ORTHOLUX II

Leitz WETZLAR

Instructions

513-180/Engl.

ORTHOLUX II

Instructions

1 Technical description

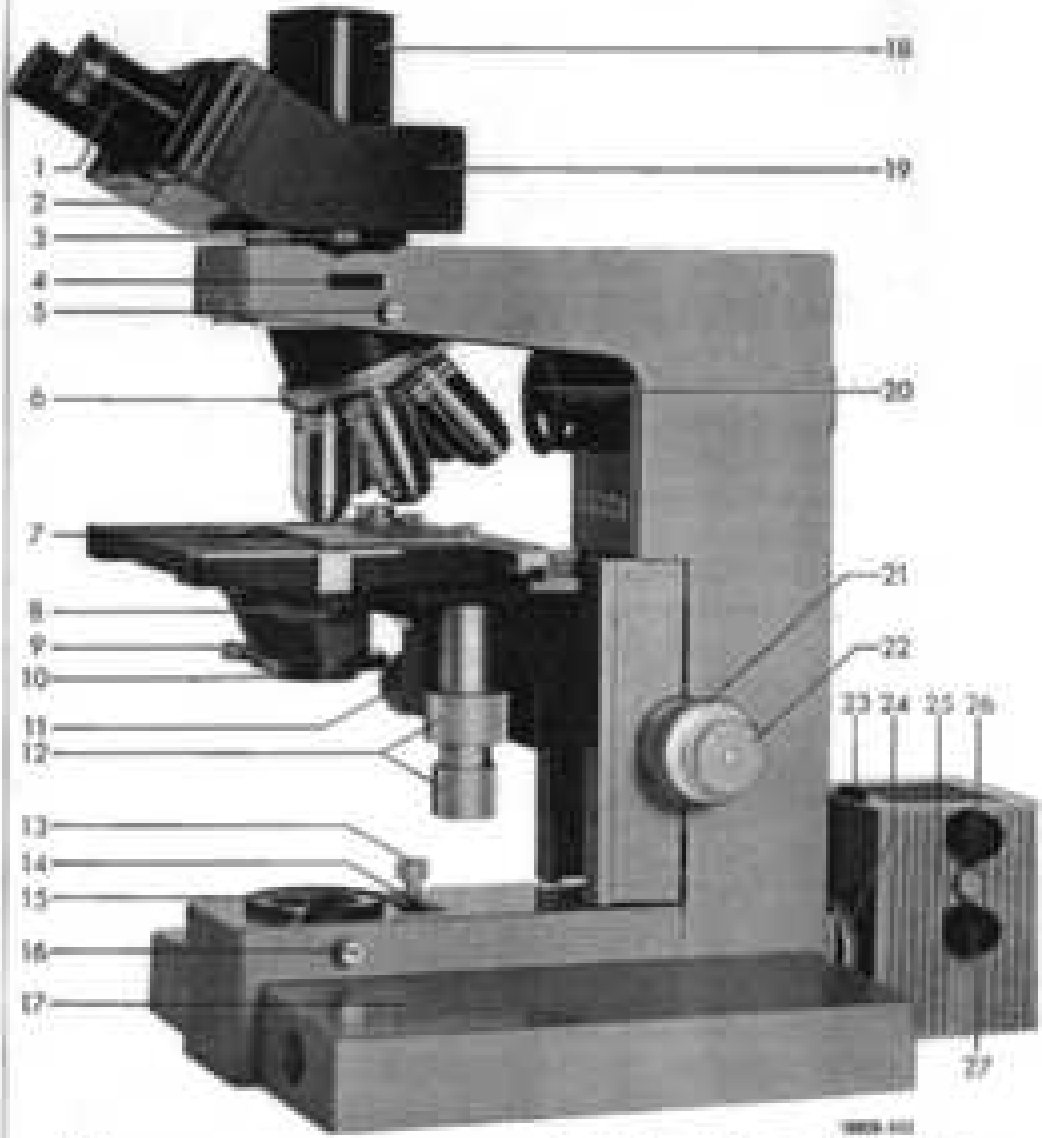


Fig. 1
ORTHOLUX® II

1. Widefield eyepieces in the eyepiece tube
2. Moulded grips for the adjustment of the interpupillary distance
3. Lever for tube change
4. Filter slot
5. Clamping screw for securing the reversing nosepiece
6. Reversing nosepiece, horizontally interchangeable
7. Mechanical stage No. 978
8. Swing-out condenser No. 902
9. Centring screws on the swing-out condenser No. 902
10. Aperture diaphragm lever
11. Knurled screw for the vertical adjustment of the condenser
12. Co-axial knobs for the adjustment of the mechanical stage

2 Unpacking the microscope

The following parts are packed in a special container:

1) Microscope stand with changing guides for the parts to be attached
2) Microscope tube
3) Object stage with changing piece and condenser carrier
4) Lamp Housing III
5) Components such as objectives, eyepieces, condenser, dust cover, etc.

Transformers and other heavy accessories are packed separately.

During unpacking carefully check the equipment against the packing note and make sure that no small components are left among the packing material.

Touching the lenses of the objectives and eyepieces must be avoided if possible. Any fingermarks on glass surfaces must be removed at once with a soft piece of chamois leather or a well-washed piece of linen. Even slight traces of finger perspiration may attack the surfaces of high-quality optical glasses within a short time.

The workroom should be as free as possible of dust, oil vapours and chemical fumes which attack optical and mechanical parts. Nor should there be any great temperature fluctuations or vibrations.

The socket for the built-in illuminator should be fused for 13 amp.

13. Knob for the swing-out lens
14. Field diaphragm
15. Dust glass with filter support
16. Clamping device for accessories
17. Hand rest
18. Photo tube with removable dust cap
19. PM tube
20. Diaphragm tube with filter slot
21. Rotating knob for coarse adjustment
22. Rotating knob for fine adjustment
23. Adjustment of the lamp condenser
24. Locking device for the Lamp Housing III
25. Lamp Housing III
26. Knurled knob for the lateral adjustment of the lamp
27. Knurled knob for the vertical adjustment of the lamp

3 Assembling the microscope

Release the clamping screw of the object stage (Fig. 2.29), insert the object stage into the changing guide (2.28) and lower it until the top of the angle bracket of the stage is flush with the top of the drive casing. Fix the stage with clamping screw (2.29).

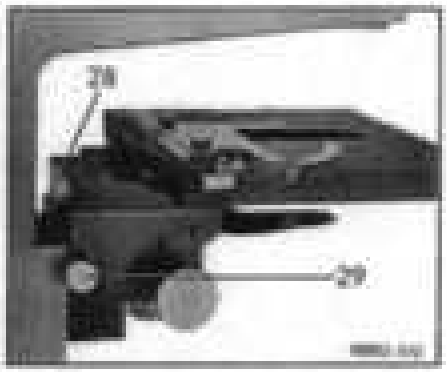


Fig. 1
Inserting the object stage

Lower the stage with the coarse adjustment until the objective revolving nosepiece or ULTROPAK® can be inserted fully into the horizontal changing guide (3.30), with the clamping screw (1.5) released. Re-tighten clamping screw (1.5).

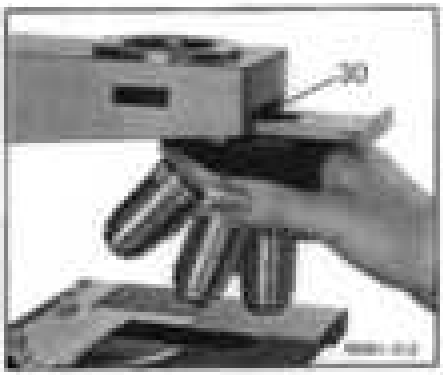


Fig. 2
Inserting the revolving nosepiece

Push the lever for the tube change to the rear and insert the tube in the changing bayonet from above. The rotation of the tube is immaterial. Release the locking lever. It must be possible to rotate the tube through 360° freely after insertion. You can clamp the tube by slightly tightening the lever (4.3) towards the front.

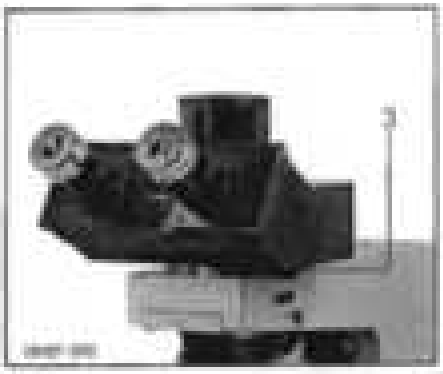


Fig. 3
Inserting the 45° binocular tube

Lower the slide changer (5.31) by means of knob (5.11) so that the swing-out condenser can be easily inserted in it as far as it will go. Ensure that the two centring screws face the observer. The rotating knob (5.32) serves for the swinging in and out of the condenser top.

11 Knurled knob for raising and lowering the condenser
29 Clamping screw for fixing the object stage
31 Swivel-f changer of the condenser
32 Knurled knob for swinging the condenser top in and out of the beam

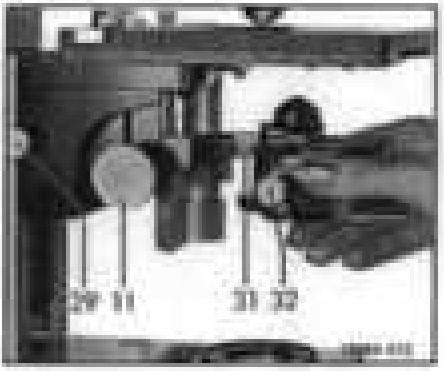


Fig. 6
Inserting the condenser

Attaching the Lamp Housing 50 for transmitted-light illumination

Slightly lift the stand or place it at the edge of the table. Turn the Lamp Housing 50 through about 80° to the left, insert it in the bayonet mount, and lock it in position by turning it to the right.

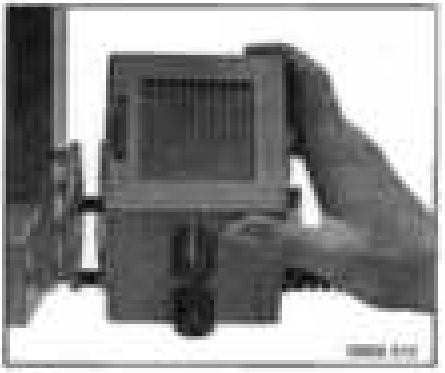


Fig. 4
Attaching the Lamp Housing 50

Inserting the 12 v 50 W lamp

Release the knurled screw (8.33) on the Lamp Housing 50 and remove the side wall from the housing.

Pull out the 12 v 50 W lamp (7.35) from the socket. Insert the new lamp with protective cover in the plug-in socket and remove the protective cover.

Insert the side wall so that holder and plugs (7.36) engage. Re-tighten the knurled screw.

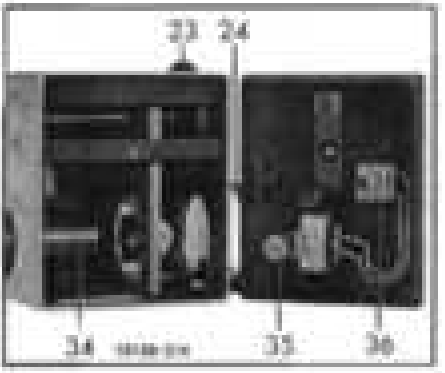


Fig. 7
Inserting the 12v 50W lamp in the Lamp Housing 50

Centring the 12 v 60 W tungsten halogen lamp in the Lamp Housing 50

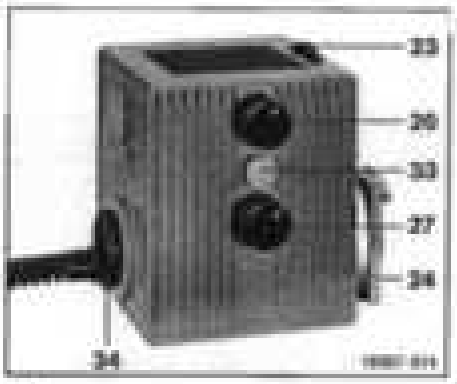


Fig. 3
Lamp Housing 30

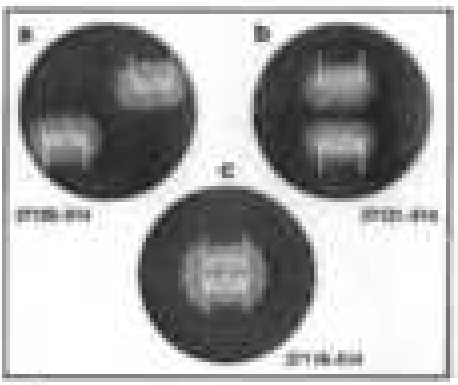


Fig. 4

(2) Lamp condenser adjustment
(20) Locking plate of the Lamp Housing 30
(33) Knurled knob for the lateral adjustment of the lamp
(27) Knurled knob for the vertical adjustment of the lamp
(34) Knurled screw for opening the lamp housing
(R) Reflector

After each lamp change the tungsten halogen lamp must be re-centred.
Fully open the field diaphragm. Place the centring disc on the dust glass of the microscope.
a) Focus the mirror image of the lamp filament by adjusting the reflector (9.34). Form an image of the filament and of its mirror image on the centring disc by rotating ring (9.23).
b) Move the image of the filament and its mirror image into the centre by rotating knob (9.26).
c) Rotate knob (9.27) for the vertical adjustment of the lamp until the image of the filament and its mirror image overlap.
After insertion of the diffusing disc and removal of the centring disc rotate the focusing knob (9.23) for the lamp condenser, simultaneously observing the image through the eyepiece tube, until the rear focal plane of the objective is evenly illuminated.

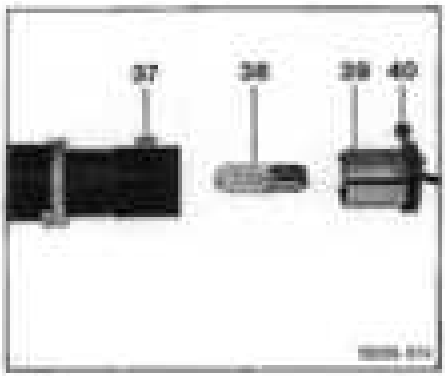


Fig. 10
Inserting the 6W lamp in the Lamp Housing 15

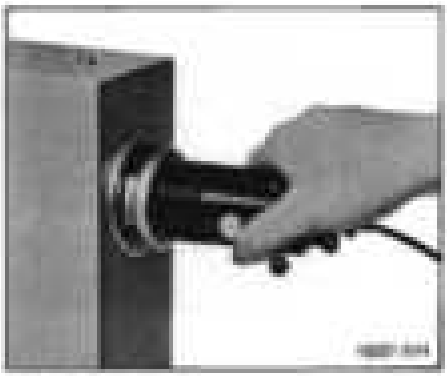


Fig. 11
Attaching the Lamp Housing 15 to the microscope

37 Clamping screw
38 Lamp
39 Socket
40 Centring screws

Insert the Lamp Housing 15 in the bayonet mount, with the arresting screw for the lamp condensor adjustment pointing about 90° to the left. Lock it by turning it to the right. The arresting knob now points upwards.

Inserting the 6 v 15 W lamp
The lamp is changed as follows:

1) Release the arresting screw (10.37) and remove the lamp socket.

2) Remove the lamp (10.38) (push the lamp into the socket (10.39) and unlock it by turning it to the left).

3) Both pre-focus or non-centred lamps can be used:

a) Insert the pre-focus lamp so that the slot of the lamp lies below the red indicating mark of the lamp socket.

b) Insert the non-centred lamp with the two lugs in the guide groove of the socket and lock it in position by pushing it in towards the right.

Centring the 6 v 15 W filament lamp in the Lamp Housing 15

Place the centring disc on the dust glass. Open the field diaphgram. Release the arresting screw and adjust the lamp socket (10.39) until the image of the light point on the centring disc is at its smallest.

Adjust the two centring screws (10.40) until the light points are in the centre of the centring disc.

After removal of the centring disc adjust the lamp socket until the rear focal plane of the objective is evenly illuminated.

4 Technical details

4.11 FSA binocular tube

The various components such as object stages, tubes, objective revolving nosepieces, etc. are described in detail in our List ORTHOLUX II. If special descriptions of individual elements are required for correct operation please consult the following paragraphs.

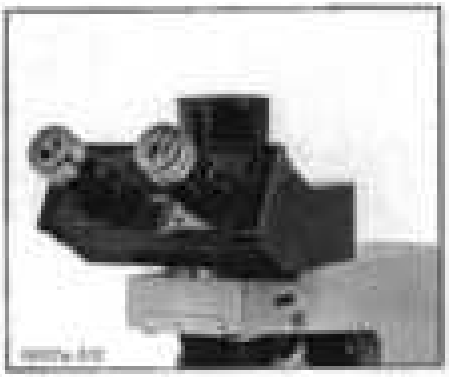


Fig. 11
FSA binocular tube

The FSA binocular tube for the ORTHOLUX II must be used for all eyepieces of 23.2mm diameter. It has a swing-out beam-splitting prism, which either splits the light intensity at a ratio of 80:20 (80% for photomicrography, 20% for visual observation) or directs the entire light (for visual observation) into the eyepiece tubes. The interpupillary distance is set with both hands laterally pulling or pushing the tubes. The optical mechanical-length compensation ensures perfect sharpness for any interpupillary distance both in the observation eyepieces and in the film plane.
Where the interpupillary distance is unknown the tube is adjusted during binocular observation until only one circular and comfortably surveyed field of view appears. Corrections of defective vision must be carried out with the aid of the focusing eyelenses of the PERIPLAN® eyepieces.

12 S binocular tube

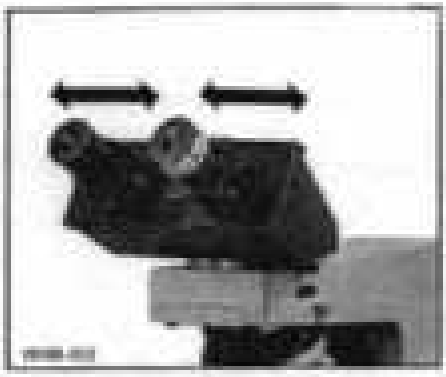


Fig. 11
S binocular tube

Fig. 11
Reversing nosepiece

The S binocular tube serves for visual observation. It is designed for eyepieces of 30.2mm diameter, both with fixed and with focusing eyelens.

The tube can be adjusted for the individual interpupillary distance of the observer. This requires an appropriate correction of the tube length, which is carried out on the eyepiece tubes as follows:

Set the interpupillary distance by pushing or pulling with both hands so that the two part images are coincident in the microscope and only one round image is seen. Read the interpupillary distance thus determined off the scale on the front plate of the tube and transfer it to the two eyepiece tubes.

Example: When an interpupillary distance of 65mm has been determined set the left and right-hand eyepiece tubes at the scale number 65. For defective eyesight one of the eyepiece tubes can be additionally adjusted for sharpness correction.

The objective revolving nosepiece can be horizontally exchanged with other optical elements such as the ULTROPAK vertical illuminator. The nosepiece has five numbered threads to receive the objectives. Each outfit is accompanied by an optical objective/eyepiece chart. This indicates, among other data, the eyepiece threads to which the individual objectives are matched.

The sharpness and the general character of the image are decisively influenced by the microscope illumination. Besides the choice of correct objectives and reproduction scale careful setting up of the illumination and its adjustment to the specimen are some of the basic conditions for optimum utilization of the microscope. The following demands must be made of microscope illumination – i.e. the optical path from the light source to the specimen:

1) The illuminator must supply the microscope with a beam cross section required by the objective/eyepiece combination in use.

2) The illuminator must make it possible to limit this beam cross section at will. Whether and to what extent the first demand is met can be seen from the complete and even illumination of the field of view in the microscope. After removal of the eyepiece the exit pupil (generally the rear lens of the objective) must also appear evenly illuminated at the bottom of the tube.

But the maximum beam cross section required by the individual objective/eyepiece combination varies. Maximum diameter is, however, not needed by the highest-power, but by the lowest-power objectives, which cover a larger object field. The condenser must therefore be computed so that it can deliver the beam cross section required by the lowest-power objectives. But this creates a surplus for higher-power objectives which cannot be fully utilized, and which may even adversely affect image quality through reduction of the contrast, causing reflections, and unnecessary heating of the specimen. It is therefore necessary to be able to reduce the beam cross section to the degree that is applicable and useful for the optical combination used in the microscope. This is achived by two diaphragms suitably arranged in the beam.

The image of one diaphragm limits the rays in the specimen. If this diaphragm is closed, it will be visible in the microscope as field diaphragm. It thus offers

the possibility of meeting the second demand, in that the light beam serving for the illumination of the field of view can be limited to the desired extent through the opening and closing of the diaphragm. The second diaphragm serves to meet this demand also for the beam cross section in the exit pupil or rear lens of the objective. Its image must therefore be visible as aperture diaphragm, i.e. as a circular boundary of the portion of the rear lens of the objective filled by light after removal of the eyepiece.

For the true Koehler's illumination realized in the ORTHOLUX II the field diaphragm is situated between the lamp condenser and the deflecting mirror, whereas the aperture diaphragm is built into the condenser. The paragraph "Centring the swing-out condenser" provides information on the use of these two diaphragms.

The achromatic aplanatic swing-out condenser No. 602, N.A. 0.90, is part of the standard outfit of the ORTHOLUX II. With this aperture the aperture of practically all dry system can be fully illuminated.

Fig. 15
Image of the aperture diaphragm in the objective (with eyepiece removed)

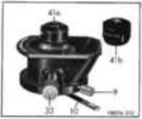


Fig. 16
Condenser bottom part No. [illegible] with the two condenser tops ACHROMAT [illegible] and APLANAT 1.20

9 Centring screws
10 Aperture diaphragm lever
33 Rotating knob for swinging the condenser top in and out of the beam
41a Condenser top No. [illegible]
41b Condenser top No. [illegible]

Immersion objectives, too, can be used with this condenser; only if the distinction of the most delicate structures is essential will a condenser aperture above 0.90 become necessary. Our achromatic-aplanatic swing-out condenser No. 603, A 1.25 is available for this purpose. The table below offers information on the properties of the condensers.

Swing-out condensers

Transmitted-light brightfield Designation	Description	Application
[illegible]	Bottom part of condenser, A [illegible], with aperture diaphragm, condenser lens for low magnification and	For objectives of up to A [illegible]. With this condenser even large fields can be illuminated at low magnification.
[illegible]	2-lens condenser top Achr. 0.90 [illegible]	
[illegible]	**Achromatic swing-out condenser Achr. [illegible], of good spherical and chromatic correction**	For more highly corrected objectives, such as fluorite systems, apochromats, phase objectives, and for photomicrography. Limited suitability for fluorescence.
[illegible]	3-lens condenser top Apl. Oel 1.25 [illegible]	
[illegible]	**Achromatic-aplanatic swing-out condenser Apl. Oel 1.25, state of correction** similar to that of condenser [illegible] at large apertures	Mainly for work with highly corrected oil immersion objectives or for colour photomicrography at large condenser apertures. Unsuitable for fluorescence.

For the investigation of tissue cultures, or very thick specimens etc., condensers of long intercept distances are used for the achievement of true Koehler's illumination.

For long intercept distances Designation	Description	Working distance	Corrected for
[illegible]	Achromatic condenser No. [illegible], A [illegible], consisting of bottom part [illegible] and condenser top [illegible], engraved Achr. [illegible]	[illegible]mm	Petri glass, water, Agar-Agar etc.
[illegible]	Non-achromatized condenser No. [illegible], A [illegible], consisting of bottom part [illegible] and condenser top [illegible], engraved [illegible]	[illegible]mm	[illegible], of which [illegible] glass etc.
[illegible]	Non-achromatized condenser No. [illegible], A [illegible], consisting of bottom part [illegible] and condenser top [illegible], engraved [illegible]	20mm	[illegible], of which [illegible] glass etc.

Objective aperture	Condenser top	Vertical adjustment of condenser
Larger than [illegible]	Remains swung in	Approximately in topmost position. A sharp image must be seen of the field diaphragm.
Smaller than [illegible]	Swung out	Lower the swing-out condenser until a sharp image is formed of the field diaphragm.

Every microscope objective has a number of data engraved in addition to our firm's emblem, which are important for recognition by the user. The following data are engraved:

170
The distance in mm from the shoulder of the objective to the rim of the tube. This distance is also called mechanical tube length. All LEITZ transmitted-light objectives are corrected for 170mm mechanical tube length. This length cannot be maintained in our tubes with inclined eyepiece tube because of the optical elements such as prisms, beam splitters, etc., they contain. Nevertheless the objectives are correctly used with such tubes, since a tube lens displaces the image into the new intermediate plane without adverse effect on the image quality. Suitable choice of the tube lens makes it possible to make the image appear at the same reproduction ratio in the intermediate plane.

Fig. 17
LEITZ plano objectives with cross section.
The Pl Apo Oel 100/1.32 objective has 14 elements

0.17
This is the thickness of the coverglass for which most of our transmitted-light objectives are computed. Instead of the figure 0.17 a dash may be engraved. This means that with these objectives specimens with or without coverglass (e.g. smear preparations) can be observed.
The table shows which objectives may be used with or without coverglass (column coverglass correction).
Below the details about tube length and coverglass correction the following data will be found in abbreviated form: the reproduction scale (size ratio = intermediate image: object e.g. 10:1) and the numerical aperture of the objective, here 10/0.30. For reproduction scale the word magnification is used in the table (this denotes objective magnification). In front of the reproduction scale the state of correction with **Fl**uorite systems, **Apo**chromats, **N**ormal-**Pl**ano objective or **Pl**ano objectives is indicated. Objectives without letters of designation are ordinary achromats. Objectives for phase contrast investigations have the additional designation **Phaco** engraved. Objectives that can be used only with an immersion medium (immersion oil) are distinguished by an appropriate engraving

(Pl Apo **Oel** 100/1.32). In addition all immersion objectives display a black ring on their mounts for instant recognition. For the protection of the specimen at the front lens of the objective all the higher-power systems have a spring-loaded front lens mount.

Objectives for transmitted-light investigation in brightfield or darkground

Designation of objectives	Magnification/aperture	Cover-glass correction [1]	Type of eyepiece	Free working distance mm	Focal length mm
Achromatic dry systems (160/–)	4/0.12	D O	P	24.8	32.0
	10/0.25	D O	P	7.6	17.0
	25/0.50	D	P	0.44	7.2
	40/0.65	D	P	0.28	4.6
Oil immersion objective (black)	Öl 100/1.30	D	P	0.30	1.6
Fluorite dry systems (?/–)	Fl 40/0.85	D 1	P	0.38	4.20
	Fl 40/0.85	D	P		
Fluorite oil immersion objective (?/–)	Fl Öl 25/0.80	D O	P	0.17	3.6
	Fl Öl 60/1.30	D 1)	P	0.10	2.6
	Iris Fl Öl 95/1.32–1.10	D	P		
Apochromatic dry systems (?/–)	Apo 10.5/0.30	D O	P	2.3	15.0
	Apo 25/0.65	D	P	0.76	7.4
	Apo 40/0.95	D 1)	P	0.08	4.6
	Apo 63/0.95	D 1)	P	0.08	3.0
Apochromatic oil immersion objectives (?/–)	Apo Öl 60/1.32	D	P	0.09	2.8
	Apo Öl 80/1.40	D	P	0.03	2.0
Plano objectives (?/–) NPl (for field-of-view ?)	NPl 6.3/0.20	D O	P	2.5	24.0
	NPl 10/0.25	D O	P	5.50	16.0
	NPl 16/0.40	D	P	0.50	11.0
	NPl 25/0.50	D	P	0.30	7.6
	NPl 40/0.65	D	P	0.70	4.0
	NPl Öl 100/1.25	D	P	0.20	1.7
Plano objectives (?/–) Pl (for field-of-view ?)	Pl 1/0.04 single lens condenser	D O	P	36.0	53.0
	Pl 2.5/0.08	D O	P	11.0	26.0
	Pl Fl 4/0.16	D O	P	14.0	40.0
	Pl Fl 10/0.30	D O	P	8.5	16.0
	Pl 10/0.40	D O	P	1.28	10.0
	Pl Apo 16/0.40	D	P	1.20	10.0
	Pl 25/0.50		P	0.65	7.6
	Pl 40/0.65	D	P	0.44	5.7
	Pl Apo 63/1.40		P	0.44	3.8
	Pl Apo Öl 100/1.30	D 1	P	0.15	2.4

1) D: to be used with coverglass d = 0.17mm (coverglass thickness should be observed within ±0.03mm); O: to be used without coverglass; DO: may be used with or without coverglass.

D1: coverglass thickness 0.17mm should be observed to an accuracy of ±0.01mm, or set to the accuracy where the objective has a correction mount if the thickness varies from this value.

2) Objectives in correction mount with achromatic sharpness compensation; its adjustment has hardly any effect on image sharpness. Ideal method of focusing when the coverglass thickness is unknown.

3) These oil immersion objectives may also be used for uncovered objects (smear preparations without coverglass). The negligible reduction of image quality can be ignored.

All LEITZ Plano- and Normal-Plano objectives have a total length of 45mm and are matched on the revolving nosepiece. This makes only negligible re-focusing with the fine adjustment necessary after a change of magnification.

The Pl 1/0.04 objective with a total length of 65.6mm is an exception! This objective can be used only in association with the low-power condenser supplied with it and with the collector lens (1.13) in the foot of the stand swung-out of the optical path.

If special circumstances require the use of objectives of an adjustment length of 57mm together with those of 45mm adjustment length, their adjustment lengths must be increased to 49mm with the adapter Code No. 319104. The consequent increase in tube length is allowed for by a lens built into the adapter.

Oil immersion objectives are distinguished by their engraving „Oel" and a black ring around the bottom rim of the objective mount. It is a characteristic feature of immersion objectives that the refraction of the rays as they emerge from the coverglass is reduced or completely eliminated and with larger angles of aperture the total reflection on the surface of the coverglass is also eliminated. This makes it possible for rays of larger angles of aperture to enter the microscope objective, which means an increase in the numerical aperture and therefore in the resolving power. The immersion oil has approximately the same refractive index $n = 1.515$ as the coverglass and the front lens of the microscope objective. Focal length and working distance of an immersion objective are usually very small. For this

Fig. 46
Objective Oel 100/1.30

reason care is indicated during work with such objectives. The coarse adjustment should be used only until the immersion objective has dipped into the oil; check by looking along the top of the coverglass. Focusing must be carried out with the fine adjustment only, and with continuous microscopic control. Ensure that the immersion oil is free from air bubbles. Use LEITZ immersion oil or, for fluorescence observation, non-fluorescent LEITZ immersion oil.

Generally the condenser No. 602 will be adequate also with oil immersion objectives. If, however, the full aperture of the immersion objective is to be used, e.g. for very delicate structures, the aplanatic-achromatic condenser No. 603, A 1.25 should be used. Here immersion oil should also be introduced between the condenser top and the underside of the object slide.

After the end of the investigation all optical surfaces wetted with immersion oil must be carefully cleaned. For this purpose a soft rag soaked in xylene is suitable. Polish with a dry rag. Alcohol (methylated spirits) must never be used for cleaning the objectives and condensers. Avoid pressure during cleaning.

In the optical system of the microscope, objective and eyepiece form a unit. In the ORTHOLUX II field-of-view indices of up to 18mm can be achieved. The eyepiece field of view is the area of the intermediate image in the tube that can be surveyed with the eyepiece.

It is magnified by the eyepiece factor. The image diameter of a PERIPLAN GF 10 x eyepiece of 18mm field-of-view diameter (field-of-view index 18) thus appears as large as a diameter of 10x18 = 180mm at a distance of 250mm from the observer. At this size the picture can also be reproduced on a groundglass screen 250mm above the eyepiece.

If the diameter of the field-of-view is divided by the objective magnification, the diameter of the object area that can be surveyed is obtained.

With the above-mentioned PERIPLAN GF 10 x eyepiece and a 25/0.50 objective an object area of

$$\frac{18}{25} = 0.72\text{mm diam.}$$

can thus be surveyed.

Eyepieces are generally constructed so that the observer's eye must be about 8 to 10mm above the top lens surface (eyelens) (pupil distance). The so-called highpoint eyepieces have a pupil distance of about 20mm and therefore permit observation through spectacles and thus compensation of astigmatism. The highpoint eyepieces have a plastic top rim; this prevents the eyepieces from being scratched.

The tables below offer a survey of the eyepieces that can be used in the ORTHOLUX II microscope:

PERIPLAN eyepiece (diameter 23.2mm)	
Magnification	Field of view
6.3 x	18
6.3 x M	18
8 x	16
10 x *	14
25 x	8
High-point	
8 x	16
10 x	15
10 x M	15
10 x MF *)	15
Graticule 10mm = 100 intervals	

Widefield eyepieces PERIPLAN NG/GF (diam. 23.2mm)	
Magnification	Field of view
NF 10 x	19
NF 10 x M	19
GF 10 x	18
GF 10 x M	18
GF 12.5 x	18
GF 12.5 x M	18
GF 12.5 x MF *)	18
GF 16 x	15
GF 25 x	10
GF 25 x M	10
Graticule 10mm = 100 intervals	

* with enlarged field of view

M = eyepiece with focusing system and suitable for accepting a pointer

MF = eyepiece with focusing system and focusing graticule

*) with graticule for ORTHOMAT-W or for attachment camera with Polaroid back or for certain attachment cameras

Special eyepieces (diameter 23.2mm)	
Magnification	Designation
H 6.3 x	Pointer eyepiece
H 8 x	Pointer double eyepiece
F 10 x *	Eyepiece with fixed pointer (* yellow engraving)
NF 10 x *	Eyepiece with fixed pointer (* yellow engraving)
GF 10 x *	Eyepiece with fixed pointer (* yellow engraving)
F 10 x	Comparison eyepiece
GF 10 x •	Eyepiece for photomicrography (engraved red dot)
12.5 x	Screw micrometer eyepiece
12.5 x	Screw micrometer eyepiece for monochromatic light

5 Operating the microscope

Attach the microscope slide to the object stage.

Choose a medium-power objective, preferably NPl 10/0.25, with PERIPLAN GF 10 x eyepiece for first examination.

Raise the swing-out condenser to its topmost position and swing the condenser top into the beam. Open the aperture and field diaphragms. Turn the swing-out lens in the foot of the stand into the beam by means of the knob; the lens remains in the beam during all examinations in brightfield and darkground. – During work with the Pl 1/0.04 objective this lens must be swung-out. Focus the specimen with coarse and fine adjustment.

Compensation for eyes of different visual acuity is carried out as follows:

Look through the eyepiece with fixed eyelens with one eye (right hand eyepiece/right eye), focus the specimen with the fine adjustment. Now look at the same area of the specimen with the other eye through the eyepiece with focusing eyelens and adjust the latter until this area also appears sharp. – The fine adjustment must not be operated during this procedure. – After centration of the condenser, this adjustment must be exactly repeated and checked from time to time.

Now the field diaphragm must be centred (Fig. 19).

The following rules apply to the use of the field diaphragm and the aperture diaphragm:

The field diaphragm protects the specimen from unnecessary heat and prevents flare. It is therefore opened only far enough to fill the field of view of the microscope objective.

The aperture diaphragm – as long as it is smaller than the aperture of the objective – determines resolving power and contrast of the microscopic image. Whereas with specimens of normal contrast range the aperture diaphragm is closed so that it only amounts to 2/3rds of the objective aperture, the following procedure is recommended with objects of low contrast range: the aperture diaphragm is opened far enough so that it is just visible in the rear lens of the objective (after removal of the eyepiece). The aperture of the condenser and that of the objective are now identical. If at this setting all details of the specimen appear at sufficient clarity, the diaphragm of the condenser is gradually closed until the less contrasty structural elements too become visible. It will in most cases be advisable to close the aperture diaphragm only far enough so that it transmits about 2/3rds of the full objective aperture. If it is closed further, the resolving power of the objective and with it the performance of the microscope rapidly deteriorate.

The aperture diaphragm must not be used for regulating image brightness. For this purpose only the transformer, or, with colour photography, neutral density filters should be used.

After the condenser has been centred, the specimen must now be fully illuminated. Remove the eyepiece from the eyepiece tube. Observing the rear focal plane (visible aperture of the objective) adjust the setting knob of the lamp condenser unit until the entire specimen is evenly illuminated.

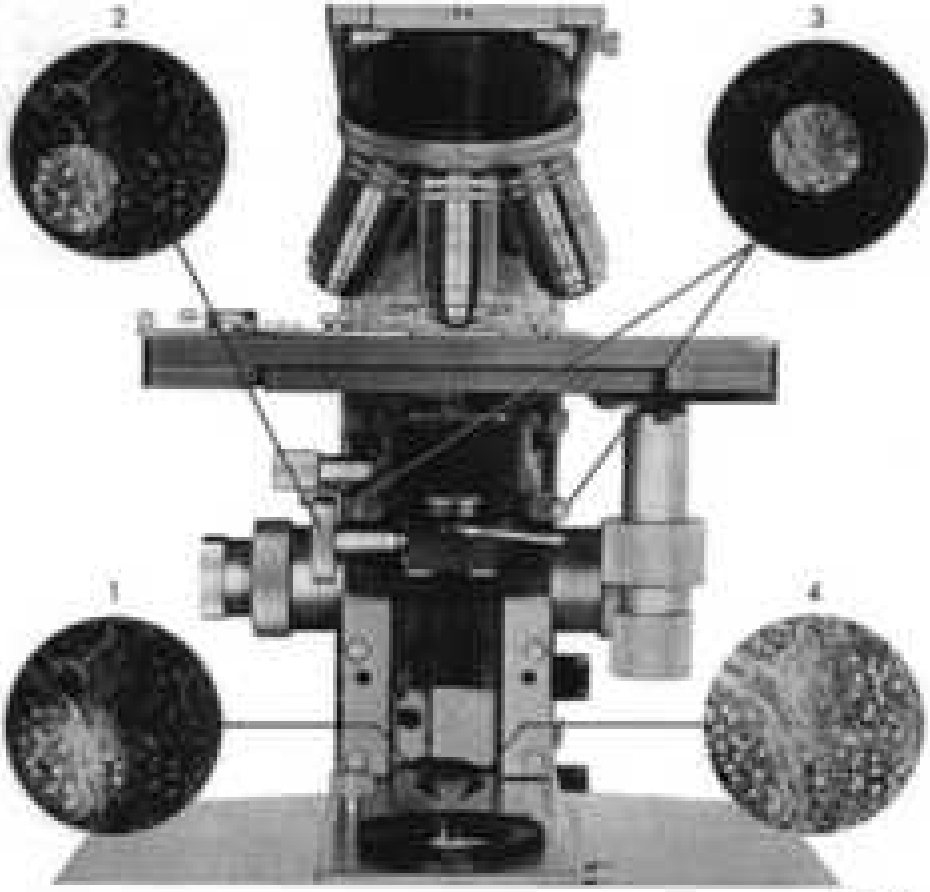


Fig. 14
Centring the field diaphragm

1 Close the field diaphragm.
2 By vertical adjustment of the condenser focus the image of the field diaphragm visible in the microscope.
3 Move the image of the field diaphragm into the centre of the field of view with the two centring screws.
4 Open the field diaphragm so that its (un-)coloured edges disappear beyond the field of view.

6 Transmitted-light-darkground

For investigations in transmitted-light darkground with the immersion darkground condenser D 1.20 mainly the special darkground objectives Oel 100/1.30–1.15 or Fl Oel 95/1.32–1.10 with built-in iris diaphragm are used. In addition, these immersion objectives can also be supplied without iris diaphragm; for darkground examination they must be used with a funnel stop to reduce their aperture, too high for the darkground condenser D 1.20, below the limiting aperture of the condenser (here 1.20). Otherwise part of the illuminating ray bundle would enter the objective and the darkground image would no longer be perfect. Naturally, dry systems of large aperture can also be used with darkground condenser D 1.20.

For darkground examination with dry systems of medium power, particularly for serial examinations, the dry darkground condenser D 0.80, which is simpler to operate, is recommended. With dry systems of an aperture larger than about 0.70 an insert stop should be placed in the condenser 0.80. This stop, however, does not change the limiting aperture of the condenser; it merely absorbs stray light.

Adjusting the darkground image with the condenser D 1.20.

1) Before inserting the darkground condenser set the centring mount roughly at the centre position (resetting with the two centring screws). Push the darkground condenser into the dovetail guide as far as it will go. Do not yet raise the condenser.

2) Place a drop of immersion oil, which must not be too small, on the condenser top surface.

3) Place specimen on the object stage and focus it with the NPl 10/0.25 objective. If brightness is inadequate, lower the condenser slightly, if necessary.

4) Raise the condenser, looking across its top, with the vertical adjustment until the oil droplet touches the underside of the microscope slide (brief lighting up of the microscope slide).

5) Looking into the tube, move the condenser still closer to the microscope slide, until the smallest possible light point (Fig. 20) is formed from the initially appearing light ring. This light point must be moved into the centre of the field of view by means of the two centring screws. Still more accurate centration can be achieved when the initially visible light ring (Fig. 20) is moved so that it is concentric with the rim of the field of view. Ensure that the specimen is in sharp focus.

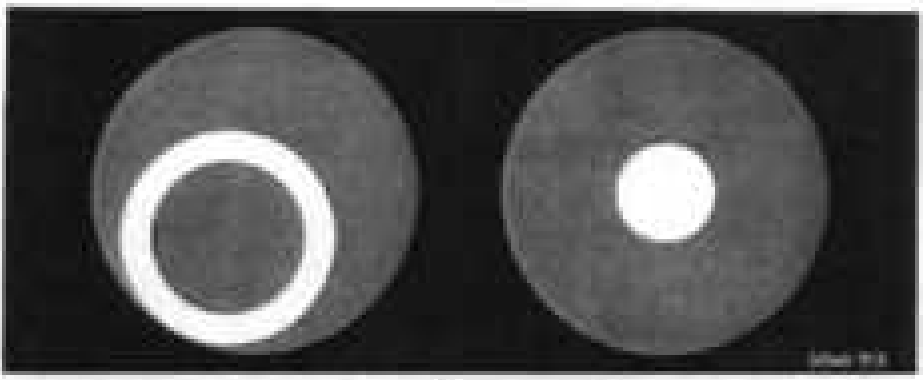
6) Only now turn in an objective of higher primary magnification. Objectives of apertures larger than 1.15 should first be fitted with the appropriate funnel stops.

7) When an immersion objective is used a drop of immersion oil must also be placed on the top of the coverglass. See also Use of oil immersion objectives in transmitted-light-brightfield.

When the special darkground objectives are used maximum brightness is set with the fine adjustment of the microscope; at the same time the structure of the specimen must become weakly visible. Now close the diaphragm on the objective until a perfect darkground image is produced: the object structures appear surrounded by a bright rim.
The «afore» points concerning the adjustment of the darkground image logically apply also to the dry darkground condenser D 0.80. Naturally immersion oil is used neither between condenser and microscope slide nor between coverglass and objective. A suitable test object for darkground adjustment is a specimen of buccal spirilla, which can always be easily obtained.
For details see instructions No. 513-31

Fig. 26
On the left the light ring as it is first seen, not yet centred

On the right the centred light slot: the darkground condenser is correctly set



7 Investigations in phase contrast

For this purpose the phase contrast equipment according to Zernike is available. It consists of:
phase contrast condenser
Phaco objectives Achr. + NPl
auxiliary focusing magnifier
two centring keys.

Operation of the phase contrast equipment

Screw the Phaco objective into the revolving nosepiece and push this into the changing guide; insert the phase contrast condenser, place the specimen on the object stage. Swing phase ring 1 in the phase contrast condenser into the beam. Swing in NPl 10/0.25 Phaco objective. Focus the specimen.

Focus the field diaphragm with condenser, and centre the condenser (see p.19 Fig.19). The aperture diaphragm remains fully open during all phase contrast investigations. Replace the eyepieces with the focusing telescope. Adjust the eyelens of the telescope until the images of the light and the phase rings appear equally sharp. Check whether both rings are concentrically superimposed. If necessary establish coincidence by means of the centring keys. Replace the focusing telescope with the eyepiece. If necessary, repeat the centring procedure after magnification change. For details see Instruction No. 517 - 84 e.

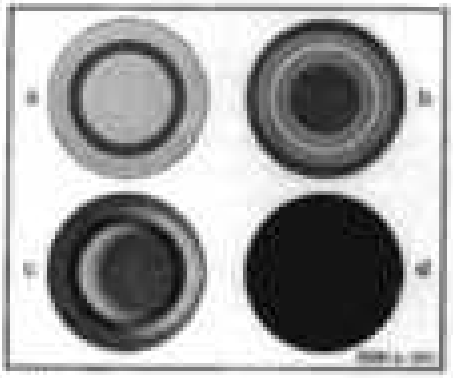
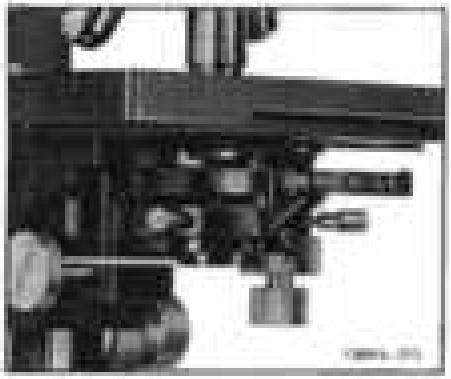


Fig. 21
Phase ring and light ring as they appear in the focusing magnifier, from left to right: a) in brightfield, b) in phase contrast in centred position, c) in phase contrast in de-centred position, d) in darkground



Phase contrast condenser in position 2 on ORTHOLUX II

Phaco objectives
NPI Phaco objectives

Condenser setting	Investigations in	Objectives
H	Brightfield	All objectives
1 2 3	Phase contrast	Phaco 100/1.25 NPI Phaco 100/1.25 NPI Phaco 160/0.90
2 3 4 5	Phase contrast	Phaco 25/0.50 Phaco 40/0.65 NPI Phaco 25/0.50 NPI Phaco 40/0.65
5 6	Phase contrast	Phaco 10/0.30 NPI Phaco 10/0.30
4	Darkground	All objectives

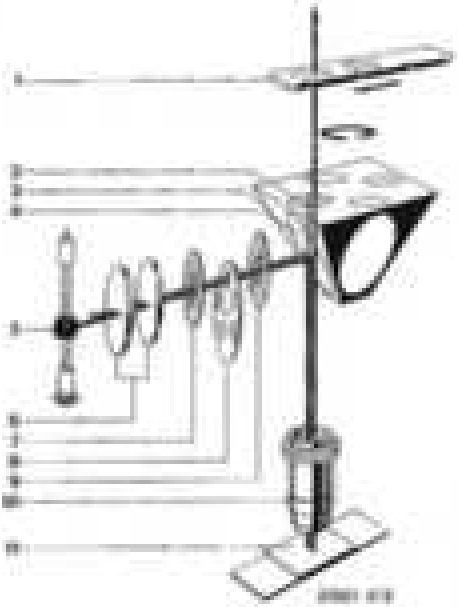


Fig. 37
Diagrammatic representation of the fluorescence vertical illuminator for the ORTHOLUX II microscope

1 Suppression filter white, 2 FD suppression filter, 3 Turret with FD suppression filter and beam splitting mirror, 4 Beam splitting mirror, 5 Light source, 6 Filter in the lamp housing, 7 Field diaphragm, 8 Exciting filter turret, 9 Strip light stop, 10 Objective, 11 Specimen

For the observation of specimens emitting primary and secondary fluorescence the following outfits are available for the ORTHOLUX II microscope:

Transmitted-light fluorescence
Mirror Housing 250 O with Lamp Housing 250 for 200W mercury lamp; darkground condenser.

Incident-light fluorescence
Mirror Housing 250 O with Lamp Housing 250 for 200W mercury lamp or Lamp Housing 100 Z with 100W mercury lamp; fluorescence vertical illuminator.

The appropriate combinations for exciting and suppression filters are described in the instructions for the Lamp Housing 250 and in the List Incident-light Fluorescence. Generally a dark background is aimed at in fluorescence microscopy. The ideal filter combination has been found when these conditions are met.

Directions: Switch on the lamp with the power unit and centre it according to instructions "Lamp Housing 250" No. 514-721. Insert the exciting filter in the filter slot in the lamp housing, the suppression filter in the filter slot in the microscope.

Appropriate exciting filters in a filter turret are built into the fluorescence vertical illuminator. Set the desired position. Focus the specimen and observe fluorescence. If necessary change exciting and suppression filters.

Note: The brightness of the fluorescence image decreases as the square of the eyepiece magnification increases and increases with the aperture of the objective. Objectives of large aperture and eyepieces of low magnification are therefore recommended.

Detailed information about the fluorescence vertical illuminator can be obtained from the instructions No. 512-92.

9 Investigations in incident-light darkground

With the ULTROPAK vertical illuminator the object is illuminated from all sides by means of ring condensers. Only the light diffusely reflected by the object structure forms the image. Observation is thus carried out in incident-light darkground. The advantages of this method are clarity and brilliance of the images. Utilization of the full objective aperture. Polarizing foils for the elimination of disturbing reflections. Illumination of irregular and unprepared surfaces.

Fig. 24
ULTROPAK vertical illuminator

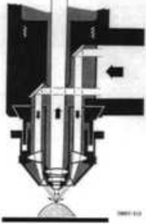


Fig. 25
Optical path in the vertical illuminator

The following ULTROPAK objectives are available:

Designation of objectives	Magnification/aperture	Free working distance in mm	Magnification with eyepieces 6.3x	8x	10x	Outfits with immersion attachments on request
Dry systems	UO 4/0.10	15.0	25x	32x	40x	4
	UO 6.5/0.16	18.0	40x	52x	65x	6.5
	UO 11/0.25	6.7	70x	88x	110x	11
	UO 22/0.45	2.1	140x	175x	220x	
	UO 32/0.55	1.6	200x	255x	320x	
	UO 42/0.60	0.80	265x	335x	420x	
Immersion objectives	UO W 50/0.85	0.38	315x	400x	500x	Staining Cuvette together with 20 cover glasses and 1 bottle of Canada Balsam
	UO Öl 55/0.85	0.36	350x	440x	550x	

W = water immersion, Öl = oil immersion

Operation of the microscope for incident-light darkground investigations

Attach lamp housing to the upper attachment tube of the microscope according to instructions p. 5, Fig. 6.

Insert the ULTROPAK into the horizontal changing guide, Fig. 26, and tighten it with clamping screw (1.5). Insert the ULTROPAK objective.

Establish the best possible illumination by adjustment of the lamp condenser. With transparent specimens or for brightening the background illumination can be used, if desired, simultaneously from a second illuminator from below via the condenser. The polarizing device serves for observation in polarized light and the reduction of surface reflections. The polarizer is pushed into the front slot of the ULTROPAK, where it can be rotated through 90°. The rear of the ULTROPAK accommodates sector stops. For colour filters two additional slots will be found in the diaphragm tube. The analyser is pushed into the filter slot (Fig. 1.4) of the stand.

Our instructions 513-90 give detailed information on further points, such as the use of the relief condenser, immersion attachments, and dipping cones.

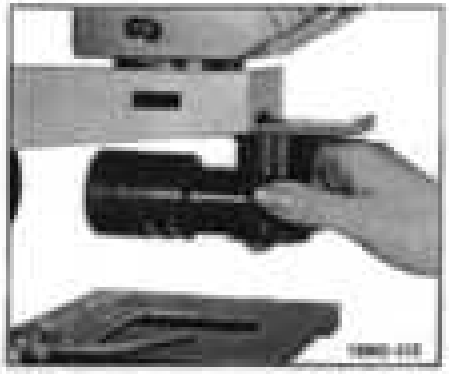


Fig. 26
Inserting the ULTROPAK in the ORTHOLUX II microscope

10 Measurements with the microscope

Linear measurements of specimens are normally carried out with a micrometer eyepiece* (graduation usually 10mm = 100 intervals), either during visual observation through the inclined tube of the binocular tube or on the focusing groundglass screen of the mirror reflex attachment.

Before measurements are begun the micrometer value of the objective used must be known. The micrometer value is the length in the object plane which is precisely accommodated by the intervals of the graticule scale in the measuring eyepiece after magnification by the objective. Although these micrometer values are laid down in tables, it is recommended to determine them with the aid of a stage micrometer, since the optical constants of the objectives are subject to slight variations. The micrometer value remains the same also with photography with the large-format camera, since the microscopic image and the graduation of the micrometer eyepiece change at the same ratio as the bellows extension.

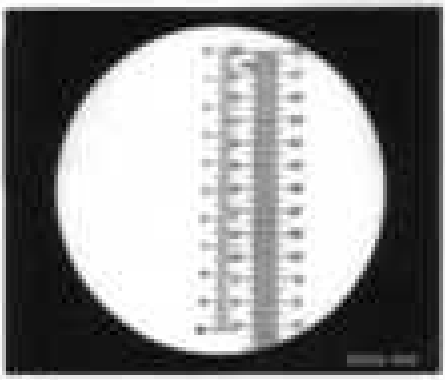


Fig. 27
Graduation of the graticule in the eyepiece and image of the stage micrometer (on the right)

Example:

Determination of the micrometer value with the aid of a stage micrometer 2mm = 200 intervals and a micrometer eyepiece with graticule 10mm = 100 intervals (visual observation): Fig. 27. Zero lines of micrometer eyepiece and stage micrometer must be made to coincide. The micrometer value is read at an unchanged setting at the end of the scale of the micrometer eyepiece. Example: If 1.220mm on the stage micrometer coincides with 100 intervals in the micrometer eyepiece, the micrometer value will be 1.220 : 100 = 0.0122mm = 12.20µm. With low-power objectives, which do not form an image of the scale of the stage micrometer across the entire scale of the micrometer eyepiece, only 10 intervals of the micrometer eyepiece are used as comparison. If, for instance, 0.36mm on the stage micrometer coincide with 10 intervals in the micrometer eyepiece, the micrometer value will be 0.36 : 10 = 0.036mm = 36µm. For the most accurate measurements under the microscope the screw micrometer eyepiece is used. Our List 913-17 supplies detailed information on this.

* The is an eyepiece which contains a circular glass plate with internal graduations, the graticule.

11 Heating stages

Heating and cooling stage 80

In place of the object stage No. 976, insert the rotating stage base No. 953. Turn the centring screws on the rotating stage fully back (without unscrewing them from the threaded hole). Push the object stage against the spring and lift it out of the centring base.

Replace it with the heating stage.

Further operation is described in our instructions 515 – 81.

Heating stage 350

Heating stage 1350

These two heating stages can be used both with the object stage 976 and with the rotating stage No. 953.

Further details on the operation of the two heating stages should be obtained from the instructions 515-89 and 515-74.

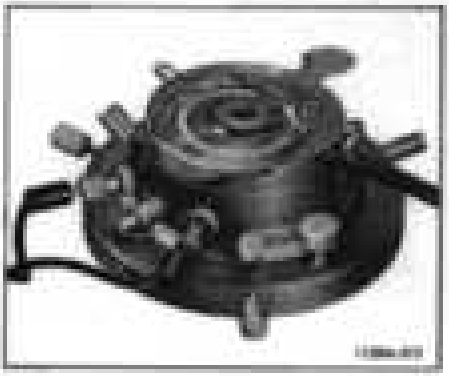


Fig. 38
Heating stage 1350°

12 Photomicrography

General notes

These instructions have been written for the correct application and use of our ORTHOLUX II research microscope and cannot be regarded as a guide to photomicrography.

We nevertheless want to point out a few important basic rules which must be followed without fail:

To obtain a good-quality photomicrograph, the following measures are essential:

1) exact setting of Koehler's illumination (para 4.3, p. 10),

2) the accurate focusing of the ground-glass screen (large format) or in the eyepiece or focusing telescope (35mm photography),

3) the accurate determination of the necessary exposure time,

4) meticulous cleanliness of all optical surfaces accessible to the user, such as deflecting mirror, dust glass, condenser, objective, tube lens, eyepiece etc., (see section Care and Maintenance).

Particular attention should be paid to useful magnification,

the choice of a light filter for the faithful reproduction (black-and-white photography) of the specimen,

the setting of the correct colour temperature of the low-voltage lamp for colour photography,

and the choice of suitable exposure material for photomicrography.

The following devices are available for photomicrography with the ORTHOLUX II:

ORTHOMAT® fully automatic microscope camera	Instruction No. 540 – 19
Micro-attachment for the LEICA® with vibration damper System attachment camera	Instruction No. 540 – 33 In preparation
CB 100 Polaroid* camera	Instruction No. 540 – 34
9 x 12cm or 4 x 5in large-format camera	In combination with the
9 x 13cm or 4 x 5in large-format camera	ARISTOPHOT® basic
with fully automatic exposure control	stand No. 540-12, 540-20

Further details can be obtained from the instructions for these instruments.

* Polaroid – Trademark of Polaroid GmbH

The range of applications of the ORTHOLUX II can be extended with the accessories listed below:

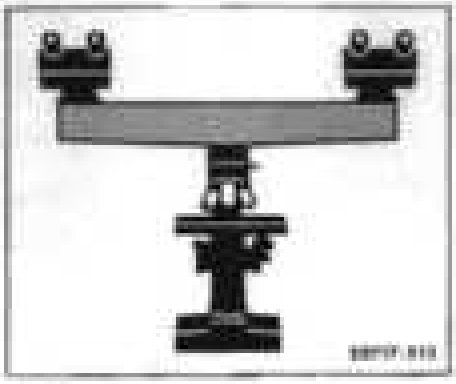


Fig. 29
Stereotaxic table

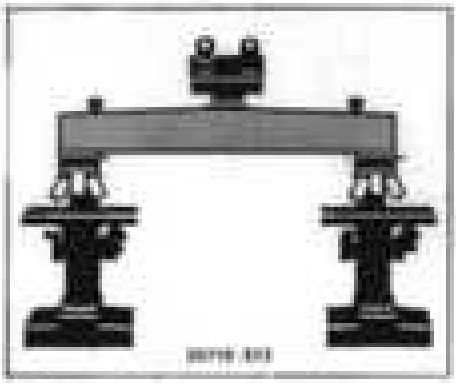


Fig. 30
Cephalostat table

Fig. 31
Camera attachment for Leica

Fig. 32
Camera with CB-MK Polaroid back

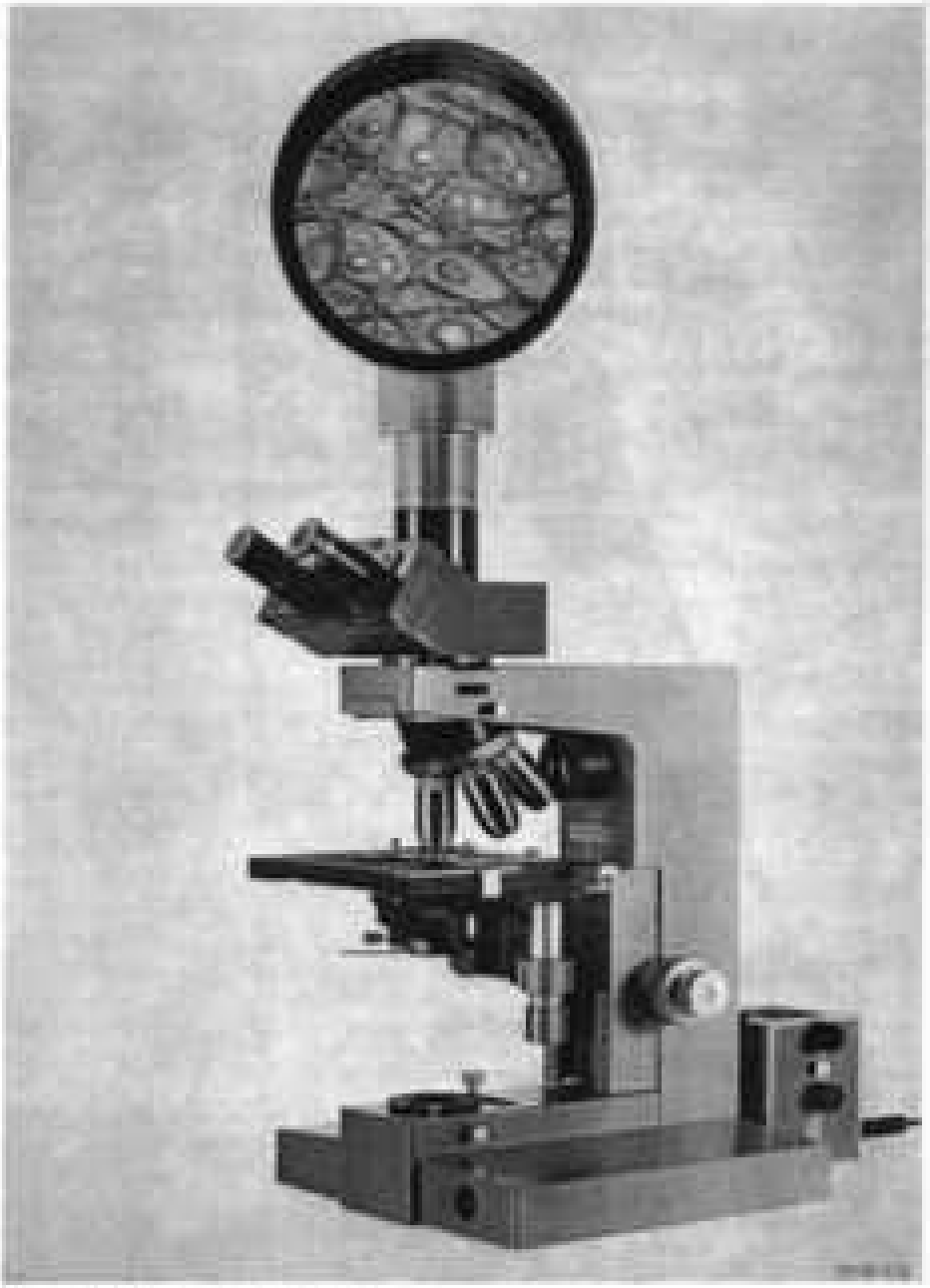


Fig. 10: ORTHOLUX II with projection attachment

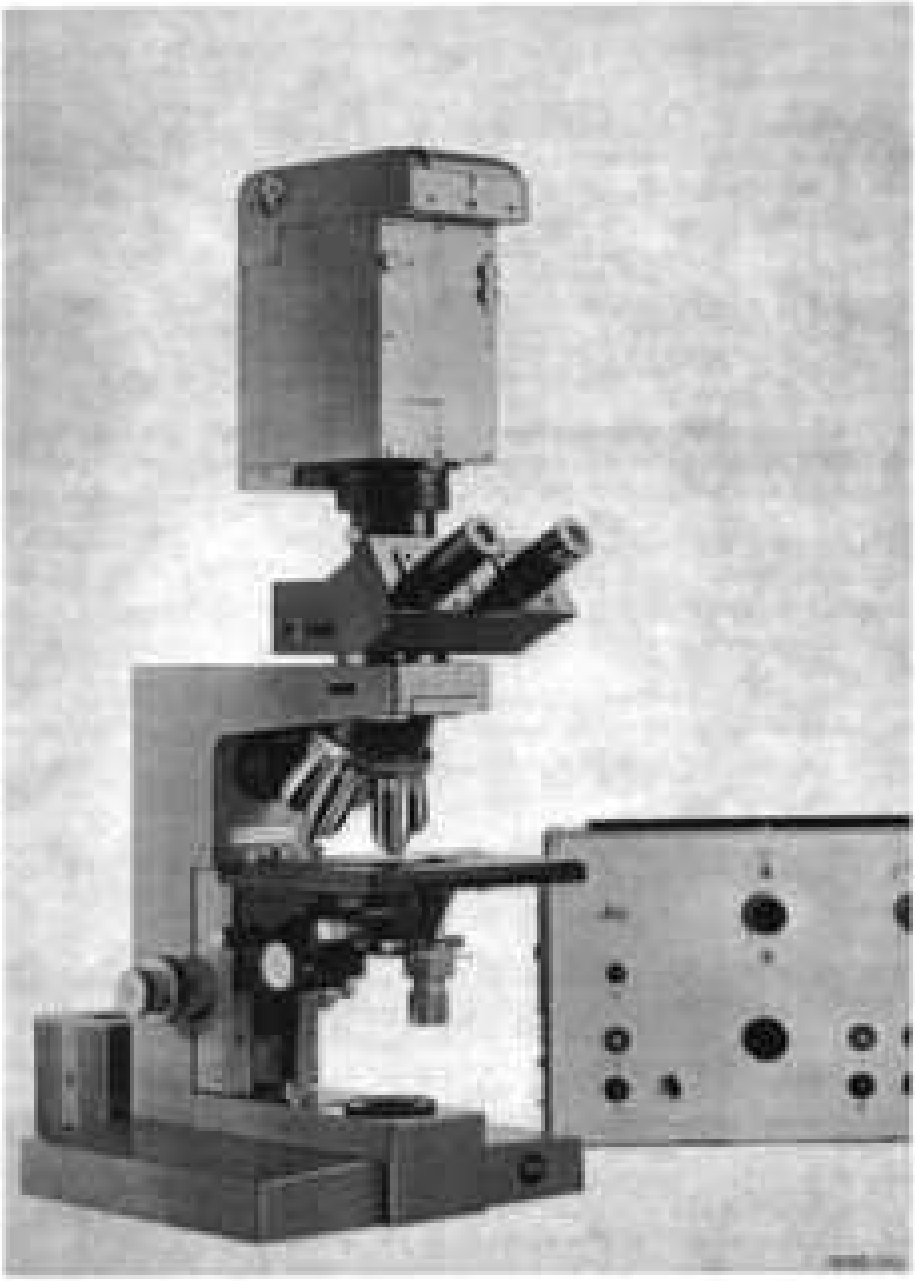


Fig. 8: [illegible]

13 Care and Maintenance

For protection the microscope is covered with the flexible dust cover after use. From time to time the stand should be cleaned with a linen or chamois-leather rag. Spirit must on no account be used, since it attacks the varnish. Petrol, however, is eminently suitable for the cleaning of varnished parts.

Bright patches on the objective stage caused by petrol can be removed by rubbing it with liquid paraffin or acid-free vaseline.

Special care is indicated during investigations involving acid (particularly acetic acid) or other corrosive chemicals. Direct contact of optical surfaces and stands with these chemicals must be avoided at all costs and all parts should be thoroughly cleaned after use.

The optical parts of the microscope must be kept scrupulously clean. Dust on glass surfaces is removed with a fine, dry sable brush; this should be assisted by gently blowing across the glass surface at the same time. If the dirt resists removal, a piece of clean linen or a soft piece of chamois leather moistened slightly with distilled water should be used. If even this does not help, petrol or xylene, but under no circumstances alcohol (methylated spirit), should be used.

For cleaning objectives should not be dismantled. If internal damage of objectives becomes evident these should be returned to the factory for repair.

Special care is indicated during the cleaning of the reflex-reducing films. The external surfaces of the eyepieces and the front lens of the objectives are coated with films about as hard glass. They should be cleaned with the same amount of care as uncoated glass surfaces. Some of the internal surfaces of the objectives and eyepieces, however, are coated with very soft films, which must only be blown across with the greatest care. For this reason cleaning of the internal surfaces of eyepieces is discouraged.

Careful maintenance preserves the performance of a LEITZ microscope for many years. If, however, an overhaul or repair of a damaged instrument is required, our agencies or our factory should be contacted.

Dimensions and weight

Height	414mm
Width	253mm
Depth	253mm
Weight	10.5 kg

14 Appendix

Magnification:

$$V = \frac{25cm}{\text{focal length objective or eyepiece in cm}}$$

Microscope magnification: $V_{Microscope}$

$$V_{Microscope} = M_{objective} \times V_{eyepiece}$$

Magnification of the microscope with built-in tube lens:

$$V_{Microscope} = M_{objective} \times V_{eyepiece} \times \text{Tube factor}$$

Photomicrography:

Large-format camera

$$V_{Micrograph} = \frac{\text{[illegible]}}{25cm}$$

ORTHOMAT-W

$M_{objective}$ x tube factor x eyepiece setting x camera factor 0.32

Projection tube:

At viewing distance 25cm $= M_{objective} \times V_{eyepiece}$ [illegible]

Projection prism:

$V_{projection\ magnification} = V_{microscope}$ x distance in m x 4

$$\text{Visual projected image} = \frac{\text{projection magnification}}{4 \times \text{observation distance in m}}$$

Useful total magnification:

$V_{useful\ magnification}$ = 500 x A to 1000 x A

or

$$V_{eyepiece} = \frac{500 \times A}{M_{objective}} \text{ to } \frac{1000 \times A}{M_{objective}}$$

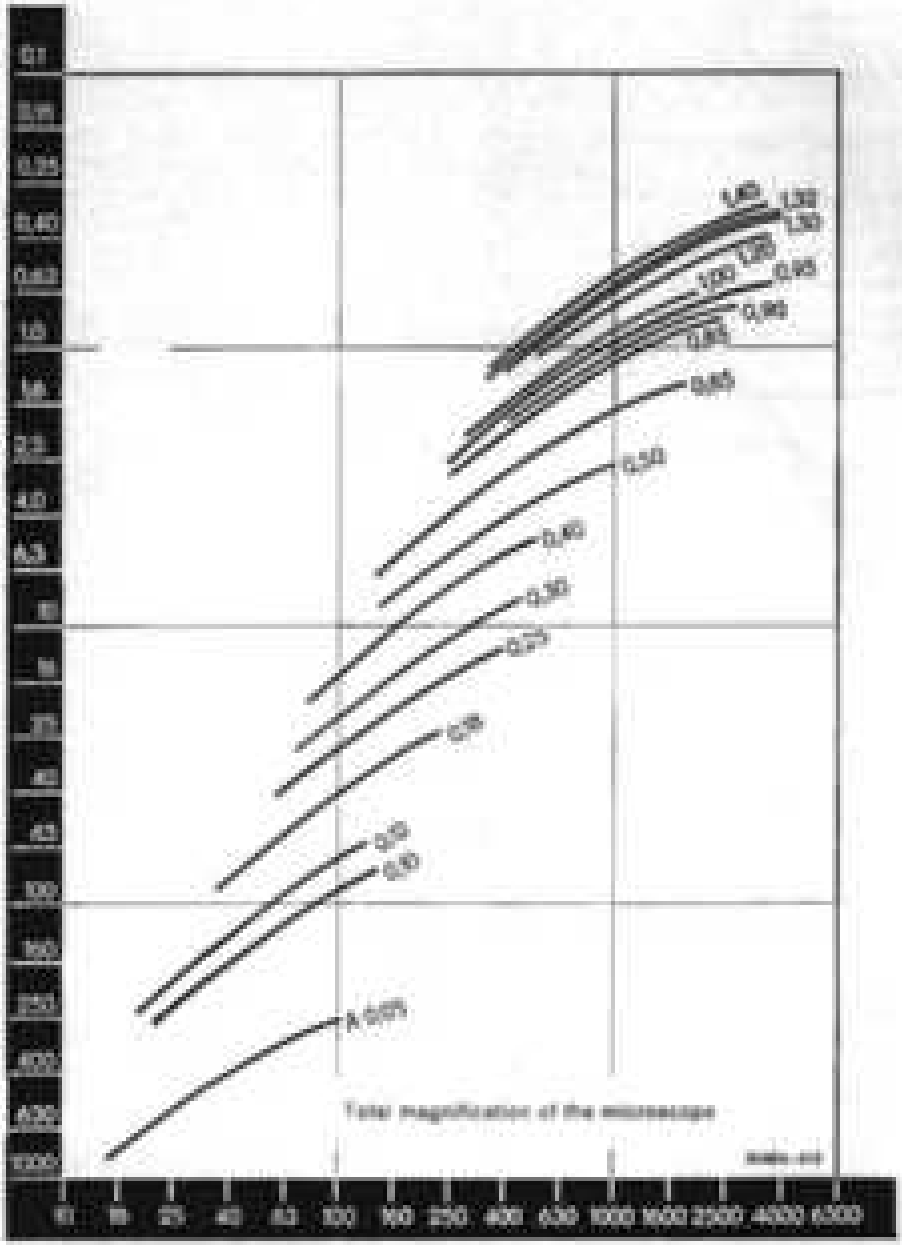


Fig. 31
Area resolving power in µm for biological specimens
A = 1.40 (water value) and 0 = 0.05

Maximum secondary magnification within the range of useful magnification:

Pl	1.5/0.04	40 x	NPl	6.3/0.20	32 x
Pl	2.5/0.06	38 x	NPl	10/0.25	25 x
Pl Fl	4/0.14	36 x	NPl	16/0.40	25 x
Pl Fl	10/0.30	30 x	NPl	25/0.50	20 x
Pl	16/0.40	25 x	NPl	40/0.65	16 x
Pl	25/0.50	20 x	NPl Oel	100/1.30	13 x
Pl	40/0.65	16 x			
Pl Apo Oel	100/1.32	13 x			

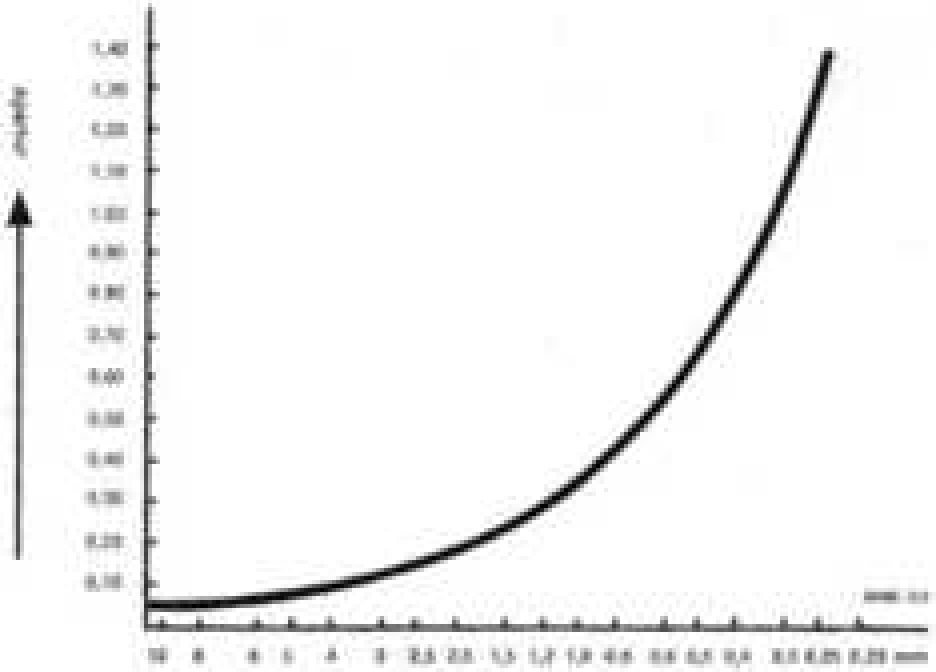


Fig. 36
Resolving power in µ and for green light
(λ = 546nm = 0.55 µm) as a function of the aperture

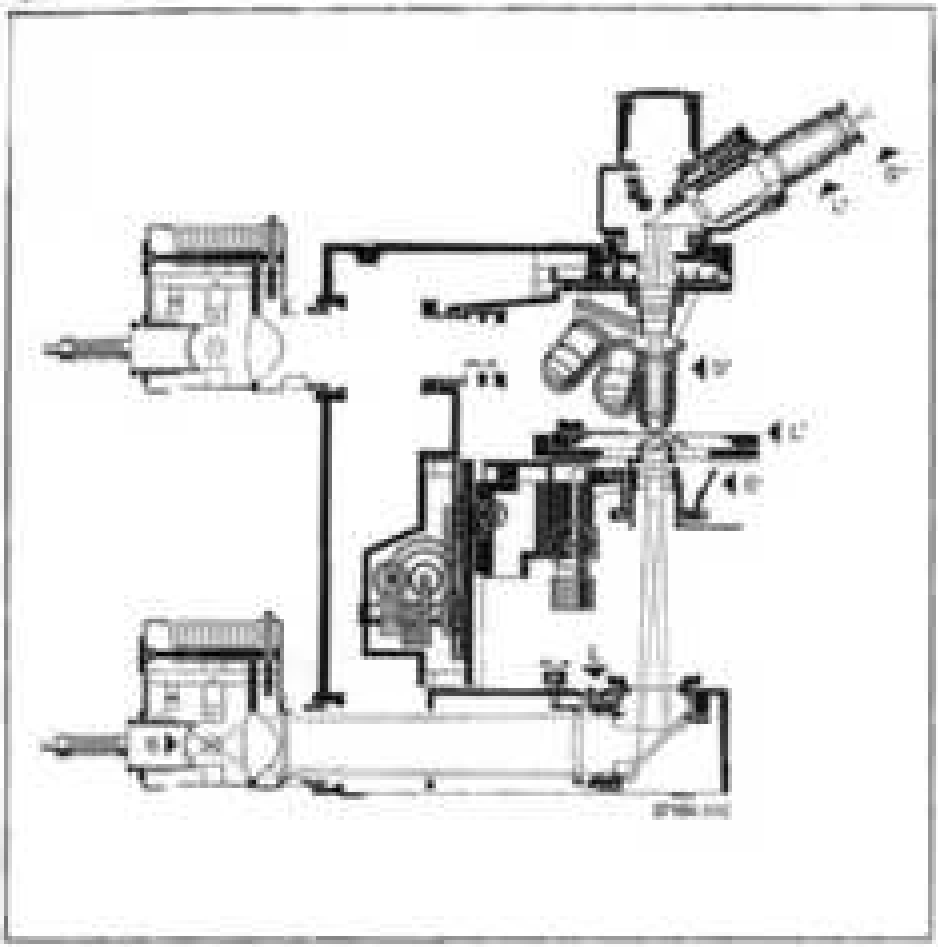
Magnification table
for the ORTHOLUX II microscope

Tube factor 1 x

Objective magnification	Total magnification with eyepieces					
	6.3 x	8 x	10 x	12.5 x	16 x	25 x
1	6.3	8	10	12.5	16	25
2.5	16	20	25	32	40	63
3.5	22	28	35	45	56	90
4	25	32	40	50	63	100
6.3	40	50	63	80	100	160
10	63	80	100	125	160	250
12.5	80	100	125	160	200	320
16	100	125	160	200	250	400
25	160	200	250	320	400	630
40	250	320	400	500	630	1000
54	340	430	540	675	900	1350
63	400	500	630	800	1000	1600
90	565	720	900	1125	1400	2250
95	600	760	950	1200	1525	2375
100	630	800	1000	1250	1600	2500

Transmitted-light beam in the ORTHOLUX II largefield microscope

Fig. 37



The beam path is based on Koehler's principle of illumination. An image of the light source E is formed in the front focal plane of the substage condenser by the lamp collector lens. The aperture diaphragm is also situated in this plane. The image E' of the light source will then be reproduced by the condenser and the objective in the latter's rear focal plane E'' and finally in the exit pupil of the eyepiece E'''. All these planes are optically conjugated. The field diaphragm L is reproduced as a reduced image in the object plane L' by the condenser. The objective forms an enlarged image L'' of the specimen in the intermediate image plane, and this image is once more viewed at a magnification through the eyepiece. The third image of the field diaphragm L''' appears together with the microscopic image on the retina of the eye.

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