



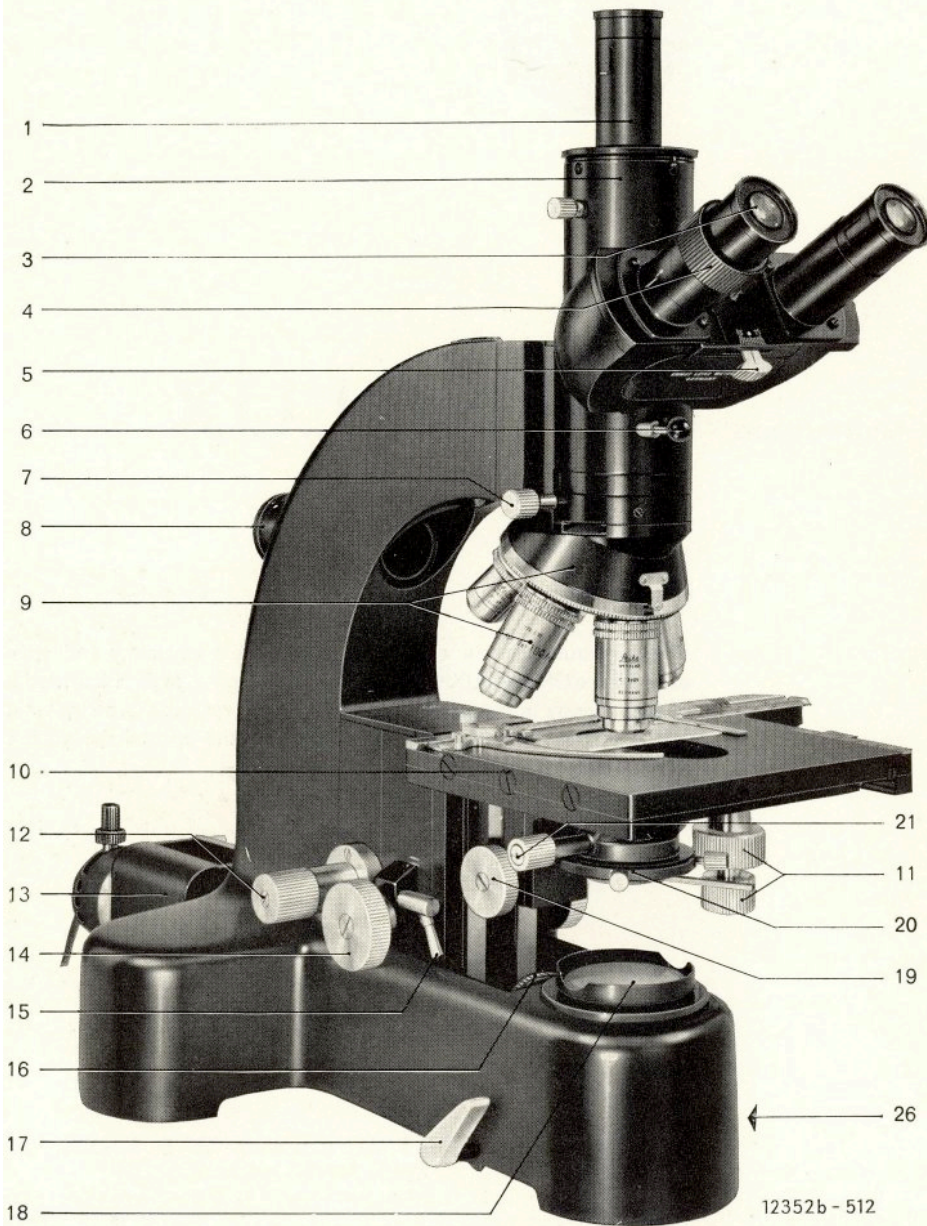
Instructions for the ORTHOLUX Microscope

# ORTHOLUX

ERNST LEITZ GMBH WETZLAR

512-76/Engl.

Fig. 1: ORTHOLUX with FS tube and quintuple revolving nosepiece.



- 1 Eyepiece tube. After removal of the eyepiece the MAKAM 9x12 cm camera attachment or the LEICA miniature camera with the MIKAS micro attachment can be inserted for photomicrography. For the ORTHOMAT the FSA tube is preferable.
- 2 Binocular tube FS
- 3 Wide field eyepiece
- 4 Knurled ring for the adjustment of the eyepiece for individual eyesight
- 5 Lever for adjusting the interpupillary distance
- 6 Prism for alternative visual observation and photomicrography
- 7 Clamping screw for the revolving nosepiece
- 8 Aperture for sleeve with lens to receive the lamp attachment
- 9 Revolving nosepiece with objectives. In the side of the nosepiece carrier is a slot for a filter slide.
- 10 Mechanical stage No. 250, coaxial, 140 x 130 mm, with object holder. Adjustment range 76 x 40 mm.
- 11 Coaxial operating knobs for the mechanical stage adjustment
- 12 Fine adjustment knob for focusing the microscope image; scale unit  $\frac{1}{1000}$  mm.
- 13 Lamp attachment
- 14 Knob for the coarse adjustment
- 15 Clamping lever for the coarse adjustment
- 16 Removable field iris
- 17 Lever for the swing-out lens
- 18 Protective disc
- 19 Vertical condenser adjustment
- 20 Swing-out condenser with screw top
- 21 Milled knob for swinging the condenser top in and out
- 26 Clamping device for the field iris (not visible in the picture)

## Unpacking the microscope

The following parts are packed separately:

- 1 Microscope stand with dovetail guides for the various interchangeable components
- 2 Object stage with carrier and condenser holder
- 3 Accessory case containing:  
Revolving objective nosepiece with tube lens and objectives in position  
tube,  
condenser,  
eyepieces.
- 4 Lamp attachment for transmitted or incident light with 6 V 30 W low-voltage lamp, daylight filter, groundglass disc, diffusion screen N, etc.

Additional equipment:

Transformer and other accessories ordered with the outfit.

During unpacking great care should be taken to check the outfit with the packing note and to remove all small items from the packing material.

Remove the wooden block inserted in the foot of the stand to protect the fine adjustment mechanism.

All mechanical and optical parts are cleaned thoroughly before dispatch; they should therefore be carefully protected from dirt and dust, above all, the glass surfaces of the objectives and eyepieces should never be touched by hand; any finger marks on glass surfaces should be removed immediately by means of a soft piece of chamois leather or a well-washed piece of lint. Even minute traces of perspiration from the fingers may attack the surfaces of high-grade optical glass very quickly.

Work room and work place:

The work room must meet some basic requirements. As far as possible it should be free from dust, and oil- or chemical fumes which may quickly attack the optical and mechanical parts of the instrument. Furthermore, the work room should not be subject to excessive variations in temperature and to vibrations.

The mains socket for the built-in light source requires a 6 amp. fuse. Check type of current and mains voltage.

## Assembling the microscope

1 Loosen clamping screw 25, insert **object stage** in dovetail guide 27, push down as far as possible and fix in position with clamping screw 25. The clamping lever 15 at the side must be released for the vertical adjustment of the stage by means of the coarse adjustment 13.

2 Before attaching the **revolving objective nosepiece or the incident light illuminator** lower the object stage by means of the coarse adjustment 28 from below after loosening the clamping screw 7. The nosepiece or illuminator should be pushed home fully, and the clamping screw 7 tightened immediately.

3 Insert the **tube** in the dovetail guide 28 from above as far as possible, lifting the clamping lever 22; release this lever. The tube is now fixed in position and aligned to the optical axis.

Inserting the **condenser**: Raise the object stage as far as possible, and with knob 19 lower the changing slide so that the swing-out condenser can be inserted in it conveniently; it should be pushed home fully. The two centring screws on the swing-out condenser should face the operator. The rotating knob 21 is used for swinging the condenser top in and out.

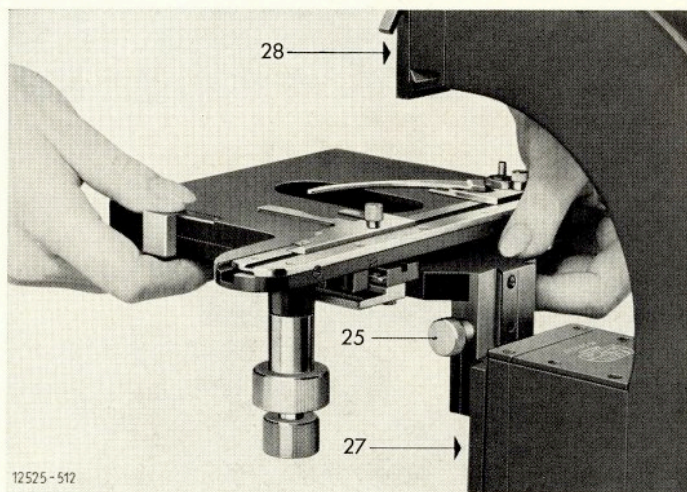


Fig. 2: Inserting the object stage

25 After loosening this clamping screw the object stage can be vertically adjusted

27 Bottom dovetail slide for the object stage

28 Top dovetail slide for the microscope tube and revolving nosepiece

## Technical details

The individual structural elements such as object stages, tubes, revolving nosepieces, etc. are described in detail in our ORTHOLUX leaflet. Special descriptions of various elements necessary for their correct use will be found in the following paragraphs.

### Tube

The binocular photo tube FS will normally be used with the ORTHOLUX. It is a combination of a binocular observation tube and a photographic tube. It can be adjusted for the interpupillary distance of the microscopist by means of a lever. Where the interpupillary distance is not known the lever is adjusted during binocular observation until a single, comfortably surveyed and circular field of view is seen. In addition, the lefthand eyepiece tube has an additional adjustment to compensate any visual defects.

Our FSA tube is also popular with the ORTHOLUX. It has an adjustable beam splitting prism dividing the light flux at a ratio of 80:20 (the photo tube receiving 80%, the eyepiece tubes 20%), or directing the entire light into the eyepiece tubes.

A Periplan 10 x MF focusing eyepiece with graticule is inserted in the eyepiece tube; the graticule outlines the picture area of the film; two small double circles in the centre simplify focusing with the focusing eyelens. Users unable to focus on the extremely fine inner double circle can make use of the outer circle. The interpupillary distance is set by means of the milled knob on the right. The automatic focusing compensation for any interpupillary distance ensures full sharpness of the image both in the eyepiece and in the film plane.

### Objective revolving nosepiece

The objective revolving nosepiece has 5 threads for the objectives. These threads are numbered. Each outfit includes an objective-eyepiece chart (magnification table). Among other facts it indicates the nosepiece threads for which the individual objectives are matched. A slot in the carrier of the objective revolving nosepiece accommodates a filter slide, e.g. for fluorescence microscopy.

### Condensers for bright field transmitted light

The achromatic condenser No. 602 with a numerical aperture of 0.90 forms part of the standard outfit of our ORTHOLUX. This aperture is completely adequate for the majority of microscopic investigations with dry systems. Only in a comparatively few cases will it be neces-

sary to illuminate the entire objective aperture. This also applies to many examinations with oil immersion objectives, and a condenser aperture above 0.90 will be called for only for the full resolution of very fine structures by high-aperture apochromatic dry systems or oil immersion objectives. For this purpose our achromatic-aplanatic condenser No. 603, N.A. 1.25 is available. The table on p. 6 contains information about the properties of our condensers.

**Note:** For objectives with numerical apertures below 0.25 the bottom part of the condenser alone is used. It must be lowered through approximately 20 mm, so that the field aperture appears in focus. This is important in photomicrography. This condenser adjustment is not necessary for the occasional use of scanning objectives; when the condenser top is swung out, sufficient, even illumination is ensured with the diffusion screen N.

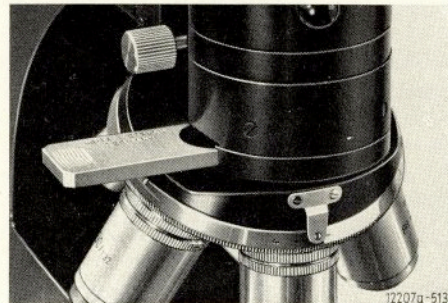


Fig. 6:  
Objective revolving nosepiece with filter slide

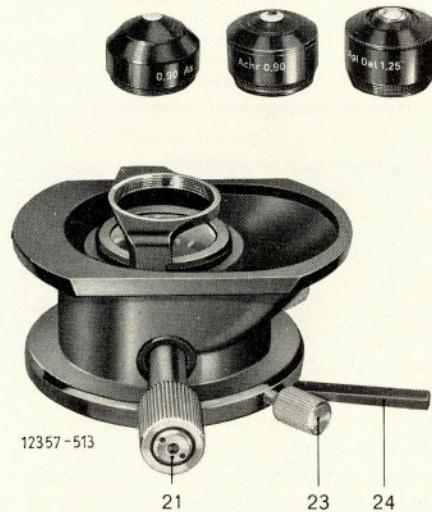


Fig. 7:  
Bottom part of condenser with three condenser tops  
21 Knurled knob for swinging the condenser top in and out  
23 Condenser centring screw  
24 Lever for the aperture iris

### Swing-out condensers for slide change

No. Code	Description	Use
600	Bottom part of condenser, N.A. 0.25 with aperture stop, condenser for low magnifications	With objectives up to N.A. 0.25. It illuminates even large fields at low magnifications
001	Condenser top with aspherical lens; engraved 0.90 As	
601	= Condenser 0.90 As, with good spherical correction	With all achromatic objectives; suitable also for fluorescence
	or	
002	three-lens condenser top Achr. 0.90	
602	= achromatic condenser Achr. 0.90 with good spherical and colour correction. This condenser produces an almost colourless image of the field diaphragm	With more highly corrected objectives such as fluorite systems, apochromats, plano objectives; and in photomicrography. Limited suitability for fluorescence.
	or	
003	4-lens condenser top Apl. Oil 1.25	
603	= achromatic-aplanatic condenser. State of correction similar to that of Condenser 602 at high aperture.	Mainly for work with highly corrected oil immersion objectives and for photomicrography in colour with high condenser apertures. In order to make full use of the high aperture of this condenser, immersion oil must be introduced between the front lens of the condenser and the underside of the microscope slide when oil immersion objectives are used. Unsuitable for fluorescence.

### Objectives

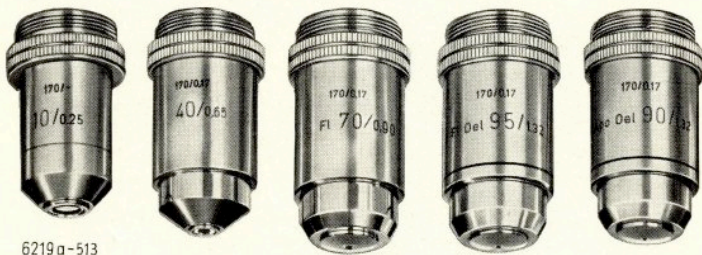
In addition to our firm's emblem, a number of data are engraved on the mount of every objective; it is important for the user to know them. Fig. 8 shows a few examples from the range of objectives available, with engravings typical of the various categories.

**170** represents the distance in mm from the shoulder of the objective to the rim of the microscope tube. This distance is also called the mechanical tube length. LEITZ objectives for transmitted light are corrected exclusively for a mechanical tube length of 170 mm. In the case of draw tubes it is essential to maintain this length. With high-power objectives, even a few millimetres' difference is sufficient to reduce the image quality considerably. With our tubes with inclined eyepiece tubes it is not possible to maintain the correct "mechanical tube length". Nevertheless, our objectives are suitable for such tubes, as a tube lens projects the image into the new intermediate image plane without impairing the image quality. This introduces a factor 1.25 by which the magnification of all objectives is increased. This factor is engraved on the tubes. It must be considered in the calculation of the final magnification.

**0,17** denotes the coverglass thickness for which our transmitted-light objectives are computed as a rule. The table on p. 7 indicates any permissible deviations (without effect on the image quality), and those objectives which can be used either with or without coverglass. These have a dash engraved in place of 0.17 (See Fig. 8, extreme left). Objectives which must be used without coverglasses at all times are marked 0.

Dry objectives of N.A. 0.95 are available in correction mounts only, which can be adjusted for coverglass thicknesses from 0.12 to 0.22 mm.

Below the figures indicating the tube length and coverglass thickness the reproduction ratio (= ratio of intermediate image and object, e.g. 40:1) and the numerical aperture of the objective are given in abbreviated form, in this case **40/0.65**. The term "magnification" is usually given in tables instead of "reproduction ratio" (it is also called primary magnification of the objective). In addition, the state of correction is indicated in the case of **Fluorite** systems, **Apochromats**, and **Plano** objectives. See also table on p. 7. Also, the type of immersion media to be used is engraved on the mount, together with a black ring. Achromatic objectives have no specific distinction mark. High-power objectives have a spring-loaded front lens mount for the protection of specimen and objective. Our leaflet "Objectives - Eyepieces" contains information about the physical definitions upon which terms such as "achromat" etc. are based.



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Fig. 8: Various objectives from left to right: Achromat 10:1, Achromat 40:1, Fluorite system 70:1, Fluorite oil immersion 95:1, Apochromat oil immersion 90:1

Description of objective		Focal length	Free working distance	Coverglass correction <sup>1)</sup>	Type of Eyepiece
Magnif./N.A.		mm	mm		
Achromatic dry systems	2.5/0.07	56.8	13.6	DO	P
	3.2/0.12	39.8	35	DO	H
	3.5/0.10	31.6	23	DO	H
	6/0.18	23.1	17.5	DO	H
	10/0.25	16.3	5.7	DO	H
	25/0.20	7.1	0.92	D	P
	40/0.65	4.5	0.67	D	P
Achromatic Immersion (W = Water Imm.)	63/0.85	2.9	0.29	D	P
	Iris 63/0.85	2.9	0.29	D I	P
Fluorite dry systems	OI + W 22/0.65	8.1	0.32	DO	P
	W 90/1.20	2.1	0.09	D	P
	OI 100/1.30	1.9	0.13	D <sup>3)</sup>	P
	Iris OI 100/1.30—1.10	1.9	0.13	D	P
Fluorite oil immersion	FI 40/0.85	4.3	0.38	D I	P
	FI 70/0.90	2.6	0.26	D I	P
Apochromatic dry systems	FI OI 54/0.95	3.4	0.22	DO	P
	FI OI 70/1.30	2.5	0.22	D	P
	FI OI 95/1.32	2.0	0.15	D <sup>3)</sup>	P
	Iris FI OI 95/1.32—1.10	2.0	0.15	D	P
Apochromatic oil immersion	Apo 12.5/0.30	13.0	2.50	DO	P
	Apo 25/0.65	7.3	0.86	D	P
	Apo 40/0.95	4.5	0.12	D I <sup>2)</sup>	P
	Apo 63/0.95	3.0	0.12	D I <sup>2)</sup>	P
Plano objectives	Apo OI 90/1.32	2.0	0.12	D	P
	Apo OI 90/1.40	2.0	0.06	D	P
Plano objectives	PI 4/0.10	41.5	15	DO	P
	PI 10/0.25	17.9	7.5	DO	P
	PI 25/0.50	7.6	0.90	D	P
	PI 40/0.65	4.6	0.58	D	P
	PI Apo OI 100/1.32	2.4	0.27	D <sup>3)</sup>	P

1) D: with coverglass, thickness 0.17 mm (adhere to coverglass thickness within  $\pm 0.05$  mm)

O: without coverglass

DI: Adhere to 0.17 mm coverglass thickness within  $\pm 0.01$  mm; where the objective has a correction mount set it at the real coverglass thickness within this tolerance.

2) These objectives are fitted in a variable correction mount. During adjustment image sharpness is almost completely maintained. Ideal possibility of optimum focusing where thickness of the coverglass is unknown.

3) These oil immersion objectives can be used also for uncovered specimens (smear preparations without coverglass); the negligible reduction in image quality is not disturbing.

4) H = Huygens eyepiece, P = periplanatic or periplanatic wide field eyepieces should be used.

All objectives of 3.5/0.10 and above are adjusted on the revolving nosepiece (see p. 8, Change of Magnification).

## Operation of the microscope

### Focusing the specimen

Clamp microscope slide on the object stage; the object holders can be adjusted for any size of slide up to 100 mm. The values appearing for a given area of the specimen on the two scales of the mechanical stage do not depend on the setting of the object holders. Choose a medium-power objective for your initial observation; preferably 10/0.25 combined with periplanatic wide field eyepiece GF 10x. Raise the swing-out condenser to its highest position, and swing condenser top into the beam path. Open aperture iris 24 and field iris 16.

Tilt swing-out lens in the foot of the stand into the beam path (Lever 17 towards the front). The lens remains in this position for all examinations in bright and dark field.

Focus the specimen with coarse- and fine adjustment.

Carry out, if necessary, corrections for faulty eyesight: look through the right eyepiece with the right eye, and focus the specimen with the coarse- and fine adjustment. Look at the same area of the specimen with your left eye, rotating the knurled ring 4 on the left eyepiece tube until the same area appears also sharp in the left eyepiece. The fine adjustment must not be altered during this operation. This setting must be accurately repeated after the condenser has been centred, and should be checked from time to time.

### Centring the lamp attachment

Open field iris 16 as far as possible.

Place groundglass disc or translucent paper on the protective glass plate in the foot of the stand. During the adjustment of the collector lens by means of lever 30 the luminous spot on the mat surface should expand or contract concentrically with the periphery of the glass plate. The centring screw 32 on the lamp mount should be adjusted, if necessary, to achieve this effect. Set the collector lens at its intermediate position.

If after the centring of the filament lamp and condenser the field of view is not evenly illuminated, the setting of the collector lens must be corrected by adjusting the lever 30. Furthermore, the possibility of an improvement in the evenness of the illumination and the brightness should be explored by pulling back the centring mount (loosen clamping screw, collector lens in intermediate position). The dimensions of the filament lamps vary. Therefore optimum illumination is not always obtained with the centring mount pushed home fully.

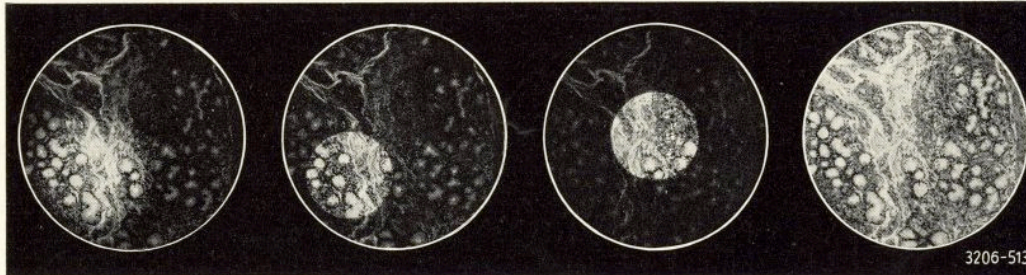


Fig. 9: Centring the field iris

- a) Field iris out of focus      b) Field iris after focusing  
 c) Field iris centred      d) Field iris open

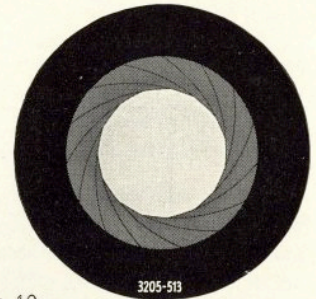


Fig. 10:

Aspect of the aperture iris in the objective (eyepiece removed)

### Centring the swing-out condenser

Turn in medium-power objective 10/0.25. Swing condenser top into the beam path. Close field iris completely and focus it by vertically adjusting the condenser.

Focus specimen by means of the coarse- and fine adjustment. Close aperture iris completely.

Align field iris in the centre of the field of view by means of the two centring screws of the condenser (centring).

Open aperture and field iris to suit the objective in use: The field iris protects the specimen from unnecessary heat and prevents glare. It is therefore opened just far enough to be clear of the field of view of the microscope.

The aperture iris – provided it is smaller than that of the objective – determines resolution and contrast of the microscope image. It must not be used for the control of image brightness; this is the exclusive task of the transformer or, in the case of colour photomicrography, of neutral density filters. Generally, the following rule should be observed when adjusting the aperture iris: Initially it is opened far enough to be just visible in the back lens of the objective (remove the eyepiece). The aperture stop of the **condenser** and the stop of the **objective** are now of approximately the same diameter. If at this position of the aperture iris all visible detail is adequately represented, the iris is gradually closed until the less differentiated structural elements, too, become apparent. In most cases it is advisable to close the aperture iris no further than to  $\frac{2}{3}$  diameter of the full objective aperture (i.e.  $\frac{2}{3}$  of the objective aperture are transmitted). Closing it beyond this point rapidly reduces the resolving power of the objective and therefore the performance of the microscope.

### Change of magnification

All LEITZ objectives of 3.5/0.10 and above are parfocal on the objective revolving nosepiece. As a result, only negligible refocusing

with the fine adjustment is required during any change in the magnification. Plano objectives should, if possible, be used on a revolving nosepiece of their own as their adjustment length\* is greater than that of standard objectives. The following basic rules should be adhered to with each change of magnification: When objectives of N.A. 0.25 and above are used, the condenser top must be swung into the beam path; with objectives of N.A. < 0.25 it must be swung out. In addition the field iris must be focused and the centring checked; recentring may be necessary.

\* If plano objectives are used on the same revolving nosepiece as other objectives, an adapter 519 164 is required for each standard objective.

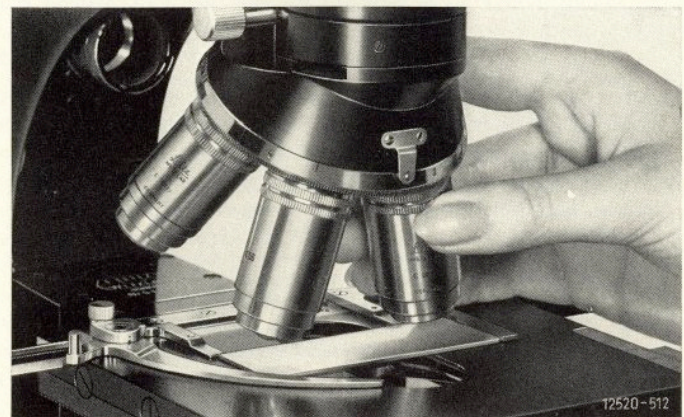


Fig. 11: Change of magnification

## Photomicrography

### Use of oil immersion objectives

If liquids (water, oil, glycerin) are present between the object (or coverglass) and the front lens of the objective, we speak of the immersion method of observation. Objectives used for this purpose are called immersion objectives. It is a characteristic feature of an immersion that the refraction of the rays emerging from the coverglass is reduced or altogether eliminated; at large angles of aperture total reflection from the surface of the coverglass is also absent. This means an increase in the numerical aperture and hence in the resolving power.

Oil is preferred in microscopy as an immersion medium. The refractive index of immersion oil,  $n = 1.515$ , is approximately the same as that of the coverglass and the front lens, so that the spherical surface of the front lens of the objective forms the first refractive interface after the object. The focal length and therefore the working distance of an immersion objective is mostly very small. For this reason care is necessary when working with oil immersion objectives. The coarse adjustment should be used only until the immersion objective has made contact with the immersion oil. This should be ascertained by inspecting it from the side. Focusing should be continuously controlled in the microscope and the fine adjustment should be used for it exclusively. Care should be taken that the immersion oil is free from air bubbles. LEITZ immersion oil, and, for fluorescence observations, non-fluorescing LEITZ immersion oil should be used.

If the full aperture (1.25) is to be used with the aplanatic-achromatic condenser, immersion oil should also be introduced between condenser top and the underside of the microscope slide.

After the end of observation all optical surfaces in contact with immersion oil must be carefully cleaned. A soft rag moistened with xylene is suitable for this purpose. Alcohol or spirit must never be used for cleaning the objectives. The surfaces should be polished with a dry rag. Pressure must be avoided, as this might push the lenses out of their mounts. Not only would this damage the front lens, in the majority of cases the next lens would also be affected.

**Tube:** Binocular tube FS, Tube FSA or monocular tube FP.

**Light source:** 6 V 30 W filament bulb or the XBO 150 W high-pressure xenon burner; the latter can, however, be used only in connection with the Universal Lamp Housing 250. The practically continuous spectrum of the xenon burner at a colour temperature of  $6000^{\circ}$  K makes it possible to use daylight colour film without filters; the colour temperature of the filament bulb, on the other hand, matches the sensitization of artificial-light colour films. The diagram illustrated in Fig. 12 shows the dependence of the colour temperature on the current load on the 6 V 30 W low-voltage lamp. It can be readily used to adapt the lamp to the artificial-light colour film in the camera by regulating the current load. However, the maximum load of 6 amp. must not be exceeded.

**Objectives:** Plano objectives or standard objectives as required.

**Eyepieces:** PERIPLAN<sup>®</sup> wide field eyepieces.

**Negative eyepieces:** Must only be used together with standard (not plano) objectives and a bellows camera. They are suitable for photomicrography only, not for visual observation.

**Filters:** As a rule, a yellow-green filter should be used for black- and white photomicrography with achromats. Neutral density filters are used in colour photography in order to cut down the intensity of the light.

**Exposure meter:** Microsix L.

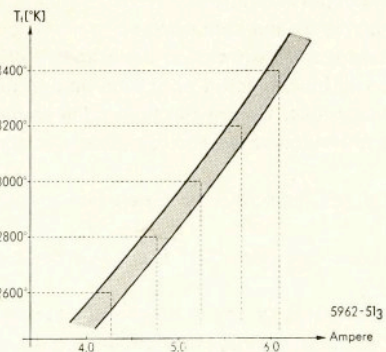


Fig. 12:  
Dependence of the colour temperature of the 6 V 30 W filament bulb on the current load



Our ORTHOLUX microscope can be used with the following photographic equipments:

ORTHOLUX with ARISTOPHOT and 9x12 cm bellows camera or bellows camera with international back (4x5") (required for the Polaroid process) or the LEICA®

ORTHOLUX with camera attachments up to 9x12 cm

ORTHOLUX with micro-attachment and LEICA

ORTHOLUX with fully automatic ORTHOMAT® microscope camera.

The operation of these photomicrographic equipments is described in special instruction leaflets.

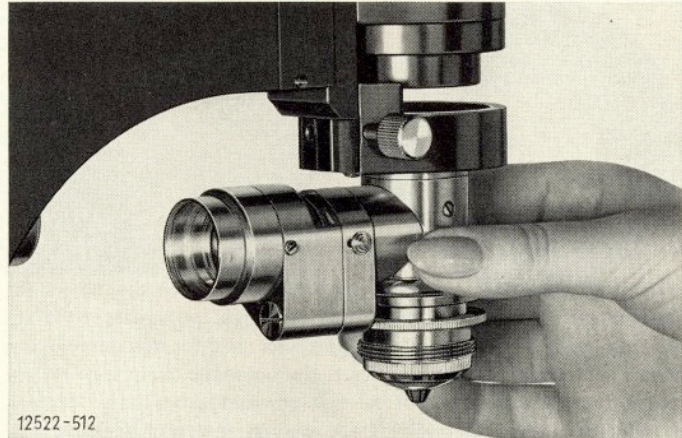


Fig. 13:  
Inserting the  
ULTROPACK

### Examinations in dark field or phase contrast

Replace bright field condenser by dark field or phase contrast condenser.

For phase contrast work, also exchange the objective revolving nosepiece for the nosepiece with matched phase contrast objectives.

Use the light source at its maximum brightness.

Further details are contained in our instructions for the use of our dark field condensers 51-31 and phase contrast equipment 51<sub>3</sub>-24.

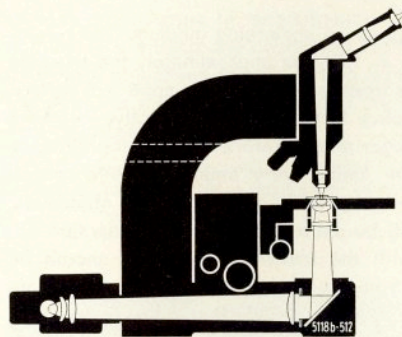


Fig. 14:  
Beam path in the  
ORTHOLUX  
(transmitted light)

### Examinations in incident light

Insert the lamp attachment in the upper aperture 8.

Remove revolving nosepiece and centre the light source:

Hold a groundglass disc in front of the light exit of the stand; a diffuse image of the light source will be projected on it, and should be focused by means of the collector lens. Centre the light cone to the light exit of the stand on the groundglass screen by means of the centring screw.

Attach incident light illuminator (ULTROPACK® or Opak Illuminator) (see Fig. 13).

**High objects:** Remove substage condenser, then the stage can be lowered further.

**Heavy objects:** Focus the image by means of the coarse adjustment. Fix the rack-and-pinion movement by means of the clamping lever.

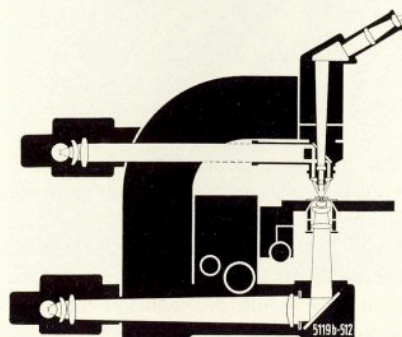


Fig. 15:  
Beam path in the  
ORTHOLUX  
(Combined trans-  
mitted and incident  
light).

### **Combined transmitted and incident light**

The two beam paths must be centred individually for combined transmitted and incident light illumination with the ULTROPAK incident light illuminator.

### **Maintenance and care of the microscope**

For protection against dust the microscope should always be covered with the flexible dust cover after use. From time to time the stand should be cleaned with a piece of linen or chamois leather. Spirit should not be used for this purpose on any account as it attacks the varnish. Benzene, on the other hand, is eminently suitable for the cleaning of varnished parts.

Light spots on the object stage caused by benzene can be removed by a thin film of neat's foot oil.

Work with acids (above all acetic acid) or corrosive chemicals requires special care as they easily spoil the appearance of the instrument and may attack metal parts and lenses.

The optical parts of the microscope should be kept meticulously clean. Dust on glass surfaces is removed with a fine, dry sable brush, blowing gently across the glass surface while applying the brush. If the dirt resists this treatment, a well-washed piece of lint or chamois leather moistened with a little distilled water is used. If even this has no effect, benzene or xylene is recommended. **On no account should spirit or alcohol be used.**

During work with chemical reagents the objectives must not be in contact with them. If they have become contaminated they must be cleaned immediately. Objectives must not be dismantled for cleaning. If internal damage appears in an objective it should be sent to our works for overhaul.

Particular care is recommended during the cleaning of the antireflex coating. The external surfaces of the eyepieces and front lenses of the objectives are coated with films of approximately the hardness of glass. They are cleaned as carefully as uncoated glass surfaces. However, internal surfaces of objectives and eyepieces are sometimes coated with extremely soft films which must be cleaned by very gentle blowing and treatment with a sable brush; they must never be wiped. For the same reason, it is not recommended to clean internal surfaces of eyepieces.

Oil immersion objectives must be cleaned after use to prevent the oil from drying on the glass surfaces. The front lens should therefore be wiped clean immediately with a piece of soft chamois leather. If necessary a little xylene should be used, **but never spirit or alcohol.**

Correct treatment maintains the excellent performance of a LEITZ microscope for many years. Any examination or repair which might become necessary should be entrusted to our factory or to one of our official agencies.

Design subject to alteration without notice.



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