



Leica DM R

Instructions

Leica

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The Leica logo is written in a red, cursive script font with a thin underline beneath the letters.

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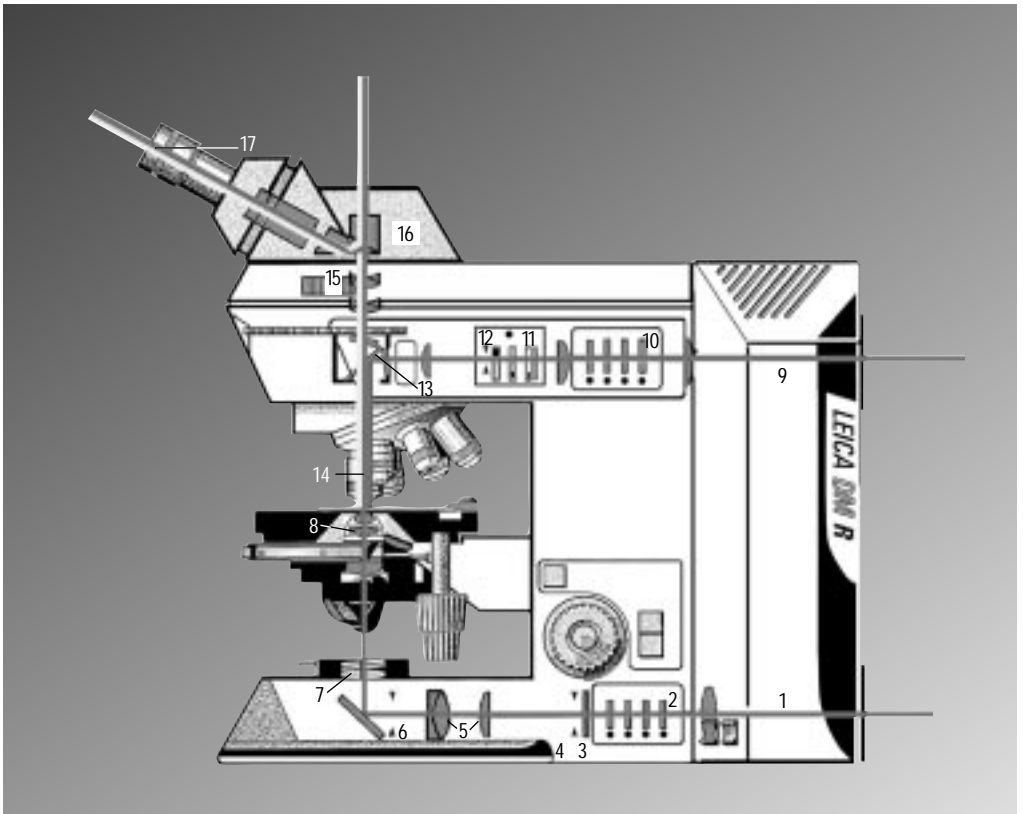
Instructions

Leica



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Transmitted light path*

1 Light source (lamphousing not illustrated), 2 Filter magazine*, 4-pos., 3 Diffusing screen, 4 Aperture diaphragm, 5 Imaging system of aperture diaphragm, 6 Field diaphragm, 7 Polarizer*, 8 Condenser

Incident light path*

9 Light source (lamphousing not illustrated), 10 Filter magazine*, 4-pos.

Diaphragm module with:

11 Aperture diaphragm* or filter and diffusing screen, 12 Field diaphragm, 13 Reflector or filter cube

Imagine light path

14 Objective, 15 Tube optics/Bertrand lens*, 16 Tube, 17 Eyepiece

* not part for all outfits

Important notes on this manual

The Leica DMR microscope series consists of several basic stands and a range of modular components allowing an almost unlimited variety of individual outfits.

Therefore this manual has been given a modular layout as well to show you other possible configurations besides your own.

The manual is divided into two main chapters:

Assembly (including a brief description of each component) and

Operation.

Any alterations or additional information are described on extra pages. There is a supplementary manual for the automatic version. The manuals are multilingual. Due to the spiral binding you can turn the language you want to the front. The manual can be filed in the supplied folder with the transparent plastic tongues.

Special manuals are supplied with some additional equipment such as photomicrography, microscope photometry (MPV), compensators, heating stages, interference attachments, etc. There are also extensive brochures on microscopy, which can be ordered, as can extra copies of this manual, from our agencies for a cover charge.

Numbers in the text, e.g. 1.2, refer to the illustrations, i.e. Fig. 1, pos. 2 in this example.



Attention:

This manual is an integral part of the product and must be read carefully before switching on and using the microscope! It contains important instructions and information for safe operation and maintenance of the product and must therefore be kept in a safe place!

Text symbols and their meaning:



*

Special safety information is marked at the edge by the lefthand symbol and highlighted by a grey background.

Warning of hot surface.

Attention! This symbol means that incorrect operation can damage the microscope or its accessories.

Explanatory note.

Item is not included in all variants of the microscope.

Assembly/General information

Unpacking

Please compare the delivery carefully with the packing note, delivery note or invoice. We strongly recommend that you keep a copy of these documents with the manual, so that you have information on the time and scope of delivery later when ordering more equipment or when the microscope is serviced. Make sure that no small parts are left in the packing material. Some of our packing material has symbols indicating environmental-friendly recycling.



Attention:

When taking the microscope out of its packing and putting it onto the desk take care not to damage the sensitive vibration-damping feet on the bottom of the microscope.



Attention:

Do not connect the microscope and peripherals to the mains yet! (see page 53).

Installation site



Attention:

Make sure that the workplace is free from oil and chemical fumes. Vibrations, direct sunlight and major temperature deviations have a negative effect on measurements and photomicrography. This and an ergonomically designed chair which can be adjusted in several positions are the basic prerequisites for fatigue-free microscopy.



Attention:

Fire hazard! Keep lamphousings at least 10 cm (4") away from inflammable objects such as curtains, wallpaper or books!

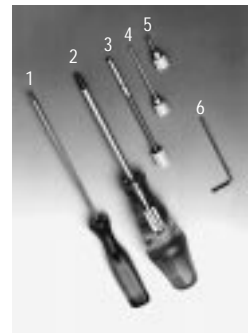
Assembly tools

You only need a few ordinary screwdrivers to assemble your microscope. These are supplied with the delivery. Replacements for lost tools can be obtained from us or from a tool shop (Fig. 1), see list of spare parts on p. 112.

Fig. 1 Assembly tools

- 1 3 mm hexagonal screwdriver
- 2 Crosstip screwdriver*
- 3 Adjustment key for Sénarmont compensator*
- 4 Pol centering key (long version)*
- 5 Centering key (short version)*
- 6 Allen key 2 mm (3 mm)*

* not part of all outfits



Setting the mains voltage

Microscopes with mechanical focusing (42.12) are automatically adapted to the local mains voltage in a range of 120 $\pm_{25}^{\pm 6}$ % / 230 $\pm_{20}^{\pm 6}$ % V. For microscopes with motor focus (RE and RXE models, Fig. 44), however, the selector switch at the back of the microscope (2.6) must be set.



Attention:

For external power units the mains voltage should always be set according to the separate instructions supplied.

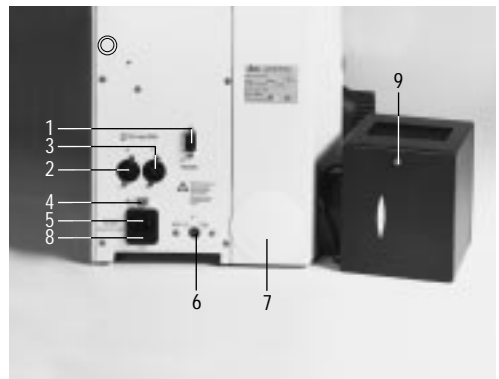
Electric safety

To ensure that the microscope and accessories are in a perfectly safe condition, please note the following advice and warnings: The mains plug must only be inserted into a grounded outlet. If an extension cord is used, it must be grounded as well. Using the ground connection (2.4), any accessories connected to the microscope which have their own and/or a different power supply can be given the same ground conductor potential. Please consult our servicing personnel if you intend to connect units without a ground conductor.

The instruments and accessories described in the manual have been checked for safety or possible risks. Before making any alterations to the equipment or combining it with non-Leica components in a way not described in this manual, consult the Leica agency for your region or the main factory in Wetzlar! Any guarantee will be rendered invalid if the instrument is opened or modified in any way by unauthorised persons or if the instrument is used in another way than the one described in these instructions!

Fig. 2 Back of microscope stand

1 RS 232 C* interface, 2 Connection for 12 V 100 W transmitted light lamp*, 3 Connection for 12 V 100 W incident light lamp*, 4 Ground connection, 5 Mains connection, 6 115/230 V** switchover, 7 Space for extra lamphousing or switchable mirror, 8 Fuses (T4A), 9 Lamphousing 106*: screw for opening lamp housing 106, ☉ Not illustrated, on the top surface of the back of the microscope: plug connection* for photomicro (lamp and shutter control)



Fuses



Attention:

The two fuses integrated in the mains connection (2.7: T4A, see spare parts list on page 112) come into action when the mains voltage selector is incorrectly set (motor focus only) or in case of internal electronic defects. For fuses for external power units please see the relevant special instruction manual and spare parts list on page 112. In the event of repeated fuse failure it is important to consult our Technical service.

Assembly of light sources

Up to 4 lamphousings can be adapted depending on the microscope configuration. If only one light source is used this is normally attached to the left side of the microscope. Only lamphousing 106 (2.8) and the microflash (see separate instructions) can be used for transmitted light).

Retrofitting additional light sources

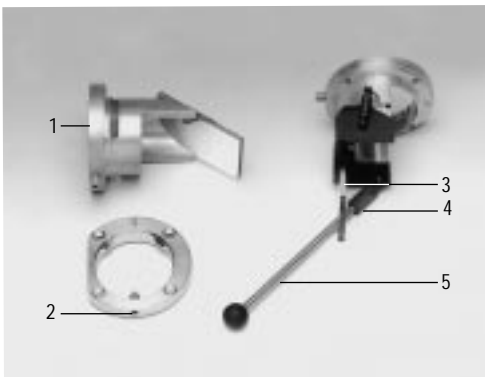
When retrofitting the incident light illuminating axis the microscope must be equipped with a deviating mirror (3.1) with lamp mount. If you want to use 2 light sources alternately in transmitted and/or incident light, a switchable deviating mirror (3.3, either manual or motor controlled) can also be retrofitted.

The non-switchable mirror (3.1) is mounted to the left, the switchable mirror (3.3) from the back. To do this, remove the cover (using a sharp object if necessary), or, if a mirror is already in place, remove it by loosening the 4 screws.

Hold the mirror you want to fit on the microscope with the flattened side of the lamp mount pointing downwards. For switchable mirrors only: before tightening the screws hold the mount for the switching rod (3.4) at an angle of about 45° to the longitudinal axis of the microscope. Remove the stopper from the hole (22.4) or (61.7) with the 3 mm hexagonal screwdriver (1.1).

Fig. 3 Deviating mirrors

- 1 non-switchable deviating mirror, 2 Lamp mount without* mirror for second lamphousing, with clamp screw,
- 3 Switchable deviating mirror*, 4 Mount for switch rod,
- 5 Switch rod*



Insert the switch rod (3.5) into the hole and screw into the mount (3.4). Screw the lamp mount without the mirror (3.2) onto the left of the microscope.

Motorized mirror only: first fix the holder with the short screw in the top right drill hole, then fix the lamp mount with the 3 long screws.

Tighten the 4 screws to fix the lamp mount(s).

Lamphousing 106

only for 12 V 100 W halogen lamp (centerable in x and y direction), focusable, two-lens collector. Without reflector, with grooved diffusing screen, heat-absorbing filter, Fig. 2.8, Fig. 4 and Fig. 48.17.

Besides lamphousing 106, the following light sources can be used for incident light:

Lamphousing 106 z

for 12 V 100 W halogen lamp and gas discharge lamps up to 100 W (Hg 50, Xe 75, Hg 100 W, spectral lamps). Like lamphousing 106, without diffusing screen, but with centerable and focusable reflector and 4- or 6-lens collector. Quartz collector on request. Fig. 5 and 48.1.

Lamphousing 252

for gas discharge lamps up to 250 W (Xe 50, Hg 200 W), centerable lamp socket, focusable 4-lens collector, focusable and centerable reflector. In preparation.

Microflash

for photography of fast-moving objects. Only in connection with the electrically switchable deviating mirror and a lamphousing (see special instructions).

Spare lamps

See page 112 for code nos.

Lamphousing 106

Disconnect from power supply (2.5), disassemble using hexagonal screwdriver (1.1 and 3.2). Unscrew screw (2.9) and remove cover. Move the collector to the front (48.19).

Remove the defect lamp and put a new 12 V 100 W halogen lamp into the lamp holder without tilting (4.1).



Attention:

Leave the protective covering on the lamp until it is in its holder. Avoid making fingerprints on the lamp or wipe off immediately.

Close the lamphousing (2.9).

Lamphousing 106 z



Important:

For incident light only (48.1)! Disassembled like lamphousing 106 (see above).

12 V 100 W halogen lamp

Disconnect from power supply (2.5).

Loosen screws (5.4 and 5.9) with crosstip screwdriver and flip up lid (5.1).

Pull cut-out plug slightly out of socket (5.11).

Unscrew screws (5.10) on the lamp holder and pull out the lamp holder (Fig. 6). Remove defect lamp and insert new 12 V 100 W halogen lamp.



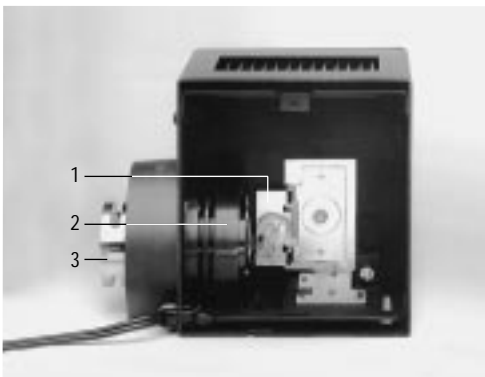
Attention:

Leave the protective covering on the lamp until it is in its holder!

Avoid making fingerprints or wipe off immediately.

Fig. 4 Lamphousing 106*, opened

1 12 V 100 W halogen lamp in holder, 2 Collector, 3 Diffusing screen



Lamphousing 106 z* Hg- and Xe lamps



Attention:

Danger: the following information is extremely important and should be adhered to under all circumstances:

Always unplug the power unit from the mains before assembly work is carried out.

Wait for the lamphousing to cool down before opening (at least 15 min.), danger of explosion!

Never touch glass parts of the burner with your hands. Remove any fingerprints or dust carefully (perhaps using alcohol).

Adjust lamps immediately after ignition (see page 90 ff.)



Attention:

Avoid switching on and off frequently, as this can impair the stability of the lamp and shorten its life.

Hot Hg lamps cannot be reignited until they have cooled down. We recommend that you let new burners burn in for several hours without interruption if possible.

It is a good idea to keep a record of the hours the lamp is in use and to compare with the manufacturer's specifications. Replace discoloured, spent lamps.

We cannot accept any liability for damage resulting from a lamp explosion.



Attention:

Always wear safety clothing (gloves and face mask) when assembling Xe burners (danger of explosion).

Fig. 5 Lamphousing 106 z*

1 Lid, flipped up, 2 Collector, 3 12 V 100 W halogen lamp with holder 4, 9 Lid screws, 5 Reflector, 6, 8 x/y centering of reflector, 7 Reflector focusing, 10 Screws for lamp socket, 11 Socket for cut-out plug

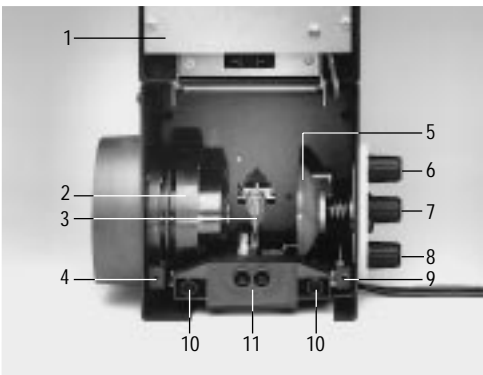


Fig. 6 12 V 100 W lamp holder (LH 106 z only)





Attention:

Protect movable interior parts with foam rubber or similar in case of shipment.

To open lamphousing 106 z and 252: undo screws (5.4) and flip up the lid of the lamphousing. Pull the cut-out plug slightly out of the socket (6.11).

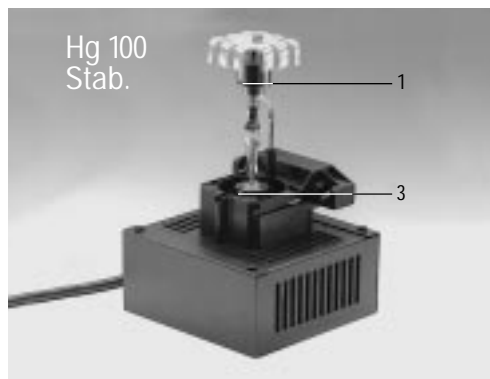
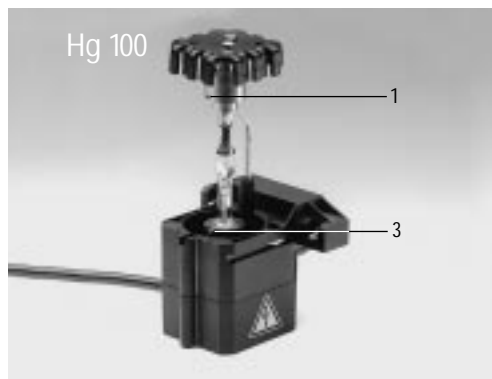
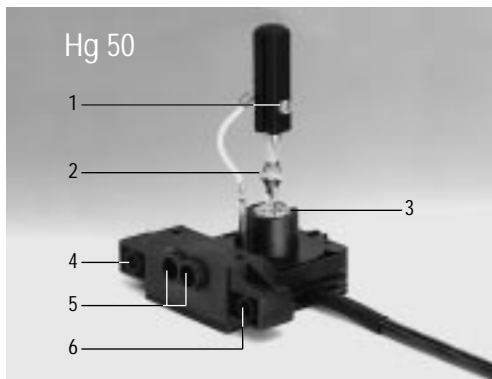
Undo the screws (5.10) on the lamp holder and remove the holder (Fig. 7). Remove the spent burner by loosening the clamp screws (7.1 and 7.3).

Insert burner as follows, adhering strictly to the above safety information:

Do not remove the protective covering yet (7.7).

Fig. 7 Lamp holders for gas discharge lamps*

1 Upper clamp, 2 Seal point of the burner, 3 Lower clamp, 4, 6 Drillholes for fixing the holder, 5 Sockets for cut-out plug, 7 Protective cover



Lamphousing 106 z, Hg- and Xe lamps



Attention:

Always insert burner so that

1. the lettering on the metal base is upright after insertion (different diameters of the metal base for the Hg 100 and Xe 75 burners ensure that these are always inserted the right way up). If one of the bases is labelled "Up", it must therefore be assembled at the top.

2. If the lamp bulb has a seal point (7.2), turn the burner so that this point will be at the side, not in the light path.

Apart from the halogen lamp the following gas discharge lamps can be used, all requiring different lamp holders (Fig. 7) and power units:

Type	Average life
Hg ultra high pressure lamp 50 W (alternating current)	100 h
Xe high pressure lamp 75 W (direct current, stabilized)	400 h
Hg ultra high pressure lamp 100 W (direct current, stabilized/non-stabilized)	200 h
Hg ultra high pressure lamp 100 W (direct current, stabilized/non-stabilized, type 103 W/2)	300 h

Put the upper pin of the burner between the clamps of the flexible power supply and clamp with screw (7.1).

Unscrew the stud (7.3) in the holder slightly, insert the lower end of the metal base and retighten the stud.

Exchanging the collector on lamphousing 106 z: Move the collector to the rearmost position with the focusing knob (48.19). Pull the focusing knob of the collector outwards. The collector can now be removed.



Attention:

Make sure that the lamp base and the power unit have the same number. If the lamp base is marked L1, for example, L1 must also be set on the power unit to make full use of the lamp and not to shorten its life.

Move the collector to the front position with the focusing knob (48.19).



Attention:

Remove the protective covering from the burner (7.7).

Put the lamp holder with burner inserted into the lamphousing and secure with the screws (8.9). Try moving the collector (48.19): it must not touch the power lead.



Attention:

When closing the lamphousing make sure that the pins of the cut-out plug engage in the sockets (8.8). Retighten the screws of the lid. Push the cut-out plug in as far as it will go.

Attach the lamphousing to the microscope (page 16) and connect to the power unit (compare mains voltage!).

Lamphousing 106, 106 z



Attention:

Only lamphousing 106 (48.1) can be used for transmitted light!

Remove the dust protection cover from the lamp mount. Unscrew the clamp screw (3.2) with the aid of the hexagonal screwdriver (1.1) so that the screw on the inner surface of the lamp mount does not protrude above the surface. Align the lamphousing so that the screw engages in the corresponding indentation on the lamphousing. Tighten the screw to fix the lamphousing firmly to the microscope.

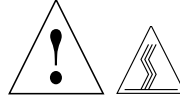
Filter mount

A filter mount (Fig. 9) taking up to four extra filters (50 mm diameter) can be assembled between the microscope and the lamphousing in the same way. When lamphousing 106 is used, only 1 thick or 2 thin filters can be inserted.

Microflash

The microflash is assembled in the same way (only in conjunction with the switchable mirror and a lamphousing).

Ventilation



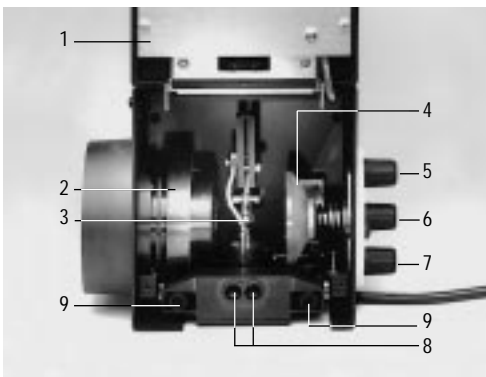
Attention:

Important: Make sure that the instrument has sufficient ventilation:

Take care not to block the air supply underneath the microscope and at the connected lamphousings or the air vents on the top of the microscope with paper, etc. Fire hazard! Minimum distance from inflammable objects 10 cm (4").

Fig. 8 Lamphousing 106 z with Hg 50 burner

1 Lid, 2 Collector, 3 Burner (Hg 50), 4 Reflector, 5, 7 x/y adjustment of the reflector, 6 Reflector focusing, 8 Sockets for safety cut-out plug, 9 Lamp holder screws



Filter holder*/lamphousing

Filters with a diameter of 50 mm can be inserted in the special filter holder (accessory, Fig. 9) next to the lamphousing or in the microflash, or placed on the microscope base (27.3) in transmitted light.

Microscope base* and condenser*

Filters with a diameter of 32 mm and holders can also be placed on the microscope base. The mount on the underneath of the condenser holder (27.6) should only be used for the polarizer or whole- or quarter-wave compensators (57a,1 and 2).



Filters situated between the microscope base and the condenser may cause disturbing reflections (this may be remedied by slightly tilting the filter) and lead to strain birefringence in polarized light and ICT.

Fig. 9 Filter holder (intermediate unit), with lamphousing for max. 4 filters, dia. 50 mm (when lamphousing 106 is used, only 1 thick or 2 thin filters can be inserted)



Filter magazine*

The best way to accommodate filters is therefore in the filter magazine (Fig. 10, 42.8 and 42.15): Loosen the 2 fixing screws to remove the filter magazine. It is easier to remove if the four controls are operated. Put the filters into the slots (without holders!) and tighten the clamp screw. Always put the diffusing screen in the position nearest the lamp. Put the label caps (10.3) onto the corresponding switch rods and align the lettering.

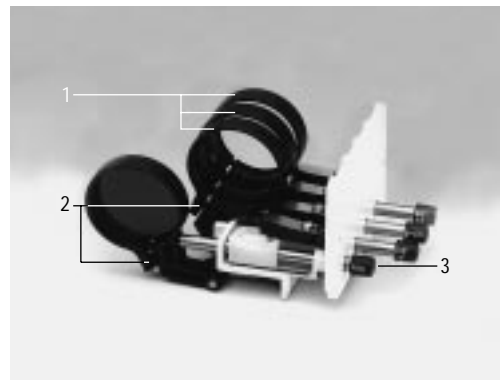
The filter magazine is more easily replaced if all 4 filters are tilted to one side first by pressing the buttons. Finally, check that all 4 filters can be switched in and out smoothly and tighten the fixing screws. If thick filters get stuck, try putting them in a different slot or altering their position in the slot.



Interference filters must be inserted with the bright reflecting side towards the light source!

Fig. 10 Filter magazine T/R (for transmitted and incident light, Figs. 42.8 and 42.15), also available with only 1 pos.

1 Filter holder (Ø 32 mm, non-mounted), 2 Clamp screw for filter, 3 Switch rod with push-on label caps



Mechanical stages* no. 1187 and 1189

Size of stage plate 200 mm x 159 mm, movement range of object guide 76 mm x 46 mm, with 0.1° verniers for registration of specimen coordinates. Removable specimen holder.

Up to 110° stage rotation, clampable. Vertically adjustable coaxial drive for specimen positioning. Maximum specimen weight 4 kg.

Stage clearance 25 mm for fixed stage, 63 mm for interchangeable stage. 2 M4 drill holes for attachment of heating stages.

The 1187 stage (Fig. 11) is especially designed for transmitted light and fluorescence microscopy, whereas the similar 1189 stage is for incident light microscopy (i.e. for thicker and heavier samples; shorter coaxial drives and sample holder without spring clip), but also for transmitted light microscopy.

Stage no. 1086 U*

with inverted stage bracket, for incident light only. Size: 160 x 150 mm, stage clearance: 123 mm. Object guide no. 12* can be adapted.

Rotary Pol stage*

Precision stage on ball bearings, stage diameter 179 mm, 360° scale division and 2 verniers reading to 0.1°, 45° clickstops, can be activated in any azimuth, Fig. 13. 3 M4 drill holes for attachment of heating stages, object guide, etc., Fig. 13.

Pol 3 adaptable object guide for specimen formats 25 mm x 46 mm, 25 mm x 75 mm, 50 mm x 50 mm. Interchangeable control knobs with clickstops at 0.1, 0.3, 0.5, and 2 mm object displacement in x and y direction.

Other stage variants are adaptable besides these standard models, e.g. the SCOPOSCAN® scanning stage.

Only for microscopes with fixed stage

The stage is protected against transit damage by 2 foam blocks (Fig. 11). Push out the upper block first. To remove the lower block, move the coarse drive* (42.12) slightly. The block can then be pushed out at the side.



Attention:

If the microscope has a motor focus: after switching on the microscope* (42.14) tip coarse focusing "Up" (44.2, page 58) 1–3 times to make the stage move upwards slightly. The foam block can then be removed at the side. Keep the foam blocks in case the microscope needs to be transported again, as long periods of vibration lead to damage!

Only for microscopes with interchangeable stage

Assemble the condenser holder* (12.10) first (see page 20). Loosen the stage clamp (12.1) and hold stage against the dovetail guide (12.4). Screwing the stage clamp only slightly, align the stage for specimens up to a thickness of about 1.3 mm (transmitted light specimens) so that the top end of the dovetail guide is flush with the top end of the stage clamp. For thicker specimens (incident light) and heating stages the stage is clamped lower down.



Then clamp the stage tightly, as otherwise it may tilt slightly when a heavy load is placed on it.

Fig. 11 Transit protection for microscopes with fixed stage*

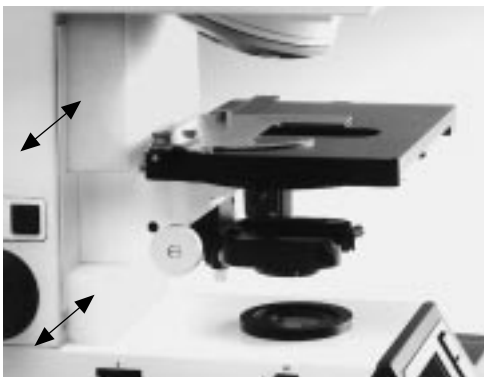
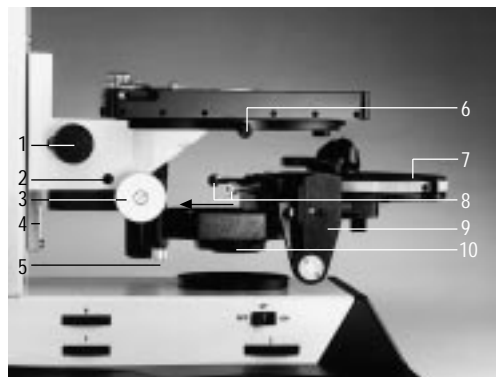


Fig. 12 Assembly of condenser holder* and specimen stage*
1 Stage clamp, 2 Drill hole for clamping the condenser holder (3 mm hexagonal screwdriver), 3 Condenser height adjustment, 4 Dovetail guide, 5 Adjustable upper stop of condenser, 6 Stage rotation clamp (no. 1187 and 1189), 7 Universal condenser with disc, 8 Centering screws for light rings/IC prisms, 9 Lever for condenser top, 10 Condenser holder (with slot for whole- and quarter-wave compensators)



Pol object guide*

Move the object guide until the fixing screw can be seen under the drill hole (13.1). Insert the object guide in the guide holes of the rotary stage and tighten the fixing screw with the hexagonal screwdriver.

Attachable object guide*

The attachable object guide can be fixed on the left, right or at the front (not illustrated) with the two clamp screws.

Condenser holder*

The microscope stage must be equipped with the condenser holder (12.10) for transmitted light work. The condenser holder enables various condensers to be changed quickly and centered and takes components for polarized light (Figs. 27.6 and 57.1). An adjustable upper stop (12.5) guarantees a reproducible vertical setting of the condenser (Koehler illumination).

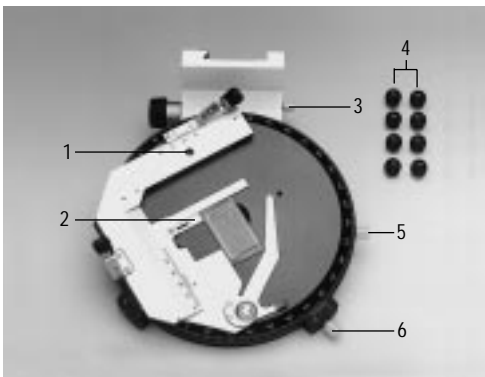
Interchangeable stages only: to assemble the condenser holder, either remove the stage or move it as far upwards as possible. Loosen the clamp screw (12.2) slightly with the 3 mm hexagonal screwdriver, slide the condenser holder onto the guide pin and retighten the clamp screw (12.2) (already assembled for fixed mechanical stage).



Important! Do not mount at an angle, note the stop!

Fig. 13 Rotary Pol stage* and Pol 3 object guide*

1 Drill hole for fixing screw, 2 Swing-in/out lever to hold specimen slides of different formats, 3 Place to keep centering keys, 4 Pairs of clickstop buttons, 5 45° clickstop, 6 Stage rotation clamp



Survey condenser

Only in combination with the Bertrand lens and survey observation (without objective!) see p. 64.

UCE* universal condenser

For objective magnifications from 1.6x (transmitted light interference contrast ICT from 10x objective) with sledge changer, swing-in/out holder for condenser tops. When the condenser top is swung out of the light path (objectives 1.6x–6.3x) the field diaphragm takes over the function of the aperture diaphragm (Fig. 14b).

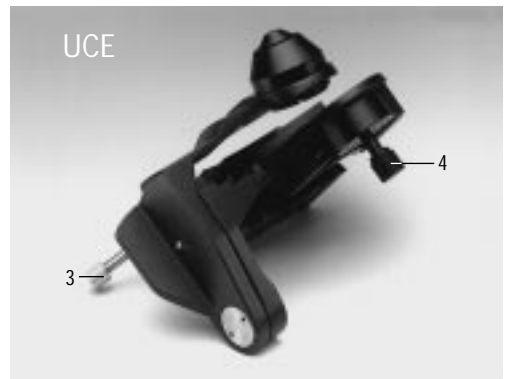
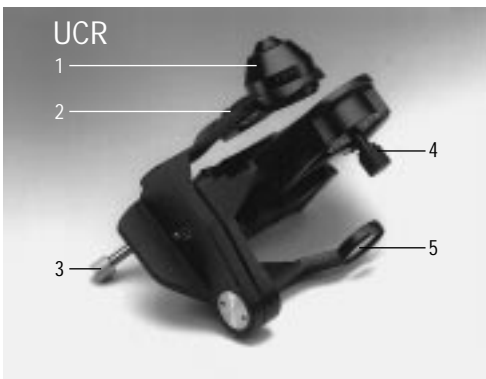
UCR and UCPR* universal condenser

For objective magnifications from 1.6x (transmitted light interference contrast ICT from 5x objective) with sledge changer, swing-in/out holder for condenser tops, coupled with 2 auxiliary lenses (14.2 and 14.5), i.e. homogeneous illumination of the specimen and Koehler illumination are guaranteed for all magnifications from 1.6x.

Fig. 14a/b UCR/UCE universal condensers

The UCPR condenser has the same construction as the UCR condenser

1 Condenser top, 2 Upper field lens, 3 Centering screw for light rings and IC prisms, 4 Fixing screw for condenser disc (removed), 5 Lower hinged lens (field lens)



Condenser discs* for contrast techniques

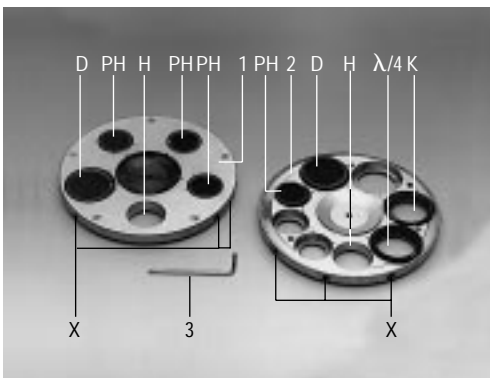
Both condensers can be fitted with discs for various contrast techniques (HF = Brightfield, DF = darkfield, PH = phase contrast, ICT = transmitted light interference contrast) (See Fig. 15).

5-position condenser turret for HF, DF, 3 PH positions (15.1).

8-position condenser turret for HF, DF, 3 PH positions, 3 ICT positions, or HF, 3 PH positions, 4 ICT positions (15.2). Whole- and quarter-wave compensators (15.3 or 17.6) can also be used instead of ICT prisms for polarized light microscopy.

Fig. 15* Discs for UCR, UCPR and UCE condensers

1 5-position disc, complete, 2 8-position disc, position 3 not yet inserted, cover plate (with label) removed, 3 Assembly keys for light rings and ICT prisms, H = hole for brightfield, PH = light ring for phase contrast, D = light ring for darkfield, K = Condenser prism K for ICT, $\lambda/4$ = compensator for polarization, X = holes for centering keys



Condenser tops* for UCE, UCR, UCPR condensers

The following condenser tops are available (Fig. 16):

0.90 S1

Dry condenser top for glass specimen slides up to about 1.2 mm. For HF, DF (up to objective apertures of 0.75), PH and ICT and polarization contrast.

P.0.90 S1

As 0.90 S1, but for polarizing microscopes.

P.1.40 OIL S1

for ultra high resolution in brightfield and for polarized light (conoscopy) and for ICT; for glass specimen slides up to about 1.2 mm.

Achr. 0.50/S15 for intercept distances up to about 15 mm, e.g. for heating stages, for BF and DF.

Fig. 16* Condenser tops for UC/UCE condensers



Condenser top

Screw the condenser top (Fig. 16) onto condenser (14.1).



Attention:

Move the stage as far upwards as possible with the coarse drive (42.12 or 44.2). Move the condenser holder downwards as far as the stop (12.3).

Securing the condenser

Align the condenser against the horizontal dovetail guide so that the two centering screws (12.8) point to the back towards the microscope; Flip the condenser top to the front (lever 12.9). Loosen the clamp screw (27.4) and carefully push the condenser to the back as far as the stop. Slightly tighten the clamp screw (27.4).

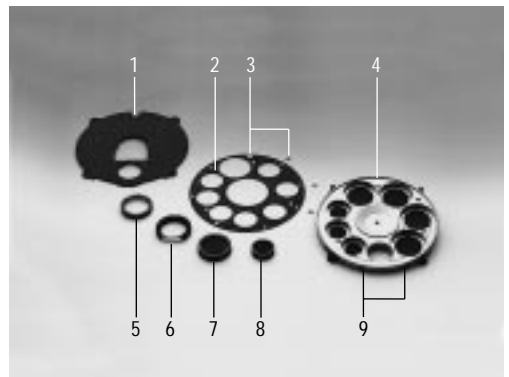
Light rings* and turrets*

For transmitted light darkfield (DF) and phase contrast (PH) the UCR, UCPR and UCE universal condensers (Fig. 14) must be equipped with a 5- or 8-position condenser disc (Fig. 15) with a set of light rings DF, PH (17.7 and 17.8). Darkfield can also be produced with the special darkfield condensers (Fig. 53). The 8-position disc with ICT prisms K is required for transmitted light interference contrast ICT.

The light rings are normally inserted into the disc at the factory, so that you can skip the following assembly instructions. You can tell that the light rings have been inserted by the fact that the four annular stops can be seen in the window when the inner plate is rotated and that the labels DF, 1, 2, 3 (17.3) appear in the reading window.

Fig. 17 Fitting the turret plates

1 Upper cover plate with reading window, 2 Lower cover plate (8-position disc only), 3 Label plates, 4 Turret (8-position in illustration), 5 ICT prisms K for ICT interference contrast, 6 Quarter- (and/or whole-)wave compensator for polarized light microscopy, 7 Light ring for darkfield, 8 Light ring for phase contrast, 9 Adjustment screw(s)



Light rings* and discs*

Remove the disc from the condenser after loosening the clamp screw (14.4). Take off the cover plate (17.1) after unscrewing the 4 fixing screws. For 8-position disc only: Also take off the second cover plate (17.2) after unscrewing the 3 fixing screws.

Insert the light rings for phase contrast (17.8, identified by the code nos. 1, 2, 3 and the intercept distance S of the corresponding condenser top, e.g. 2 S 1) into the small holes (Fig. 15/PH) of turret as follows:

- Unscrew both centering screws (15.X) slightly with the supplied Allen key (15.3) so that the light rings can be inserted.
- When the light rings are inserted, their labels must be visible, i.e. pointing upwards.
- Keep to the order 1, 2, 3. Insert the large light ring for darkfield DF into the large hole (15.D, with centering facility). The darkfield ring can only be inserted into 2 of the 4 large holes on the 8-position turret.
- Using the Allen key, readjust the centering screws until they do not protrude outside the outer edge of the disc and the light rings cannot fall out.

- Fit ICT condenser prisms if used (see below).
- For 8-position turret only: Lay the cover plate (17.2) on the disc so that all drill holes coincide and fix with the 3 screws. Push the plastic labels (17.3) into the cover plate as follows:
 - On the side opposite to the axis of rotation, corresponding to the light ring, i.e. ② for light ring 2 S 1, ① for darkfield, ③ for brightfield, etc.
 - So that the lettering is not upside down when read, i.e. reading in a direction away from the outer edge of the turret.
 - Label unoccupied positions with blank white plates if desired.

Screw the upper cover plate back on with the 4 screws and fix the disc back onto the condenser (14.4). Make sure that the disc can be rotated by 360°.

λ - and $\lambda/4$ -compensator

Model for 8-position condenser disc (17.6): Insert so that the notch engages in the spring fin; fix with an Allen key (15.3).

ICT condenser prisms*

Remove the 8-position disc (15.2) by unscrewing the fixing screw (14.4) (the 5-position disc is not suitable for ICT). Take off the upper and lower cover plates after removing the 4 (3) fixing screws.

Insert the ICT condenser prisms K (17.5) into the large holes (15.K) in the order of their code numbers (i.e. K1, K2, K3). Insert the prisms so that the code, e.g. K1, is on the outside. Turn back the adjustment screw (15.X) if necessary, turn back both adjustment screws in positions 3 and 4. Press the prism against the spring clip and engage the catch on the underneath in the guide groove. Tighten the left-hand adjustment screw if necessary (the additional right-hand adjustment screw in positions 3 and 4 is for darkfield or phase contrast only and must therefore stay screwed back for ICT so that the adjustment of the prism with the left screw is not obstructed).

Mount the light rings for phase contrast and darkfield if appropriate (see page 23). First lay the round cover plate on the disc so that all drill holes and windows coincide and then push in the corresponding labels (17.3, e.g. $^{10}/_{20}$ for 10x and 20x objectives), as follows:

- On the opposite side (i.e. on the other side of the axis of rotation).
- So that the lettering is not upside down when read, i.e. reading in a direction away from the outer edge of the disc.



- Different labels may be necessary for different objective classes (e.g. N PLAN, PL FLUOTAR, HC PL FLUOTAR, PL APO), so always refer to the supplied optics chart for prisms!
- Label unoccupied positions with blank white labels if desired.
- Carefully wipe any fingerprints or dust off the prisms.

Replace both the cover plates with the 7 screws and attach the whole disc to the condenser. Mount the condenser top 0.90 S 1 or P 0.90 S 1 or P 1.40 OIL S 1 (other condenser tops are not suitable!).

**Incident light reflectors*/
fluorescence filter systems***

Remove the front cover of the microscope (Fig. 19) by strong pressure upwards at an angle. Insert the filter system (combination of

excitation filter, dichroic mirror and suppression filter) or the incident light reflector or the adjusting reflector (Fig. 18) into the turret (Fig. 20) with the angled end of the dovetail guide first as far as the stop.

Fig. 18* Incident light reflectors* and filter systems*

1 45° BF reflector with neutral density filter* N, 2 DF darkfield reflector, 3 Adjustment reflector (DMR series only), 4 Fluorescence filter system, 5 Bertrand lens module, 6 ICR module, 7 POL system, 8 Smith reflector



Fig. 19* Front plate with incident light turret

Sticker with filter positions 1 – 4

Stickers of corresponding filter systems or reflectors



Fig. 20* Incident light turret

1 Display of position in the light path, 2 Display of filter system or reflector, 3 Marking of assembly position, 4 Filter system or reflector or adjusting reflector

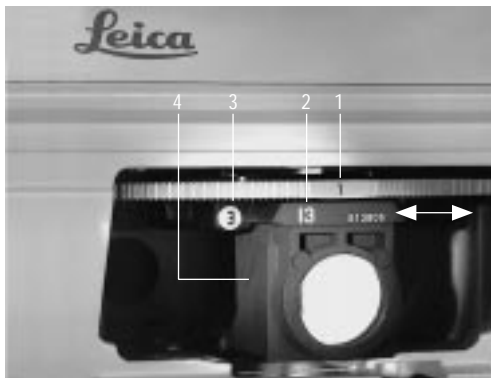
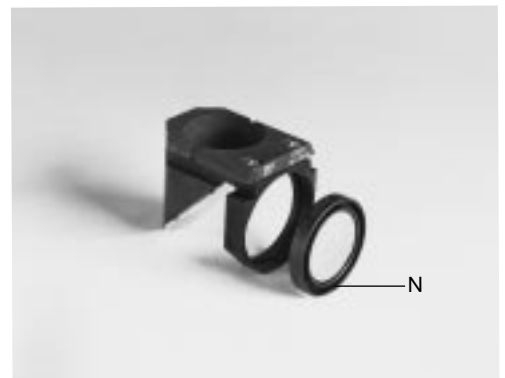


Fig. 21* Slot-on neutral filter N for BF reflector



Up to 4 positions can be occupied by rotating the turret.

In combination with incident light darkfield, a neutral filter (Fig. 21) can be slotted onto the BF reflector (for brightfield, polarized light and interference contrast) to avoid glare when switching between illumination techniques.



The adjusting reflector, Smith reflector and DF reflector can only be placed at **opposite** positions. The 4 turret positions are each marked on the left of the dovetail guide with the numbers 1 – 4 (20.3). In addition the position currently in the light path is indicated on the outside of the turret (20.1). Self-adhesive labels indicating the positions 1 2 3 4 and the abbreviations for the filter blocks and the reflectors (e.g. D) are enclosed with the filter systems and reflectors. Stick the label 1 2 3 4 in its place in the upper line on the front plate (Fig. 19).

Then stick the labels with the abbreviations in the corresponding fields underneath according to the marking on the systems (20.2) and the number indicated on the left on the filter wheel (20.3). The Smith reflector (with two reflecting surfaces and lenses, Fig. 18.4) and the DF reflector (with ring mirror, Fig. 18.3) do not have a label.

Push the front cover hard until it locks back into place.

Retrofitting the incident light axis*

Microscopes that were not fitted with the HC RF 4 IL* module at the factory can have it retrofitted as follows:

The following components are necessary for fluorescence (for IL-BF/DF/ICR additional components are required from the Technical Service):

- HC RF 4 IL* module, incl. 4 mm Allen screws (22.2)
- Deviating mirror with mount for lamphousing incl. 4 4 mm Allen screws (3.1) or switchable mirror (3.3)
- Cover plate for the side of the stand (22.10)
- Lid for filter magazine mount, incl. 2 cross-head screws (22.8) or filter magazine (Fig. 10)
- Ground glass disc for lamp centration in mount (22.5)
- Adjustment aid (22.9 or 18.2)
- Front cover with hole (22.12)
- Diaphragm module (see p. 29 – 30)
- 2 centering keys (1.5)
- Lamphousing 106 or 106z, power unit(s) if required.

* IL = incident light

Remove the front cover of the microscope (22.12); it is no longer required.

Using the supplied 3 mm screwdriver unscrew the 4 fixing screws (22.1) and remove the cover with built in tube optics from the microscope.



Caution:

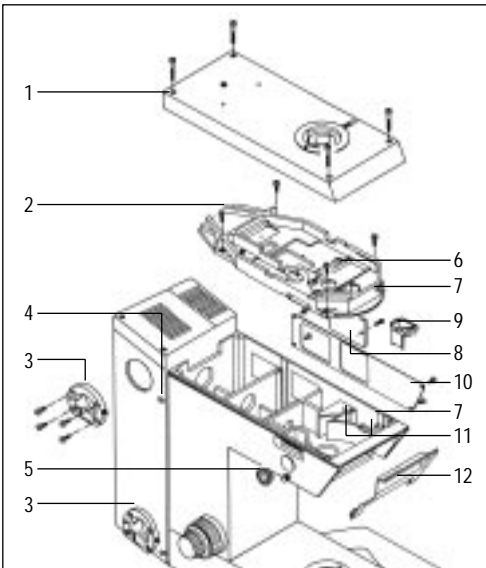
Store upside down so as not to damage the optics. Protect from dust!

Using the crosstip screwdriver, unscrew the 4 fixing screws (22.11) of the analyser mount and remove it (this component will not be required again as the analyser mount is integrated in the HC RF 4 IL module, 22.6).

Using the 2 mm screwdriver, unscrew the 4 Allen screws on the lateral cover plate (22.10). This plate is no longer needed. Please keep the Allen screws.

Fig. 22* Retrofitting the incident light axis (only for BF, DF and fluorescence! Pol and ICR components can only be retrofitted by the Technical service)

1 Cover plate (tube optics) with 4 fixing screws, 2 RF 4 incident light module with 4 fixing screws, 3 Lamp mount (with or without reflector), 4 Mount for switch rod (for switchable mirror only), 5 Ground glass screen for lamp centration, 6 Analyser mount, 7 3 control points for assembly, 8 Cover plate or filter box, 9 Adjustment aid (reflector), 10 Lateral cover plate with 4 fixing screws, 11 Analyser fixture (only before conversion), 12 Front cover with hole



Push out the cover cap from the inside and clip the holder with the ground glass screen (22.5) for lamp centration in its opening in the stand.

Insert the HC RF 4 IL module (22.2) into the stand from above, with the turret pointing to the front and downwards, as follows: Holding the HC RF 4 module in the longitudinal axis, tilt it slightly forwards. Carefully put the module into the stand with the turret as high up in the front hole as possible.

Put four 4 mm Allen screws into the bore holes in the HC RF 4 module, move the module to the right and to the front so that it pushes against the stops (22.7) and tighten the screws with the screwdriver.



Attention:

Put the cover back on the microscope (caution: built-in optics!), align by moving to the front and to the right (22.7) and secure with the Allen screws.

Fix the metal cover (22.10) to the side of the stand with the 4 Allen screws (2 mm screwdriver).

Close the mount for the IL filter magazine with cover (22.8) and screw down the cover with 2 cross-head screws or attach the filter magazine (Fig. 10).

Hold the front cover (22.12, with slit) against the microscope and push slightly so that it clicks in position.

Assembly of deviating mirror on page 10, lamp-housing on page 16.

Diaphragm modules

The diaphragm module HC F has a centrable aperture (23c.6 and 8) and field diaphragm (23c.3 and 4), an engageable BG 38 red attenuation filter (23c.11) and a switch for blocking the incident light path (23c.12). Main application: fluorescence microscopy.

The diaphragm module HC RF has an additional decentrable aperture diaphragm for oblique illumination (23b.6 and 7); instead of the BG 38 filter and the light path blocking switch it has a light-blocking neutral density filter (23b.5), interchangeable diffusing screens (23b.9) and an optional focusing graticule* (23b.10).

Main applications: all incident light techniques especially bright field and darkfield, polarized light and ICR reflected light interference contrast.

There is also a special MPV diaphragm module HC for microscope photometry, and the reflection contrast module HC RC (see separate manuals).

Assembly of diaphragm module HC F*

Push into the slot (63.5) from the left as far as possible.

Functions → p. 93.

Assembly of diaphragm module HCRF*

Insert the focusing graticule in the mount* (23a/b.10), first slackening the clamp screw (23a.10) if necessary, making sure that the **smooth** side of the mount points inwards, the rotatable mount with slit points outwards, see p. 64. Tighten the clamp screw only slightly.

The diffusing screen set A (23b.9) can be turned over and interchanged with set B. Turn the slit of the screw (23b.1) so that it is horizontal. Insert the diaphragm module HC RF into the slot in the stand (65.9) as far as possible. Turn the screw slit (23b.1) to a vertical position; the diaphragm module is now locked in position. Functions → p. 93 and 96.

Fig. 23 Diaphragm modules HCRF (a, b) and HCF (c)

1 Fastening screw, 2 Grip for pulling module out, 3 Field diaphragm, 4 Centering screws for field diaphragm, 5 Neutral density filter N in/out, 6 Aperture diaphragm, 7 Decentration of aperture diaphragm, 8 Centering screws for aperture diaphragm, 9 Diffusing screen set A and B, 10 Focusing graticule with clamp screw, 11 BG 38 filter, 12 Interruption of light path, 13 Lever for additional lens

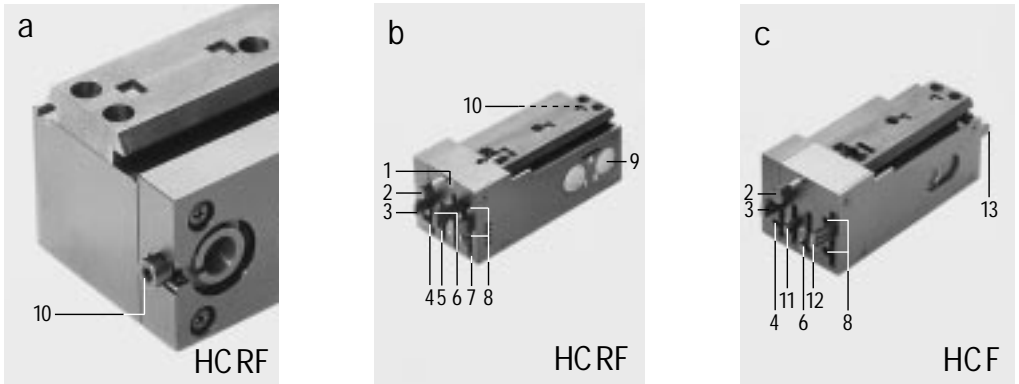
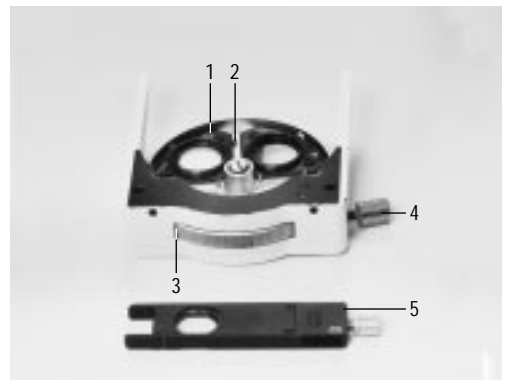


Fig. 24 IC objective prism turret and slide

1 IC prism with code letter, 2 Stop pin, 3 Adhesive label with code letters (for opposite position!), 4 Adjustment screw, 5 IC prism in slide (only ICR reflected light with Pol objective nosepiece)



Objektive prisms* for interference contrast ICT/ICR

The prisms are already fitted into the turret at the factory in various configurations. If you should want to change the prisms yourself: make sure to push the prism mount against the guide pin (24.2) and do not screw the fixing screws too tightly (use washers!) to avoid strain. The code letters, e.g. A, must be visible, cf p. 48 and 86. Stick on adhesive label (24.3) corresponding to lettering of opposite positions, e.g. A.

The turret is assembled in its mount to the objective nosepiece as follows*: Unscrew the two fixing screws (25.2 and 25.3) on the underneath of the nosepiece with the 3 mm hexagonal screwdriver, remove the cover plate (25.3), put the IC turret in position and press hard against the two stops (25.1). Fix in position with the two longer screws. It is practical to take interchangeable nosepieces off the microscope for this conversion.

* When the IC device is ordered as a complete outfit, these components are generally assembled at the factory.

Fig. 25 Conversion of objective nosepiece

1 Stop pins in objective nosepiece, 2 IC prism turret with 2 fixing screws, 3 Cover plate

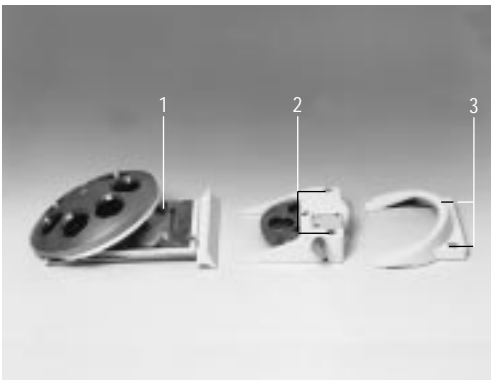


Fig. 26 Objective centering nosepiece*:

Screws for tube slit/IC objective prism turret changeover. The other screws must not be loosened under any circumstances.



On the Pol centrable nosepiece (Fig. 26 and 38.2) the tube slit (compensator module, 38.6) must be removed instead of the cover plate. This is done by unscrewing the 2 fixing screws on the top surface (Fig. 26).



Attention:

Important: Do not unscrew the other 4 fixing screws or the centration of the nosepiece axis will be lost!

Alternatively, single objective prisms in slides (not illustrated) can be inserted into the centrable objective nosepiece (54.13), but only for incident light interference contrast ICR.

Transmitted light polarizers*

The polarizer for polarization contrast (27.3) can either be placed directly on the window in the microscope base or inserted from the right into the mount on the underneath of the condenser holder (27.6).

ICT/P polarizer (Fig. 28) only:

Remove the black plastic cover ring (42.7) from the microscope base by exerting strong pressure.

Fig. 27 Condenser and transmitted light polarization contrast*
 1, 5 Condenser centration, 2 Fixing screw for the turret plate, 3 Polarizer (Ø 32 mm), 4, 5 Condenser clamp, 6 Mount for whole- or quarter-wave compensator or polarizer (Ø 32 mm)

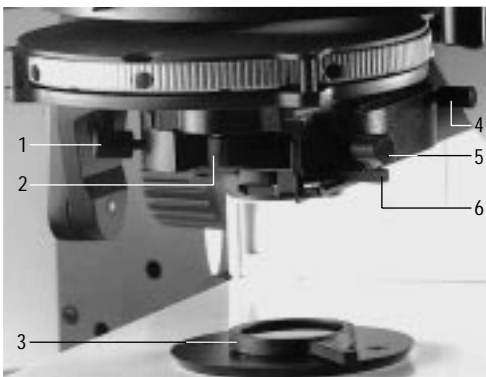
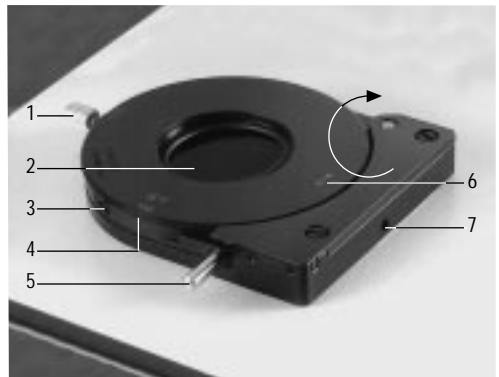


Fig. 28 ICT/P polarizer*

1 Clamp screw for rotation, 2 Polarizer (at an angle), 3 Index adjustment, 4 Index reading, 5 Lever for disengaging the polarizer, 6 Vibration direction of the polarizer ↕, 7 Fixing screw



Slightly unscrew the clamp screw (28.7) if necessary with the Allen key (1.5 or 1.4). Place the transmitted light polarizer on the microscope base with its straight outside edge parallel to the right outside edge of the microscope base.

When you notice the orientation slot click into position (left) retighten the clamp screw.

Reflected light polarizers*

One of the following polarizers is used, depending on the area of application. They are inserted as far as possible into the stand from the right (29 and 65.4) see also p. 99.



Attention:

Hg and Xe lamps can destroy the polarizer, so use protective filter (29.6)!

Polarizer R/P

For qualitative and quantitative reflected light polarization (29.1). The interchangeable Pol filter can be taken out and inserted in two positions:

± parallel to the longitudinal axis of the mount: for polarized light microscopic examinations with the analyser 360 (30.1). The analyser must be set at 90.0° at the crossed position (see page 77).

◇ vertical to the longitudinal axis of the mount: this position is always used with analyser IC/P (30.5) 45° , analyser 360 only. For ICR up to fov 20 only!

Polarizer with whole-wave compensator

For qualitative reflected light polarization (29.2). The rotatable whole-wave compensator permits extremely sensitive colour contrast, e.g. for microscopy of anisotropic ores and metals such as aluminium.

Polarizer ICR

With fixed vibration direction (N–S) (29.5), due to built-in MgF_2 plate up to fov 25, but not for polarized light. For reflected light interference contrast ICR the ICR reflector with polarizer, analyser and MgF_2 plate can be used instead.

Fig. 29a Reflected light polarizers*

1 Polarizer R/P (switchable vibration direction), 2 Polarizer with whole-wave compensator, 3 Polarizer rotation, 4 Whole-wave compensator rotation, 5 ICR polarizer

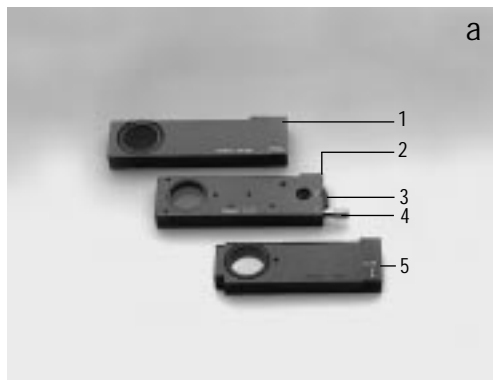
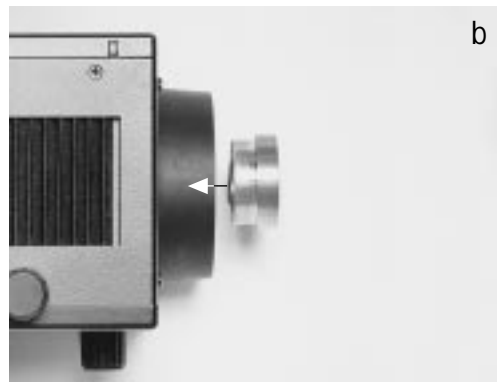


Fig. 29b Protection filter* for Hg and Xe lamps in polarized light*



POL filter system Reflector ICR

The polarizer and analyser are in a fixed crossed position and combined with a 45° reflector. Inserted like filter systems and reflectors (see p. 26). The ICR reflector has a built-in MgF₂ plate as well: better homogeneity (fov 25) but not for colour contrast. Polarizer and analyser are not required in this case.

Protective filter



Attention:

When using Hg and Xe lamps, the polarizers must be protected by a special protective filter!

Analysers*

There are two different types of analyser for reflected and transmitted light polarization and interference contrast techniques:

Assembly: remove cap and insert analyser from the left (48.2 or 54.3) as far as possible.

Analyser IC/P

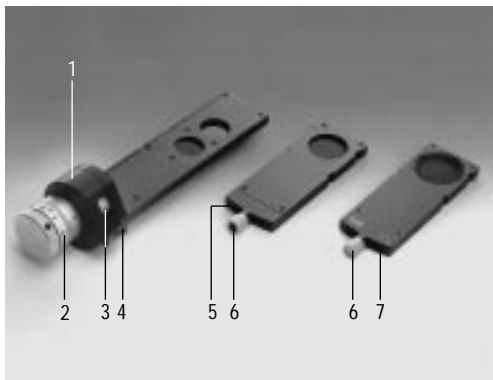
Polarization direction E – W, rotatable through approx. $\pm 7^\circ$ (30.5). Combined with a whole-wave compensator (λ) on its upper surface, so when the analyser is inserted the other way up, red I becomes active (30.7), see also colour chart on p. 80.

Analyser 360

Rotatable through 360° and reading to 0.1° (30.2), vibration direction in 90° setting according to DIN: N – S. Engageable (30.4) neutral density filter in empty slot to prevent glare when the analyser is switched off. A whole-wave compensator is not integrated, so colour contrasting is only possible for ICR reflected light interference contrast with a polarizer ICR from the “DML” range.

Fig. 30 Analysers

1 Analyser 360, 2 Precision scale with 0.1° vernier (clamp screw on the back), 3 Orientation scale (90° intervals), 4 Neutral density filter switch, 5 Analyser IC/P, with whole-wave compensator inactive, 6 Clamp screw and index, 7 Analyser IC/P turned the other way round for use of whole-wave compensator



Functional description

In all microscopes with infinite tube length (∞) the objective theoretically forms the image at infinity, which would be of no use to the microscopist.

Therefore microscopes with infinite tube length always need a tube lens that projects the intermediate image into the eyepiece. The magnification of an objective for tube length ∞ thus depends not only on the focal length of the objective, but also on the focal length of the tube lens, which is 200 mm. The magnification of this system, i.e. objective + tube lens, is engraved on the objective, while the tube factor is defined as 1x and therefore does not need to be engraved (according to DIN and ISO standard). Infinity objectives that comply with these conditions are identified by the code nos. beginning with the figure 506..., 556..., 557..., 566..., 567.

Objectives for ∞ microscopes with conventional reference focal length $f_B = 250$ mm can also be used, but the engraved magnification factor must be corrected with the value $200 : 250 = 0.8x$. However, as the visible field is then enlarged by the factor 1.25x, the edges of the image may be blurred. The code nos. of these objectives for tube lens focal length 250 mm begin with 559..., and 569...; an adapter (spacer ring 32/RMS or 25/RMS is also necessary due to the RMS objective thread (see Fig. 39). The mount (labelled collar) may also require modification.

Another important function of the tube lens is correction of chromatic and other image aberrations, such as astigmatism. This used to be performed by the eyepieces in former microscopes. Additional correction by the tube lens, however, has proved to be far more advantageous. Optimum colour correction cannot be carried out by one single lens – a system of several lenses, some of them cemented, is used, so that it is more accurate to speak of a tube lens system. The tube lens system is permanently integrated in the top plane of the stand (22.1), designated as cover plate in the instruction manual, except for the tube module HCL (\rightarrow p. 36). This module is available in interchangeable versions.

Conversion of tube optics

Remove the 4 fixing screws (22.1) using the hexagonal screwdriver, remove the tube optics by pulling upwards and mount the module of your choice with extreme care.



Attention:

Make sure the components are completely clean – it is particularly important to check that there is no dust or fingerprints on the underneath of the tube lens. Screw in the four fixing screws loosely, so that you are still able to move the module.

In the opened upper part of the stand there are 3 stop points (22.7), with corresponding points in the tube module and in the incident light module. Carefully pull the tube module forwards and simultaneously to the right to ensure that there is precise fitting at these three points. Carefully tighten the 4 fixing screws.

The following versions of the tube optics are available:

Tube optics HC E

With tube factor 1x

For brightfield, darkfield, interference contrast ICT and ICR, polarization contrast, fluorescence. An auxiliary telescope (51.1) with adapter (51.3) is also required for phase contrast, but for this the tube optics HC B (or HC V) with Bertrand lens is recommended.

Tube optics HC B with Bertrand lens

With tube factor 1x, engagable and focusable Bertrand lens.

Specially for the adjustment of darkfield, phase and interference contrast and for survey observation (p. 65) and observation of very fine bores. For all other techniques, including polarization contrast, but not for quantitative polarization microscopy (42.2 and 50.2).

Tube optics HC V:

Magnification changer with Bertrand lens

With tube factors, 1x, 1.25x, 1.6x and focusable Bertrand lens (adjustment DF, PH, ICT and for survey observation), see p. 64.

Tube optics HC P 1x/1.6x with Bertrand lens

With tube factor 1x, switchable to 1.6x, engagable focusable and centerable Bertrand lens. Iris diaphragm in intermediate image for isolation of small grains (15 μm for 100x objective). Specially for polarized light microscopy, but can also be used for all other techniques (54.1, 54.2; 58), see p. 77.

Integrated depolarizing quartz plate: prevents the formation of interference colours due to polarization effects of tube prisms (pseudo-dichroism) when the analyser is disengaged and the polarizer engaged. Only effective with tube factor 1x, however. Not for spectral photometry. When using tube factor **1.6x**, remember that at high objective magnifications and apertures the useful magnification (objective aperture x 1000) may be exceeded, causing blurred images. Quartz plate inactive.

Tube module HC L 4/25

Without tube optics, only for adaption of HC L tubes from the DML microscope range in which the tube optics are integrated.

Tubes (DM R series)

A wide range of tubes for various applications is available for the LEICA DM series of microscopes.

The abbreviations in the names of the tubes mean:

HC = Tube system HC, only with HC PLAN and wide field eyepieces, HC photo adapter components, HC TV adapters.

E = Phototube, i.e. apart from the binocular observation part the tube also has a vertical photo exit for adaption of photomicrographic equipment, video cameras and microscope photometers.

B = Binocular tube, for visual observation only.

SA = Automatic focus compensation: if the binocular viewing port set to the individual interpupillary distance of the user (p. 67), changing optical path length (which would cause a blurred image when the magnification was changed and during photography) is automatically compensated.

P = This tube is also fully suitable for polarized light microscopy, as the crosslines in the right-hand eyepiece are automatically aligned together with the tube to the polarized light microscope.

E = Provision for lateral adaption of overlay device (p. 40 and 101).

R = Back reflection of format outlines and measuring spot possible for photomicrography and photometry.

25 = Eyepieces up to field of view index 25 can be used (e.g. L PLAN 10x/25)

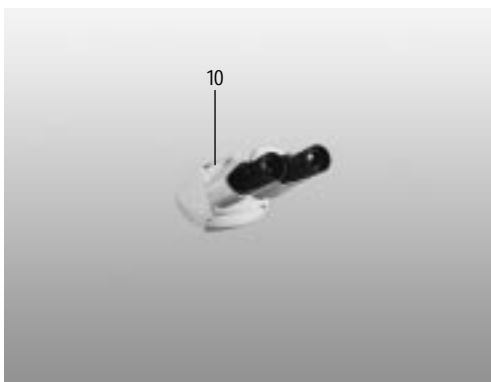
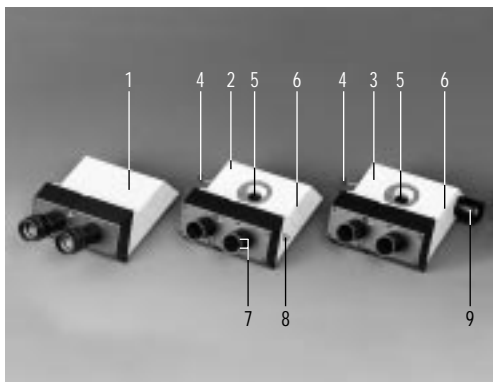
Outer diameter of eyepieces: 30 mm

V = Variable viewing angle.

L = DM L tube range with integrated tube optics.

Fig. 31 Microscope tubes

1 BSA 25: binocular tube with focal compensation (shown with pair of eyepieces), **2** HC FSA 25 PR and HC FSA 25 P: binocular phototubes with (PR) or without (P) back reflection, **3** FSA 25 PE: binocular phototube with provision for adaption of lateral overlay device, **4** Switch rod for beamsplitter, **5** Mount for photo adapter, **6** Photo adapter clamp, **7** Clickstop for Pol eyepieces, **8** Socket for light trap control cable (PR tube only), **9** Connection for lateral overlay device, **10** Example from HCL tube range with integrated tube optics (tube HC LVB 0/4/4)



BSA 25




Binocular observation tube 25, Fig. 31.1

Viewing angle 30°, not for polarized light microscopy.

HC FSA 25 P

Binocular observation and photo tube (31.2).

Viewing angle 30°, also for Pol microscopes, with 3 clickstop positions of the beamsplitter in the tube:

Switch rod (31.4)	Visual	Photo
	100 %	0 %
	50 %	50 %
	0 %	100 %

HC FSA 25 V

Binocular observation and photo tube (31.10) with variable viewing angle from 0–35° and image erection, i.e. image of object appears the right way up and the right way round. 2 switching positions: 100 % light to binocular port or 20 % visual and 80 % vertical. Not for polarizing microscopy.

HC FSA 25 PR

Binocular observation and phototube (31.2).

Like HC FSA 25 P, but with additional back reflection for the MPV microscope photometer. Switchable light trap of the binocular port for microphotometry. Back reflection only at the beamsplitter setting 50 % / 50 %.

HC FSA 25 PE

Binocular observation and phototube (31.3).

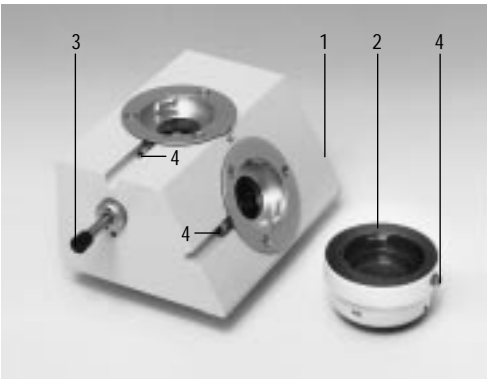
Like FSA 25 P, but with additional provision for the overlay of transparent (diapositive overlay) or non transparent (macro device) masks, see pages 40 and 102.

Photo adapter tube HC FSA and HCL

Interchangeable photo adapter tube with vertical exit (32.2) or with vertical **and** horizontal* exit (32.1) for all HC FSA tubes, with 2 clickstop positions for switchable beamsplitter (100% to the top exit or 100% to the back). The photo adapter tube HCL* (not ill.) with fixed beamsplitter ratio 50 % / 50% is available as an option for the HC L3T phototube (DM L series).

Fig. 32 Photo adapter tube for FSA HC tubes

1 Switchable photo adapter tube*, 2 Vertical photo adapter tube, 3 Beamsplitter switch rod (not for HC L3T tube), 4 Clamp screw



Assembly of photo adapter tubes

Slightly loosen the clamp screw (42.1) on the side with the 3 mm screwdriver, remove black cover, place tube on microscope and align edges parallel to the microscope. Retighten clamp screw (42.1).

The supplied vertical photo adapter tube (32.2) can be used instead of the photo adapter tube with two exits (32.1) on any of the photo tubes. This is attached by loosening the clamp screw (31.6) with the 3 mm hexagonal screwdriver and then retightening.

Eyepiece adapter tube HC, TV adapter HC

Photo eyepieces and HC TV adapters can be inserted into the photo adapter tubes.



Make sure you are using the right combination, depending on the type of eyepiece, photo system (LD or MPS) and TV chip size!

Phototube Leica DM RD HC

Automatic microscope camera system with integrated observation tube and 0–35° variable viewing angle, automatic focus compensation, overlay of measurement field and format outlines, image erection; also for Pol eyepieces (field of view index 28 for zoom setting 0.9x); zoom eyepiece system 0.9x to 2.5x for all exits, motor-driven; external overlay facility; one additional exit each for a second 35 mm camera and a TV camera; intermediate image plane access for graticules in slide for documentation purposes; with control electronics (Fig. 33 and special instructions).

Fig. 33 Leica DM RD HC phototube



Lateral overlay*

The devices for diapositive overlay and macroscopy can only be adapted to the HC FSA 25 PE tube (31.9) and Leica DMRD HC phototube (Fig. 33).

These tubes have a side flange (31.9) to allow attachment of the reflection optics (Fig. 34 and 35).

The reflection optics are used for the mechanical and optical adaption of the diapositive overlay device and the macro dual zoom system.



Attention:

If reflection optics are not adapted to the microscope (34a.1 and 35.3), an image cannot be obtained.

Diapositive overlay device

The diapositive overlay device consists of the reflection optics, the illumination unit with 6 V / 4 W halogen lamp (34.8), the standard 5 x 5 cm slide frame (34.6) and the control for focusing the transparencies. The halogen lamp is fed by a separate transformer.

Assembly of the diapositive overlay device

Align the reflection optics to the tube flange (34.1) with the coupling ring (34.2) and fasten with screws. The guide pin must latch into the groove of the mount. Screw the diapositive overlay device onto the reflection optics with the coupling ring (34.2) in the same way. Again, make sure the guide pin latches into position.

Multi-viewing attachment

This is attached between the tube and the microscope (not illustrated). Max. fov 25, see also separate manual.

Fig. 34a Diapositive overlay device on the HC FSA 25 PE

1 Tube flange, 2 Coupling ring for reflection optics, 3 Reflection optics, 4 Coupling ring for diapositive overlay device, 5 Knurled ring for focusing, 6 5x5 cm slide frame, 7 Filter slot, 8 Illumination tube of lamphousings



Fig. 34b Transformer



Changing the halogen lamp in the illumination

Disconnect from power supply.

Screw out the Allen screw at the back and remove the lamp unit from the lamphousing.

Take the lamp out of the socket and replace, making sure that the contact paths of the lamp lie on the contacts in the socket.

Do not touch the lamp bulb with your fingers due to the danger of perspiration burning in.

After the lamp unit has been replaced in the lamphousing, the lamp holder can be adjusted vertically by about 2 mm with the Allen screw from beneath.

Looking through the microscope eyepiece, adjust the lamp to the height where the greatest image brightness is achieved.

Macroscopy device

This consists of the reflection optics (35.3), the macro adapter (35.5) and the macrodual zoom.

Assembly of the macro device

Screw the reflection optics (35.3) onto the tube flange with the coupling ring (35.2).

Align the macro adapter (35.5) against the macrodual zoom and secure with the threaded ring (35.6).

Fasten the macro adapter and the macrodual zoom to the reflection optics with the coupling ring (35.4). Watch the guide pin.

Fig. 35 Macro device on the HC FSA25 PE tube

1 Tube flange, 2 Coupling ring, 3 Reflection optics, 4 Coupling ring, 5 Macro adapter, 6 Threaded ring, 7 Zoom setting ring 1 : 4, 8 Zoom factor scale, 9 Scale for magnification factor of the working distance, 10 Scale for distance of object from the lower edge of the mirror housing, 11 Mirror housing



For direct visual observation (see page 37–38 for tubes) only eyepieces of the type **HC L PLAN** can be used. Fitting diameter = 30 mm.



L PLAN type eyepieces may only be used on microscopes of earlier series (= DMR label on the right side of the microscope in **black**, not red!).


PERIPLAN eyepieces, eyepieces from stereo-microscopes or of manufacturers may not be used, as the full performance of the objectives would then not be utilized. Exceptions to this are the Leica/Wild 16x/14 B and 25x/9.5 B eyepieces, for which a special adapter ring is required, which is pushed onto the eyepiece (37.2).

Fig. 36 Eyepieces

1–4 Eyepieces ready for use by viewers without eyeglasses (anti-glare protection 10 mounted or pulled up), 5 PHOTO eyepiece, 6 10x/25M eyepiece disassembled, 6 Upper part, 7 Lower part, screwed off (applies also for 10x/22M, 2.5x/6M, but not for 10x/20 and 10x/20M), 8a, b Retainer ring for eyepiece graticules, can be screwed out, 9 Eyepiece graticule*, 10 Anti-glare protection, removed for viewers wearing eyeglasses (it can be pushed back with eyepieces 10x/20 and 10x/22, insertable and remove pos. 8a or 8b). The 12.5x/6M model is basically the same as the 10x/25M eyepiece



Eyepiece labelling

Example: **10 x/20**  **M** (Fig. 36)

This name is put together as follows:

10x

Magnification of the eyepiece, i.e. the magnified intermediate image produced by the objective is additionally magnified by the eyepiece by the engraved value (= eyepiece magnification).

Total magnification of the microscopes = $M_{ob} \times M_{eye}$
(Reproduction scale of the objective x eyepiece magnification)

Example: Objektiv **25x/0.50**, Eyepiece **10x/20**
 $25 \times 10 = 250x$ total magnification

If the tube factor is not 1x, the result must be multiplied by tube factor as well. In the above example, the total magnification after switching to tube factor 1.6x would be $250x \times 1.6 = 400x$.

Fig. 37 Widefield 16x/14 B  eyepiece

1 Clamp screw, 2 Spacer ring for Leica microscopes (must be pushed upwards as far as the stop)



The tube factor is only engraved on the microscope if it is not 1x. The HC P (Pol) tube system has 2 switchable tube lenses, 1x and 1.6x, whereas HC V tube optics have 3 switchable tube lenses. The Leica DMRD HC phototube allows a continuous variation of the tube factor.

Useful magnification

The total magnification for visual observation should not be more than **1000x** the objective aperture. In the above example (n.a. = 0.50) this would be the case for a total magnification of about 500x using tube factor 2x.

When this threshold value is exceeded, e.g. with 100x/1.30 Oil objective, 10x eyepiece and tube factor 1.6x the image may appear out of focus (empty magnification).

/20, /22, /25

Field number (fov) of the eyepiece. The field number represents the diameter (in mm) of the intermediate image that can be viewed through the eyepiece. This appears magnified by the eyepiece factor. The microscope image in a 10x/20 eyepiece therefore appears to be as large as a circle of 200 mm diameter, observed from a distance of 250 mm (250 mm = reference viewing distance).

The field number of the eyepieces used must correspond with the field performance of the objectives. If the eyepieces have too high a field performance for the field flattening of the objective, part of the field of view, e.g. the edge, may appear out of focus.

Objectiv series

max. recommended eyepiece field of view

15 20 22 25 28¹⁾



Object field diameter: If you divide the eyepiece field of view by the objective magnification, you will get the real diameter of the observed object field. The eyepiece magnification is not part of the calculation. For example, with the 10x/25 eyepiece and a 50 objective an object field of 25 : 50 = 0.5 mm can be viewed.

¹⁾Fov 28 at zoom factor 0.9 with photo system DMRD HC

If the tube factor (TF) is not 1x, this value must be divided by the tube factor as well. Example: Polarized light microscope or zoom system with TF = 1.6x

Objectfield = 0.5 : 1.6 = 0.3 mm.

M

The eyepiece has a focusable eyelens (36.4) and therefore allows individual focusing of the edge of the field of view, inserted graticules or overlaid markings. Adjustment range = ± 4 dioptres.* The light-coloured ring (36.5) that becomes visible under the adjustable mount marks the setting for a person with normal or corrected eyesight when used without a graticule (when a graticule is inserted the standard setting is about 0.5 mm above this mark).

Assembly of graticules* in M eyepieces

Important: Be extremely careful to avoid dust and fingermarks, as these will be visible in the field of view. The graticule diameter is always 26 mm for HC L PLAN eyepieces.

10x/25 and 2.5x/16 eyepieces only: Screw the retainer ring of the underneath of the eyepiece (36.6). 10x/22 and 10x/25 eyepieces only: Screw out the bottom part of the eyepiece (36.8) and screw out the retainer ring with a blunt blade. Insert the graticule with the coated side downwards (in the direction of the objective) so that any lettering is seen the right way round when later observed in the viewing direction. Screw the retainer ring and the bottom part of the eyepiece back in.



The eyepiece can be used both with and without spectacles. When wearing spectacles, pull off or push back the anti-glare protection (36.7), as otherwise part of the field of view may not be visible.

Photoeyepieces*

The HCLPLAN eyepieces (fitting diameter 30 mm) are designed for direct visual observation only. Special eyepieces with fitting diameter of **27 mm** and the engraving **HC...PHOTO** are used for the adaption of photomicrographic equipment with a fixed magnification factor, e.g. DMLD and MPS systems and for special TV adaption systems.

Assembly of eyepieces

Only use identical eyepiece types (left-right)! Exception: polarized light microscopy: The right-hand eyepiece on polarized light microscopes has lines and a scale division (e.g. for length measurements, see page 105). Due to a double clickstop (31.7) the right hand eyepiece can be set with the crosslines aligned at the north south/west position (horizontal/vertical) or at an angle of 45°. The crosslines then show the transmission directions of the polarizers or the vibration directions of the object in its brightest orientation (diagonal position).

* It is possible to extend the dioptre compensation by having an ophthalmic optician center antireflection coated spectacle lenses (2 – 3 dioptres) and inserting them into the glare protection ring (36.7). However, this method is not generally recommended by Leica.

Widefield 16x /14 and 25/9.5 eyepiece pair: push the spacer ring (37.2) on to the lower part of the eyepiece as far as it will go secure with the clamp screw (37.1).

Objective nosepiece

Depending on the type of microscope, the objective nosepiece is either fixed or interchangeable (Fig. 38 and 48.5).

The following types of nosepiece are available:

Septuple objective nosepiece, M25 objective thread, changeable and interchangeable
dto. coded, not interchangeable and interchangeable

Centerable sextuple Pol objective nosepiece, interchangeable only

Sextuple objective nosepiece (BD), for incident light bright-/darkfield

Objectives with M32 thread, interchangeable and non-interchangeable

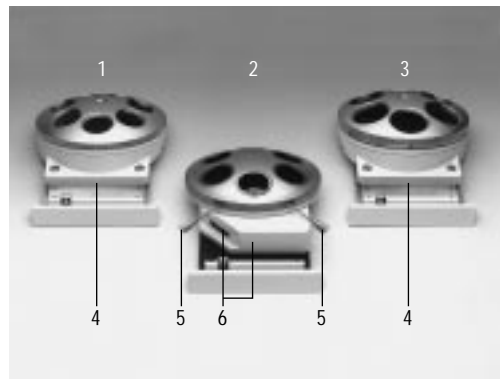
dto. coded, non-interchangeable and interchangeable

Objective thread and objective spacer rings*

Incident light bright- and darkfield objectives B (40.1) have an **M32** x 0.75 thread and can only be used on the objective nosepiece with M32 thread. These objectives have the letters **BD** after the aperture, e.g. HC PL FLUOTAR 10x/0.30 **BD**. Objectives with thread **M25** x 0.75 can be screwed onto all nosepieces. An adapter ring (M32/M25), Fig. 39, is available for using these objectives on nosepiece **BD** with M32 thread.

Fig. 38 Objective nosepieces

1 Septuple objective nosepiece (M25), **2** Sextuple centerable objective nosepiece (M25) with tube slit and centering keys in place, **3** Sextuple nosepiece (BD, M32), **4** plate, interchangeable with IC turret (Fig. 25 – 26), **5** Objective centering keys in place, **6** Tube slit, interchangeable with IC turret



Adaption of objectives with **RMS** thread (**R**oyal **M**icroscopical **S**ociety W 0.8x 1/36"): objectives with this classical thread size can only be used on all nosepieces under certain circumstances and together with the spacer ring M32/RMS or M25/RMS (Fig. 39):



Objectives with tube length **160 mm** are not adaptable at all due to optical reasons. These are identified by the engraving 160 and the missing multiplication sign after the magnification, e.g. PL FLUOTAR 40/0.70. In the case of incident light objectives whose engraved code number has a 9 in the third position from the left, e.g. 559 678 or 569 678, the engraved magnification must be multiplied by 0.8, as these objectives are designed for incident light microscopes with tube lens focal length 250 mm. The aperture and the working distance are not affected.

Code numbers with a 6 or 7 in the third position, on the hand, indicate objectives for tube lens focal length 200 mm which is used without exception in your microscope so that the engraved magnification applies.

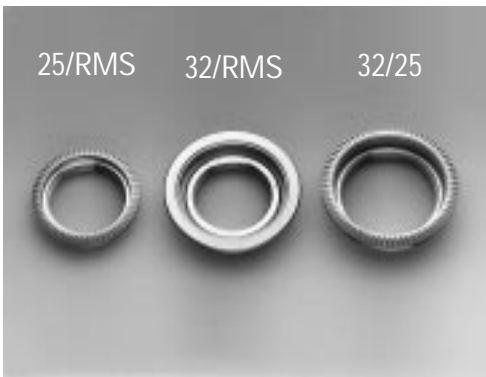


Caution:

When using objective spacer rings:

Objective spacer rings are manufactured with a thickness tolerance of about 1/500 mm to ensure the parfocality of the objectives. They must therefore be treated with extreme care. When adapting objectives with RMS thread it may be necessary to shorten the upper edge of the objective collar by about 1.5 mm (this is done at our factory) as otherwise the objective cannot be screwed on properly, so that parfocality is not guaranteed and the objective collar cannot be rotated. Please consult our agency in this case.

Fig. 39 Objective spacer rings (adapters)



Objectives/Assembly

For microscopes with fixed nosepiece: lower stage as far as possible (42.12 or 44.3). If you have a motor focus, press keys 44.5 and 44.6 simultaneously to display an already stored magnification (page 64).

Microscopes with interchangeable nosepiece: loosen the clamp screw on the left (48.5), pull out the nosepiece towards the front and place upside down on a clean flat surface.

Screw in the objectives carefully as far as possible in order of ascending magnification, corresponding to the order of the light rings (PH 1 – 3) or the IC prisms in the condenser.

Once you have assembled the objectives and nosepiece, rotatable objective collars should be turned so that you can easily read the lettering.

Lettering

Example:

$\infty/0.17/A$ N PLAN 10x / 0.25 PH1 506 088

∞

Infinite mechanical tube length for which the objective is designed (there are also microscopes and corresponding objectives with tube length 160 mm), cf. Fig. 40 and 41.

0.17

Stipulated specimen coverglass thickness. In the case of dry objectives, the higher the aperture, the more important it is to keep to the cover-glass thickness of 170 μm . For an aperture 0.85 the coverglass thickness should only deviate a few μm at the most from 170 μm to

Fig. 40 Examples of objectives

1 Brightfield objective, 2, 3 POL objectives, 4 Phase contrast immersion objective, 5 Immersion objective with iris diaphragm, 6 CORR objective for inverted microscopes, 7 BD objective for incident light brightfield and darkfield (M25 thread)

Some immersion objectives with a knurled ring have a front part which can be pushed up and "locked" with a small rotational movement. This device must be unlocked for observation! The sleeve of PL FLUOTAR and PL APO objectives can be rotated so that the engraving can be read more easily.



achieve the full performance of the objective. We recommend coverglasses no. 1H (high performance, $0.17_{-0.02}^0$ mm) which comply with DIN 58878/ISO 8255/1. The thickness of the embedding medium layer between the specimen and the coverglass should be as thin as possible. However, if you have a high dry aperture and a non-standard coverglass thickness, the aperture can be reduced by integrating an iris diaphragm (41.7) to make deviating coverglass thicknesses uncritical. Alternatively, an objective with correction mount (CORR) can be used.

0

Coverglass thickness 0, i.e. specimens must not be covered with a coverglass. These objectives are primarily designed for reflected light specimens, but can also be used to great advantage with transmitted light specimens without a coverglass, e.g. blood smear specimens.

–

The specimen can either be covered or not. A maximum aperture of about 0.25 is considered the threshold value for dry objectives for universal use with or without a coverglass; for oil immersions this upper threshold is 1.25.

A, B, C, D, E

Pupil position in the objective: the exit pupil of most Leica microscope objectives has 4 standard positions A, B, C and D, the so-called pupil blocks. When using the ICT and ICR interference contrast devices make sure that the IC prism (25.3 and 60.7) used above the objective has the same letter, see "Optics" data sheet.

The most important performance criteria of microscope objectives (apart from aperture and magnification, see below) are field performance and chromatic correction. Field performance is understood as the diameter of the focused intermediate image formed in the eyepiece (cf page 43). As regards chromatic correction, there are three main types: achromats, semiapochromats (or fluorites) and apochromats.

C PLAN

Achromatic objectives with a field performance up to 20 mm (eyepiece fov max. 20).

N PLAN, PLAN

Planachromatic objectives with a field performance of at least 20–22 mm. For visual observation eyepieces with a field performance of 20 or 22 mm are recommended, e.g. HC PLAN 10x/20. However, eyepieces up to 25 field of view can be used if you are prepared to accept slightly blurred edge definition.

PL FLUOTAR®, HC PL FLUOTAR, HCX PL FLUOTAR

Semi-apochromats with a field performance of at least 25 mm. The improvement in field performance and colour correction compared with the achromats is particularly important for photomicrography.

PL APO, HC PL APO, HCX PL APO

Plan apochromats with a field performance of over 25 mm, the best objectives in the Leica range.

PLAN L, N PLAN L

Achromats with particularly long free working distances, specified in the Leica objective charts. L objectives with apertures over 0.25 are designed for use without a coverglass. Field performance over 20 mm.

PLAN H

Achromats for use with heating stages which have a 1.80 mm thick quartz window and with interference attachments. Field performance over 20 mm, e.g. 10x/0.25 PH 1.

10x

Magnification of the objective, which is also indicated by colour of the lower edge of the objective collar (see chart).

0.25

Numerical aperture of the objective, derived from the angular aperture of the ray cone penetrating the objective. The aperture influences a number of image factors and is therefore just as important as the magnification. It influences: resolution, which also depends on the wavelength λ of the light. A general rule for a medium wavelength $\lambda = 0.55 \mu\text{m}$ for visible light is:

$$\text{resolution} = \frac{\lambda}{2 \text{ n.A.}} = \frac{0.55}{2 \text{ n.A.}}$$

Example: aperture 0.50

resolution (opt.) = 0.55 : 1.0 = 0.5 μm

Depth of field (axial resolution)

Image intensity: This increases quadratically with the aperture, so objectives with high apertures, especially immersion objectives, are preferred for fluorescence microscopy, for example.

Coverglass sensitivity (cf 1st line 0.17!)

1.25 – 0.65

Objective with built-in iris diaphragm to adjust the aperture (41.3), e.g. for darkfield immersion.



Attention:

Objective with built-in diaphragm!

The knurled may only be used for adjusting the diaphragm, **not** for screwing the objective in or out.

Risk of damage!

PH 2

Phase contrast objective, with phase ring no. 2 built in. For phase contrast observation, the corresponding light ring 2 in the condenser must be selected, see page 72.

Phase contrast objectives all have green engraving.

P

Extremely low-strain objective for polarized light microscopy, with red engraving.

BD

Dry objective with M32 thread, for BF and DF (incident light).

↑

Leica objectives with infinite tube length can be used for both transmitted and incident light. However, objectives corrected for coverglass thickness 0.17 are only used in transmitted light, as incident light specimens, of course, are never covered (except for fluorescence specimens). The upwards arrow ↑ indicates that this objective for use with or without a coverglass should only be used in transmitted light, as disturbing reflections may occur in incident light. This is indicated by the letter T instead ↑ of arrow in the objective charts.

OIL

Oil immersion objective: it may only be used with DIN/ISO standard optical immersion oil. For apertures over 1.25 the engraving 0 or 0.17 shows whether the objective should be used with or without a coverglass. The coverglass thickness should be adhered to as exactly as possible ($\pm 5 \mu\text{m}$) for apertures larger than 1.32. Immersion objectives with an aperture greater than 1.35 should only be used in a temperature range of 20 – 25 °C. the refractive index of liquids varies considerably at different temperatures, the optical coordination between the objective and the oil changes during major temperature fluctuations. The quality of the image may suffer in the same way as for the wrong coverglass thickness. Also remember that if specimens are stained in strong colours, the temperature of the immersion oil may rise by a few degrees due to the object absorption. The illuminated object field should therefore be strictly limited to the area observed (Koehler illumination, page 69) and the illumination intensity reduced if necessary using a neutral density filter or the lamp supply.

The immersion oil is applied with the stage lowered or the objective turned out of the light path, taking care to avoid air bubbles. It is later removed with a clean cloth and ethyl alcohol, cf p. 111.

First read the safety data sheet (available on request from your Leica agency).

W

Water immersion objective. Use distilled, or at least demineralized water, if possible, as it is often difficult to remove the sediment from drops of water that have dried on the objective.

IMM

Universal immersion objective for water, salt water, glycerine, oil.

Locking of objectives

The front part (41.1 and 41.2) of certain immersion objectives can be pushed in by about 2 mm and slightly rotated. This stops any remaining drops of immersion liquid from wetting objects and other objectives when the nosepiece is turned.



Attention:

This locking device must be released before the immersion objective is used again, as otherwise the spring mechanism protecting the specimen and the objective is inactive and the other objectives are not parfocal with the immersion objective.

CORR Objectives

Special objectives with adjustable matching to the coverglass thickness: Set correction mount (not illustrated) approximately by turning the knurl to the average or estimated value: focus the B specimen (→ Fig. 25).

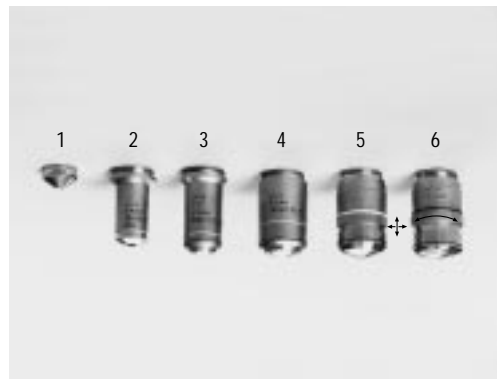
Adjust the correction mount until you achieve optimum contrast, refocusing with the fine control if necessary. This setting may be very difficult for specimens with low contrast or weakly pronounced structures.

Lens attachments

Can be pushed onto the front of some objectives, or are readymounted at the factory:

Fig. 41 Examples of immersion objectives

1 Immersion cap for N PLAN 10x objectives (pos. 2), 2 N PLAN 10x dry objective, 3 Achromat, 4 Planachromat, 5, 6 Objectives with push-in locking device at front



Push-on cap CG and IMM

This can be used with some objectives with long working distances to achieve optimum image quality with coverglasses (CG) of different thicknesses. Cap CG 0.4, for example, is recommended for windows of vessels or for LCD displays with a thickness between approx. 0.25 and 0.55 mm. Without CG cap 0.4 an optimum image is achieved, for example, at a wall thickness of 0.95 to 1.25 mm (C PLAN L 40x/0.50 objective). Immersion cap IMM for enhancing contrast and observing inner reflections in incident light brightfield and POL (Fig. 41).

Reduction of reflections

A rotatable birefringent plate attached in front of the front lens can suppress reflections for certain incident light objectives and thus improve image contrast. Used only with crossed polarizers or Pol filter system.

Interference attachments

For quantitative measurement of roughness, film thickness, etc. See special instruction manual.

Colour code rings on objectives

In accordance with German and international standards (DIN/ISO) the magnification of each objective is additionally indicated by a colour ring above the knurl (41.4):

100x 125x 150x 160x	63x	40x 50x	25x 32x	16x 20x	10x	6.3x	4x 5x	2.5x	1.6x	1x 1.25x
white	dark blue	light blue	dark green	light green	yellow green	orange	red	brown	grey	black

Immersion objectives have a second coloured ring further down (41.6):

black Oil or IMM
(= universal objective oil, water, glycerine)
white water or IMM
orange glycerine

Engraving order code no. e.g. 506 001

Six-digit factory code number of the objective. Please always state this code number as well as the full engraving of the objective when making technical or commercial enquiries. Objectives whose code numbers begin with 569... and 559... can be used under certain conditions if they have the engraving ∞ , see page 45. However, the engraved magnification value must be multiplied by the correction factor 0.8x. Objectives of tube length 160 or 170 (engraving 160 or 170) cannot be used at all.

Operation

Switching on

Turn on mains switch (42.14).

Set selector switch to transmitted or incident light (42.13). If using a gas discharge lamp: turn on external switch and check lamp adjustment immediately (see page 90).



Caution:

Leica power units are immune to interference. Nevertheless we recommend you ignite gas discharge lamps before switching on the other components, particularly if your power unit is not from the Leica range.

Switchable mirrors (3.3, 61.7) only: Switch to left or rear lamphousing.

Engage or disengage neutral density filter* (42.8, 42.15, 48.23, 65.10, 30.4, Fig. 9), depending on required brightness.

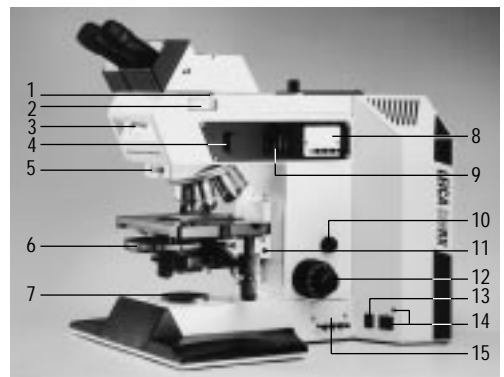
Adjust brightness with dial (48.24). The numbers are not absolute values, but merely enable reproducible settings. The light-coloured dot on the dial indicates the setting for about 3200 K for photography on indoor colour film and TV microscopy. See page 61 for DM RXE stand.

Tube optics

Disengage Bertrand lens (42.2), Switch on tube factor **1x**. If you have HC P (PoI) tube optics, just switch to tube factor 1x (page 83). See page 67 for how to set tubes and eyepieces.

Fig. 42

1 Tube clamp screw, 2 Bertrand lens* in/out, cf Fig. 50, 3 Reflector/filter system turret*, 4 Incident light polarizer*, 5 IC objective prism disc*, 6 Condenser disc*, 7 Coverring for base of stand, 8 Filter magazine*, 9 Incident light diaphragm module* cf Fig. 23, 10 Stage adaption*, 11 Place to keep centering keys* (interchangeable stage only), 12 Mechanical coarse and fine focusing, 13 Transmitted/incident light selector switch, 14 Mains switch with pilot lamp* (not for motor focus), 15 Filter magazine* for transmitted light



Analyser*

Disengage analyser (48.2) by pulling it out part way.

Reflector*/filter system*

For transmitted light only:

Disengage reflector (48.3) or filter system. Turn condenser disc (48.14) to pos. H (brightfield).

For incident light only:

Engage HF or Smith reflector (Fig. 18; 19; 48.3). For incident light fluorescence examinations of transparent objects it is advisable to set transmitted light mode first.

Adjustment specimen

For initial microscope adjustment we recommend you use a specimen that has both high and low contrast areas. Non-plane parallel reflected light specimens must be aligned on a specimen slide with a handpress and plasticine.

Mechanical stages*

Individual setting of specimen clamp:

Stage no. 1187: Push down the knurled ring (48.7) on the joint of the specimen holder and turn to the left (tighter clamping) or to the right (looser). Then pull upwards so that it clicks into position.

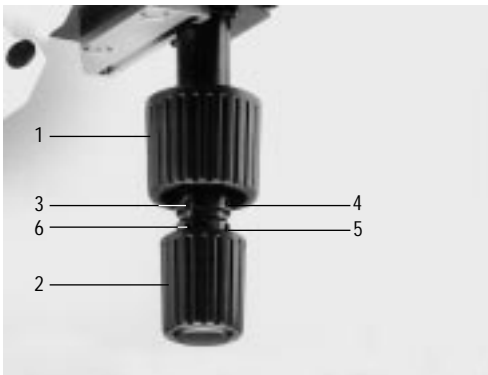
Stage no. 1189: The clamping jaws can be moved after the knurled screws have been loosened. In addition a incident light object guide (code no. 563 546) with movable sample platform for direct sample positioning and the tilting stage (code no. 563 294) can be adapted.

Individual setting of the x-y drive (Fig. 43):

Lengthening and shortening: First pull the lower control (for x adjustment, 43.2) downwards, then pull the upper control (for y adjustment, 43.1) in the same direction.

The coaxial drive is shortened by pushing the controls upwards in the opposite order.

Fig. 43 x-y specimen adjustment on the mechanical stage
1 y adjustment, 2 x adjustment, 3, 6 Clamp screws 4, 5 Rotatable rings for torque setting



Torque setting: The torque has already been optimally adjusted at the factory, but you can change this setting as follows: move the lower control (43.2) to the “long” position (see above). Push the upper control (43.1) upwards.

Loosen the 1.5 mm Allen clamp screws (43.3 or 43.6), using either an offset screw key (1.5 mm socket-head) or one of the two centering keys (1.4 or 1.5). The threaded hole for the clamp screw of the upper ring is at an angle.

After 1 – 2 rotations of the rings (43.4 or 43.5) the x and y adjustment can be set tighter or looser, respectively; move the x- and y-adjustment as far as the stop if necessary. When you have set the torque, fix the ring with the clamp screw (43.3 or 43.6) and pull the upper control down.

Stage rotation: Loosen the clamp screw (12.6).

Pol rotary stage*, Pol object guide*

The specimen is fixed to the stage either with two spring clips or preferably, with the Pol 2 multi-format object guide (Fig. 13). For specimen slides with a width of approx. 26 mm (1”), swivel out the metal plate (13.2) and insert the object as shown in the illustration. If ordinary specimen slides with a width of 26 mm are inserted vertically to this, the movement range of the object guide of about 30 x 40 mm is not fully utilized. The supplied set of pairs of clickstop buttons enables clickstops at intervals of 0.1,

0.3, 0.5, 1 and 2 mm. These are replaced by a strong axial pulling movement. Note the correct orientation of the catch pins inside when pushing on the new clickstop button. The stop screw on the underneath must be moved inwards by about 2 mm to limit the vertical travel on smaller types of microscope.

The two verniers permit angle measurements with a reading accuracy of 0.1.

45° clickstop: Screw in the rotary knob (13.5) until you feel slight resistance, then turn the stage to the next noticeable clickstop. Loosen the rotary knob, look for the position of the next clickstop (e.g. extinction position of object) and retighten the rotary knob. The stage can now be rotated at clickstop intervals of 45°.

Light filters*

Light filters can be built into the intermediate filter holder (Fig. 9, filter diameter 50 mm), the filter box (Fig. 10, Ø 32 mm) or can be placed on the dust protection glass of the microscope base (27.3). Filters should not be used between the polarizer and the specimen in polarized light and ICT interference contrast (possibility of birefringence due to strain caused by heat). Besides the standard filters listed below there are also various special filters, Optics data sheet and interference filters for measurement purposes, e.g. the MPV microscope photometer.

Filters	Application
Grey filter	<p>Grey filters (neutral density filters) are used to attenuate light without influencing the colour temperature. The engraved value, e.g. N16, indicates the attenuation value. N 16, therefore, means reduction to $1/16 = 6.3\%$ transmission.</p> <p>Integrated grey filters can be switched: in the microscope base (48.23) (T = 6.3 %), in the RF reflected light diaphragm module (23.5), T = 5%, in the empty slot of the analyser 360 (30.4) T = 25%, Various grey filters can also be inserted at the places described.</p>

Green filter,	Contrast enhancement for black-panchromatic and -white photography.
DLF 2 (blue)	Conversion filters for colour photography with daylight film.
ALF	dto. for artificial light film.
BG 20	Highlights red in Polaroid exposures.
VG 9 (green filter)	Contrast enhancement for chromosome photographie.
546 nm interference filter	Pol compensator measurements, interference attachments.
BG 38 (blue filter)	Suppression of red in fluorescence (is integrated in diaphragm module F (23.8).
Diffusing screen	For more homogeneous illumination at objective magnification 1.6x and conoscopy and incident light pupil illumination.
Grooved diffusing screen	Lamphousing 252 with 150 W Xe lamp.

Stage clamp*

Stage height setting (interchangeable stage only*)

The following chapters describe how to focus the specimen. The stage height can also be adjusted with the stage clamp (48.9). The stage should be clamped at the level where the thinnest specimens just touch the objective with the highest magnification at the highest possible setting of the coarse/fine drive. As high-power objectives always have telescopic front spring loading, there is hardly any risk of damaging the specimen or microscope.



Attention:

Don't forget to release the locking mechanism on immersion objectives (page 51).

Loosen clamp screw (48.9) on the left of the stage bracket. Supporting the stage with both hands, carefully move it up or down.



Attention:

Make sure the condenser does not touch the microscope base.

Temporarily retighten the stage clamp.

Put the thinnest specimen you are going to examine (e.g. transmitted light object) on the stage and move the stage up to the stop using the coarse drive (42.12 and 44.2). Loosen the stage clamp again (48.9) and carefully move the stage upwards in the dovetail guide until the specimen just touches the objective with the highest magnification, or an image can be focused.

If working with ordinary transmitted light specimens of 1 – 1.2 mm thickness you can also clamp the stage so that the stage bracket is flush with the upper end of the dovetail guide (12.4) after setting the upper stage stop.

Focusing, mechanical dual knob drive*

The smaller dial (42.12) is for fine focusing; one division of the scale represents a vertical movement of approx. 2 μm (see page 107). The larger dial is for coarse focusing.

Motorized* focusing



Attention:

Before using the motor focus, read the instructions* carefully to eliminate the risk of damage due to operation errors. If you have an interchangeable stage, set the clamp (48.9) so that specimens just touch the front lens of the higher-power objectives when the vertical adjustment of the stage is at its highest position.

1.1 Switching on

After you turn on the power supply with the mains switch (42.14) the display (44.7) will still show the data set before the microscope was switched off last time, except for the "coarse drive" setting on the focusing wheel (44.4), which is not stored.

If neither the display nor one of the LEDs lights up, the microscope is probably not properly connected to the power supply (check mains cable connections).

1.2 Focusing

The position of the stage can be adjusted with

- the focusing wheel (44.4) and
- the "Up" (44.2) and "Down" (44.3) keys.

On some models the interchangeable stage can also be vertically adjusted with the clamp (48.9).

These controls are situated on both sides of the microscope, giving you a choice of left- or right-handed operation.

1.2.1 Fine and coarse focusing with the focusing wheel

Like the mechanical coarse and fine focus, the motor focus also translates a rotary motion of the focusing wheel into a vertical motion of the stage. One main difference, however, is that there is only one focusing wheel.

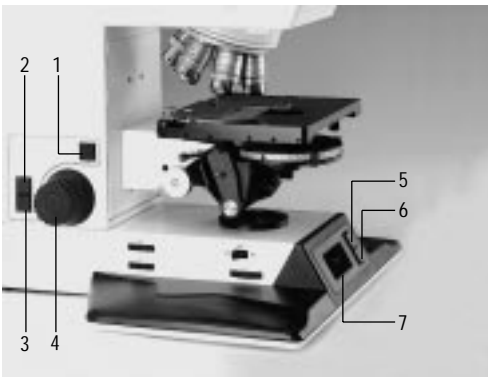
Instead, the translation from the rotary to the vertical movement can be effected by keystroke (see below 1.3).

With the focusing wheel the stage can also be moved over a set upper threshold (see section 1.4) but a lower threshold setting can only be overridden by one step.

Fig. 44 Motor focus controls

Controls 1–4 are situated on both sides of the stand in the same layout.

1 Stepwidth, 2 "Up", 3 "Down", 4 Focusing wheel, 5 "Upper threshold", 6 "Lower threshold", 7 Display



1.2.2 Stage height adjustment by keystroke

The stage can be moved up and down at a maximum speed of about 6 mm per second with the "Up" (44.2) and "Down" (44.3) keys. At first, the acceleration is deliberately retarded to allow fine vertical movements by keystroke.

If the upper threshold has been set (see section 1.4), the stage can be repositioned at this setting with the "Up" key (with an accuracy of $\pm 1 \mu\text{m}$).

A set upper threshold cannot be overridden with the "Up" key, but this can be done with the focusing wheel. If the z drive is above the upper threshold, the stage will be lowered to the upper threshold when the "Up" key is pressed.

If no thresholds are set, the stage travels to the mechanical end-switch position.



Attention:

Risk of damage, particularly to the condenser, the objectives and the specimens.

1.3 Stepwidths, Focusing wheel

Fine focusing

The motorized vertical movement of the stage is not continuous, but by extremely fine reproducible steps. These are chosen, depending on the objective, so that the stepwidth is smaller than the depth of focus, giving the effect of continuous focusing. The stepwidth for the focusing wheel can be set with the "Stepwidth" key (44.1). This alternates between three possible settings when the key is pressed and is

indicated in the display (44.7). Each objective position can be individually stored on the coded objective nosepiece, see page 60.

The three possible stepwidth settings for the fine focusing are:

1 = $0.1 \mu\text{m}$ 2 = $0.7 \mu\text{m}$ 3 = $1.5 \mu\text{m}$

Coarse focusing

By simultaneously pressing the "Up" and "Down" keys (44.2 and 44.3) you can switch from the set stepwidth to the "coarse drive of the focusing wheel" function. When the coarse drive is activated, numbers 1–3 on the left-hand side of the display light up simultaneously.

With the coarse drive the stage can be moved up or down by about 1 mm per rotation of the focusing wheel.

The keystroke function of repositioning at set thresholds is retained with full accuracy for the coarse drive.

You can switch back to fine focusing by pressing keys (44.2 and 44.3) simultaneously again.

1.4 Setting/deleting z thresholds

A threshold can be set at the current stage position by pressing and sustaining (≥ 1 sec) the "Upper threshold" (44.5) or "Lower threshold" (44.6) keys.

You can delete a threshold whenever you like by pressing the same key.

The relevant key must be kept pressed down until the corresponding symbol in the display field "z status" has switched over. The display then shows the active function:

- "Set ↑" setting of the upper threshold,
- "Del ↑" deleting of the upper threshold,
- "Set ↓" setting of the lower threshold,
- "Del ↓" deleting of the lower threshold.

If you see the display "Err"! with flashing LED ↓ or ↑ while you are trying to set a threshold, the position of the threshold is not acceptable.

Examples: lower threshold = upper threshold
lower threshold > upper threshold.



Attention:

When viewing specimens of different thicknesses, the upper threshold must be readjusted every time the specimen is changed (risk of collision!).

1.5 Coded objective nosepiece*

The coded objective nosepiece enables several parameters to be allocated and stored for each objective position.

These parameters are:

- Stepwidth of the focusing (see section 1.3),
 - Objective magnification (see page 64),
 - Offset of objective focal plane ("parfocality"),
- p. 62

The objective magnification and the offset to the focal plane must be "read in" once (see page 62, Calibration).

The stepwidth last used at a nosepiece position is automatically stored.

The setting of the stored stepwidth, the display of the magnification and the compensation of the focus offset are done automatically while the nosepiece is rotated.

If 2 coded, interchangeable nosepieces are available, these can be labelled "nosepiece A" and "nosepiece B" by operating a switch. As the system is capable of storing up to 14 objective positions, the data allocated to each objective are automatically called up or displayed every time. When you screw out an objective and turn the nosepiece, you can see the switch inside the nosepiece. This switch has to be switched to the left for one nosepiece, and to the right for the other (not illustrated). This can be done with a thin wooden stick or similar.

1.6 Display

Stage height

When an upper threshold is set, the height of the stage in relation to the upper threshold is indicated in the display, e.g. “-012”.

The unit is displayed automatically with the two LEDs μm and mm (i.e. 12 μm or 12 mm below the upper threshold). Positive values signify stage positions above the upper threshold.

If the upper threshold is not set, you will see “Set?” in the display. If the **lower** threshold is not set, the downwards arrow \downarrow will not be displayed.

Magnification display

Regardless of the threshold status, you can switch between a display of the stage height and a display of the objective magnification (see page 64) by simultaneously pressing the keys “Upper threshold” and “Lower threshold” (44.5 and 44.6).

Switching over the display influences neither the thresholds nor the stage height.

1.7 Collision and overload protection



Attention:

If the electronics register overload or a collision while the motor focus is being operated with “Up” or “Down”, the motor is actively braked and switched off, and the display flashes.

In this case the stage should be immediately moved clear in the opposite direction.

We cannot accept any liability for damage due to operation errors.

1.8 Leica DM RXE microscope only: Lamp voltage setting

With the exception of the Leica DMRXE microscope the lamp voltage is adjusted directly with the dial (48.24).

On the Leica DMRXE microscope, the dial (48.24), which acts as a switch, must be slightly turned clockwise until the voltage value of the lamp (5 – 12 V) appears in the display (44.7); the lamp voltage can be controlled with the focusing wheel (44.4), if this position is sustained.

When the switch is in the home position the handwheel takes over the z drive control again.

This setting allows interactive adjustment of the lamp voltage when a PC is connected. See separate instructions for further details.

1.9 Parfocality

The depth of field (axial resolution) depends on the objective aperture and the magnification; it is under 1 μm for highest magnification objectives.

In principle, it is possible to achieve absolutely perfect parfocality (identical focusing) of all objectives used on the nosepiece by mechanical and optical means, but this is extremely complicated. It would be noticeably impaired even by the torque and any dust particles on the objective shoulders when the objectives were screwed in. All the same, the parfocality on Leica microscopes with mechanical focusing is so precise that only slight refocusing is necessary after each objective change. Using the motor focus, this parfocality can even be perfected with automatic focus correction through the motor focus and coded nosepiece for each objective after one calibration.



Attention:

Please read the following important information before storing the objective focus offsets:

Screw all objectives into the nosepiece with about the same torque. If the nosepiece is interchangeable, make sure it fits properly in the microscope and keep the contacts clean. The eyelenses of the eyepieces must be exactly focused on the intermediate image. This is only possible by inserting a (random) graticule in the eyepiece or the Vario tube.

Another suitable focus indicator for the eyepiece eyelenses is any overlay of a photomicro device or the MPV microscope photometer.

However, it is not sufficient to focus on the edge of the eyepiece field diaphragm or on diapositive overlays (see page 101).



Attention:

When the viewer changes his glasses or when a different person looks through the microscope the focusing of the eyelens(es) should always be checked and corrected if necessary. When the eyelens is not properly focused the focal plane of the objectives varies by different amounts, which can cause focusing errors and even collisions between specimen and objective.

Adapted TV cameras may have a different focal plane compared with that for direct observation. This may be caused by tolerances in the flange focal length of the objective of the camera; the flange focal length can be adjusted for some TV cameras.

Objectives with coverglass information "0" must not be used for covered specimens; only use objectives with the engraving "-" (i.e. for use with or without a coverglass, see page 48) and "0.17" (only with 0.170 mm coverglass). For heating stages with an observation window, H PLAN heating stage objectives with engraving 1.8 Q (i.e. for 1.8 mm quartz glass window) and "-" objectives can be combined.

Objectives with engraving "0" (i.e. without coverglass) and "-" are suitable for uncovered specimens. If the microscope is used for both covered and uncovered specimens, objectives with the engraving "-" can be combined with "0" as well as "0.17" objectives, without the focal plane having to be reprogrammed, with the exception of immersion objectives.

To store the focus data, always use a high-contrast specimen where the same area is suitable for all objective magnifications. For transmitted light the specimen used for storing the focus data should be as thin as possible in order to have a defined focal plane even at highest magnifications, e.g. a Leica stage micrometer.

1.10 Storing the objective focus offsets

Focus the specimen with the objective with the highest resolution (i.e. max. aperture/magnification).

! Attention:

When using immersion objectives (OIL, W, IMM): release the locking mechanism of the front part of the objective (page 51) to give the objective the standard parfocalizing distance of 45 mm!

Accuracy can be enhanced by setting variotubes and switchable tube lenses to a higher magnification factor or by putting the auxiliary telescope (Fig. 51) on the eyepiece.

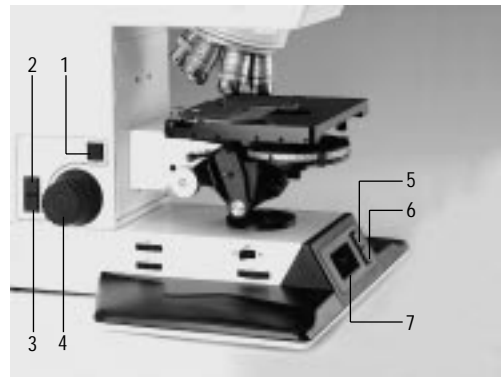
Then set the upper threshold at this position with the key (44.5) (display 0 μm !) and switch off the microscope (42.14).

Pressing key (44.5) at the same time, switch the microscope on again. "OK!" appears in the display as long as the key (44.5) is pressed. After the key has been released "Call!" appears in the display to indicate the storage of the focal plane of the first objective. "0" is now stored as offset for the focused objective. Now all the objectives on the nosepiece can be focused.

Fig. 44 Motorfocus controls

Controls 1–4 are situated on both sides of the stand in the same layout.

1 "Stepwidth", 2 "Up", 3 "Down", 4 Focusing wheel, 5 "Upper threshold", 6 "Lower threshold", 7 Display



After focusing you only need to press key (44.5) until "OK!" is output in the display to store the offset. Finally, switch off the microscope briefly (42.14).

1.11 Storage of objective magnifications

As well as the offset values, the magnification of each objective screwed in the nosepiece can be stored during the calibration. First store the offset values of at least two objectives.

By pressing the key (44.6) and simultaneously turning the focusing wheel you can set the magnification value of the objective currently in the light path. It is automatically stored when key (44.6) is released.

During calibration it is not possible to set or delete thresholds. To conclude the calibration the microscope must be temporarily switched off.



The first time it is switched back on again the upper threshold for the focal plane (objective with highest magnification only) must be deleted and reset. This also applies when the specimen is replaced by a specimen of different thickness.

Survey observation without an objective*

In transmitted light, the focusable Bertrand lens⁺ can also be used together with the survey condenser (Fig. 45) as a survey objective with ca. 1x magnification, making it possible to scan objects with a diameter of about 25 mm (= width of specimen slide). Not generally suitable for photographic documentation. The DMRD HC photomicro system can only be used from factor 1x, pronounced marginal fall-off (vignetting) is to be expected.

Fit the survey condenser (cf Fig. 12, p. 23). Remove objective or objective nosepiece.

Focus the Bertrand lens* (50.3), open the aperture diaphragm = (48.21), the field diaphragm (48.22) can now be used as aperture diaphragm. For a more even illumination, a diffusing screen can be used in the filter magazine (42.15) or in the condenser holder B (27.6). See also p. 80 and 102).

Incident light focusing graticule*

Focusing can be made easier by inserting a graticule (23.11) into the diaphragm module HC RF*, see page 29–30. After pulling out the diaphragm module part way (= channel II) this graticule is projected onto the specimen surface and imaged together with it. This is particularly useful for exact focusing of specimens lacking in structures or contrast, e.g. for photomicrography or topological measurements.

Adjustment:

Attention! Only with Smith reflector! Set the microscope exactly, particularly the eyepieces and the aperture diaphragm. Exactly focus a flat, contrasty focusing object (e.g. incident light stage micrometer or mirror with scratches or other structures). Pull out the HC RF diaphragm module slightly = channel II, so that an image is formed of the graticule.

^{+) Max. SFZ = 25, not at tube optics HCP (Pol 1x/1.6x/Bertrand lens)}

If this image is not absolutely sharp: remove the diaphragm module (see page 30) and slightly pull out or push in the mount of the graticule (slit on one side for screwdriver). After replacing the module, check exact focusing and repeat the process if necessary!

Objectives

See page 47 for detailed information on how to use objectives. The main points are described again below:

Objective engraving

Only use objectives with “infinite” tube length (∞ engraving).

∞ 0.17 0 –

Note coverglass specifications (objective engravings 0.17, 0 or –).

Immersion

For all immersion objectives: before focusing, make sure that the front part of objective is not pushed in and locked (pull out telescopically, page 51).

Only use OIL objectives with Leica DIN/ISO standard immersion oil. Clean with ethyl alcohol only.

IMM objectives can be used with water, glycerine, oil, etc.

W objectives should be used with distilled water.

To immerse: Lower the stage or turn the objective slightly out of the light path, apply 1–2 drops of immersion oil to the specimen, taking care to avoid bubbles. Focus carefully, as the working distance of immersion objectives is usually extremely short. Be careful with objectives with front locking device!

Centration

Only for polarized light microscopes: objective centration*

The objectives are centered by adjusting them with two Allen keys (1.4) until the optical axis of the objective (and thus the centre of the image) coincides with the axis of rotation of the stage. When the objective is properly centred, a focused area of the specimen does not drift out of the field of view when the stage is rotated. A specimen point in the centre of the crosslines therefore remains in this position for a whole stage rotation. It is advisable to use a high-contrast specimen full of detail for objective centration.

Fig. 45 Survey condenser



Disengage the analyser (54.3), tube lens 1.6x (54.11) and Bertrand lens (54.2). Greatly narrow the aperture diaphragm (54.9). Insert the two objective centering keys above the objective you want to centre (38.5). Focus the object. There are two similar methods of objective centration:

Method I (Fig. 46a)

Rotate the stage and note the point on the specimen that remains stationary. This point corresponds to the mechanical axis of rotation of the stage.

Now move this prominent point of the specimen to the centre of the crosslines with the two centering keys. Rotate the stage and fine-adjust the centration if necessary.

Method II (Fig. 46b)

Move the prominent point on the specimen (46a) to the centre of the crosslines M. Rotate the stage until the point on the specimen is furthest away from the centre of the crosslines M (position A, Fig. 46b). Point A (= maximum distance of the specimen point from the centre) may even be outside the field of view. Turning the centering keys, adjust the image until the specimen point A is midway (= pos. B) between pos. A and the centre of the crosslines M (46c). Move point A to M and check that A stays at M when the stage is rotated (46d). Repeat the centering process if necessary.

Each objective must be centered separately. If an objective is screwed out of the nosepiece, e.g. for cleaning, and screwed back in the same place, its centration is more or less retained. If the stage height is altered by a few centimetres with the coarse drive or stage clamp (e.g. for specimens of different thickness) the fine centration may be slightly lost for all objectives.

Fig. 46a
Centration method I

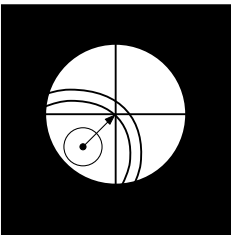
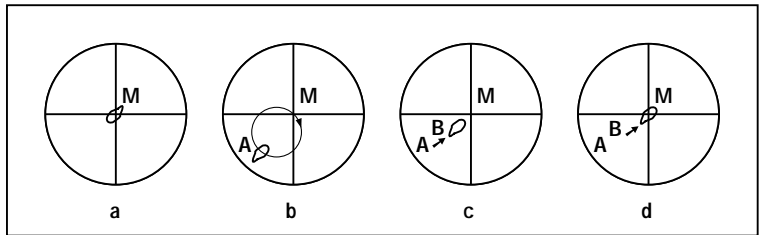


Fig. 46b
Centration method II



Tube and eyepiece setting

Set the beamsplitter in the phototube to the viewing position by fully or partly pushing in the rod (31.4).

The meaning of the switching positions is shown by symbols on the left face of the tube and described on p. 38.

For eyepieces with graticule inserted only: Defocus the specimen or remove from the light path and exactly focus the graticule by adjusting the eyelens (Fig. 37.4) with a relaxed eye. (The eye relaxes best if you look out the window at a far distant object for a moment). See also page 62. Only focus the specimen through the eyepiece with graticule. Then close your eye and focus the specimen by adjusting the second eyepiece only.

Only if neither eyepiece has a graticule inserted: When you adjust the eyelens a white line (36.5) becomes visible round the basic part of the eyepiece. This indicates the correct position of the eyelens for viewers with normal or corrected eyesight.

Spectacle wearers must remove the glare protection, but viewers not wearing spectacles must always put it on (36.7).

Set the interpupillary distance by pulling apart or pushing together (50.1) the eyepiece tubes until only one image can be seen with both eyes. Note your personal interpupillary distance, e.g. 65.

Close any tube exits (31.5, 31.9, 32 and 33) that are not in use, as otherwise stray light can disturb the image.

Transmitted light lamphousing 106*

Remove any diffusing screen(s) and filters from the light path (Fig. 9 and 10).

Method I:

UCR and **UCPR** condenser (Fig. 14a):

turn in a **10x** objective.

UCE condenser (Fig. 14b):

turn in a **5x** objective.

Raise the condenser to its highest position (48.12).

Focus the specimen and find an empty area.

Switch the condenser disc (48.14) to position **H** (= brightfield).

Disengage the condenser top (48.15).

Open the aperture diaphragm (48.21).

Slightly narrow the field diaphragm (48.22).

Remove one eyepiece from the tube and look

into the open tube from a distance of a few cm.

Adjust the collector (48.19), looking through the eyepieces at the same time, until the reflected image of the lamp filament (Fig. 47a) can be seen.

Adjust the centering screw (48.18) for the horizontal lamp adjustment with a screwdriver until the blurred, bright, vertical line (= overlapping of image and reflection of the filament) is in the centre of the bright circle. Reduce lamp brightness to do this if necessary.

Adjust the centering screw (48.17) until the image of the filament is in the centre of the field in vertical direction as well (Fig. 47). Put the eyepiece back on the tube and put the filters and diffusing screens back in the light path.

Alternatively, you can focus on the image of the filament with a Bertrand lens or auxiliary telescope (Figs. 50 and 51, condenser top swung in, use objective 40 x to 63 x, swing out polarizer 48.25).

Method II:

Lay the adjustment device* (Fig. 47a) on the window in the microscope base and adjust the image of the filament visible inside, as with method I, using the collector and centering screws (48.19, 48.17, 48.18).

Fig. 47a Lamphousing 106

Reflection of the lamp filament, greatly schematized: in reality the reflection is extremely low in contrast. In incident light the bright overlap area is wider and less defined.



Fig. 47b Adjustment device for transmitted light source



Brightfield, Koehler illumination

Setting of UCE, UCR, UCPR condensers and the field diaphragm (Köhler illumination)

Turn in a 10x objective or higher and focus the specimen. Correct the upper stage stop for the E focus (44.5) if necessary. The best position is just above the set focal plane.

Condensers, Field diaphragm

Close the field diaphragm (48.22).

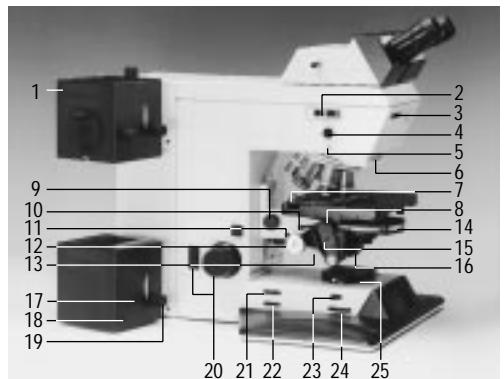
Slightly narrow the aperture diaphragm (48.21).

Swing in the condenser top (48.15).

Turn the condenser stop screw (48.13) clockwise and move the condenser to the top position with the height adjustment (48.12). Switch the disc (48.14) to the H position (= brightfield). The disc is not necessary for brightfield.

Fig. 48*

1* Lamphousing 106z for reflected light, 2* Analyser, 3* Rotatable reflector turret, 4* Window for incident light lamp adjustment, 5* Clamp screw for nosepiece change, 6* ∞ Turret for objective side Wollaston prisms, 7* Knurled knob for adjusting the object holder, 8 Stage rotation clamp, 9* Stage clamp, 10 Centering keys for condenser disc, 11 Fixing screw for condenser holder, 12 Condenser height adjustment, 13 Adjustable upper stop of condenser, 14 Condenser disc, 15 Lever for condenser top, 16 Condenser centering screws (hidden, cf 27.1 and 27.5), 17, 18 Centering screws for lamp holder, 19 Collector adjustment, 20 Focusing, 21 Aperture diaphragm, 22 Field diaphragm, 23 Grey (neutral density) filter, 24 Illumination intensity control (12 V 100 W lamp), 25* IC/P polarizer



The items marked with an asterisk are not part of every outfit.

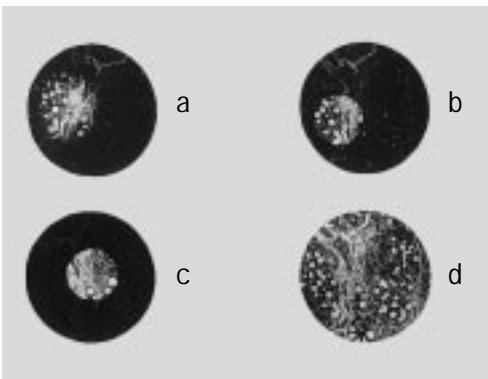
Turning the condenser stop screw (48.13) or the condenser height adjustment (48.12), lower the condenser until the edge of the field diaphragm is sharply focused (49b) and also centre the image of the field diaphragm with the two centering keys (48.16 or 27.1 and 27.5) (49c).

Open the field diaphragm (48.22) until it just disappears from the field of view (49d). When the objective is changed the condenser centration may need slight correction. Adjust the collector (48.19) until the image is homogeneously illuminated.

The field diaphragm (48.22) protects the image from unnecessary heat and keeps all light not required for imaging away from the specimen so that contrast can be enhanced. It is therefore only opened far enough to just illuminate the viewed or photographed object field. A change of magnification thus always necessitates adjustment of the field diaphragm.

Fig. 49 Koehler illumination

a Field diaphragm not focused, not centered, **b** Field diaphragm focused but not centered, **c** Field diaphragm focused and centered, but diameter too small, **d** Field diaphragm diameter = object field diameter (Koehler illumination)



Aperture diaphragm

The aperture diaphragm (48.21) determines the lateral resolution, depth of field and contrast of the microscope image. The best resolution is obtained when the apertures of the objective and the condenser are roughly the same.

When the aperture diaphragm is stopped down to be smaller than the objective aperture, resolving power is reduced, but the contrast is enhanced. A noticeable reduction in the resolving power is observed when the aperture diaphragm is stopped down to less than 0.6x of the objective aperture and should be avoided where possible.

The aperture diaphragm is set according to the viewer's subjective impression of the image, the scale on the dial is just to allow reproducible settings and does not represent absolute aperture values. In principle you can do a calibration yourself by comparison with the apertures of various objectives. Visual comparison of the apertures of the objective and the condenser can be made as follows: Remove the eyepiece from the eyepiece tube or engage an auxiliary telescope (Fig. 51) or Bertrand lens (50.2 or 54.2/54.11) and focus. Close or open the aperture diaphragm until its image is just visible in the objective pupil (brighter circle). This is considered the standard setting, i.e. condenser aperture = objective aperture.

Replace the eyepiece or disengage the Bertrand lens. For objectives with low contrast the aperture diaphragm can be stopped down further to highlight faint specimen details. In polarized light microscopy narrowing the aperture diaphragm usually results in brighter colours except for conoscopy, see page 82.



Attention: The aperture diaphragm in the illumination light path is not for setting the image brightness. Only the rotary brightness adjustment knob or the neutral density filters should be used for this.

An aperture diaphragm in the objective (41.3) is normally fully opened. The reduction in image brightness caused by stopping down results in:

- Greater depth of field
- Less coverglass sensitivity (p. 47)
- Suitability for darkfield (p. 75)
- Change in contrast

Condenser top 0.90 S 1/P 0.90 S 1

The condenser top (48.15; 16) increases the illumination aperture, which should be about 0.6x – 1x of the aperture of the objective used. The condenser top may therefore only be swung out for low-power objectives. The following rule of thumb applies for condenser tops 0.90 S 1 and P 0.90 S1:

out/in

<u>Objective magnification</u>	<u>Condenser top S 1</u>
< 10x	swung out
≥ 10x	swung in



For brightfield observation the condenser top can also be swung out for 10x objectives. However, DF, PH and ICT would not work with the condenser top swung out.

When the condenser top is swung out, the UCR, UCPR and UCE condensers remain in the same vertical position as when the condenser top is swung in. When the condenser top on the UCE condenser is swung out, the field diaphragm takes over the job of the (variable) aperture diaphragm. However, the “aperture diaphragm” must be fully opened with low magnifications and the UCE condenser. There is no exact setting of the illuminated field.

With the UCR and UCPR condenser the field and aperture diaphragm functions are retained when the condenser top is swung out (Koehler illumination).

0.50 S 15:

The Condenser top 0.50 S 15 is used from objective magnification 5x. It has an intercept distance of 15 mm when there is no glass, etc. in the light path between the condenser and the specimen. The intercept distance is lengthened when plane-parallel glass windows or liquids are introduced into the light path by about a third of the thickness of the glass or liquid, e.g. for a 3 mm glass window the intercept distance is about 16 mm.

P 1.40 OIL S 1

The Condenser top P 1.40 OIL S 1 is used when maximum resolution is required with immersion objectives with an aperture > 1.0, or for polarization-optic conoscopy (page 82) of large shaft angles. About drop of Leica immersion oil is applied to the front lens of the condenser, taking care to avoid air bubbles. The groove round the mount can pick up any superfluous oil.

The oil condenser top and condenser top 0.50 S 15 are not intended for phase contrast and ICT interference contrast.

Diffusing screen, collector

Image homogeneity can be optimized by adjusting the collector (48.19) and maybe engaging 1 – 2 diffusing screen(s) (Fig. 9 and 11).

Possible errors

Wrong coverglass thickness (see page 47) or wrong objective. Specimen has been placed on the stage with the coverglass downwards instead of upwards.

Aperture diaphragm (48.21) too wide or too narrow. Incorrect height or centration of condenser.

Lamp not adjusted (page 68). Dirty optics.

Phase contrast

Like transmitted light darkfield and transmitted light interference contrast ICT, phase contrast is used to produce high-contrast images of unstained specimens.

Turn the phase contrast objective (engraving PH) with the lowest magnification (generally 10x) into the light path and focus the specimen. If you have trouble finding the specimen plane: temporarily stop down the aperture diaphragm (48.21) or use a stained specimen, setting the condenser disc at H (48.14).

Set Koehler illumination (see also page 69): sharply focus the field diaphragm together with the specimen by adjusting the condenser in x, y and z. Swing in the condenser top (48.15).

Set the light ring corresponding to the objective engraving (e.g. light ring 1 for objective PH 1) on the condenser disc (48.14).

Fig. 50 Tube and tube lens system with Bertrand lens

1 Interpupillary distance setting of the observation tube, 2 Dial for Bertrand lens (B) or tube lens (1x), 3 Focusing of Bertrand lens



Fig. 51 Auxiliary telescope

1 Adjustable eyelens, 2 Clamp ring for fixing the focus position



Open the aperture diaphragm (= pos. PH).

Engage the built-in Bertrand lens* into the light path by turning the knurled wheel (50.2) = pos. B, and focus the annular structures (Fig. 52) with the lever (50.3). See page 83 for how to operate the Bertrand lens on the polarized light microscope.

If your microscope does not have a Bertrand lens: insert an auxiliary telescope* (Fig. 51) into the observation tube in place of an eyepiece. Slightly loosen the clamp ring (51.2) and focus the annular structures by adjusting the eyelens (51.1). Retighten the clamp ring.

Push in the two centering screws at the back of the condenser (48.10 or 14.3) and rotate until the dark ring (phase ring in the objective) coincides with the slightly narrower bright ring (light ring in condenser).

Disengage the Bertrand lens and watch the quality of the phase contrast image. If using the auxiliary telescope, watch the image with one eye through the eyepiece. Then repeat the centering process for the other objective light ring combinations.

Possible errors

Specimen: too thick, too thin, too brightly stained; refractive index of mounting medium and specimen identical so that there is no phase jump.

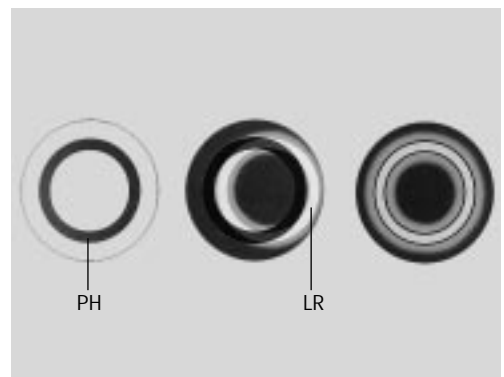
Specimen slide too thick, so Koehler illumination not possible.

Wedge-shaped coverglass position, so centering of light and phase ring is no longer effective.

Wrong light ring, or light ring has been put in the turret upside down (see assembly on page 23).

Aperture diaphragm not open. Wrong condenser top (only 0.90 S 1).

Fig. 52 Centering process for phase contrast, observed with a Bertrand lens or auxiliary telescope
a Condenser in brightfield position (H), **b** Condenser in PH position, light ring LR not centered, **c** Light ring and phase ring centered



Transmitted light darkfield with UCE, UCR and UCPR condensers

Darkfield is possible with all objectives from 10x magnification; the image background may be inhomogeneously illuminated at lower magnifications. Solution for 5x objective: Use light ring 3 with condenser top swung out (UCR/UCPR condenser only) or use condenser top 0.50 S 15 (condenser UCR/UCPR and UCE). The highest possible objective aperture is 0.75, although objectives with higher apertures can be used if it is possible to reduce the aperture with a built-in iris diaphragm. These objectives can be identified by the fact that the maximum and minimum apertures are given in the objective engraving and in our lists, e.g. 1.30 – 0.60, (Fig. 41.3).

Rotate the condenser disc to position H (= brightfield). Focus the specimen (10x objective). If you have trouble finding the specimen plane, temporarily close the aperture diaphragm (48.21). Swing in condenser top 0.90 S 1.

Set Koehler illumination (page 69) (sharply focus the centered field diaphragm together with the specimen).

Open the aperture diaphragm as far as the stop (= pos. PH) and turn the disc to pos. D (= darkfield ring). Optimize image homogeneity by slightly adjusting the height of the condenser and collector (48.19).

Transmitted light darkfield with special darkfield condenser

Whether the DF condensers (Fig. 53) can be used depends on the aperture of the objectives. Objectives with built-in iris diaphragm (41.3) have adjustable apertures.

DF condenser:	max. objective aperture
D 0.80 – 0.95	0.75
D 1.20 – 1.44 OIL	1.10

Compared with brightfield objectives, phase contrast objectives do not produce such good imaging results for critical specimens in darkfield.

Move the upper stop of the condenser to its highest position by unscrewing screw (48.13) in clockwise direction. Put a specimen on the stage.

Carefully clean the upper and lower surface of the specimen.

Traces of dust and oil film on the glass surfaces or air bubbles in the mounting medium seriously impair the quality of the darkfield image!

n.b.: Open the aperture diaphragm (48.21) = pos. PH.

Focus the specimen with the 10x objective, open the field diaphragm (48.22).

Adjust the condenser in x, y and z direction (48.12 and 48.16) until the field is homogeneously illuminated, narrowing the field diaphragm (48.22). You can now switch to a higher-power objective. Make sure only the observed field of view is isolated by the field diaphragm.

Immersion darkfield

Assemble the immersion condenser (see above). Before putting the specimen on the stage, apply a drop of oil to the front of the condenser, making sure there are no air bubbles. Set as for "darkfield with UC/UCR condenser", p. 75.

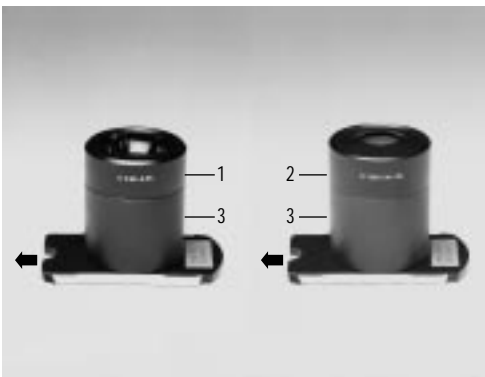
Possible errors

Darkfield illumination is very sensitive to the slightest inhomogeneities in the specimen. As dust particles and fingermarks on the upper or lower surface of the specimen and the front lens of the condenser also cause scattering and diffraction of light, it is essential to keep specimen surfaces and neighbouring lenses absolutely clean.

If the objective aperture is larger than the threshold values listed above of 0.75 or 1.10, you will get an image similar to brightfield. This will also happen if the condenser is greatly decentered.

Fig. 53 Special darkfield condensers

1 D 0.80 – 0.95 (dry), 2 D 1.20 – 1.44 OIL, 3 Condenser bottom



Transmitted light polarization*

See page 65 for objective centration (polarized light microscopes only).

Adjust the light source, diaphragms and condenser as for transmitted light brightfield (page 69); the following description applies for polarized light microscopes (Fig. 54) and for other microscopes retrofitted with polarizers (polarization contrast, Fig. 27).

Crossing the polarizers

Focus an empty area of the specimen or remove the specimen from the light path.

Remove any compensators (50.13; 27.6), Bertrand lens (54.2 or 50.2) and incident light reflectors (54.12) from the light path.

Rotate the condenser disc (54.16) to pos. H.

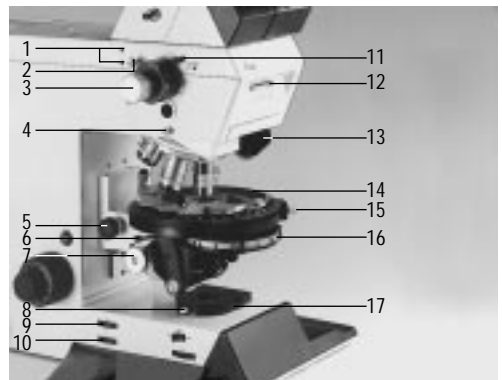
Rotate the objective nosepiece (60.7) to pos. H. Insert the analyser (54.3) and preadjust as follows, corresponding to the polarizer used:

<u>Analyser IC/P</u> (30.5) Make index coincide exactly, the λ mark must point downwards	<u>Polarizer Ø 32 mm</u> (27.3) insert from the right (27.6) or place on the window in the microscope base (27.3) or <u>Polarizer IC/P</u> (54.17) IC setting = 90° (i.e. vibration direction N – S (54.17↓))
<u>Analyser 360</u> (30.1) Set exactly at <u>90.0°</u> pos. (DIN standard)	<u>Polarizer IC/P</u> (54.17) 0° setting (vibration direction E – W ↔)

Looking at the empty field of view, set the optimum extinction position by rotating the polarizer (never the analyser!)

Fig. 54 Controls on polarized light microscope

1 Centration* of Bertrand lens, 2 Bertrand lens* on/off, focusing, 3 Analyser, 4 Objective nosepiece clamp screw, 5 Stage clamp, 6 Centration of PH light rings and ICT prisms, 7 Condenser height adjustment, 8 Polarizer rotation clamp, 9 Aperture diaphragm, 10 Field diaphragm, 11 Tube lens 1x/1.6x*, 12 Quadruple* turret for incident light techniques, 13 Compensator slot (tube slot), 14 45° clickstop (hidden), 15 Stage rotation clamp, 16 Condenser disc, 17 Index adjustment of transmitted light polarizer



Make sure the specimen, the condenser lenses and polarizers are clean, as this will affect the accuracy of the setting.

A particularly accurate method of setting this position is to use the built-in Bertrand lens (54.3 with 54.11) on the polarized light microscope as follows:

Turn a high-magnification objective into the light path (e.g. 40x, 50x, 63x).

Open the aperture diaphragm (54.9) (pos. PH).

Focus the Bertrand lens or auxiliary telescope so that the slightly brighter circle in the centre of the field of view is sharply defined. If you slightly adjust the polarizer you will see 2 dark stripes that close to form a cross when the polarizers are exactly crossed (55a). If objectives and condensers without the engraving "P" are used, the cross usually does not completely close.

Index adjustment on IC/P polarizer

If the two index marks on the mount of the polarizer (28.4) do not exactly coincide when the polarizers have been crossed: alter the index adjustment with the centering keys (28.3 or 54.17) until the index marks coincide. After this adjustment the crossed position of the polarizers can be set reproducibly or checked.

Examinations in polarized transmitted light

The following section is only intended as a rough guide to the various examination methods. Further details are to be found in the Leica booklet "Polarized light microscopy", code no. 923009, and in many books on the subject.

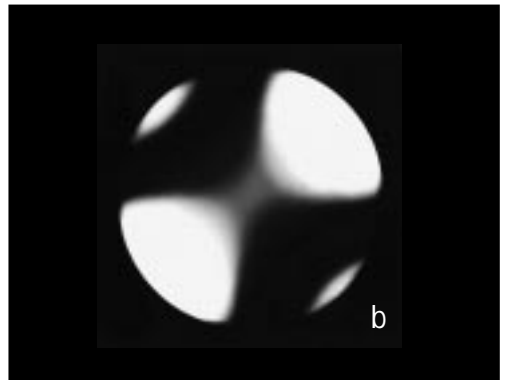
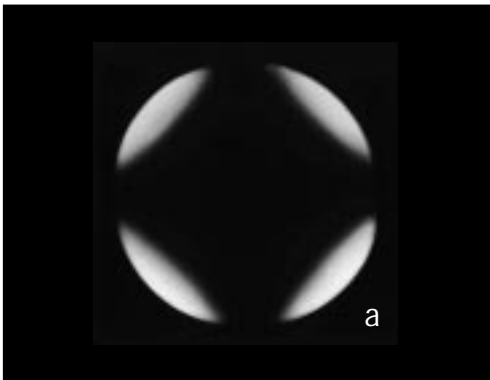
Examinations

Only one polarizer

If you want to examine specimens with other transmitted light techniques such as brightfield, phase contrast and darkfield instead of with crossed polarizers, it is usually sufficient to disengage either the analyser or the polarizer.

Fig. 55 Crossing the polarizers, viewing with a Bertrand lens and a high-aperture objective
a exactly crossed, **b** not exactly crossed

Pos. a cannot be set at all if there is strain in the condenser or objective



If the image is not bright enough, both the polarizer and the analyser should be disengaged. A neutral density filter (30.4) can be used in the empty slot of the analyser 360 (30.1) to protect the viewer from glare when the analyser is disengaged. Coloured birefringent specimens may exhibit differences in brightness and colour when the stage or polarizer is rotated (when the analyser is disengaged). This phenomenon is called dichroism or pleochroism and is an important indication for crystal examinations. However, this effect can also be simulated on non-polarized light microscopes, as these have no built-in depolarizing quartz plate, or if an incident light reflector has been left in the light path when transmitted light is switched. This also applies for the use of the tubelens 1.6x on the polarized light microscope (54.11).



Incident light reflectors or fluorescence filter cubes should disengaged during examinations in polarized transmitted light and transmitted light interference contrast ICT.

Examinations

Crossed polarizers

The DIN and ISO standard vibration directions are shown in the chart on page 77, but when the polarizers are crossed the same polarization-optic effects are observed when the polarizers are transposed by 90° .

If the specimen contains many non-birefringent or opaque particles, the analyser is frequently turned out of the crossed position by a few degrees so that these particles show up at least

faintly (they remain dark when the polarizers are exactly crossed). It is not customary to examine specimens with the polarizers parallel, as this method of identifying birefringence is not sensitive enough.

Change in brightness when birefringent objects are rotated

When the stage is rotated, the brightness of birefringent (anisotropic) objects changes periodically. During a full rotation the object disappears four times after each 90° interval. The four dark positions are called extinction or normal positions. Exactly between each of these extinction positions the object can be observed with maximum light intensity. These are the four diagonal or 45° positions. In the extinction positions the object vibration directions run parallel to the transmission directions of the polarizers, at maximum intensity the object vibration directions represent the angle bisectors of the polarizer directions. The crosslines in the (right-hand) eyepiece of polarized light microscopes can either be aligned at N–S/E–W, i.e. in the polarizer directions, or at 45° angles, i.e. corresponding to the object vibration directions in the diagonal position.

Insert quarter-wave compensator (57.5) in the tube slot.

Push the quarter-wave compensator (57.1) into the slot underneath the condenser (27.6) and rotate until the empty field of view appears at its darkest position (first cross polarizers exactly!).

Compensators for quantitative measurements

Only in conjunction with polarized light microscopes in transmitted light. Adjustable compensators are used for exact measurements of phase differences. For a known specimen thickness d and the measured phase difference Γ the birefringence $\Delta n'$ can be worked out using the following formula:

$$\Gamma = d \times \Delta n' \text{ [nm]} \text{ or } \Delta n = \frac{d}{\Gamma}$$

To perform the measurement, the compensator is introduced into the tube slot and adjusted until the object to be measured is in its maximum extinction position. For this purpose the object has to be moved into a certain diagonal position. With HC P tube optics the measurement areas can be isolated with an iris diaphragm (58-I with 54.11) Further details are given in the instructions for the use of the compensators. The following compensators are available:

Elliptical Brace-Koehler compensator (57.9)

Rotary compensator with compensator plate of about $\lambda/10$ difference. Measurement is carried out in white or in monochromatic light. Measurement range up to approx. 50 nm.

Fig. 57a/b Compensators

1, 2 $\lambda/4$ and compensator for pos. 27.6. Only for polarized light microscopes: **3** $\lambda/4$ and λ compensator for 8-position disc (54.16), **4, 5** $\lambda/4$ and compensator for tube slot (54.13), **6** Rotatable $\lambda/4$ compensator (Sénarmont compensator), **7** Quartz wedge, **8** Tilting compensator, **9** Brace-Koehler compensator

Elliptical Sénarmont compensator (57.6)

($\lambda/4$ compensator in subparallel position)

Measurement is executed in monochromatic light (546 nm), and a 360° rotatable analyser (30.1) is necessary. Normally this compensator is used to measure phase differences of up to the first order, although higher phase differences can be measured, too. However, the compensator does not produce the entire phase difference but only the amount that is in excess of a whole wavelength or a multiple thereof. Whole wavelengths must be determined with a tilting compensator, quartz wedge, or estimation of the interference colour. Accuracy is higher than with the tilting compensator alone.

Tilting compensator B (Berek compensator) measuring up to 5 orders

Compensator (57.8) with MgF_2 plate or measurements in monochromatic or white light of up to 5 orders phase difference. The phase difference can be read directly from the sum of the two angles of compensation produced when the compensator plate is tilted in both directions, from a supplied calibration chart.

Tilting compensator K, measuring up to 30 orders (57.7)

For the measurement of phase differences in white or monochromatic light up to the maximum phase difference mentioned above. The compensator plate is made of calcite; evaluation is based on simple calculation by means of enclosed tables and the stated calibration constant. A programmed computer can be used for evaluation of measurements taken with tilting compensators. The necessary formulae and parameters are given in:

Kornder, F. and W. J. Patzelt: The use of minicomputers to evaluate polarization-optic compensator measurements. – Leitz Scientific and Technical Information IX/1, 30–32, 1986.

Conoscopy of crystals

Only with the Leica DMRXP polarized light microscope: Birefringent crystals cause interference patterns (Fig. 59a/b) in the exit pupil of the objective (i.e. inside the objective). These are also called conoscopic images. The shape of these interference patterns and the way they change when compensators are used supply information on the number of the crystal axes (uniaxial or biaxial crystals), the orientation of these axes and the plus or minus sign of the birefringence (positive or negative birefringent crystal).

As these interference patterns occur in the pupil they are not normally visible during normal microscopic observation (orthoscopy). Their observation can be improvised by removing one of the eyepieces and looking into the tube with one eye from a distance of a few centimetres. Observation is better with the auxiliary telescope for phase contrast (Fig. 51). Other crystals in the field of view disturb the interference patterns of a crystal in the centre, so that this needs to be isolated. This can only be done with the polarized light microscope (tube optics HC P, with Bertrand lens and iris diaphragm). This module also has a second tube lens allowing additional magnification by a factor of 1.6x.

Setting the microscope for conoscopy

The most suitable object areas for conoscopy are those that show the lowest possible phase differences (chart in Fig. 56).

Exact objective centration and exactly crossed polarizers are essential for perfect conoscopical observation. Turn an objective with as high an aperture as possible (e.g. 40x, 50x or 63x) into the light path. Open the aperture diaphragm (54.9). Move the crystal you want to examine as near to the centre of the field of view as possible.

Turn in tube lens 1.6x.

Narrow the iris diaphragm (Fig. 58, pos. I) to match the size of the crystal, stopping down the field diaphragm (54.10) as well if necessary.

Push in the Bertrand lens (58B) and focus by rotating the control until the interference image or the circular bright-dark edge of the pupil is focused. Centre the Bertrand lens if necessary. This is done by inserting the hexagonal screwdriver (1.1) into the two holes (54.1) in succession. Align the right-hand eyepiece so that the crosslines roughly correspond to the directions of movement for the centration process.

Fig. 58 Functions of the der Pol tube optics HC P

Controls	Orthoscopy 1x	Orthoscopy 1.6x	Conoscopy
Tube lense (54.11)	1x	1.6x	1.6x
Iris diaphragm	doesn't matter as	matched of the field of view	> object
Bertrand lens (54.2)	not in light path	out	in
Polarizers (54.3 and 54.16)	in or out	in or out (not for dichroism/pleochroism)	crossed

Adjust the collector (48.19) to an optimal setting, using a diffusing screen (42.15) if necessary.

Determination of optical character

Uniaxial crystals (Fig. 59a)

Uniaxial crystals observed in the conoscopic (divergent) beam show a dark cross, whose centre indicates the position of the optical axis. The cross is surrounded by coloured interference fringes*. When a variable compensator (quartz wedge or tilting compensator) is operated the rings drift towards the centre or outwards in two opposite quadrants of the cross. The optical character is determined from the direction of movement of the rings as in Fig. 59. Cutting directions in which the optical axis of the crystal is inclined to the direction of observation are suitable for the determination of the optical character, which can mostly be determined even when the centre of the cross is outside the field of view. Fig. 59 shows that fixed instead of variable compensators can also be used for the determination of the optical character.

The optical character can usually be identified even when only one of the optical axes is in the viewing direction of the observer. In the orthoscopic beam the brightness of specimens orientated in this way changes little if at all during rotation. In the conoscopic beam, only one of the two isogyres will then be visible.

Biaxial crystals (Fig. 59b)

For the determination of the optical character cutting directions are particularly suitable in which the bisectrix of the two optical axes is parallel to the viewing direction (section vertical to the acute bisectrix).

In the divergent beam a dark cross will be seen which opens up into the two branches of a hyperbola, the so-called isogyres, when the stage is being rotated. The cross and the branches of the hyperbola are surrounded by interference fringes. According to Fig. 59 or the rule mentioned below the optical character can be determined from the displacement direction of these fringes after operation of the compensator. The symmetry plane of the isogyres (axial plane) must be vertical to the γ direction of the compensator.

* Only the cross is visible for thin specimens or specimens with low birefringence.

Biaxially positive crystals:

The interference fringes move from the convex to the concave side of the isogyres when the compensator is operated.

Biaxially negative crystals:

The interference fringes move from the concave to the convex side.

Possible errors

Polarizers damaged (discoloured) by powerful light sources or dirty.

Objectives or condenser strained through mechanical damage.

Beamsplitter or filter between the polarizers.

Mounting medium for transmitted light specimens is birefringent.

Further sources of error on page 72.

Fig. 59a Determination of the optical character of uniaxial structures

Left: Positively uniaxial crystal, cut vertically to the optical axis

Right: Negatively uniaxial crystal, cut vertically to the optical axis

1 Diagram of the vibration directions in the object and in the compensator

2 Change in the interference pattern when a quarter-wave compensator is used

3 Change in the interference pattern when a whole-wave compensator is used

Fig. 59b Chart for determination of the optical character

Condenser

Important: Only use the condenser tops 0.90 S1 or P 0.90 S1 and P 1.40 OIL. The condenser top with long working distance 0.50 S15 (p. 22) is not designed for ICT work. See pages 25 and 30 for assembly.

Crossing the polarizers

To obtain a good quality ICT image the analyser must be set exactly at 0 and the polarizer must be exactly crossed (extinction position)!

Push the analyser (50.1) into the microscope as far as the second clickstop. The lambda sign (λ) must be on the underneath (not visible), see also page 34.

Loosen the analyser clamp (30.6) and adjust so that the two index marks are exactly opposite each other. If using the 360° rotatable analyser (30.1), set the 0° position with the coarse and fine scale and vernier (30.2 and 30.3), clamp on back. Retighten clamp.

Turn the objective nosepiece turret (60.7) and the condenser disc (60.8) to pos. H = brightfield. The IC prisms are then disengaged. Disengage the incident light reflector (60.6).

Fig. 60 Controls for ICT transmitted light interference contrast
1 Analyser, cf Fig. 30, 2 Stage rotation clamp screw,
3 Condenser top lever, 4 Polarizer rotation clamp, 5 Polarizer index adjustment (cf Fig. 28), 6 Incident light reflector turret, 7 Objective-side prism turret with fine adjustment, 8 8-position disc for condenser-side Wollaston prisms, 9 Mount for λ or $\lambda/4$ compensator (hidden, cf Fig. 27.6)

Focus the specimen. It may be easier to focus a stained specimen first or the edge of the cover-glass. Set Koehler illumination exactly (see page 69), then find an empty area of the specimen or remove the specimen.

Turn the polarizer (60.4) round the IC position until the optimum extinction position is observed through the eyepiece. This setting can be found particularly accurately with a high-magnification objective (40x or 63x):

Open the aperture diaphragm (48.21) as far as the stop, engage the Bertrand lens (50.2) and focus (50.3) or use the auxiliary telescope (Fig. 51) instead. The polarizers are exactly crossed when the two branches of the hyperbola are as near to each other as possible – or form a cross (55a).

Fix this crossed position with clamp screws (60.4 and 30.6).

Put the centering key (1.5) in the index adjustment (60.5) and make the two index marks (28.4) coincide; you will now be able to reproduce the current polarizer setting later.

Adjustment of the condenser prisms

If the equipment was delivered together, the condenser prisms will already have been adjusted at the factory, but it is advisable to check the adjustment from time to time, especially after transport:

Disengage the objective side ICT prisms (60.7) (pos. H).

Swing in the condenser top (48.15). Engage the Bertrand lens (50.2) or use the auxiliary telescope (Fig. 51).

Engage the condenser-side prisms (60.8) in succession and focus the dark diagonal compensation stripe. The whole-wave compensator must be inactive, i.e. the engraving must be on the side of the analyser that points downwards.

The dark stripe should be in the centre of the brighter circular area. If not, proceed as follows: push in the right-hand centering key on the back of the condenser until it clicks into position and rotate it until the stripe is in the centre of the circle. The left-hand key is not required. However, for the 3rd and 4th prism position make sure that the left-hand centering screw for the light rings is not rotated too far inwards or it may obstruct the movement of the prism with the right-hand key.

Objectives for ICT

Transmitted light interference contrast is possible with the brightfield and phase contrast objectives which have the code letter of the pupil position in the first line of engraving, e.g. A and which are listed under the objectives suitable for ICT on the optics data sheet. Transmitted light interference contrast is also possible with certain incident light objectives (see separate objective table). A condenser prism, e.g. K₁ must also be available for the objective.

Choice of prisms

Choose the objective side prism (60.7) with the letter indicated in the top line of the objective engraving* (page 48 and on Optics data sheet), e.g. A for pupil position A.

Additional numbers e.g. B₂: Prism with greater beamsplitting than the standard version (= B₁), for higher detection sensitivity.

Choose the condenser side prism (60.8) that corresponds to the magnification of the objective used, e.g. pos. 20/40 for objective 20x (and 40x).

Swing in condenser top 0.90 S1, only swing out condenser top for the 5x objective (only with UCR/UCPR condenser; ICT is only possible with the UCE condenser from objective 10x upwards). Exactly set Koehler illumination (see page 69). This is made easier by temporarily focusing a stained specimen or the edge of the coverglass.

Setting ICT contrast

Carefully turn the objective-side prism turret (60.7) to the left and right. Also adjust contrast with the aperture diaphragm (48.21). Particularly sensitive setting is possible with the $\lambda/4$ compensator (57.1), which is inserted in the holder under the condenser (60.9) and rotated

(objective prism roughly at the centre position). Optimum contrast for specimens with parallel structures can be obtained by rotating the stage (48.8).

Colour contrast: Turn over the analyser, so that the sign can be seen on the top. If using the 360° rotatable analyser (30.1) colour contrasting is carried out by placing a rotatable whole-wave compensator (57.1) on the polarizer or pushing it into the mount underneath the condenser (27.6) and rotating.

Specimen preparation

ICT gives best results for unstained, relatively thin, non-birefringent specimens. Interpretation of birefringent specimens can be extremely difficult, if not impossible. It may be helpful to rotate the specimen to an optimum azimuth position.

Specimen slides, coverglasses and embedding resins of birefringent material may not be used.

Preparation errors

Possible sources of error if ICT image is unsatisfactory

Embedding medium, specimen slide (Petri dish) or object (e.g. crystals, fibres) are of birefringent material. The phase shifts caused by birefringence disturb the interference contrast image. This can sometimes be remedied by rotating the specimen.

The specimen is too thick or too thin.

Specimen slide or coverglass are too thick or the coverglass is missing (except for HC PL FLUOTAR 5x/0.12 and 10x/0.25, which can be used either with or without a coverglass).

The difference in the refractive indices of the specimen and the embedding medium is too small (this often happens when uncovered specimens are observed with an immersion objective). Inhomogeneous mounting medium.

Errors in instrumentation

Polarizers not engaged, or rotated too far out of the crossed position or, though crossed, turned out of the zero positions.

Polarizer has been damaged by powerful light sources. Check this by holding the polarizer against a window or light source. Damaged polarizers then show distinctly uneven colouring.

The IC prisms in the condenser are in the wrong position or upside down. This is checked by combining an IC prism with all available objectives and seeing if the interference contrast image is optimal at corresponding magnifications of the objective and the condenser.

Condenser top in wrong position.

Wrong condenser top (only 0.90 S1 or P 0.90 S1 or P 1.40 OIL may be used).

Koehler illumination not set (image of field diaphragm in the specimen plane).

Aperture diaphragm too wide or too narrow.

Dirty optics or polarizer.

Dust protection: Turn the condenser prism out of the light path if it is not being used for a long time.

For specimens with parallel texture: specimen is in wrong azimuthal position (remedy: rotate specimen with stage 60.2; 54.15).

The following description applies for fluorescence, brightfield, darkfield, polarized light and interference contrast techniques.

Attention:

Never look into the direct light path! There is danger of glare when switching to the brightfield reflector (BF) or the Smith reflector (6.4; 6.5)!

Imaging the light sources to check adjustment

There are several different ways of imaging the lamp filament or discharge arc; the field diaphragm (63.6 or 65.8) is first narrowed and the aperture diaphragm (65.12) opened. Switch to the light source you want to use (61.7*).

Using the centering aid

To do this the left side of the microscope stand must be equipped with the adjustment window (61.9) for imaging the light source. Put the centering aid (reflector for lamp adjustment, 18.2) into the reflector turret instead of a filter cube or reflector (see page 26). Rotate the turret until the centering aid is in the light path. Alternatively:

Projection on the microscope base

Remove the specimen and the condenser. Turn in a 5x (or 2.5x) objective. Put a piece of blank paper or card on the microscope base: the bright circle projected onto it represents the (unfocused) projection of the objective pupil (in principle the condenser could be left on the microscope and the adjustment carried out through the condenser at a certain setting, but this method is not recommended due to the necessity for exact condenser adjustment).

Or:

Back reflection via the specimen

Focus a well-reflecting incident light specimen (e.g. surface mirror) (this is not possible for fluorescence). Remove an eyepiece from the tube, or engage Bertrand lens (50.2 or 54.2/11) and focus, or use an auxiliary telescope (51.1). The light source can be observed inside the bright circle (objective pupil) through the tube.

Centration of reflected light lamps

Lamphousing 106 (Fig. 4, page 12) with 12 V 100 W lamp

Adjust the collector (48.19) until you see the lamp filament (Fig. 47, page 68).

Using a screwdriver (1.1) adjust the vertical position (48.17) of the lamp holder until the slightly brighter stripe in the reflection of the lamp filament is in the centre of the brighter area (Fig. 47). Then move the reflection of the lamp filament with the horizontal adjustment (48.18) to the centre of the range of movement (Fig. 47).

Lamphousing 106 z with halogen lamp and gas discharge lamp (Fig. 5, page 13 and Fig. 61)

The image of the light source is focused with the collector (61.6) and the holder with the light source adjusted vertically and horizontally (61.1 and 61.2). The reflector is also focusable (61.4) and centerable in x and y direction (61.3 and 61.5).

The adjustment principle is similar for all light sources:

Move the reflection of the lamp filament or discharge arc to the side or completely out of the light path (62a) by turning the adjustment screws on the back of the lamphousing (61.3 and 61.5). Focus the direct image of the filament or discharge (61.6) and adjust as follows (61.1, 61.2 and 61.6):

Halogen lamp: just below or above the imaginary line through the centre of the brighter circle (62b).

First focus the reflection (61.4) and then move it symmetrical to the direct image inside the brighter circle (62c), or superimpose it on top of the direct image.

Mercury- (Hg) and xenon lamp (Xe)

Move the direct image (62a) to the centre of the brighter circle with the horizontal (61.2) and vertical (61.1) adjustment of the holder. Focus the reflection (61.4) and adjust the mirror until the reflection coincides with the direct image (62c).

Caution:

Caution with Hg and Xe lamp:

Be careful not to project the reflection on the electrodes for long, as there is a risk of explosion if they overheat. The two electrodes can just be seen in the extension of the symmetry plane of the discharge arc.

Replace spent burners in good time and dispose of in an environment-friendly way. Do not open the lamphousing until the lamp has cooled down and you have disconnected it from the mains. Wear protective clothing (gloves and mask) when using Xe lamps. Hg lamps take a few minutes to reach their full intensity; they do not ignite when hot.

Fig. 61 Lamphousing 106z

1 Vertical adjustment of lamp, 2 Horizontal adjustment of lamp, 3, 5 Vertical and horizontal adjustment of reflection, 4 Mirror focusing, 6 Collector (focusing of lamp image), 7 Aperture for switch rod* (switchable mirror only), 8 Analyser*, 9 Adjustment window*

Collector setting, diffusing screens

Halogen, Hg and Xe lamps:

Adjust the collector (61.6) until the bright area is uniformly illuminated. Switch the microscope back to object observation; the diffusing screen(s)

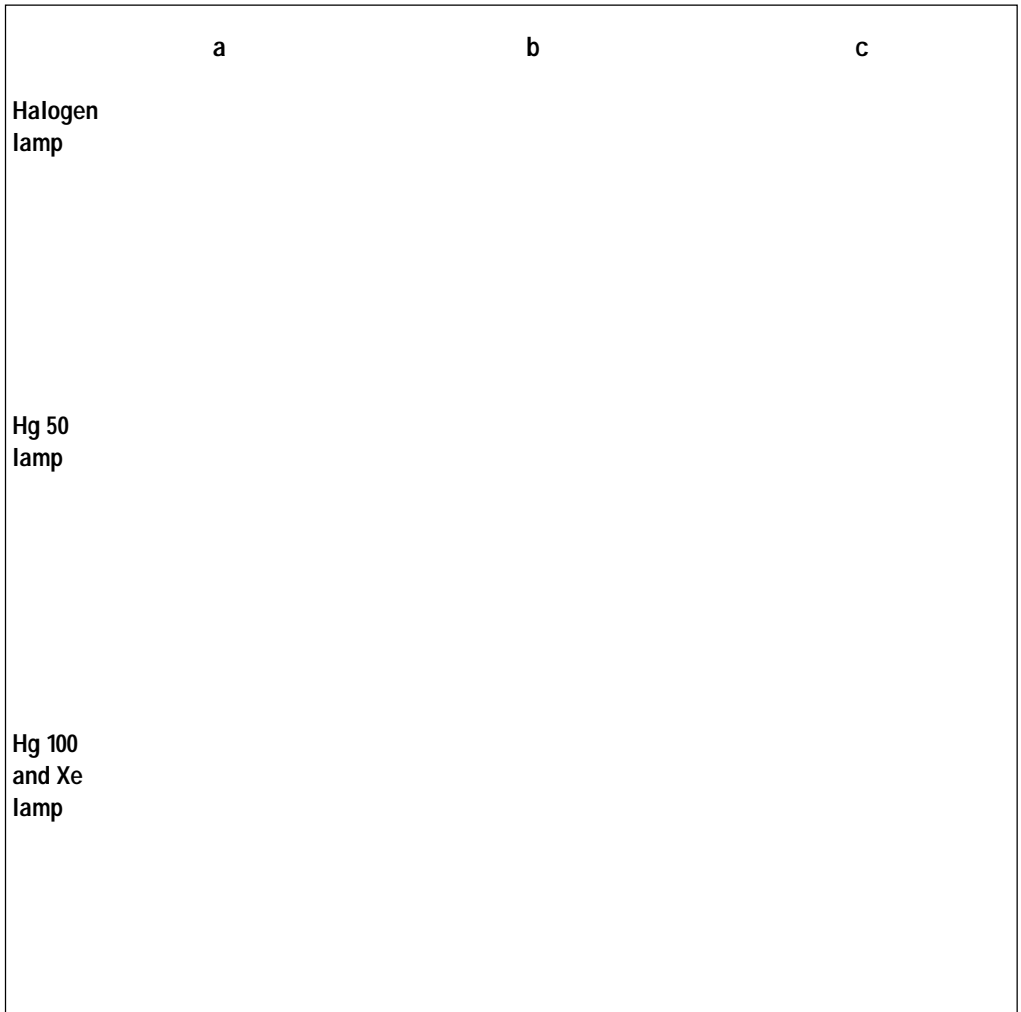
can be engaged (only with halogen lamp) to check whether the image is homogeneously illuminated (use a homogeneous specimen section if possible and a low-power objective). Use a grooved diffusing screen for the Xe 150 W lamp.

Fig. 62 Lamp adjustment

a direct filament image focused, but decentered

b direct filament image in the right position

c reflected and direct filament image in the right position



Filter cube, objective, tube factor

Focus the specimen in transmitted light first if possible. Select a filter cube to suit the excitation and emission spectrum of the specimen and switch it into the light path (63.1), see page 26 for assembly. Use high-aperture objectives (immersion) to obtain optimum image intensity; open the iris diaphragm in the objective if applicable (41.3). Switch the tube system* to factor 1x. Protect the immersion oil from impurities to avoid disturbing fluorescence.

Diaphragm module HC F

Push the diaphragm module in fully (Fig. 63.5 – 10). Unblock the incident light path (63.8), focus the specimen and switch off or cover transmitted light (Fig. 64).

Set the field diaphragm: Close (63.6) until it is visible in the microscope field of view (49b).

Insert the two centering keys (1.5) in their holes (63.5) and turn until the image of the field diaphragm is in the middle of the field of view (49c). Open the diaphragm until the entire field of view is homogeneously illuminated (49d). If you have an interchangeable stage the centering keys can be kept on the right side (as in 13.3). Disengage the BG 38 filter (63.7) if there is no disturbing red background. Always engage the filter for photography, however. Always disengage the incident light polarizer (63.4).

Set the aperture diaphragm: Remove the objective and focus a light source on dark paper (specimen stage). Narrow and open the diaphragm: if the image of the diaphragm lies eccentric to the circle: insert the centering keys (23b.8) and center the diaphragm image. Open the aperture diaphragm in fluorescence mode, only narrowing it in special cases to influence contrast. Position of the lever (23.13) for auxiliary lens (can only be set after pulling the diaphragm module HC F out of the microscope):

Push in: Optimisation for fov 20 and 22,
gain in intensity (TV!)

Pulled out: Optimization for fov 25

Fig. 63 Controls for fluorescence with diaphragm module HC F
1 Turret for 4 filter systems/reflectors, 2 Interference contrast prism turret*, 3 Tube lens 1x/Bertrand lens (B)*, 4 Incident light polarizer* mount, 5 Holes for centering keys (field diaphragm), 6 Field diaphragm, 7 BG 38 filter, 8 Interruption of the incident light path, 9 Hole for aperture diaphragm centering keys, 10 Aperture diaphragm, 11 Filter magazine

Fluorescence with diaphragm module HC RF: As a BG 38 filter is not integrated here, it must be built into the filter magazine (65.13) if needed. The light path can be blocked by pulling out the diaphragm module part way. Intensity can be increased by interposing the illumination telescope ("booster", not illustrated).

Light trap

To avoid stray light from the underneath of the specimen: remove the condenser and put the light trap (Fig. 64) in its place. Alternatively, a black metal plate can be pushed into the stage.

Fig. 64 Light trap for fluorescence microscopy (instead of the condenser). Instead of the light trap, a dark metal or plastic strip can be used, which is inserted between the upper and lower part of the x/y stage under the specimen.

Possible errors

Weak fluorescence, weak image intensity due to:

Incorrectly stored, too old or faded specimens; fast specimen fading (e.g. with FITC); inspecific filter combination, numerical aperture of objectives too low; eyepiece magnification too high; spent lamp; room too bright.

Low contrast image due to:

Excitation bandwidth too great; inspecific staining; fluorescing inclusion medium; auto-fluorescence of the objective or immersion oil.

With double fluochroming, green and red image details visible at the same time due to:

Filter cubes unsuitable for selective observation.

Inhomogeneous illumination due to:

Incorrect lamp centration or flickering lamp.

Brightening of image background or red background due to:

Absence of BG 38 red attenuation filter in light path.

Metal staining

The case is different for reflecting objects, such as in the immunogold technique (IGS). Here the POL filter system (crossed polarizers for contrast enhancement) is used for incident light polarization instead of a fluorescence filter cube, and contrast, resolving power and depth of field can be influenced with the aperture diaphragm.

Reflection contrast*

The following equipment is required (see separate manual):

Reflector system POL (Fig. 18), special objective RC with rotatable λ and $\lambda/4$ compensator mounted in front, reflection contrast module HC RC, with additional annular diaphragms for optimising contrast.

Incident light brightfield*, alignment of polished sections

Homogeneous illumination and uniform definition over the whole field of view can only be guaranteed if the surface of the specimen is aligned at exactly 90° to the optical axis. A precisely horizontal position of the object is particularly important at high magnifications, as the depth of field decreases as magnification increases.

Polished specimens can be pressed plane-parallel onto a metal specimen slide (code no. 563014) with the special handpress (code no. 563035) and plasticine. The handpress has an adjustable stop so that all specimens can be aligned to the same height. Then only slight refocusing is required with the fine control during serial investigations.

Objects that do not lie flat and that cannot be levelled with the handpress can be aligned by autocollimation. The object is focused on the tiltable specimen slide, for example, (code no. 562294) at a low magnification (5x or 10x objective).

Fig. 65 Controls for incident light brightfield, darkfield, polarized light, ICR interference contrast, see also Fig. 23

1 Analyser (hidden, on the left of the microscope, cf Fig. 30), **2** Tube lens 1x/Bertrand lens*, **3** 4-position turret* for reflectors/filter system, **4** Incident light polarizer*, **5** Turret for objective-side Wollaston prisms* or Pol compensator slit, **6** Contrast adjustment ICT and ICR, **7** Holes for centering keys (field diaphragm), **8** Field diaphragm, **9** Switch lever on diaphragm module HC RF, **10** Grey filter, **11** Aperture diaphragm (hidden), **12** Aperture diaphragm decentration (oblique light illumination), **13** Centration of aperture diaphragm, **14** Filter magazine*

The field diaphragm must also be exactly centred in the field of view (65.7) and the aperture diaphragm (65.11/12) closed.

Remove the objective or objective nosepiece: the reflection of the field diaphragm is reflected in the centre of the field of view if the object surface is aligned exactly horizontal.

Diaphragm module HC RF

With 2 illumination channels and interchangeable diffusing screens for incident light BF, DF, POL, ICR, FLUO.

Illumination channel I

(Diaphragm module fully pushed in)

For use in all incident light modes with

- variable iris aperture and field diaphragm
- oblique illumination
- switchable neutral density filter

Illumination channel II

(Diaphragm module pulled out as far as the 1st clickstop)

For use in all incident light modes with

- fixed aperture and field diaphragm
- mount for focusing graticule

Illumination channel II offers the additional advantage of fast, reproducible switching between open and closed diaphragms e.g.:

If the diaphragm setting is to be retained when switching between brightfield and darkfield.

For fast switching between high and low objective magnifications.

Channel II is also advantageous for measurements with fixed diaphragms, for colour assessment of coatings and oxide films and for work with the focusing graticule.

Diffusing screen pairs A and B

The diaphragm module HC RF is equipped with an interchangeable pair of diffusing screens (23b.9) to obtain optimally geneous illumination both for visual observation and for video and digital image processing.

Diffusing screen pair A is included in the standard delivery and contains diffusing screen 1 with dense distribution for even mination over a large field of view of 25 mm or 28 mm with the DM RD HC.

Diffusing screen 2 with low distribution for maximum illumination homogeneity, but over a reduced field of view of max. 20 mm (video and digital image processing). Diffusing screens 1 and 2 can be used in either of the two illumination channels. To do this, remove the diffusing screen pair, which are held by magnetism, and turn by 180° so that the labelling A, 1 2 is upside down.

As well as diffusing screen pair A, 1 2 diffusing screen pair B, order no. 565 502, can be supplied. Diffusing screen pair B contains 2 identical diffusing screens and is recommended when the same illumination conditions are required on both channels.

Incident light brightfield

Set the microscope illumination to medium intensity (42.14 and 42.8).

Turn in a low power objective (e.g. 10x). Make sure the front lenses of the objectives are clean! Push in the diaphragm module (65.9) as far as the stop (= channel I).

Close the **field diaphragm** (65.8). Open the aperture diaphragm (65.12).

Using the stage clamp (48.9) and the coarse focus control (42.12) or (44.2 and 44.3), position the sample surface roughly in the focal plane (= 45 mm below the objective thread, see page 57). Focus the object. The image of the closed field diaphragm (65.8) makes it easier to find the object surface.

See page 67 for tube and eyepiece setting.

Setting the field diaphragm:

Close the field diaphragm (65.8) until its edge can just be seen within the observed object field (49b). Put the two centering keys (1.5) into the holes (65.7) and adjust until the edge of the field diaphragm is concentric with the edge of the field of view (49c). Centration of the closed field diaphragm can also be performed with a graticule e.g. with crosslines.

Open the field diaphragm (49d) until it just disappears from the field of view.

This setting of the field diaphragm is retained for all objectives.

If the diaphragm module HCRF is pulled out as far as the stop clickstop (= channel II), the field and aperture diaphragms are fixed, see chart on p. 96.

The field diaphragm only has to be readjusted when eyepieces with different field numbers are used, when the secondary magnification is altered with a magnification changer or zoom system, or for photography and filming. Narrowing the field diaphragm usually improves the contrast.

For interchangeable stages only: The centering keys can be kept in the stage bracket (42.11 or 13.3) after use.

The **aperture diaphragm** (65.12) affects resolution, contrast and depth of field of the microscope image.

It must be set carefully and must not be used to adjust image intensity.

Engage the Bertrand lens (50.2) and focus (50.3) or remove an eyepiece and look into the tube from a distance of a few centimetres. Mount the centering keys (23.8) and adjust so that the closed diaphragm lies in the centre of the brighter circle (= objective pupil). Open the aperture diaphragm until it is just visible in the brighter circle (= objective pupil). The illumination aperture is then equal to the observation aperture.

After returning to the normal observation mode (Bertrand lens disengaged) image contrast can be individually adjusted.

If the aperture diaphragm is stopped down too far – especially at low and medium objective magnifications – the object structures will exhibit pronounced diffraction phenomena.

The aperture diaphragm can be stopped down further for high-power objectives to improve contrast and depth of field. Fine-adjust the aperture diaphragm, watching the structure and topography of the object, to obtain the best contrast and resolution.

Incident light darkfield

Special darkfield objectives (BD, Fig. 40) with built-in annular mirror or annular lenses are required for incident light darkfield. These objectives have a greater external diameter and screw thread M32 x 0.75.

High light intensity is necessary for darkfield, as this type of illumination is produced by diffracted and scattered light. Therefore, remove all filters, polarizers, IC prisms, etc. from the light path and set maximum intensity.

Make sure the front lens of the objective is clean as this has a great influence on the imaging quality in darkfield.

Pull out the incident light diaphragm module HC RF (65.9) as far as the first (= channel 2) stop. The aperture and field diaphragm functions are then set.

A neutral density filter (65.10) can be engaged in the light path to adjust the image intensity when switching to brightfield.

This neutral density filter is only in channel I. It saves the user from having to reduce the lamp intensity and is particularly useful when switching quickly between techniques DF ↔ HF.

Oblique light

For brightfield illumination the illuminating cone is rotation-symmetrical to the optical axis. For oblique light the aperture diaphragm (65.11 and 65.12, for channel I only) is moved to the side and stopped down so that the illuminating cone hits the sample at an angle, highlighting the surface topography.

Incident light interference contrast ICR

Cross polarizers.

Exactly crossed polarizers are an essential requirement for perfect ICR quality!

Insert the ICR polarizer (29.5) (65.4). Never use a different incident light polarizer. Switch on brightfield reflector BF or Smith reflector (Fig. 18); push in diaphragm module (65.9, channel I). Turn the turret* (65.3) to pos. H (= brightfield). Align and focus a homogeneous and well-reflecting specimen.

Insert the IC/P analyser so that the lambda (λ) engraving is showing (65.1 and 30.5). If using the 360° rotatable analyser (30.1) set the 0 position.

Swivel the analyser round the 0 position until you obtain the darkest possible setting.

Instead of the polarizer and analyser, the ICR module (= crossed polarizers) can be used

Choice of IC prism

Choose the prism in the turret (65.5) that corresponds to the objective you are using, see objective engraving, p. 48, or "Optics" data sheet. Optionally an IC prism in slide mount can be inserted in the Pol compensator slot (54.13).

Prisms with an additional number, e.g. D₁, B₂, split the beam than the D or B₁ prism and are therefore more sensitive for detecting fine topological details. However, the B₁ prism is used for obtaining the highest possible resolution.

Contrast setting

Carefully move the turret round the centre position, additionally operating the aperture diaphragm (65.12) to optimize contrast.

The interference contrast technique gives a relief-like and three-dimensional image of the specimen surface.

The contrast of linear structures can be improved even more by rotating the specimen with the stage rotation control (48.8).

For observations in ICR colour contrast, remove the analyser slide, rotate by 180° and push back in with the λ engraving showing. In this position a whole-wave compensator is effective in front of the analyser, producing colour interference contrast. With the analyser 360 and the ICR module, colour contrast is only possible with the "turnaround" polarizer L ICR with whole-wave compensator.

To switch from interference contrast to bright- or darkfield, turn the prism turret to position H = brightfield, and pull out the polarizer and analyser by one clickstop.

Quantitative interference attachments

Surface roughness and topography are depicted as interference fringes by the various interference techniques. These are evaluated similar to the way contour lines are interpreted on maps. The measurement accuracy is up to 30 nm, the maximum height difference is about 30 μm . See special instructions.

Fibre-optic, light guides

Illumination with flexible fibre-optic light guides with ball-jointed arms (VOLPI intralux 6000), rotatable round the optical axis of the objectives. Colour glass filter(s), slip-on iris diaphragms, auxiliary lenses, polarization device (polarizer and analyser).

Incident light polarization

Adjustment

Set the light source and diaphragms as for incident light brightfield (page 90 and 95).

Reflector: BF or Smith, the Smith reflector is better from a polarization optic point of view and should be used for slight anisotropy (polarization) (see Fig. 30).

Crossing the polarizers

Important: The polarizers should be exactly vertical or horizontal as a deviation of even 1° may lead to impaired extinction.

Setting the R/P polarizer (29.1):

↓ When combined with IC/P analyser (30.7)

↔ When combined with 360 analyser (30.1)

Focus an isotropic specimen that fills out the whole field of view, e.g. a mirror, open the aperture diaphragm (65.12) and turn the analyser (65.1) until the maximum extinction position can be seen. For the IC/P analyser (30.5) the λ engraving must point downwards (compensator inactive).

As for transmitted light (page 77) a particularly precisely crossed position can be achieved with a Bertrand lens or auxiliary telescope.

Polarizer with rotatable whole-wave compensator (29.9)

Set the analyser exactly at 0° or 90° .

Turn the whole-wave compensator (29.3) roughly to the centre position.

Turn the polarizer until the object appears as dark or as highly contrasted as possible, turn the whole-wave compensator (29.4) until colour contrast is obtained.

Filter system POL (p. 33 and ICR)

This does not need adjusting, as polarizer, analyser and 45° flat glass reflector are combined as a fixed unit.

Possible errors, brightfield, darkfield, ICR

Fall-off of focus, one-sided:

Sample surface not aligned at exactly 90° to the optical axis.

Sample has round edges.

Stage not clamped tight.

Fall-off of focus on both sides:

Sample surface greatly inclined.

Fall-off of focus, partial:

Pronounced relief zones in the sample beyond the range of the depth of focus of the objective.

Fall-off of focus, concentric:

Sample surface is round.

Image is unusually flat:

Poor sample quality.

Fingerprints or dirt on front lens of objective.

Sample covered by other layers.

Illumination aperture not exactly matched to the sample (Close aperture).

Objective in use is not suitable for reflected light (see DELTA optics objective data sheet).

Inhomogeneous image illumination:

Lamp not adjusted.

Sample not aligned flat.

For bright-/darkfield:

Oblique illumination lever not in exact position.

Diaphragm module not in exact position.

Polarizer/analyser slide not exactly positioned.

Tube beamsplitter at incorrect setting.

For ICR interference contrast:

IC prism in light path.

Wrong IC prism engaged.

Polarizer discoloured due to overheating.

Incorrect polarizer setting (see page 100).

Object marker

The object marker is screwed in place of an objective (not illustrated). When rotated, a diamond is lowered onto the coverglass or object surface, where circles of variable radii can be scribed to mark objects.

Multi-viewing attachment: see separate manual.

Diapositive overlay

The diapositive overlay device (Fig. 66) is used to reflect measurement and comparison masks, μm marks, marker arrow, company logo, charge and quality data, etc. into the microscope image so that they can be recorded together with the image. Only with tube HC FSA 25 PE and with DM RD (Fig. 31 and 33)!

The following diapositives are available:

- Marker arrow
- 10 mm measurement scale with 100 divisions
- μm marks for 2.5x – 100x objectives
- 10 x 10 mm grid division in 100 fields
- Test circle and measured length for grain size measurements
- Picture series for ASTM-E 112 grain size measurements.

Individual masks with any measurement and comparison patterns, quality data, company logos etc. can be made by the user.

To do this, the original must be photographically reproduced on a 35 mm negative, i.e. bright lines on a dark background and framed in a standard 50 x 50 mm slide frame. The best film to use is fine-grain "document film".

The diapositive is imaged in the intermediate image plane of the microscope at a scale of 2 : 1. A distance of e.g. 5 mm in the diapositive overlay is magnified to 10 mm in the intermediate image plane of the microscope. The overlay device only works when the beamsplitter in the tube (31.4) is set at 50/50 (switch rod in middle position). The framed diapositive is inserted in the integrated holder (66.6) (white side of diapositive with lettering facing microscope).

Fig. 66a Diapositive overlay device on the HC FSA 25 PE tube
1 Tube flange, 2 Coupling ring for reflection optics, 3 Reflection optics, 4 Coupling ring for diapositive overlay device, 5 Knurled ring for focusing, 6 5 x 5 cm slide frame, 7 Filter slot, 8 Illumination tube of lamphousings

Fig. 66b Transformer

The holder is adjustable on all sides, so the overlay can be moved to different areas of the microscope image. Remember that when you move the diapositive, the overlay will move in the opposite direction. This takes a bit of getting used to.

You can give the bright lines a coloured background by putting 32 mm colour filters in the filter slot (66.7).

Macro device

Like the diapositive overlay device, the macro overlay (Fig. 67) only works in the 50/50 beamsplitter position (switch rod at middle position) of the HC FSA 25 PE tube.

The microscope illumination is left switched off to avoid disturbing image brightening.

The object is placed on the stage under the mirror housing of the macrodual zoom (67.11) and illuminated.

Stand lamps, cold-light illuminators and fibre-optic lamps, etc. are suitable light sources for macroscopy.

The image is observed in the microscope tube and focused by turning the knurled ring.

The magnification can be changed continuously in a range of 1 : 4 by adjusting the knurled ring (67.7).

When changing the magnification with the zoom control the object may change position slightly and go out of focus. It must then be refocused and moved back into position.

The zoom magnification factors can be read on the scale (67.8). The magnification also changes when the distance between the object and the macro attachment is varied.

Fig. 67 Macro device on HC FSA 25 PE tube

1 Tube flange, 2 Coupling ring, 3 Reflection optics, 4 Coupling ring, 5 Macro adapter, 6 Screw ring, 7 Zoom setting ring 1 : 4, 8 Scale of zoom factor, 9 Scale of magnification factor of the working distance, 10 Scale of object distance from the bottom edge of the mirror housing, 11 Mirror housing

The total magnification in the microscope, the reproduction ratio on the photograph or TV image can be quickly and easily measured with a scale and calculated.

Important: For normal viewing without the macro mirror housing or macrodual zoom, put on the cover to avoid disturbing overlay effects.

The mirror housing (67.11) can be rotated through 360°, for example to alter the angle at which the photograph is taken. This is done by loosening the Allen screw.

The intermediate image magnification M_1 the macro object can be worked out from the eyepiece field of view (see page 43) and the diameter of the object field (measured with a graduated ruler) as follows:

$$M_1 = \frac{\text{field of view } \emptyset}{\text{object field } \emptyset} \quad \text{e.g.} \quad \frac{10\text{x}/25 \text{ eyepiece}}{\text{object field} = 200 \text{ mm}} \quad M = 0.125$$

Viewed with a 10x eyepiece, this intermediate image of 0.125x gives a total magnification of 1.25x in the microscope eyepiece ($0.125 \times 10 = 1.25$).

The total magnification in the film plane of a camera is derived from multiplying the intermediate image magnification M_1 by the magnifications of the photo eyepiece and camera attachment, e.g.:

intermediate image magnification 0.125x

photo projection lens 8x

large-format attachment 1.25x

$$0.125 \times 8 \times 1.25 = 1.25x$$

The total magnification at the 4 x 5" large-format camera of the DM LD would therefore be 1.25x.

The total magnification can be roughly worked out using the scale divisions on the macrodual zoom:

The following factors are multiplied:

- magnification factor of the working distance (scale 67.9, e.g. – 0.11x)
- zoom factor (scale 67.8, e.g. 1x)
- correction factor of the reflection optics (without engraving 1.17x)
- eyepiece magnification (e.g. 10x)
e.g. $0.11 \times 1 \times 1.17 \times 10 = 1.29$

The total magnification in the eyepiece would therefore be 1.29x.

Using the macrodual zoom as a drawing device

Drawing microstructures under the microscope has the advantages over photomicrography that significant details can be high-lighted and that structures can be depicted in three dimensions. Apart from this, drawing with the superimposed image method is a valuable didactic exercise.

It is done by superimposing the drawing area (the area of the stage under the mirror housing of the macrodual zoom) onto the microscope image. The drawing area or sheet of paper is homogeneously illuminated with a stand lamp or table lamp.

The microscope illumination and illumination of the drawing area are matched providing the lamps are adjustable; otherwise the brightness of the drawing area can be varied by altering the proximity of the lamp.

The exact magnification of the object in the drawing is most easily determined by means of a stage micrometer, by transferring the length measured by the stage micrometer onto the drawing. The magnification can also be calculated as follows:

$$M_{Ze} = \frac{M_{Obj}}{F_{Zoom} \times F_D \times F_E} \text{ e.g. } \frac{5x}{4 \times 0.11 \times 1.176} = M_{Ze} 9.6x$$

M_{Ze} = magnification in the drawing plane

M_{Obj} = objective magnification

F_{Zoom} = magnification factor of the zoom optics, scale 67.8.

F_D = magnification factor of the object distance, scale 67.10

F_E = correction factor of the reflection optics (1.176x)

The magnification can be altered by changing the zoom setting (scale 67.8) or the level of the drawing plane.

At the smallest zoom setting the drawing area has a diameter of approx. 190 mm, at the highest zoom setting approx. 48 mm with an eyepiece field of view of 25 mm. For different fov numbers the correction value is fov/25.

Auxiliary lens 2x

An auxiliary lens 2x can be screwed in under the mirror (67.11) to magnify the field that is to be imaged. This must be taken into account for the above formula. This auxiliary lens 2x is recommended for microscopic tracing as object structures are shown twice as large.

Overlay of data and code numbers with the VARICODE systems

The VARICODE system can be supplied together with the macrodual zoom.

It allows code numbers, micron measurement bars, ASTM grain size pictures and 35 mm negatives to be overlaid on the microscope image.

Further details on how to use this system can be found in the manual of the manufacturer, Leica AG, Vienna. Not illustrated.

VARIMET digital measurement system

The VARIMET measurement system can be connected to the reflection optics for the measurement of microstructures. An adapter is available on request. See manufacturer's manual (Leica AG, Vienna) for further details.

Linear measurements

The following are required for linear measurements:

- Graticule with scale division in eyepiece (Fig. 68) or Variotube DMRDHC (Fig. 33) or diapositive overlay device (Fig. 66) or a digital linear measuring eyepiece.
- Transmitted or incident light stage micrometer for calibration.

The micrometer value of the objective-eyepiece combination used must be known before the measurement, i.e. the distance in the specimen that corresponds to the length of a division on the graticule used.

Calibration:

Align the stage micrometer and the graticule parallel to each other by rotating the stage or the eyepiece and adjust the zero marks of both scales to exactly the same height.

Read how many scale divisions of the stage micrometer correspond to how many on the microscope scale (graticule) and divide the two values.

Example:

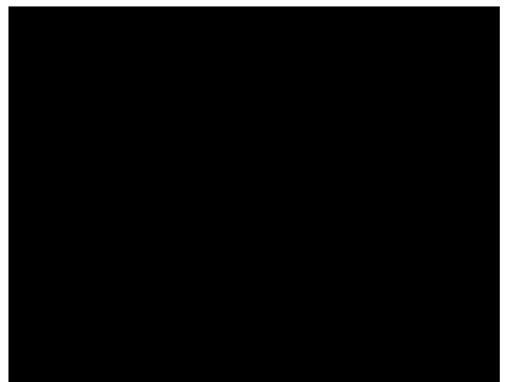
If 1.220 mm of the stage micrometer corresponds to 50 divisions of the measurement scale, the micrometer value is $1.220 : 50 = 0.0244 \text{ mm} = 24.4 \text{ }\mu\text{m}$. For extremely low objective magnifications it may be that only part of the measurement scale can be used for calibration.

Important: If using a Variotube or variable tube factor:

Remember to take the additional magnification value into consideration! We strongly recommend you calibrate each objective separately instead of extrapolating the micrometer values of the other objectives from the calibration of one objective. Measurement errors may occur if the eyepiece is not pushed into the tube as far as the stop.

Particularly large object structures can also be measured on the stage with the verniers (0.1 mm); the distance to be measured could be calculated from a combined x and y measurement.

Fig. 68 Graticule division in eyepiece (left) and image of the stage micrometer (right)



Microscopic measurement and comparison in metallography

Linear measurements with measurement grati-
cules

The size of the line patterns and the length of the divisions are designed for the standard magnifications customary in metallography. For standard magnifications one graticule division has the following rounded values in the object plane:

standard magnification:

100x – 1 division approx. 10 μm

standard magnification:

200x – 1 division approx. 5 μm

standard magnification:

500x – 1 division approx. 2 μm

standard magnification:

1000x – 1 division approx. 1 μm

The exact proportions of measurement divisions in the microscope can be checked by using stage micrometers, calibration standards or microscopes.

Graticules for grain and particle size determination

The graticules for the standard series and Snyder Graff methods contain a test circle which the viewer sees as having a diameter of 80 μm at standard magnification. Its size therefore conforms with the standard picture series charts, facilitating size comparison.

These graticules also include a measured length to allow the Snyder Graff line sectioning method. This and similar methods involve counting the number of grains cut by the measured distance. An average grain size can be worked out by taking several measurements.

The graticule for ASTM-E 112 grain size measurements is divided into eight segments with numbered grain sizes. The pictures conform with grain size plate no. 1 of the ASTM-E 112 standard. We refer to the ISO/DIS 643, Euronorm 103/71, DIN 50 601 and ASTM-E 112 standard specifications for taking grain size measurements with the named graticules.

Digital length and height measurement using TV technology: see separate Leica MFK 2 manual.

Thickness measurements

In principle, thickness measurements can be carried out if both the upper and the lower surface of the object can be clearly focused. The difference in stage height setting (mechanical dual knob focusing; distance between two divisions = 2 μm) gives a value for transmitted light objects that is falsified by the refractive index of the object (which has been “transfocused”) and perhaps immersion oil. The true thickness of the object detail measured in transmitted light is given by the vertical stage movement (focusing difference) d' and the refractive indices n_o of the object and n_i of the medium between the coverglass and the objective

$$d = d' \frac{n_o}{n_i}$$

Example

The upper and lower surfaces of a thin polished specimen have been focused with a dry objective ($n_i = 1.0$) scale readings of the mechanical fine drive (division spacing = 2 m) : 19.0 and 12.5. Therefore $d' = 2 \times 6.5 \mu\text{m}$.

The refractive index of the object detail was taken to be $n_o = 1.5$.

Thickness $d = 2 \times 6.5 \times 1.5 = 9.5 \mu\text{m}$

TV microscopy

Various adapters are available for the connection of TV cameras with c-mount or B-mount objective thread (Fig. 69).

C-mount adapters listed in the following table can be used on all phototubes and on the Leica DMRD photomicroscope. The picture area on the monitor depends on the adapter used and on the chip size of the camera (s. table).

	Recorded picture diagonal with			
	1 inch camera	$\frac{2}{3}$ inch camera	$\frac{1}{2}$ inch camera	$\frac{1}{4}$ inch camera

Without zoom magnification for 1 chip cameras

c-mount adapter 1x HC	16	11	8	6
c-mount adapter 0.63x HC	-	17.5	12.7	9.5
c-mount adapter 0.5x HC	-	-	16	12
c-mount adapter 0.35x HC	-	-	-	17.1
c-mount adapter 4x HC	4	2.8	2	1.5

Without variable magnification, for 1–3 chip cameras

in conjunction with TV optik 0.5x HC (screwed connection)

c-mount adapter 1x	-	-	16	12
B-mount adapter 1x	-	-	16	12
B-mount adapter 1.25x	-	17.5	-	-
F-mount adapter 1x	-	-	16	12
F-mount adapter 1x	-	17.5	-	-

With zoom magnification (Vario TV adapter) for 1 chip cameras

c-mount, 0.32–1.6x HC	-	-	19 ¹⁾ –5	18–3.8
B-mount, 0.5–2.4x HC (SONY)	-	-	16–3.3	-
B-mount, 0.5–2.4x HC (SONY)	-	-	-	12–2.5

¹⁾ from zoom factor 0.42x!

Fig. 69 c-mount adapter and B-mount (Vario)

1 TV camera, 2 Adapter with c-mount thread, 3 Clamp screw in tube head

a c-mount adapter for 1 chip cameras

b Vario TV adapter

TV cameras with bayonet mount

Cameras with the standard Sony bayonet mount can also be connected to all phototubes, the Leica DMRD photomicroscope and the Variotube 28 VPE. A/B-mount adapter 0.55x and a Vario B/C-mount adapter 0.55x – 1.1x are available for this purpose. The recorded field sizes can be seen in the table.

Calculation of the magnification on the monitor

For all FSA tubes the magnification on the monitor can be calculated with the following formula:

$$V_{TV} = \text{objective magnification} \times \text{tube factor} \times \text{TV-adapter magnification} \times \frac{\text{monitor diameter}}{\text{chip diameter of camera}}$$

If using the magnification changer or the Leica DMRD HC photomicroscope the above formula must also be multiplied by the factor of the magnification changer or zoom.

Possible errors

Picture too dim (noisy TV picture, poor contrast)

Remedy: Increase lamp intensity, swing filter out of light path, switch over beamsplitter in tube system, switch TV camera to higher sensitivity.

Picture too bright (TV picture glare)

Remedy: Switch neutral density filter, switch over beamsplitter in tube system, reduce camera sensitivity.

Picture area too small

Remedy: Use a TV adapter with a smaller factor.

Incorrect colour rendering

Remedy: Vary illumination intensity, carry out white balance for TV camera according to manufacturer's instructions, use a conversion filter, e.g. CB 12.

Disturbed picture frame

Remedy: Earth the microscope, Variotube and camera. Avoid parallel laying of mains and signal cables; connect camera and microscope to the same mains phase (socket).

Picture spoilt by inhomogeneous glare and/or spots. Lamps or windows are reflected in through the eyepieces.

Remedy: Switch over the beamsplitter or cover eyepieces or remove the disturbing light source. Dirt particles in the light path, lamphousing not centered (TV systems are generally more sensitive to inhomogeneous illumination).

Care and maintenance

Attention:

Disconnect from the mains before cleaning and servicing!

Dust protection

Protect the microscope and peripherals from dust by putting on the flexible dust cover after each work session. Dust and loose particles of dirt can be removed with a soft brush or lint-free cotton cloth.

Solvents

Obstinate dirt can be removed with a clean cotton cloth moistened with any ordinary hydrous solution, benzine or alcohol. Do not use acetone, xylol or nitro dilutions. Cleaning agents of unknown composition should be tested on an inconspicuous part of the microscope. Painted or plastic surfaces must not be tarnished or etched.

Acids, alkaline solutions

Particular care should be taken when working with acids or other aggressive chemicals. Always avoid direct contact between such chemicals and the optics or stands. Thorough cleaning after use is strongly recommended. Keep the microscope optics absolutely clean.

Dust/optics

Remove any dust from glass surfaces with a fine, dry, grease-free artists' hair brush, or by blowing with a bellows ball or by vacuum

suction. Any remaining dirt can be removed with a clean cloth moistened with distilled water. Failing this, use pure alcohol, chloroform or benzine.

Oil

First wipe off immersion oil with a clean cotton cloth, then wipe over several times with ethyl alcohol.

Attention: Fibre and dust residue can cause disturbing background fluorescence in fluorescence microscopy.

Objectives must not be opened for cleaning. Only the front lens can be cleaned in the ways described above and the upper lens by blowing dust off with a bellows ball.

All Leica instruments are manufactured and tested with extreme care. If you do have cause for complaint, however, please do not try to repair the instruments and their accessories yourself. Contact your national agency or our central servicing department, the Technical Service in Wetzlar direct.

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Please direct any questions on application to our Produktmanagement Mikroskopie.

Main wearing and spare parts, tools

Code no. Part no.	Component	Used for
<u>Spare lamps</u>		
500 974	halogen lamp 12 V 100 W	Lamphousing 107/106 z
500 296	halogen lamp 6 V 4 W	ORTHOMAT E, Leica DM RD, Diapositive overlay
500 137	max. pressure Hg lamp 50 W	Lamphousing 106 z
500 138	max. pressure Hg lamp 100 W	Lamphousing 106 z
500 321	max. pressure Hg lamp 103 W/2	Lamphousing 106 z
500 139	max. pressure Xe lamp 75 W	Lamphousing 106 z
500 186	Na spektral lamp	Lamphousing 106 z
<u>Tools/adjustment key</u>		
553 407	Centering keys, short 1.5 x 23 mm	Diaphragm module F/RF
020-422.573-053	POL centering keys, long version (1.5 x 51 mm)	Pol microscope
553 143	Centering keys, square	Sénarmont compensator
016-500.020-006	3 mm Allen key	Assembly for light rings and ITC condensor Wollaston prisms
150-000.100-129	2 mm Allen key, angled	
150-000.100-128	1.5 mm Allen key, angled	Torque setting of x/y-stage movement
<u>Screw cover for unoccupied nosepiece positions</u>		
020-422.570-000(4)	Screw cover M25	Septuple objective nosepiece and sextuple centrabre objective nosepiece
016-016.005-200	Screw cover M32	BD objective nosepiece (sextuple)
<u>Spare eyecups (anti-glare protection) for HC PLAN eyepiece</u>		
021-500.017-005	Eyecup HC PLAN	10x/25 eyepiece
021-264.520-018	Eyecup HC PLAN	10x/22 eyepiece
021-264.520-018	Eyecup HC PLAN	10x/20 eyepiece
<u>Immersion oil, DIN/ISO standard, fluorescence-free</u>		
513 787	10 ml	OIL and IMM objectives and oil condenser tops
513 522	100 ml	
513 788	500 ml	
<u>Spare fuses, primary</u>		
846-205.000-000	IEC 127-2 T 4 A	all microscopes
832-493.000-000	IEC 127-2 T 2.5 A for 90 – 140 V	Xe 75 Hg 100 power unit, stabilized (500 311)
827-902.000-000	IEC 127-2 T 1.25 A for 90 – 140 V/187 – 264 V	Xe 75 Hg 100 power unit, stabilized (500 311)
824-716.000-000	IEC 127-2 T 0.16 A for 90 – 140 V	Xe 75 Hg 100 power unit, stabilized (500 311)
826-095.000-000	IEC 127-2 T 0.08 A for 187 – 264 V	Xe 75 Hg 100 power unit, stabilized (500 311)
825-347.000-000	IEC 127-2 T 2 A	100 power unit, non-stabilized (500 299)
The following external power units are without fuses:		Hg 50 (500 277)
		Xe 150 (500 298)
		Hg 200 (500 235)

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EU-Confirmaty declaration

EU-Confirmity declaration

We hereby declare that the product specified below conforms in its design and construction as well as the model we have put on the market to the relevant safety and health regulations laid down by the European Union. This declaration will cease to be valid if the instrument is modified without our consent.

Product names: DMR, DM RE, DM RX, DM RXE,
DM RXP

Instrument type: Light microscope

Instrument no.: 020-525.701 to 020-525.780

EU directives: Low voltage: 73/23/EWG
Electromagnetic
compatibility:
89/336/EWG

Harmonised EN 50 081-1
standards EN 50 082-1
applied: EN 61 010-1

Wetzlar, den 18. 4. 1998

Prof. Dr.-Ing. habil. M. Jacksch,
Director of Technology and Development
planning