to allow simultaneous observation and photography (→ *Microscope Software*).
- Select illumination technique, objective magnification and condenser setting as usual.
- Set the brightness required for observation (3200 K color temperature) on the stand (Fig. 3/4).
- Load the correct film into the film cassette and attach the Photo module.
- Select the film type used under **Film data** in **Photo** program module. Set the required exposure metering technique (normally center-weighted averaging) and the illumination technique (FL/D, H/Ph/DIC, H) under **Mode**.

The display field of the **Photo** menu now shows the exposure time, the correct data of the loaded film, and the frame counter.

- Switch on the luminous frame.
- Set the image frame and focus.

Proceed carefully. If the focusing cross and the specimen are visible in focus simultaneously, imaging on the film will also be in focus.

In the case of a low objective magnification, the use of the Optovar 2.5x or the **Focus Finder** function are absolutely required as a focusing aid.

- Click **START** to take the photo.

The following is then performed automatically:

- luminous frame disappears
- new, current exposure metering
- exposure
- data projection
- film advance
- luminous frame appears again

The next exposure can be released.

Film selection

Reversal films (films for slides) are used for color photomicrography. In general, we would recommend reversal films for artificial light (3200 K).

If daylight film is used, the conversion filter 3200/5500 is required.

Films marked “professional” feature closer tolerances in sensitivity and color balance, i.e. homogeneous results are obtained. Always use DX-coded films in their original cartridges.

We would advise against the use of long films, since light entry, scratches on the film, dirt, etc. might impair the quality or damaged cartridges can result in defective film advance.

Use of yarded films

We do not recommend the use of yarded films, since unintended illumination, scratches or dirt on the film etc. might impair the quality of the film. The use of damaged cartridges can result in defective film advance.

If you wish to work with a yarded film, please be sure to follow these instructions:

Only use DIN 4335 or ISO 1007-1977 cartridges. Make sure not to exceed the given maximum measurements.

Film cartridges are not suitable for continuous operation. Discard the cartridges after 10 loads to the most.

The front part of the film needs to be cut in accordance with DIN 4536 or ISO 1977 (→ Fig. 53).

- Do not cut through a perforation hole when cutting the front part of the film.
- The cut needs to be as long as 7 ... 9 P and must run parallel to the edge of the film.
- The corners must be rounded in order to prevent the film from jamming the cartridge opening or parts of the cassette.
- The end part of the film must be cut off at a right angle and secured tightly with adhesive tape.
- Avoid using very long films (some cartridges cannot be used for 36 exposures with every type of film). This could result in defective film advance.

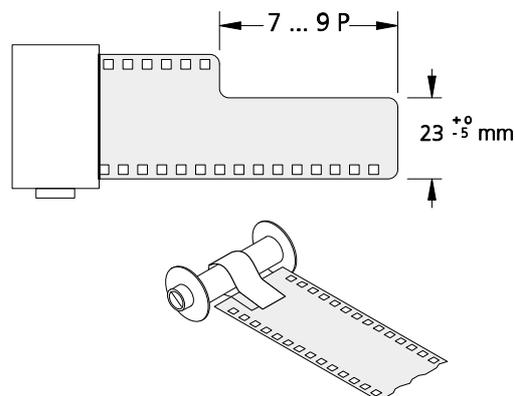


Fig. 53 Use of yarded films

Microscopy Techniques

Correction of the color balance of color reversal films

The color balance of a type of color reversal film can differ from batch to batch.

Both these deviations and influence from the optical system on the color can be compensated using commercially available color compensating (CC) filters.

The filter density is indicated by a 2-digit number and the color by its initial letter.

Examples: 05 - B (blue, 10 - G (green), 20 - R (red))

Assessing the color balance

- View slides on a standard light box, the light source of which has the correct illuminance and the spectral energy distribution of 5000 K.
- Take test exposures of an object area with as much empty background as possible in transmitted-light brightfield.
- The empty background of an exposure series should range from dark gray, medium gray and light gray to white.

Correction the color balance

- Place CC filters in the complementary color of the color tinge on the slide to be corrected.

Color tinge	Color of the CC filter
blue	Yellow Y
green	purple (magenta) M
red	bluish green (cyan) C
yellow	blue B
purple	green G
bluish green	red R

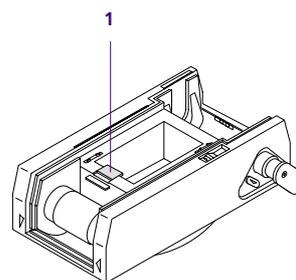
If the required color balance is obtained during observation using a filter with density 10, for example, a CC filter of half th is density, i.e. density 0.5, should be used for the exposure to follow. Normally, filters of density CC-05 to CC-10 are sufficient for correction.

Note: Perfectly corrected color exposures make it necessary that the microscope setting, the developing procedure and the film batches remain unchanged.

Data projection for 35 mm photography

The data projected into the image may be poorly legible against bright object structures. We would therefore recommend you to slide the data shield (Fig. 54/1) on the film support into the frame window until stop. This shield masks out a field of 2.5 mm x 14 mm at the edge of the format, and the data are then clearly legible on the black background.

The data shield can be moved only before insertion of the film; it is not possible to move it while a 35 mm cassette is attached!



1 Data shield

Fig. 54 35 mm film cassette mot

Compensation of reciprocity failure

The automatic compensation of the reciprocity failure (RECI value) may become effective in the case of exposure times of more than 1 second.

When the film type is entered, the relevant data are automatically called from the database of the software and the exposure time is corrected.

When using films not included in the database, determine the correct value as follows:

No test exposures are required if the film manufacturer indicates the extension of the exposure time, e.g. +2 values for an exposure time of 10 s. "+ 2 values " means that the exposure time must be quadrupled, i.e. 40 s.

- First, set your microscope in so that the automatic system indicates 10 s with RECI set to 0 (in this exceptional case, you may use the aperture diaphragm to reduce the brightness).
- Now change the RECI value and you will quickly find the one which most closely approximates 40 s. This will then be the value for your film (8 in the above example).
If no data have been indicated by the manufacturer, test exposures in the range of the required exposure time must be made using the RECI values 0 ... 9.

Microscopy Techniques

Fluorescence photography

The following specialties apply compared to photomicrography:

- The often low brightness requires long exposure times. Before taking the exposure, therefore select the option **100% of light to film** under **Set Exposure Function**. Reduce the brightness of the luminous frame in a useful way.
At lowest brightness, set the beam splitting to 100 % of light for observation and again select the function **100% of light to film** before taking the exposure.
- Fluorescence light is neither daylight nor artificial light, but is generated in the specimen itself. Normally, better results are obtained in fluorescence microscopy when daylight films are used.
- Don't be afraid of using high-speed films. The graininess of such films rarely impairs the quality of fluorescence images.
- The dark or black background will often cover a large part of the measuring field of the automatic exposure metering system (also with spot metering). Especially in the spot area, the brightness/darkness ratio can be easily estimated and corrected via exposure correction.
If a typical measuring area of a specimen shall not remain in the image center during the exposure, you can store the appropriate exposure time, move the requires specimen section into the center and then release the exposure.
- The exposure margin is considerably large due to the high "contrast" because luminous structures in front of a dark background always stand out clearly even in different illumination.
However, if an exact color rendition of the fluorescence dyes is important to you, an exposure series with different exposure times is recommended.
- Some fluorescence dyes bleach out quickly, especially under pronounced, high-energy excitation radiation. To protect the specimen, you can reduce - at least temporarily - the excitation intensity via the aperture diaphragm.

Note: Always remember that weak fluorescence is more visible in a dark workroom.

Exposure times and filters

The Axiophot 2 Photo module covers the following longest exposure times for 35 mm film with exposure correction 0:

Sensitivity of the film	Longest exposure time
100 ISO	960 s
400 ISO	240 s
1600 ISO	60 s
6400 ISO	15 s

Filters for Photomicrography	∅ 32 mm	∅ 18 mm
gray filter 0,50 (50 % transmission)	467840-0000-000	
gray filter 0,12 (12 % transmission)	467841-0000-000	
gray filter 0,03 (3 % transmission)	467842-0000-000	
neutral-density filter 0,25 (25 % transmission)		467856-0000-000
neutral-density filter 0,06 (6 % transmission)		467855-0000-000
conversion filter 3200-5500 K	467847-0000-000	467854-0000-000
blue filter CB 6	467851-0000-000	
blue filter CB 3	467852-0000-000	
interference-green filter	467803-0000-000	

Care, Maintenance

General

The instrument back may be removed only by service personnel.

Please ensure that your instrument is not exposed to inadmissible climatic influences (humidity and temperature) for longer periods of time.

Always protect the instrument against dust and humidity. Therefore, always drape the dust cover over the instrument after use. Do not forget to switch off the lamps first.

Remove dust on optical surfaces using a natural hair brush and a squeeze-blower device.

To remove stubborn dirt or fingerprints, use commercially available cloths for cleaning optics and eyeglass lenses.

For moving your Axioplan 2 imaging to another location on your premises, ensure that all mobile parts are secured in position or transport them separately. Also protect your instrument against toppling, cover it and ensure under all circumstances that it is not subjected to knocks or mechanical shock. If you are in doubt, please contact our customer service staff.

Lamp change

Prior to every lamp change, ensure that you read the special instructions and safety regulations of the lamp manufacturer.

Allow the lamp to cool down appropriately before you change it.

Even at room temperature, lamps filled with xenon gas are subjected to considerable internal pressure. Therefore, always wear gloves and face protection for your personal safety.

Never touch the glass of the lamps with your bare hands. Even the slightest trace of grease may impair the intensity and service life of the lamp.

Ensure that the used lamps are disposed of in a proper way.

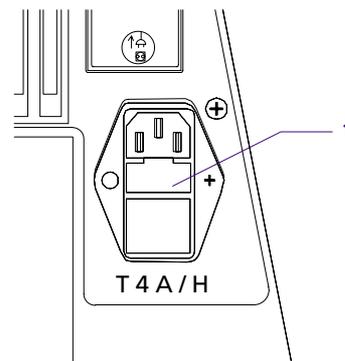
For further information, please see the information leaflet enclosed with the mercury short-arc lamp, e.g. the HBO 103.

Line supply

Your Axioplan has been designed for use with a line voltage ranging from 100 to 240 V and 50 to 60 Hz.

Changing the fuses

Compartment (2) for mounting the instrument fuses is also located on the rear of your microscope. It contains two T4 AH fuses for 250 V.



1 Fuse compartment

Fig. 55 Rear of stand - Fuse compartment

- Pull out the fuse compartment in a forward direction (use a small screwdriver, if required), remove the defective fuse and insert a new one.
- Press the fuse compartment back into the rear panel of the instrument.

Technical Data

Range	Description
Ambient conditions	
Room temperature	+10 ...+ 35°C
Humidity	max. 75% at + 35°C
Storage temperature	– 40 ... + 70°C, humidity 10 ... 30%
Weight	depending on configuration used
Safety	
Equipment class	I
Degree of protection	IP 20
Radio disturbance characteristics	conforming to EN 55011 (Class B)
Electromagnetic immunity	conforming to EN 50082-2 The instrument meets the requirements of the EC directive 89/336/EEC and EMV/Nov. 1992
Degree of contamination	2 (Altitude 2000 m)
Overvoltage category	II
Electrical supply	
Power connection	100 ... 240 V ± 10 %, 50 ... 60 Hz; no conversion required
Power consumption	approx. 225 VA
Data for connection of Axiophot 2	
Voltage supply	+5 V, +15 V
Data for connection of 12V/100W microscope lamp	
DC voltage, stabilized	3 ... 12 V; suitable for photometry (continuous light)
12V/100 W halogen lamp	
Lamp voltage	12 V
Power	100 W
Color temperature at 11.5 V	3200 K
Luminous flux	3100 lm
Mean service life	50 h
Luminous surface	3.1 x 3.1 mm ²
Fuses	
Power inlet	F1/F2: 115 V / 230 V; T 4 AH / 250 V according to IEC 127

Interface Description



WARNING!

Destruction of the electronic system

Wrong interface configurations can destroy the electronic system. This interface description is therefore meant for personnel trained in the use of hardware and software only.

General

Every microscope unit, described as a CAN-node in the following, has its own address which may occur only once within the network.

(Range A...Z, switch position 1...x).

This address enables individual activation of every CAN-node via the RS 232 interface.

During communication, the target address must be sent first, then the source address and then the actual command!

A uniform BIOS (Basic Input Output System) containing the communication via RS232 and CAN is used for all CAN-nodes on the basis of the 80C517A uP.

Furthermore, the BIOS also contains a Download Routine which allows the firmware of a CAN-node, if EEPROM is present, to be changed during operation.

Further commands are available for test purposes, e.g. to test memory and ports.

CAN-BUS

The CAN-bus (Controller Area Network Serial Communication Bus) has been developed in the automotive industry especially for high data security and fast reaction times. It allows the use of approx. 26 instrument units (CAN-nodes) in a network.

Technical Data:

- Transfer rate max. 1000 k Baud (100x faster than RS232 with 9600 Baud)
- single-wire transfer (can also be used for light guide transfer)
- automatic error recognition and transfer repetition
- multimaster
- real time
- approx. 40 m cable possible with 1000 k Baud and
- approx. 600 m cable with 100 k Baud

Communication via RS 232

Protocol

Communication between the CAN-network and the PC (host computer) is performed via RS232. The PC can be connected to any required CAN-node.

(For test purposes, it is also permitted to connect several PCs to the CAN-network).

Prior to transmission of each individual character, each communication partner must check whether the other partner is ready for reception. The RTS and CTS handshake lines are used for this.

Normally, the Interrupt-controlled reception routine of a CAN-node is always active. If functions are performed where time is a critical factor, or if the input buffer is full, the handshake is switched to "not ready for reception".

At 9600 Baud, overfilling of the input buffer is not possible in the practice, since the interpreter normally deals with the data faster than it can be supplied by the RS232 interface.

Data of the RS232 interface:

9600 Baud, 8 data bits, 1 stop bit, no parity.

The pin assignment of the RS 232 C interface is identical to that of the PC (see → Fig. 56).

The lines in the cable connecting the Axioplan 2 imaging and the PC have been crossed.

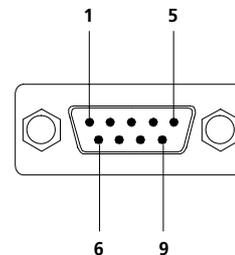


Fig. 56 Pin assignment of RS 232 C interface (view on connector front)

Interface Description

Communication via CAN

Protocol

Communication between the connected CAN-nodes is performed via CAN-bus.

In every CAN-node, incoming data is stored in the reception buffer via an interrupt routine.

Transmission is made without interrupt in the base loop with the lowest priority.

If transmission cannot be made via the CAN-bus, the trial is interrupted after a time-out of approx 1 sec and the error byte is set.

The interrupt-controlled reception routine of a CAN-node is always active and cannot be switched off on the CAN-bus.

Data of the CAN interface: 100,000 Baud. (Can controller parameters: 4, 1C, AB).

Statuses

In the basic status, a CAN-node waits for commands from the host/control panel and simultaneously performs incremental part functions of so-called continuous functions. Continuous functions are functions which require the CPU CAN-nodes to be permanently active or which need some time and are therefore processed incrementally so that control can be forwarded to the command interpreter in the meantime. (Example: blinking of the green LED).

If a complete command has been received (via CR), it will be passed on to the command interpreter and the system adopts the status Function Performance.

The functions activated by the command interpreter can take data from the input buffer or write data in the output buffer. (Substatuses Data Reception, Data Transmission). The stand control returns to the basic condition from all statuses by activation of Time Out, if need be.

Command structure (input format)

A command consists of a sequence of ASCII-characters which is finished with Hex 0D (CARRIAGE RETURN).

Only printable ASCII-characters, and no binary-coded data (except the carriage return as end character), are permitted. A command consists of a name and parameters. A name always consists of two letters.

The first letter (always a capital letter) normally designates the function group, the second letter (capital = WRITE, small = READ) the actual function.

All parameters are numeric (INTEGER, 8-bit) and strings are permitted.

String parameters are always last and are delimited by quotation marks (""). Numbers are transmitted in different fixed formats.

When a command line is executed, the syntax is checked. If a syntax error occurs, all characters up to the next CARRIAGE RETURN are bypassed!

Output format

Each numeric or non-numeric output is made in the ASCII-format. Strings are transmitted without any special delimiters (quotation marks).

The target address and source address is given before every number or every string, and the CARRIAGE RETURN character (Hex 0D) is then entered as the end character. It is therefore possible at all times to see which CAN-node the message is coming from.

Formats		
0 ... 255	0 ... 255	preceding zeros can be deleted
0 ... 255	00 ... FF	2 characters must always be transmitted (TB)
0 ... 65535	0000 ... FFFF	4 characters must always be transmitted (TD2)
0... ± 8388607	000000 ... FFFFFFFF	6 characters must always be transmitted
"Str1"		string format, max. 4 characters between 2 quotation marks

Important:

A message may contain max. 8 byte of information, plus 2 byte of addresses and 1 byte with the end character (CR). This means that a message is 11 byte long.

Interface Description

General functions

Error statuses in a CAN-node can have different causes:

Communication errors occur if transmittance on the RS232 lines is disturbed or if data is continued to be sent in spite of the CAN-node not being ready for reception (displayed by handshake).

Timeout errors occur if a CAN-node function wants to transmit data which is not accepted by the recipient or if a function waits for receiver data which have not been transmitted.

Syntax errors occur if, for example, an incorrect command name is transmitted (fail character, name too long or too short, command not existing), or if a command is transmitted with the wrong number of parameters or wrong parameter types.

Errors in function execution occur if transferred function parameters or combinations of parameter values result in fail values.

Hardware errors occur if there is a defect in the hardware (blocked motors, defective components, etc.).

Where errors can cause damage to the instrument, measures are taken immediately by the CAN-node (if possible) to avoid this. (For example, the motor current is switched off in the case of blocked motors).

Otherwise, it is the job of the activating program to enquire the error status and to provide suitable remedy.

Errors are displayed as follows:

- an error byte which can be read using the Eb command;
- a red LED which is normally off, but permanently switched on in the event of an error until backout of the error status by reading the error byte.

The following error bits occur in the error byte:

- 0 - Overrun Error (Input-Buffer Overflow)
- 1 - Transmit Error (Output-Buffer Overflow)
- 2 - Timeout on send (function waits in vain for host to be ready to receive data)
- 3 - Timeout on read (function waits in vain for data from host)
- 4 - Reserved
- 5 - Syntax error (detected by the command interpreter. Can also be caused by a transmittance error.)
- 6 - Not yet assigned
- 7 - Error during executing of function (cause: wrong parameter value or hardware error)

Test functions

To test the hardware, functions are available which permit data to be written byte-by-byte on any position in the memory address range (TB) or to be read from any position (Tb).

Furthermore, (Trn) and (Tpn) permit the A/D converter and ports of the uP 517A to be read, and (Tpn) enables the ports to be written.

The use of commands permitting a change of the memory range require detailed knowledge of the hardware because wrong application may result in a system crash.

A green LED blinking in 0.5 s intervals indicates that the processor does not yet run properly, even when the red error LED is switched on.

Start sequence

Every CAN-node runs through the following sequences during startup:

- Initialization of the processor and the hardware and checking the EPROM application, approx. 3 s.
- Waiting time of 1 s until all CAN-nodes are initialized
- Checking whether further CAN-nodes are connected to the network (the CAN-bus status is set accordingly)
- Waiting time of 1 sec until all CAN-nodes have sent queries to each other
- Starting of application program

During this start sequence, the red LED is on continuously, the green LED blinks in 0.5 s intervals.

Note: For further information, please order the detailed programming instructions from our service department.

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