# The Microscope

## and Its Application

ERNST LEITZ GMBH WETZLAR

512–69 a / Engl.

## Preface

Since their foundation in 1849, the Ernst Leitz Optical Works at Wetzlar have remainted faithful to their original field of activities, the manufacture of microscopes and accessories; to-day, in spite of the expansion of their manufacturing programme to related fields, they have maintained their leading position as makers of microscopes among the large optical manufacturers with more than half a million Leitz microscopes in use in every part of the world.

For research work the microscope has become both versatile and indispensable. Not only by the pure scientist and the medical man, but also in industry microscopical methods of investigation are extensively used to-day. As a result of their increasing importance, microscopes are now no longer the exclusive preserve of the trained microscopist; a daily growing number of technical assistants without thorough experience in microscope techniques have to learn how to use them. This little book is addressed to them as a guide and a source of advice. Obviously it cannot replace existing handbooks of microscopy and microscope technique, or the operating instructions which are enclosed with the various instruments. But it does offer the novice a summary of what he needs to know, and a number of practical hints which, if he follows them carefully, will soon teach him how to handle his microscope. In addition, it contains useful information for the advanced worker, too.

ERNST LEITZ GMBH WETZLAR

**We shall be pleased to make blocks available for scientific publications. Illustrations contained in this pamphlet are not binding in all details, since our instruments are continuously developed to conform to the latest advances of science and technology.**

## Contents

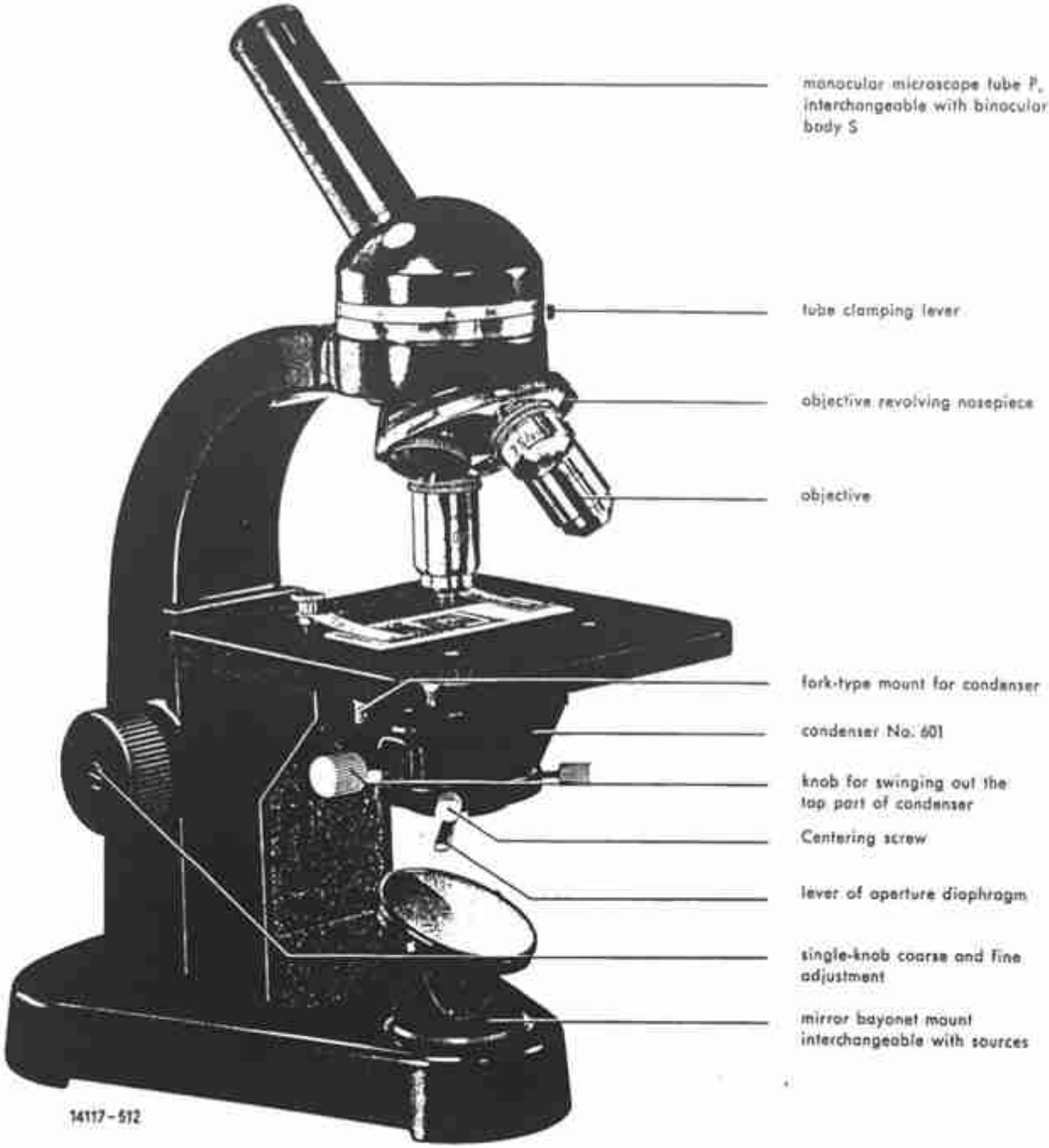



Fig. 2a SM Microscope

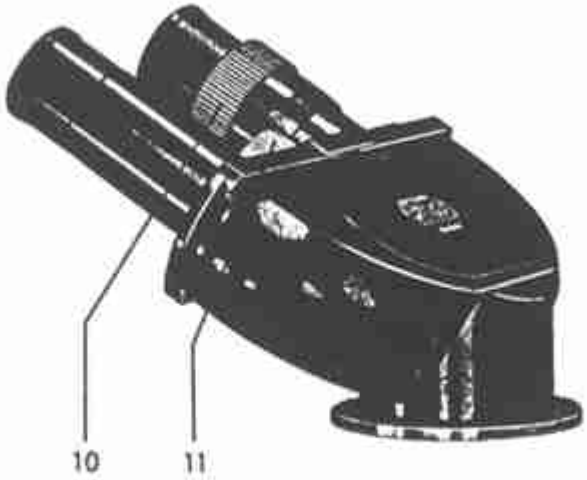



Fig. 2b

10 adjustable eyepiece tube for the compensation of different visual acuity

11 setting knob for the interpupillary distance

## The Components of the Microscope

Although the various microscopes may at first glance appear vastly different from each other, they invariably consist of the following components: –
Stand with stage supporting the specimen,
Microscope tube with eyepiece,
Objective revolving nosepiece,
Condenser.
Illuminating device.

These parts are explained with the aid of the **illustration** of a laboratory microscope (fig. 2a). The functions they have to full are basically the same as in more advanced models.

The stand consists of a sturdy foot and an upper part to which the tube and the objective nosepiece are attached. The illustration shows a monocular, inclined tube; the eyepiece is inserted into the upper end of the tube. Here, the objective nosepiece is of the quadruple, revolving type; four objectives are screwed clockwise into its threads.

The distance between the flange of the objective thread and the top of the tube is 170mm; this is called the mechanical tube length, for which our objectives are corrected.

The objective, which is turned into the optical path by rotating the revolving nosepiece, forms an inverted and side-reversed image of the object on the stage in the diaphragm of the eyepiece; this image is viewed through the eyepiece as through a magnifying glass. In order to focus this image accurately the distance between objective and object must be correctly adjusted. This may be done by means of two separate drives, the coarse adjustment with its large knobs on both sides of the instrument, and the fine adjustment, also on both sides, with which the final focusing is carried out (fig. 1c, d). In the SM stand shown here both drives are combined (p. 14), but operated separately by means of a single knob (see also fig. 1a, b). Whether these drives actuate the tube, as in some types of microscope, or the stage is quite immaterial for the focusing. The stage shown in fig. 1a can be rotated and centred; however, a **plain stage** (fig. 2a) or, for the systematic scanning of a specimen, a **mechanical stage** (fig. 1b, c, d) can be fitted, depending on the equipment of the microscope.

An **attachable specimen guide** (fig. 3) can be used on the two first types of stage. The illuminating device which concentrates the light (daylight or light of a separate microscope table lamp) on the specimen is fitted below the stage. It consists of a mirror which can be tilted in all directions (plane and concave mirror) and a vertically adjustable condenser. Further details about the various types of condenser follow on p. 23.

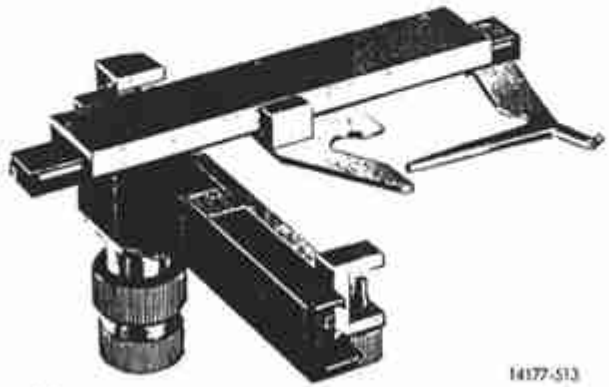


Fig. 3
Attachable specimen guide No. 21

14177-513

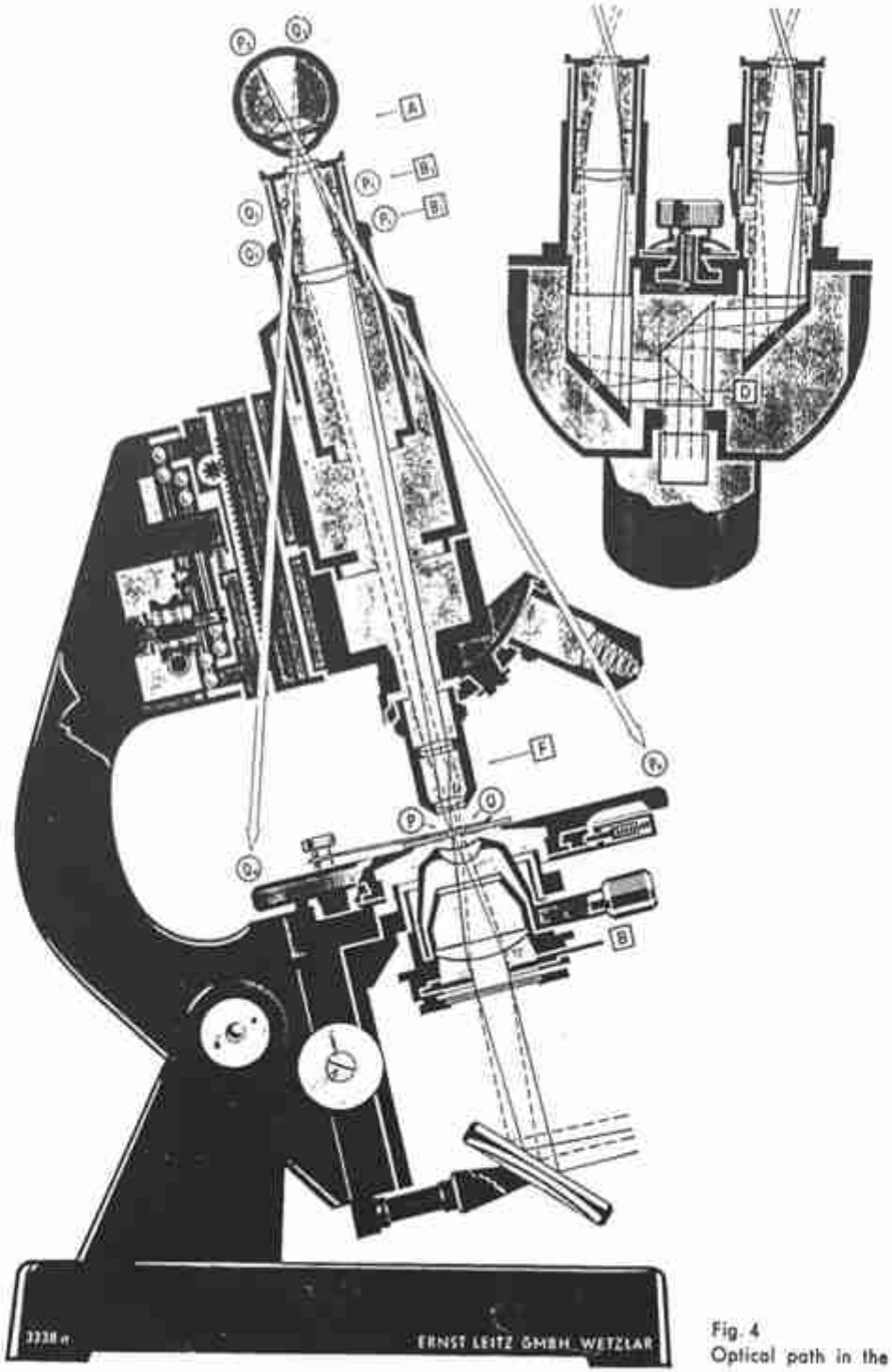



Fig. 4
Optical path in the microscope

The **cross section** (fig. 4) shows the **optical path in the microscope** on a tube-focusing stand with a straight, monocular tube.

An image of the two points P and Q representing a microscope object is first formed by the objective, as already mentioned, in $P_1$ and $Q_1$, in the plane $B_1$, 18mm below the top of the tube. However, in most eyepieces, being inserted in microscopes the so-called field lens is situated so far inside the tube that it affects the optical path before the image $P_1$ $Q_1$ is formed. As a result, the image is displaced into $P'_1$ $Q'_1$, in the plane $B'_1$, which coincides with the plane of the eyepiece diaphragm.

Hence, $P'_1$ $Q'_1$, as well as $P_1$ $Q_1$ – the latter when the eyepiece is removed – are real, magnified images of the object P Q. They are called intermediate images, and their plane is called the intermediate image plane. They could, for instance, be demonstrated by means of a piece of tissue paper or groundglass in the tube, and thereby made directly visible. However, the size of the image $P_1$ $Q_1$, which has not yet been changed by the field lens of the eyepiece, alone determines the effect of the objective. The eyelens of the eyepiece forms the image of the intermediate image $P'_1$ $Q'_1$ practically at infinity, so that finally the image points $P_2$ and $Q_2$ are formed on the retina of a relaxed, i. e. infinity-accommodated eye looking into the microscope. To the eye, the image-forming rays appear to come from the direction $P_x$ and $Q_x$.

Thus, the observer sees the object considerably larger through the microscope than he would with direct observation without instrument. The degree of magnification will be discussed on p. 10. At this point it will be sufficient to state that P and Q have changed sides in the image and the object. $Q_x$ occupies the side of P, and $P_x$ that of Q. The same applies to the section vertical to the plane of the diagram. Hence, compared with the object the microscope image is side-reversed and inverted.

An examination of the illumination beam path shows that the cross section of the beam illuminating point P can be regulated by means of an iris diaphragm located in plane B. An image is formed of this diaphragm by the condenser and the objective in or near the plane F, the rear focal plane of the objective, and again by the eyepiece in A. The diagram shows that this applies to any number of rays illuminating an object point. Thus, all the rays forming images of the various object points converge in A, the exit pupil of the eyepiece, which is also the exit pupil of the microscope as a whole. This exit pupil can easily be demonstrated on a piece of paper, and during observation must coincide with the pupil of the eye.

Hence, if the diameter of the diaphragm B is altered, the aperture of the beams illuminating the individual object points is also altered. This diaphragm is therefore called the aperture diaphragm of the microscope, in which generally an image of the light source is formed. The image of this diaphragm, produced by the objective, is best seen by the observer looking down the tube after the eyepiece has been removed (see fig. 5). When the diaphragm is opened and closed a bright circular spot appears in the objective, changing in size in conformity with the aperture stop. The importance of this will be further discussed on p. 26.


Fig. 5 Appearance of the aperture diaphragm in the objective. It is visible on looking down the tube after eyepiece has been removed.

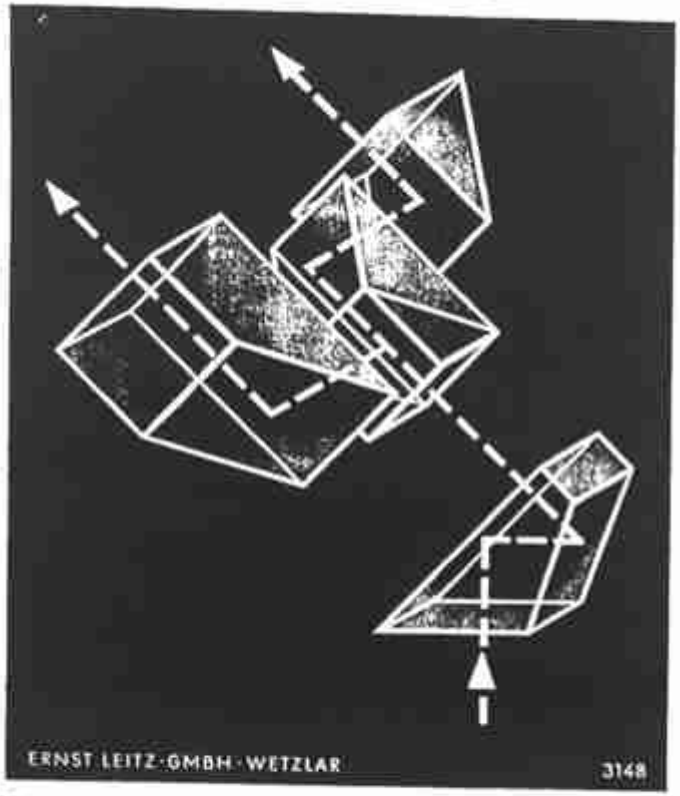



Fig. 6 Binocular inclined observation tube: – beam path in the set of prisms.

In the **binocular tubes** the bundle of rays is split into two equal parts by a semi-silvered prism surface (fig. 4, surface D, and fig. 6), so that the same image reaches both eyes. Usually such binocular tubes have a tube length in excess of 170mm, the standard length of the LEITZ microscope tubes. A lens built into the tube (**tube lens**, formerly also called intermediate optics) has the task of displacing the image $P_1 Q_1$ in the longer binocular tube to the same position with respect to the top of the tube as in the simple monocular tube, but without changing the focus of the objective for the tube length of 170mm required for its optimum performance. However, this image displacement causes a change in the magnification which in the new tubes is prominently engraved (tube factor); for the older tubes the catalogue should be consulted.

## Magnification of the Microscope

It is important to bear in mind that the term "magnification" used in everyday language covers the effect of the magnifying glass (angular magnification) as well as the "scale" (more comprehensively "scale of magnification"). It is wise to distinguish between these two conceptions in microscopy.

The **magnification V** represents the ratio of the visual angle under which the microscopical image of an object appears (in the case of fig. 4 the angle between $Q_s$ and $P_s$ with the apex at A) and the angle under which the object itself would appear to the naked eye at a distance of 250mm. This distance is called the "standard distance of comfortable vision" which we encounter in all formulae concerning magnifications.

Thus, the magnification of an eyepiece or of an objective computed for an image distance at infinity is

$$V = \frac{250\text{mm}}{\text{Focal lenght objective or eyepiece in mm}}$$

i. e. a factor, which is written, for instance,

$$V = 6.3\,x.$$

The **scale**, reproduction ratio, or briefly **magnification M** is the ratio of a distance in the image to the corresponding distance in the object, a fact well known in map making. This term is thus used whenever a real image (which can, e. g., be made visible on a groundglass screen) is to be compared with the original object. Therefore, for a magnified image of an object a scale of, for instance,

$$M = 20 : 1,$$

and, for a reduced image, a scale of, say

$$M = 1 : 3$$

is obtained.

In the first case we often speak of a magnification scale, in the second case of a reduction scale.

In the microscope we find: – The image $P_1 Q_1$ formed by the objective at a certain scale is observed with the eyepiece as with a magnifying glass, and once again magnified. Hence,

Magnification of a microscope =
scale of the image formed by the objective × Magnification of the eyepiece.

$$V_{\text{microscope}} = M_{\text{intermediate image}} \times V_{\text{eyepiece}}$$

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

These values are engraved on the objective and eyepiece mounts (p. 20, 22); the total magnification of the microscope can therefore be found easily by simply multiplying them.

In a microscope with a built-in tube lens the **tube factor** must be considered. Here, the scale of the intermediate image = the scale of the image formed by the objective (without the tube lens) in the original intermediate image plane, i. e. the already used "**scale** of the image formed by the objective" × tube factor

$$M_{\text{intermediate image}} = M_{\text{objective}} \times \text{Tube factor.}$$

Besides objectives computed for a finite tube length there are those computed for an infinite tube length (tube ∞). Here, the real intermediate image in the tube is always formed with the aid of a tube lens, and the following formula applies: –
scale of the intermediate image = *Magnification* of the objective × Tube factor

$$M_{\text{intermediate image}} = V_{\text{objective}} \times \text{Tube factor.}$$

In this case, too the magnification of the microscope is
Magnification of the microscope = scale of the intermediate image × magnification of the eyepiece.

$$V_{\text{microscope}} = M_{\text{intermediate image}} \times V_{\text{eyepiece}}$$

regardless of the origin of the intermediate image scale.

These consideration apply to the magnification of the microscope during visual observation.

In **photomicrography and microprojection** we are concerned with the scale of the image formed by the eyepiece on a photographic plate or a projection screen instead of on the retina of the eye. Here, we must also measure the image distance, which is the distance between the exit pupil A of the microscope and the image plane. For approximate measurements it is sufficient to determine the distance from the rim of the tube. We find that at an image distance of 250mm (standard distance of comfortable vision) the scale is numerically identical with the magnification of the microscope, and calculate it generally according to

$$\text{scale of the microscope image} = \text{magnification of the microscope} \times \frac{\text{image distance in mm}}{250\text{mm}}$$

$$M_{\substack{\text{projection or}\\ \text{photographic image}}} = V_{\text{microscope}} \times \frac{\text{image distance in mm}}{250\text{mm}}$$

This kind of calculation applies if no additional camera with built-in optical system such as our camera attachments is used for the formation of the final real image on a groundglass or projection screen. In microprojection, for instance, enormous scales of magnification are sometimes obtained for the picture on the screen. However, it must be borne in mind that it is the viewing distance which determines the size at which the picture appears on the screen or the photograph, since these real images are viewed by the eye, and are observed under a certain visual angle. Therefore, the magnification at which the image appears compared with its original is numerically identical with the scale of the projection image or photograph viewed at a viewing distance of 250mm only. This is relatively unimportant in photomicrography because here the main purpose is to compare several photomicrographs of either the same, or of known, different, scales, and because the viewing distance does not materially differ from 250mm.

The scale of photomicrographs is usually given as, e. g. scale 200 : 1 or 200 : 1.

A better method, especially in publications, is to enter a distance ——| in the picture and to indicate the appropriate measurement in the object alongside. This avoids errors frequently caused by the reproduction of photomicrographs at reduced scales.

In microprojection the projected image appears under a different visual angle to each viewer, depending on his position. The magnification at which the individual viewer sees the object in an image projected on a screen at a known scale is
$V_{\text{projected image}} = M_{\text{projected image}} : (4\times$ distance of viewer from the projected image in m).

In cameras with a fixed extension and built-in camera lens, the magnification depends on the focal length of this lens. The so-called **camera factor** is obtained by dividing the focal length in mm of the camera lens by 250mm. The camera factor is always engraved and the scale of the photomicrograph is found by

$$M_{\substack{\text{projected image}\\ \text{or photograph}}} = V_{\text{microscope}} \times \text{Camera factor.}$$

It should be borne in mind that negatives of **miniature photographs** are usually enlarged.

Here we have the very convenient rule according to which the enlargement of the 24×36mm negative to the 9×12cm (approximately ¼ pl.) format roughly cancels the camera factor ⅓ or 0.32×; in such cases the scale of the paper print is numerically identical with the magnification of the microscope.

## General Hints for the Use of the Microscope

Before the microscope is taken out of its case it should be noted how the instrument and its accessories are arranged inside, so that they can be stored correctly after use. The stand is housed in the case so that the limb, designed for easy carrying, is readily accessible.

If the microscope is taken from a cold into a warm room it should not be used until the optics are free from the condensation caused by the change in temperature.

The table supporting the microscope should be rigid, not too high, and large enough to accommodate the necessary microscope accessories and other utensils. If daylight is used for illumination the table should be placed not too far from a window.

The microscope is then arranged so that sufficient light enters the mirror. Diffused light reflected from white cloud is more favourable than light from the blue sky; direct sunlight should be avoided, and a north-facing window is therefore preferable.

The microcopist should make it a rule to carry out his observations sitting down, in a comfortable posture, with head and shoulders slightly inclined. An uncomfortable position of the head and neck may adversely affect the observer's blood circulation and eyes.

On a monocular microscope both eyes should always be used alternately, and the "resting" eye should be kept open. After a little practice the microscopist will quickly become used to seeing the image in the microscope only, and to letting the other eye look into "empty space".

It is particularly important to work with relaxed, not **close-accommodated** eyes; in this state they become rapidly fatigued and after prolonged strain may even suffer permanently. To avoid this it is necessary to place the intermediate image P', Q', (see p. 8) very accurately in the diaphragm plane of the eyepiece by means of the fine adjustment, so that it will be projected at a long distance by the eyelens. This setting corresponds with the maximum possible distance between objective and object or the top of the coverglass, its numerical value is given in the tables of objectives in the column "Free working distance".

The use of a binocular tube is very much recommended. The **LEITZ Binocular Tubes** (figs. 1b, c, d; 2b; 7) permit the type of observation corresponding to normal vision with eyes relaxed and parallel-directed. Fatigue is therefore avoided even after prolonged use of the microscope. In addition the image quality appears improved, since the optimal characteristics of both eyes are utilized simultaneously, complementing each other. This is also the reason why with binocular observation the details of an object are usually recognized more quickly, better, and with less eyestrain than with monocular observation. Individual differences in the interpupillary distance and visual acuity can be compensated on the binocular tube by adjusting the eyepiece tubes, so that any observer accustomed to using both eyes can also use a binocular microscope without difficulty.

Fig. 7
Interchangeable inclined binocular body

**The interpupillary distance is adjusted on the interchangeable binocular body by means of this knob or lever. In order to obtain equal focus with both eyepieces, the microscope image is critically focused for the right eye with the fine adjustment of the microscope (keep left eye closed), and the left tube rotated (keep right eye closed) with the fine adjustment undisturbed, until for the left eye, too, the microscope image appears in sharp focus.**

The **light source of a microscope** may, as already pointed out, be daylight, or, as in the vast majority of cases, artificial light, which makes the worker independent of the changing nature of daylight. Among the artificial light sources we distinguish between the normal halfwatt bulbs (fig. 8a), and the low-voltage lamps (figs. 8b and c) particularly suited for microscopy on account of their high and variable intensity which permits their use for all types of work including darkfield observation and microprojection at short distances.

If a microscope lamp with built-in focusing condenser is used, it is important to centre its light on the microscope mirror without inserting a groundglass screen into its path. The condenser stops are opened, the eyepiece removed from its tube, and the mirror adjusted until the rear plane of the objective appears brightly illuminated.

The object is placed, cover glass upwards, across het aperture of the microscope stage. If the stand is inclined or immersion oil used for the observntion the object must be fixed by means of the stage clips.

All examinations should begin with a low-power objective, with which it is possible to scan a large object field; the magnification is then methodically increased by using either a stronger eyepiece or by turning in a stronger objective until the required resolution and optimum reproduction of the desired detail have been reached. It should be borne in mind that eyepieces of 6.3x to 12.5x magnification should be used for normal microscopical observations. The total magnification of a microscope should never be increased beyond what is needed for the recognition of the desired detail, since the brightness and the diameter of the object field decrease as the magnification is increased. Also, the magnification is limited by the thickness of the object. The thinner the object, the higher the magnification which can be used to advantage. (See also the chapters on lateral and axial resolving power, pp 29–34, and on eyepieces, p. 21).

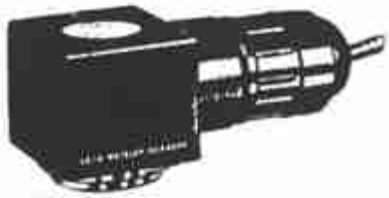


Fig. 8a
15w mains lamp attachment for transmitted light

Fig. 8b
MONLA 6v, 5amp. microscope lamp

Fig. 8c
6v, 2.5amp. lamp attachment

## Focusing on the Object

With the aid of the column "Free Working Distance" in the table on p. 15 it will be found easy to focus low power objectives with a fair amount of accuracy. The higher the power of an objective, the shorter its free working distance generally, i. e. the smaller the distance between objective and object when the image is in focus.

In order to avoid damage to the object or the front lens, springs have been built into the mounts of the higher power objectives; their front part is buffered as soon as it bears down on the object.

When high-power dry systems and particularly oil immersion objectives of the old type without springs are used, great care must be taken to prevent their front lens from bearing down on the object with force. Apart from being a source of potential damage to the object, this negligence may cause the tiny front lens of a high aperture oil immersion objective to be pushed back into the mount. The following method of focusing largely eliminates this danger: –

As a first step, the stage is carefully raised by means of the coarse adjustment until the front lens of the objective almost touches the cover glass of the object, (carefully watch the adjustment from the side); the stage is then slowly lowered with the coarse adjustment; the field is closely observed through the eyepiece until the image appears. Final focusing is now carried out with the micrometer fine adjustment. On stands with tube focusing, the tube is first carefully **lowered** until the distance between the objective and the object is as small as possible, and then **raised** with the coarse- and fine adjustment until the object is focused.

On most stands the sense of rotation of the fine and the coarse adjustment is the same; however, with the single-knob control whose function is described in fig. 9, the fine adjustment is always operated in the direction opposite to that of the coarse adjustment. Since the worm gear of the latter results in a considerably slower movement than the direct rack-and-pinion drive, this, combined with the springloaded front lens mount of the objectives almost completely eliminates the danger of damaging the specimen and the front lens. If the micrometer screw has a scale division, the fine adjustment can be read off. On the simple microscopes the circumference of the fine adjustment drum is divided into 50 parts, on the large stands into 100 parts. Each interval corresponds to a tube displacement of 0.002 and 0.001mm respectively. Further details concerning the suitability of the

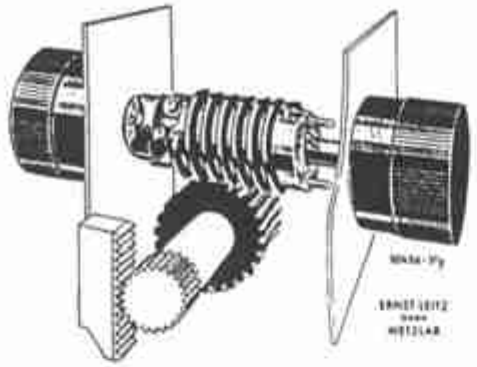


Fig. 9 Single-knob control for coarse and fine adjustment.

The bilateral single-knob control of coarse- and fine adjustment (Fig. 9) also contributes towards very fast and reliable operation in all ranges of magnification. When the knob is turned in one direction only, the mechanism acts as a coarse adjustment. When the direction of rotation is changed, the fine adjustment will be automatically engaged. Its range covers about ⅓ turn of the control knob. If it is turned beyond the slight resistance of the fine adjustment, the coarse adjustment will again come into operation.

micrometer screw for exact measurements will be found on p. 36. After approximately 25 rotations of the 100-interval drum the fine adjustment has reached the end of its travel. Two index lines show the extreme positions. Before the microscope is used the micrometer screw should be set so that the single index line points approximately at the centre between the two opposite lines to make the adjustment range equally large in either direction.

To allow the accurate focusing of the most powerful objectives the play of the fine adjustment must be less than the wave length of light. Any "giving" of the micrometer screw must also be less than this value so that the focusing is not disturbed for prolonged periods; this is particularly important for photomicrography. The LEITZ Micrometer Motion on Ball Bearings which is a feature of all LEITZ microscopes with stage focusing meets this condition in an especially effective way; its mechanism is not affected by the state of the lubricants and atmospheric influences. Also, these micrometer movements are not sensitive to additional strain due to microscope accessories such as camera attachments or the heavy binocular tubes. Any strain on the fine adjustment by such loads is excluded if the mechanism actuates the stage, however, here, too, the LEITZ fine adjustment mechanisms meet all the other requirements fully.

To focus an oil immersion objective a little immersion oil is placed on the front lens of the objective **and** on the portion of the object to be examined; the stage is raised by means of the coarse adjustment (observing the movement from the side) until both drops merge. The object is now focused carefully in the microscope with the micrometer screw. Air bubbles in the oil film, which occasionally form between the cover glass and the front lens, impair the image quality. They can be recognized as small, luminous globules or points when looking down the tube after the eyepiece has been removed. The air bubbles can be pushed off the object field by slightly turning the objective nosepiece, or by gently passing a flat wooden stick across the front lens of the objective. Dry objectives must never be used with water or oil, immersion objectives never without their immersion fluid.

If corrosive substances, such as acetic acid, lyes, dyes, canada balsam, varnish, etc., have been placed along the edges of the cover glass, the object must not just be pulled out from underneath high power objectives; in view of their short free working distance their front lens might come in contact with the substance. It should therefore, be made a rule to lower the stage or raise the tube according to the design of the microscope by means of the coarse adjustment before the specimen is removed.

## Objectives

Microscope objectives are little optical and mechanical marvels and must be treated accordingly. They contain up to 15 individual lenses of which the smallest have a diameter of approximately 1mm, and whose position relative to each other must be maintained within fractions of 1/100mm. Hence, such objectives must never be taken apart by the user himself; at most, the front lens and, if necessary, occasionally the rear lens should be cleaned with a very soft, often-washed piece of linen. Solid dirt or dried immersion oil should be dissolved with xylene or benzene; spirit and alcohol must be strictly avoided, as they attack the cement of the lenses.

However, in order to achieve the utmost in the performance of a microscope objective, attention should not only be paid to the mechanical aspects of its use; knowledge and consideration of the optical data given below are equally important.

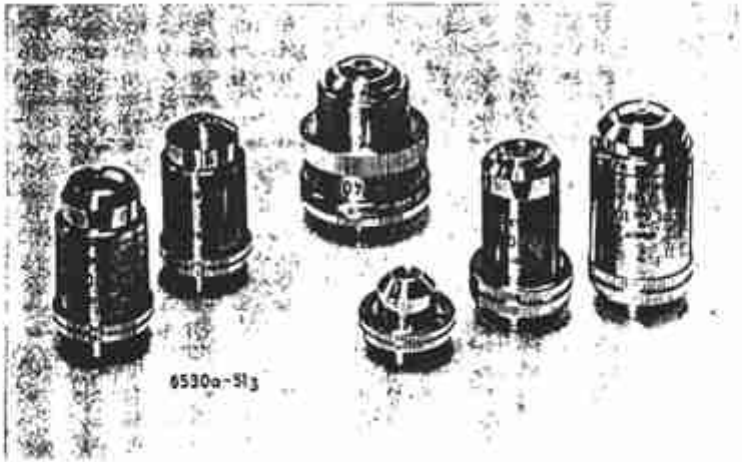



Fig. 10 Typical microscope objectives.
From left to right: – objective oil 100/1.30; 45/0.65; Apo 40/0.95 with correction mount; 3.5/0.10; Pl 10/0.25; Pl oil 100/1.32.

| Designation of objectives | Scale/Aperture | Focal length mm | Free working distance mm | Cover glass correction [1] | Type of eyepiece [2] |
|---|---|---|---|---|---|
| Achromatic dry systems | 2.5/0.07 | 57 | 14 | DO | P |
| | 3.2/0.12 | 40 | 35 | DO | H |
| | 3.5/0.10 | 32 | 23 | DO | H |
| | 6/0.18 | 23 | 17 | DO | H |
| | 10/0.25 | 16 | 5.7 | DO | H |
| | 25/0.50 | 7.1 | 0.92 | D | P |
| | 40/0.65 | 4.5 | 0.67 | D | P |
| | 63/0.85 | 2.9 | 0.29 | D1 | P |
| | Iris 63/0.85 | | | D | P |
| Achromatic immersion objectives (W = water immersion) | Oil + W 22/0.65 | 8.1 | 0.32 | DO | P |
| | W 90/1.20 | 2.1 | 0.09 | D | P |
| | Oil 100/1.30 | 1.9 | 0.13 | D [4] | P |
| | Iris Oil 100/1.30–1.10 | | | D | P |
| Fluorite dry system | Fl 40/0.85 | 4.3 | 0.38 | D1 | P |
| Fluorite oil immersion objectives | Fl Oil 54/0.95 | 3.4 | 0.22 | D | P |
| | Fl Oil 95/1.32 | 2.0 | 0.15 | D | P |
| | Iris Fl Oil 95/1.32–1.10 | | | D | P |
| Apochromatic dry systems | Apo 12.5/0.30 | 13 | 2.5 | DO | P |
| | Apo 25/0.65 | 7.3 | 0.85 | D | P |
| | Apo 40/0.95 | 4.5 | 0.12 | D1 [3] | P |
| | Apo 63/0.95 | 3.0 | 0.12 | D1 [3] | P |
| Apochromatic oil immersion objectives | Apo Oil 90/1.32 | 2.0 | 0.12 | D | P |
| | Apo Oil 90/1.40 | 2.0 | 0.06 | D | P |
| Plano objectives [5] | Pl Fl 4/0.14 | 40 | 14.4 | DO | P |
| | Pl Fl 10/0.30 | 18 | 7.1 | DO | P |
| | Pl 25/0.50 | 7.6 | 0.90 | D | P |
| | Pl 40/0.65 | 4.6 | 0.58 | D | P |
| | Pl Apo Oil 100/1.32 | 2.4 | 0.27 | D [4] | P |

All objectives from 3.5/0.10 are adjusted to the revolving nosepiece for parfocality.

[1] D = with coverglass thickness d = 0.17mm (adhere to coverglass thickness within ± 0.05mm).
O = without coverglass DO = suitable for use with or without coverglass.
D1 = Adhere to coverglass thickness within ± 0.01mm; where the objective has a correction mount, set it for the actual coverglass thickness at this accuracy.

[3] These objectives have an adjustable correction mount with automatic sharpness compensation. During adjustment the sharpness of the image remains almost unaffected. Ideal opportunity of optimum setting when the coverglass thickness is unknown.

[2] Use H = Huygens, P = PERIPLAN® or PERIPLAN widefield eyepiece.

[4] These oil immersion objectives can be used also for preparations without coverglass (e.g. smear preparations); the negligible loss of image quality can be ignored.

[5] Plano objectives are matched to 45mm instead of 37mm adjustment length on the revolving nosepiece.

## Tube length and Cover Glass Thickness

As we have already pointed out during the discussion of the optical path in the microscope, adherence to the correct tube length, i. e. the distance between the top of the tube and the screw flange of the objective is an important factor in microscope design. The LEITZ microscope objectives for investigations in transmitted light are corrected for a mechanical tube length of 170mm. (Special attention must be paid to maintain this tube length with monocular instruments with drawtubes. These have a ring marked 17 (cm) engraved at the correct tube length). If for structural reasons a microscope tube has to be longer, a built-in tube lens will ensure that the optical effect of the tube corresponds to that of the standard mechanical length.

Deviations from the tube length have the effect of a progressive loss of contrast and increasing veiling of the image as the numerical aperture of the objective increases. Thus, with an objective of N. A. 0.95, a difference of only a few mm from the correct tube length is sufficient to produce a completely unsatisfactory image. In addition, the objective magnification changes at the same ratio as the tube length. In the case of the low-power objectives an incorrect tube length will have an appreciable effect on the parfocality on the revolving nosepiece, so that sharpness is no longer ensured after a change-over of objectives.

Differences in the thickness of the cover glass used for the object have a similarly unpleasant influence on image quality. The objectives are corrected for a cover glass thickness of 0.17mm, and the table on p. 15 indicates the maximum deviation from the standard thickness possible without deterioration of image quality.

In this table, low-power objectives are listed with the designation **DO**, denoting that they can be used with or without cover glass, i. e. within a thickness range from 0 to 0.22mm. An **!** behind **D** indicates that with such objectives the cover glass thickness must be adhered to within a tolerance of ± 0.01mm. A cover glass gauge or a simple micrometer screw is used to determine the thickness of cover glasses.

For use in cases where cover glasses of the correct thickness of 0.17mm are not available, we supply the objectives with the designation D I in correction mounts which can be adjusted within a range of 0.12–0.22mm for the known or measured thickness of the cover glass. This always ensures that the objective gives its optimum performance. Where the cover glass thickness is unknown, the knurled ring on the objective is turned until the index line 17 corresponding with the assumed standard cover glass thickness is aligned with the fixed index mark and, after accurate focusing, the quality of the microscope image carefully noted. The setting of the correction mount is then slightly (1–2 divisions) altered in either direction; the image is refocused as accurately as possible, and attention paid to any increased contrast and sharpness. This will immediately show direction in which the adjustment has to proceed, and with some practice the optimum setting of the correction mount and hence the real cover glass thickness will be found quickly. Some of our objectives have a correction mount with automatic sharpness compensation. When this correction is adjusted, focus is preserved almost completely, so that only a slight readjustment is needed, facilitating the finding of the optimum focusing position.

Although immersion objectives, particularly oil immersions, are less sensitive to deviations from the standard cover glass thickness – thickness differences in the glass are largely compensated by corresponding changes in the thickness of the oil film – excessively thick cover glasses may prevent accurate focusing of the objective altogether: – The working distance of a powerful immersion objective is normally below 1/10mm, and the remaining distance is not enough to allow for the focusing movement.

(In microscopes with a drawtube deviations from the prescribed cover glass thickness can be compensated within narrow limits by changes in the tube extension. Cover glass thickness below 0.17mm require an increased tube length, above 0.17mm a decreased tube length. Proof of the correct drawtube adjustment is the best possible image. It must, however, be borne in mind that the magnification changes with the tube length).

## The Numerical Aperture

The performance of the microscope depends chiefly on its objective. E. Abbe was the first to show that the ability of a microscope to render the most minute details as separate features, to **resolve** them, does not depend on the scale of the objective, but on its Numerical Aperture. The importance of this term merits a more detailed discussion.

The numerical aperture

$$N.A. = n \cdot \sin \alpha$$

where α is the angle formed by the outermost ray of light (or its projection) admitted by the objective and the optical axis, and n the refractive index of the optical medium (e. g .air, water, immersion oil) through which this ray has to pass on its way between cover glass and front lens.

The decisive role played by the numerical aperture in determining the lateral resolving power is described on p. 29. The following three diagrams show the maximum aperture values possible in air, water, and oil.

Light emerges in all directions from an object point on the underside of the cover glass. If, as in fig. 11a, the space between cover glass and front lens contains air (refractive index n = 1.000), according to the law of refraction only those rays on the top of the cover glass are able to pass into the air space above, which are not totally reflected inside the glass, i. e. they must be inside the angle of total reflection, in this case 41° 30'. In air, the so-called "glancing exit", i. e. 90°, would correspond to this limiting angle. By the formula given above, this would result in the value 1.0 as the theoretical maximum numerical aperture for **dry systems.** However, it will be seen without difficulty that it is impossible to utilize this angle fully, since this would require the front lens to be in contact with the cover glass. Due to the distance necessary between cover glass and front lens and the limited size of the latter only a maximum angle of 72° is accepted; hence, the practically attainable maximum numerical aperture is 0.95 for this type of objective.

Fig. 11b shows a **water immersion** (n = 1.333) objective. Here, the angle of total reflection is 61° 30', which raises the theoretical limit of the numerical aperture to 1.333. However, the some technical conditions which we have met with in the preceding para-

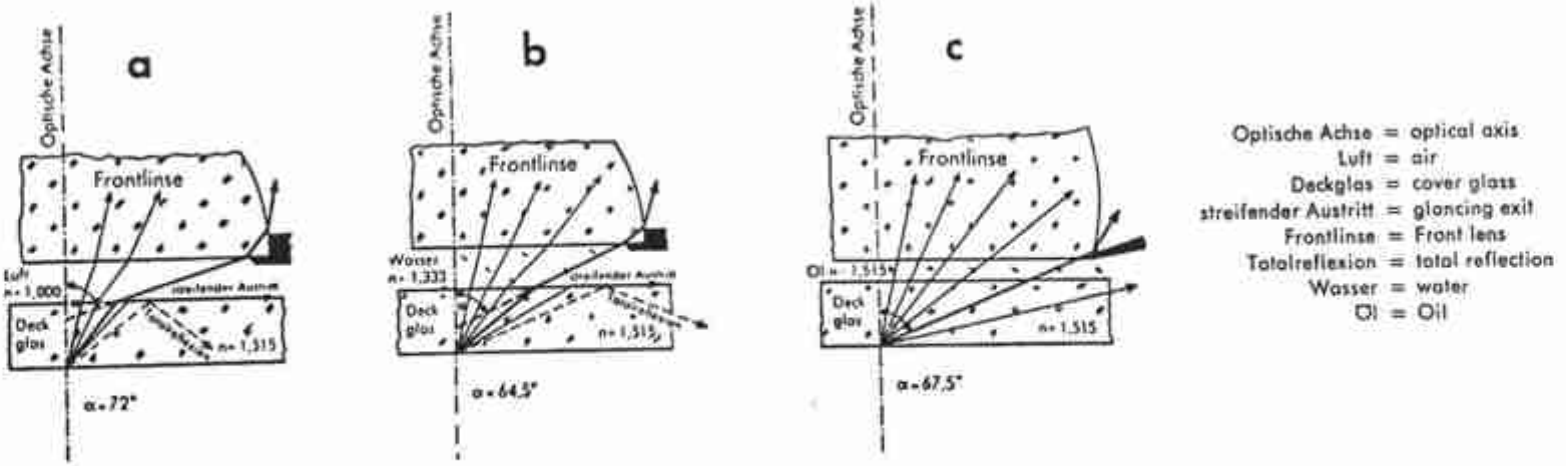



Fig. 11 Schematic representation of the aperture values.

graph restrict the useful angle in water to 64° 30', and therefore the practical maximum of the numerical aperture to **1.20.**

Finally, fig. 11c demonstrates the ray path in an **oil immersion** objective. Total reflection does not occur here, since cover glass and immersion oil have the same refractive index of $n = 1.515$. Thus, if only the front lens could be made large enough, all light proceeding within an angle of up to 90° with the optical axis would reach the front lens without deviation. However, the short focal lengths of the high power immersion objectives result in front lenses of diameters hardly more than 1mm; thus, in spite of small working distances they can only accept a maximum angle of 67° 30' in extreme cases; this corresponds to an upper limit of the numerical aperture of **1.40.**

The three limiting cases of practically attainable apertures are shown in figs. 11a–c by the rays drawn in bold lines. Between the cover glass and the objective their inclination is approximately the same, however, inside the cover glass the corresponding rays form a considerably larger angle in the case of an oil immersion (67° 30') than in that of a water immersion (52° 30') objective, let alone a dry system (39°). Incidentally, the numerical apertures can be calculated just as easily from these angles shown in brackets; one merely has to multiply the sine of each angle with the refractive index of the cover glass. This consideration shows with particular clarity how the numerical aperture and hence the resolving power of an objective increase directly with the cone of rays able to reach the objective from the object. It also becomes apparent how progressively less space remains for the front lens mount as the numerical aperture increases. The more than hemispherical front lenses of the high power immersion objectives can therefore be held in place by very narrow metal rims only, and it is obvious that the greatest care is necessary during use and cleaning lest the front lens is pushed back into its mount. In most such cases not only the front lens, but also the one immediately behind it would be liable to damage.

Every group of objectives contains some which do not have the maximum possible aperture. This does not place them in a category inferior to that of the first-class objectives; they have their own uses and are necessary in examinations calling for relatively low magnifications. According to considerations discussed on p. 31 the final magnification should generally remain within 500x and 1000x the numerical aperture of the objective. An excessively high numerical aperture would therefore not be utilized in such cases.

## The Components of the Objectives

The components of a microscope objective vary greatly, depending on whether it is a high-power or a low-power system, on the flatness of the image, and on the degree of its correction.

Fig. 12 shows a few characteristic examples designed to demonstrate that the very low-power objectives and especially those with the highest numerical apertures require a considerable number of lenses. They are reproduced at ⅔ natural size, and even the diagrammatic representation conveys clearly the difficulties of producing such objectives. Particular attention is drawn to the more than hemispherical front lenses of the high-power oil immersion objectives. Low-power systems raise the difficulty of limiting their working distances, i. e. the distance between the front lens and the surface of the cover glass, so that they can still be matched with the other objectives on the revolving nosepiece. Objectives are matched if their distance between the screw flange and the object is identical. This makes it unnecessary to use the coarse adjustment every time the objectives are changed; a slight turn of the micrometer screw is all that is required.

The objectives listed in the table on p. 15 are matched on the revolving nosepiece to a distance of 37mm from the 3.5/0.10 objective on wards.

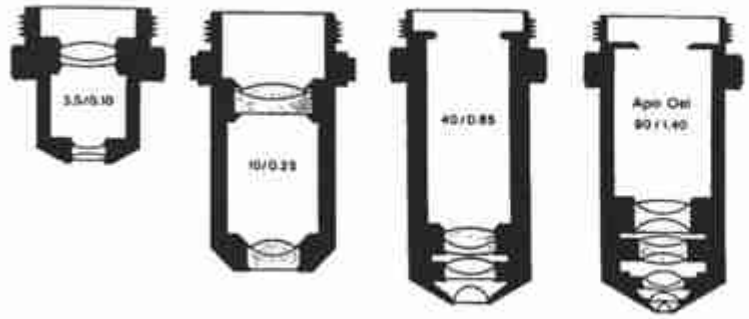
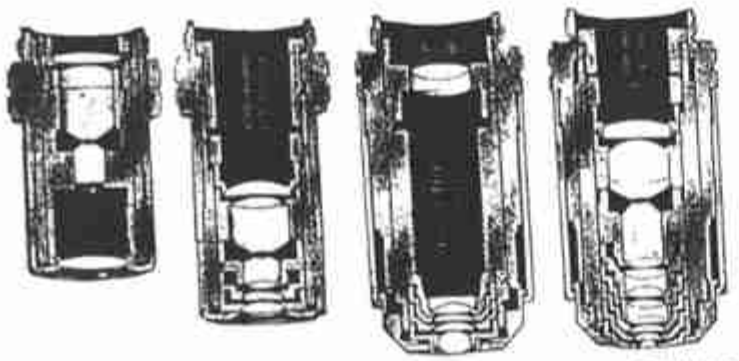



Fig. 12 Longitudinal section through characteristic normal objectives.



Pl 4/0.10 Pl 10/0.25 Pl 40/0.65 Pl Apo Oil 100/1.32

Fig. 13 Longitudinal section through plano objectives.

The plano-objectives (see following paragraphs) as well as several other new series of objectives are matched to 45mm instead of 37mm. Shorter objectives to be used together with the longer ones on the same nosepiece must be fitted with an 8mm adapter ring.

As collecting lens systems, microscope objectives form images on a surface which is curved towards the objective. This "field curvature" becomes more noticeable as the power of the objective increases. The difficulties of removing it are considerable, but have been surmounted in the design of the so-called **plano** (or flatfield) **objectives,** (fig. 13), in which the curvature of the image is practically eliminated for the largest fields visible in the microscope. As a group they are distinct from the **normal objectives** (fig. 12).

With a plano objective, a plane object can therefore be examined directly across the entire image field, whereas with a normal objective the same field must be refocused for the centre or the periphery of the image, and only part of the field is seen in sharp focus at any one time. Often, especially during routine work, refocusing will not be found disturbing, since the fine adjustment must in any case be used for the examination of thick specimens; it may even be possible to obtain an image which is sharp from edge to edge with extremely thick objects.

However, the situation is different, for instance, in photomicrography and with thin specimens. Here, plano objectives will generally show clear advantages.

The classification of the objectives according to their other characteristics of correction does not depend on the flatness of their field.

**Achromats,** made entirely of glass, are the most commonly used microscope objectives for general scientific and practical investigations. It has been possible to improve their state of correction progressively due to advances in glass technology. Of these objectives, only the highest apertures cannot be completely corrected.

**Fluorite systems.** The fluorite systems represent an improvement in this direction; fluorspar lenses are used in their construction.

The designation of these systems is "Fl". Thus, Fl Oil 95/1.32 is the particularly popular high-power fluorite immersion objective, with a scale of 95 : 1, and a numerical aperture of 1.32.

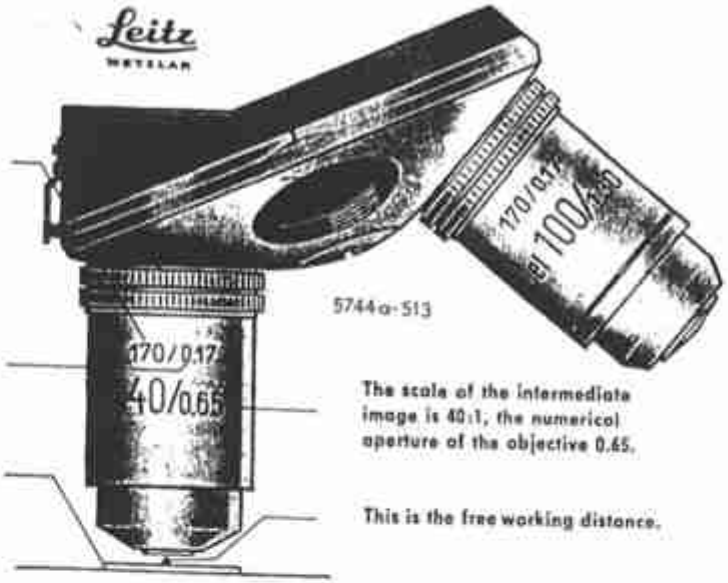
Fluorspar or fluorite is a naturally occuring, and, recently, also synthetically produced mineral of very low refractive power and negligible colour dispersion not yet achieved with ordinary types of glass. These qualities make it possible to improve colour correction and spherical correction beyond that of the achromats.

**Apochromats.** The apochromatic objectives, designed "Apo", e. g. Apo 40/0.95 (an apochromatic dry system with a scale of 40 : 1, and a numerical aperture of 0.95) have the highest degree of correction. The images they produce fascinate through their outstanding brilliance. The characteristic feature of apochromatic design is the liberal use of fluorite or similar material for the lenses.

The high price of these objectives is due to their complex structure as well as to the high cost of optically suitable fluorite.

When choosing objectives of a certain power it is important to realize that apertures generally increase from the achromats through the fluorite systems to the apochromats; this affects resolving power (see p. 29) and resolution in depth (see p. 32).

## The engraving of the objectives




The objective is corrected for a tube length of 170mm.

The cover glass of the preparation should be about 0,17mm thick. A dash (–) engraved instead of this number indicates that the objective can be used with or without cover glass.

The scale of the intermediate image is 40:1, the numerical aperture of the objective 0.65.

This is the free working distance.

Oil immersion (designated by a black ring): – A drop of immersion oil should be inserted between front lens and cover glass before observation. The reproduction scale of the intermediate image is 100:1, the aperture of the objective 1.30.

Fig. 14 Engraved objective data.

The objectives are classified according to the scale of the image they form and their numerical aperture. In addition any immersion fluid with which they have to be used, and their type of correction is stated. In the absence of any information about the last two points the objective is an achromatic dry system used without immersion fluid. A 10/0.25 objective is an achromatic dry system with a scale of 10 : 1 and a numerical aperture of 0.25.

In addition to these data, our more recent objectives have two numbers engraved near the top, of which the first indicates the tube length, and the second the cover glass thickness with which the objective should be used. If a cover glass is essential, the number 0.17 is engraved. A dash is shown on objectives which can **also** be used **without** cover glass, while objectives which should **always** be used **without** cover glass bear the number 0. Attention should be paid to the tube lengths 170, 185, 215, and ∞.

## Immersion oil

Optically, the immersion oil which fills the space between cover glass and the front lens of a suitable objective is a part of that objective. It is therefore of great importance that the oil used should have precisely the qualities stipulated during the computation of the objective. For our own objectives we require a refractive index of precisely $n_D = 1.515$ at 20 °C for the D line of sodium light, and a dispersion $\nu = 49$. $\nu$ is a well-known Abbe number defined as $(n_D - ) : (n_F - n_C)$.

These values apply to the formerly popular natural cedarwood oil, which was diluted with xylene but thickened easily, as well as to our new synthetic oil, which does not thicken.

Since many products are marketed under the name of "immersion oil" the optical data of which, particularly their dispersion, differ from those of our oil, it is urgently recommended to use LEITZ immersion oil from original bottles only.

## Eyepieces

In order to make full use of the properties of objectives it is of great importance to insert always the most suitable type of eyepiece. Information on this point will be found in the column "Type of eyepiece" in the table on p. 15.

There is no advantage in using eyepieces weaker than 6x, because even the simplest eyepieces of this power make it possible to scan the entire cross section of the tube; a further reduction in the power of the eyepiece does not result in any increase in the field to be observed. Eyepiece of less than 6x magnification can be usefully employed for projection purposes only.

For **measuring purposes,** special eyepieces with focusing eyelenses are produced; they allow focusing on a scale division mounted in the plane of the eyepiece diaphragm (see pp. 34, 35).

Fig. 15 Typical eyepieces: – Pair of eyepieces for spectacle wearers; pair of PERIPLAN eyepieces (designation B for paired eyepieces in binocular tubes); micrometer eyepiece with focusing eyelens.

**Huygens eyepieces** are the simplest and also the cheapest. This type is best suited for the objectives marked "H" in the column "Type of eyepiece" in the table on p. 15. Due to their low price, they are often used with other objectives, too. But it should be borne in mind that they do not do complete justice to the high image quality of the plano objectives, normal fluorite systems, and apochromats. They are distinguished from other types of eyepiece by having only their magnification and the manufacturer's name engraved on their mount. The magnifications of Huygens eyepieces generally range between 6.3x and 16x.

Fig. 16 Longitudinal section through eyepieces.

PERIPLAN 8x

Huygens 8x

**Eyepieces PERIPLAN®** are made up of cemented members and so adapted to the state of correction of the high-power achromats, fluorite systems, and apochromats, that the good qualities of these objectives are utilized to the full.

Their correction is an improvement on that of our earlier compensating eyepieces, which they have replaced. The group of eyepieces PERIPLAN covers magnifications from 6.3x to 25x.

The various series of periplanatic eyepieces are distinguished by their fields of view and the plane of their exit pupil.

The field of view of an eyepiece is the area of the intermediate image in the tube covered by it. It appears magnified at the magnification of the eyepiece. Thus, the image diameter of a 10x eyepiece with a field of view diameter of 18mm will, since the magnification is referred to the standard distance of comfortable vision of 250mm, appear as large as an area with a diameter of 10 x 18 = 180mm, situated 250mm in front of the observer.

The image can be made visible at this size on a groundglass screen 250mm above the eyepiece.

If the diameter of this field of view is divided by the scale of the intermediate image, the diameter of the object area reproduced is obtained. Thus, with the 10x eyepiece mentioned above with an 18mm field of view a 40:1 objective and a 1.25x tube lens, a field in the object with a diameter of 18mm: (40 x 1.25) = 0.36mm can be examined.

The **normal eyepieces PERIPLAN** have fields of view appearing to the observer at a size from 110 to 150mm when he looks down the eyepiece.

The **PERIPLAN wide field Eyepieces, PERIPLAN GF,** have considerably larger fields of view, making it possible to scan larger fields of the object; these appear at a diameter of 180 to 250mm through the PERIPLAN GF eyepieces.

Still larger fields can be surveyed with **eyepieces PERIPLAN for large fields and wide tubes** (diameter 30mm), **PERIPLAN GW,** but only in combination with special tubes, e. g. on the large metallographic microscope or on the binocular tube FSA with automatic interpupillary distance compensation.

While the eyepieces described so far are designed for the observer's eye to be about 8–10mm above the topmost lens surface (pupil distance), the high-point **(spectacle wearers') eyepieces PERIPLAN, PERIPLAN** 60, permit observation through spectacles, compensating visual astigmatism. Here, the pupil distance is approximately 20mm.

## Tube lenses

As we have already mentioned during the discussion of binocular observation, a third optical component, the tube lens, contributes towards the formation of the final picture in large microscopes. In order to determine the final magnification of the microscope correctly (see p. 11), it is important to consider the primary magnification of the tube lens, which is prominently engraved on the new tubes; in the case of older models the catalogue should be consulted. In universal stands on which various types of special objectives computed for other mechanical tube lengths (185, 215, and ∞) can be used, the tube lens systems can sometimes be interchanged. Great care should be taken to match these systems with their appropriate series of objectives corresponding to their designation; confusion here would have the same effect on image quality as a deviation from the correct tube length.

## Condensers

Condensers are systems of lenses and mirrors with which the object is illuminated; the term condenser covers the lens system proper, while the illuminating device includes mirrors and stops. According to their purpose, one distinguishes between bright field and dark field condensers.

In **bright field illumination** light from the condenser passes through the object, and enters the objective. Thus, a silhouette, as it were, is seen of the less transparent, or completely opaque, portions of the object against a bright field of view. In contrast, the illuminating rays in **dark field illumination** are directed towards the object at a very oblique angle, by-passing the objective. Only the light deflected by the object or accidentally reflected into the objective forms the image here, showing the outline of the object under observation in brilliant bright light against a dark background. Since the only basis of this method of observation is the difference in the refractive powers of the object and its surroundings, dark field illumination is recommended whenever thin, unstained specimens such as bacteria are to be examined.

Fig. 17
Bright field condensers Nos. 601, 65 upper row and Nos. 72, 66 lower row

**Bright field condenser.** It is quite obvious that the size of the angle under which the illuminating rays pass through the object must correspond with that of the angle the objective is able to accept. In addition, contrary to our merely diagrammatic representation on p. 8, it must be possible to transilluminate widely varying portions of the specimen according to whether a tiny field is to be examined under the highest power, or a large field under a low power. Thus, the size of the field changes in the inverse ratio to the magnification. If we scan, e. g., a field of 10mm under a final magnification of 20x, the field will only be 0.2mm under a magnification of 1000x. These very divergen conditions should, if possible, be met by a single condenser.

Fig. 18 Dark field condensers D 1.20 A and D 0.80.

Simple condensers allow a changeover from high- to low-powe objectives with artifical light sources; all that is necessary is t unscrew the front lens in order to enlarge the illuminated fiel and to decrease the aperture. With other condensers the len nearest the object is removed from the light path by means of lever in order to change from high- to low-power observation Generally the dividing line is an objective magnification of 10: and a numerical aperture of 0.25.

The condenser apertures required depend on the type of observ ation and usually amount to ½ to ⅔ of the objective aperture For research purposes one must be able to choose the condense aperture as large as the objective aperture. It must, however, b borne in mind that apertures larger than about 0.90 can b utilized only if condenser and object carrier are optically joine by a drop of oil. It is a well known fact that total reflection wil occur at the front surface of the condenser at N. A. = 1.0, o which, however, an aperture of only 0.90 will become effectiv due to the thickness of the object carrier. This effect has alread been explained during the discussion of the apertures of dr objectives.

Normally the condensers are fitted with a diaphragm which con trols the aperture only when the front lens is screwed (or turn ed) in.

The two-diaphragm condenser after Berek will be described i column 2 of this page; condensers Nos. 601, 602, and 603 see p. 2

## Condenser apertures above 1.0

For condenser apertures, as for objective apertures, above 1.0 it is essential to use an immersion fluid with the optical component, in this case with the condenser. As a rule, the apertures of the microscope condensers do not exceed 1.20. However, for special purposes condensers are available with which apertures of up to 1.40 are possible. The immersion fluid, normally immersion oil, is placed on the condenser front lens and the condenser raised until the fluid touches the underside of the object carrier so that an optically homogeneous unit is procuded.

## The Two-diaphragm Condenser after Berek

This condenser is distinguished from the conventional types by having two diaphragms, of which one controls the aperture, while the other serves to limit the illuminated field (so-called Koehler's Illumination). The normal model permits apertures of up to 0.95 However, with a special screw-on immersion condenser cap apertures of up to 1.40 can be obtained.

For powerful objectives from N. A. 0.25 upwards the front lens of the condenser is turned in. The lower iris diaphragm (knurled ring) acts as a field diaphragm, the upper, lever-operated iris diaphragm as an aperture diaphragm. A scale division indicates the diameter of the stop set in mm.

For low-power objectives below N. A. 0.25 the front lens of the condenser is turned out. The lower iris diaphragm will then function as an aperture diaphragm; the upper, lever-operated iris diaphragm has lost its effect and must be open.

When the illumination is adjusted the condenser is raised as far as possible with its rack-and-pinion movement, and the front lens turned in. The condenser diaphragms should be open. The upper diaphragm is adjusted by the lateral lever, the lower by the knurled ring. After a preliminary centration of the microscope lamp, to be carried out in the normal way (p. 12) and the focusing of the specimen with an objective of about 10x magnification the field diaphragm (knurled ring) is closed (without regard to the full illumination of the field) so that it will be completely visible in the field; the condenser is now lowered a little with the lateral rack-and-pinion drive until the diaphragm is also in sharp focus. This is the position in which the condenser must henceforth be kept even if the objectives are subsequently interchanged (a negligible adjustment to focus the field diaphragm will become necessary only if a new specimen mounted on a slide of different thickness is placed on the stage. If the slide is thicker than 1.2mm, the field diaphragm can no longer be focused sharply; this does not, however, impair its function. In this case, the condenser remains in its topmost position against the stop). If the field stop does not appear in the centre of the field, the condenser must be centred by means of its two centring screws, and the stop opened just far enough to clear the entire field seen in the eyepiece. The light source is now adjusted for the optimum illumination of the field.

The condenser is lowered, and a fair-sized drop of immersion oil placed on top. The specimen is focused with a low-power (preferably 10/0.25) objective. The condenser is now raised until the drop of oil touches the underside of the microscope slide. In the field of view appears a slightly unsharp bright ring which contracts into a light circle as the condenser is further raised. This bright spot, or, easier still, the light ring is readily moved into the centre of the field of view by means of the condenser centring screws.

For the examination proper an immersion objective is turned in which must be stopped down to N. A. 1.15 by means of a funnel stop or a built-in iris diaphragm in order to obtain a perfect darkfield. A dry objective of large aperture such as the Apo 63/0.95 can be used instead of an immersion objective.

Preparations to be illuminated with the darkfield condenser D 1.20 must be embedded between coverglass and slide in a medium of a higher refractive index than 1.20.

During the changeover to more powerful objectives the field diaphragm is readjusted so that again the field is just fully illuminated. If the diaphragm is no longer concentric with the field of view, the condenser must be recentred.

During the changeover to weaker objectives, below N. A. 0.25, the front lens is simply turned out and the upper iris diaphragm opened, while the aperture of the illumination is controlled by the lower iris diaphragm (which, with the front lens turned in, had functioned as field diaphragm). A field aperture is no longer required with these low-power objectives, since the field illuminated by the condenser with the front lens turned out is adapted to the diameter of the field of view of the low-power objectives.

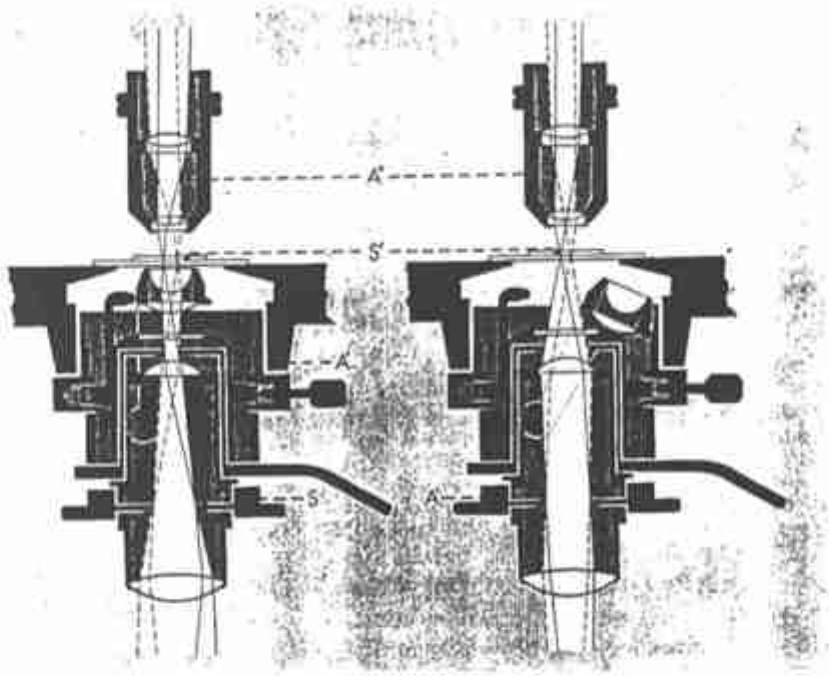
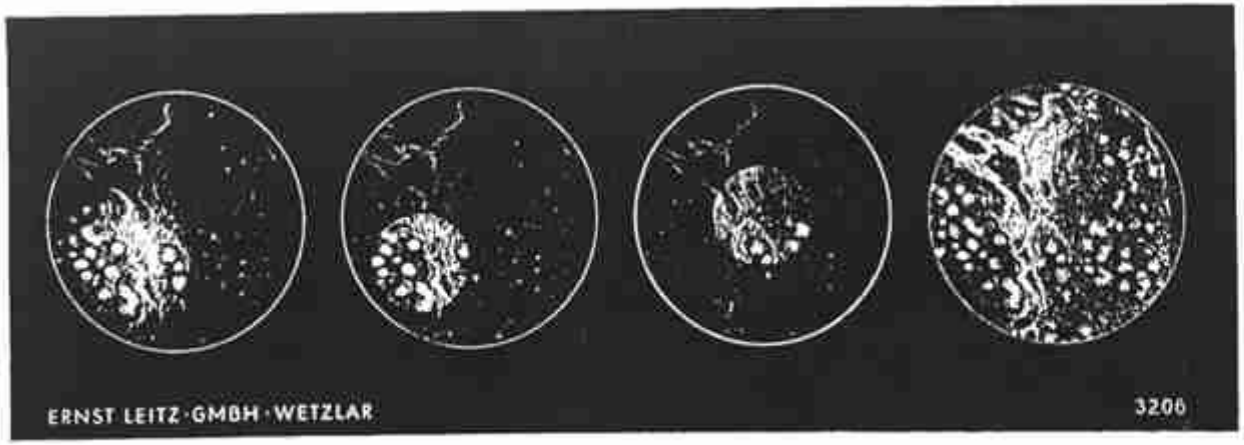



Fig. 19 Beam path in the two-diaphragm condenser after Berek.

The field diaphragm, which at first appears out of focus (picture on the left) is focused by lowering the condenser (left centre). The image of the diaphragm is moved into the centre of the field by means of the centring screws on the condenser (right centre) and the diaphragm opened until its image just clears the field of view (picture on the right).

Fig. 20 Centring the field aperture.



## Two-diaphragm illumination with the Field Diaphragm built into the base of the Microscope

(Koehler's Illumination).

Whereas in the two-diaphragm condenser after Berek aperture- and field diaphragm are combined, the field diaphragm can also be mounted in the base of the microscope. The installation of the field diaphragm in the microscope base calls for condensers which must be matched for the fixed distance of the field diaphragm in the foot, so that the best possible use can be made of all the facilities offered (Fig. 21). At the same time this arrangement has the advantage of greater freedom in the correction of the condensers.

Our condensers 601, 602 and 603 have been specially computed for our stands with built-in field diaphragm; they provide correct illumination and offer facilities of the best possible spherical and chromatic correction. They are readily distinguished from the two-diaphragm condenser through the absence of the lower diaphragm ring; they have an aperture diaphragm only.

The lower part with aperture diaphragm and centring device is identical for all three condensers and has a numerical aperture of 0.25. The condenser tops 0.90 As and Achr. 0.90 combine with the lower part to form the aspherical swing-out condenser No. 601 and the achromatic swing-out condenser No. 602 respectively. Both condensers have an aperture of 0.90. The condenser top Apl Oil 1.25 is mounted to obtain the aplanatic swing-out condenser No. 603, with an aperture of 1.25. It can only be used with immersion oil between the condenser top and the object slide.

For its operation generally the rules for setting up Koehler's Illumination apply; these have already been discussed in connection with the two-diaphragm condenser after Berek. However, since the controls and the distances in the case of illumination with the iris diaphragm in the base of the microscope differ somewhat from those with the two-diaphragm condenser after Berek, the basic rules of setting up this illumination are once more given below: –

1.) Swing in field lens in the base of the microscope, open field diaphragm. Check with a piece of white paper, groundglass disc, etc., on the dust glass whether the lamp is centred and remains so when the built-in focusing eye condenser on the lamp housing is moved.

2.) Swing in front lens of the condenser, open aperture diaphragm, raise condenser as far as possible, focus specimen with a low-power objective; after adjusting the field diaphragm (closing it completely if necessary) vertically adjust the condenser until the edge of the now visible field diaphragm is focused sharply without colour fringes.

3.) Turn in a medium power objective; the field diaphragm must remain visible. When it is opened, its image must disappear uniformly behind the edge of the field of view; otherwise, the condenser must be recentred with the centring screws.

4.) Adjust contrast, resolution and image brightness by means of the aperture diaphragm and the regualting transformer for optimum visual observation.

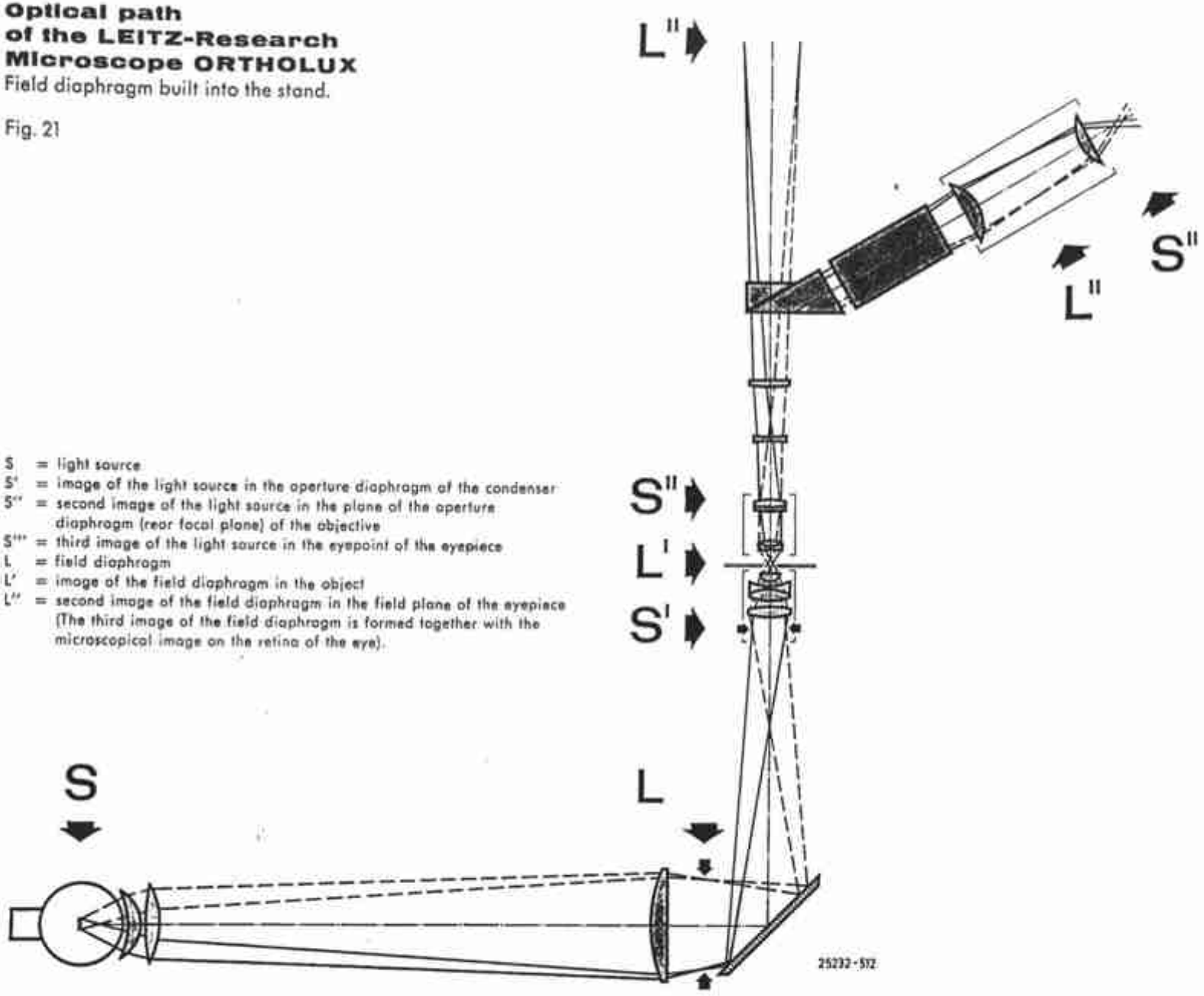
5.) Whenever an objective is changed (change of magnification) the field diaphragm must be readjusted to match the new field of view, and the optimum illumination obtained by operating the aperture diaphragm.

6.) With **low-power** objectives (general views, objectives up to 10x) it is also possible to form a sharp image of the field diaphragm in the object plane, although with visual observation this is usually omitted. In photomicrography, however, it is of advantage to eliminate stray light, as it increases brilliance. It is therefore advisable in this case to lower the entire condenser through about 20mm with the condenser top swung out and the aperture diaphragm fully open, until the field diaphragm appears sharp in the field of view. The function of the aperture diaphragm affecting contrast and brightness is fully preserved in both positions of the swing-out condenser top.

**Optical path of the LEITZ-Research Microscope ORTHOLUX**

Field diaphragm built into the stand.

Fig. 21




S = light source
S' = image of the light source in the aperture diaphragm of the condenser
S'' = second image of the light source in the plane of the aperture diaphragm (rear focal plane) of the objective
S''' = third image of the light source in the eyepoint of the eyepiece
L = field diaphragm
L' = image of the field diaphragm in the object
L'' = second image of the field diaphragm in the field plane of the eyepiece (The third image of the field diaphragm is formed together with the microscopical image on the retina of the eye).

## Adjustment of the Microscope Illumination

Suitable illumination is an essential requirement for all microscopic examinations. Particular attention must therefore be paid to the adjustment of the illuminating device.

With low-power objectives the simple microscope mirror without condenser will be quite adequate. Whether the plane or the concave mirror is preferred depends on the type of light source used and on its distance. Since the focal point of the concave mirror lies near the plane of the object, a condenser is essential with a concave mirror where the light source has a definite structure, unless the light is made sufficiently diffuse by the insertion of a groundglass screen.

The aperture of the concave mirror is 0.30.

In order to adjust the illumination, a low-power objective is used together with a medium-power eyepiece, as has already been pointed out, and the object focused approximately. The eyepiece is now removed from the tube and the microscope mirror tilted so that the rear lens of the objective appears at its brightest and most evenly lit. The eyepiece is replaced in the tube and the specimen focused sharply. Excessive light intensity may be cut down by the insertion of a groundglass screen or adjustment of the transformer.

When medium- and high-power objectives are used the correct adjustment of the condenser iris diaphragm to vary the condenser aperture is of the greatest importance. It is best to determine the degree to which the illuminating rays are stopped down by the graduate closing of the iris diaphragm while looking down the tube after the removal of the eyepiece. (see p. 9).

The image of the iris diaphragm is seen in the rear lens of the objective. If possible, every observation should start with a condenser aperture of the same diameter as that of the objective, i. e. the iris diaphragm must be opened just wide enough fully to reveal the rear lens of the objective to an observer looking down the tube without the eyepiece. The entire rear area of the objective should be evenly filled with light. Otherwise, e. g. with artificial light, the centring of the light source may have to be checked, or a groundglass screen placed in front of the mirror or inserted in the lamp attachment. With illumination at full aperture, only object detail well set off against its surroundings by differences in absorption will be clearly visible. Such detail, provided a top-quality objective is used, will then be reproduced at optimum resolution. However, most specimens also contain some detail the absorption of which differs little from that of their surroundings, and also features the refractive index of which is almost the same as that of their surroundings. This detail, too, must be rendered visible. This is achieved by a very gradual procedure: – when everything visible at full aperture has been sufficiently registered by the observer, the iris diaphragm is closed a little, say to about $^2/_3$ of the diameter of the free rear lens of the objective, then to $^1/_2$, and finally to $^1/_4$. During this gradual closing of the iris diaphragm the structural detail which at first showed little differentiation from its surroundings will stand out progressively more clearly, and can easily be fitted into the previously seen pure absorption image. The resolution of the detail reproduced by absorption alone steadily decreases when the iris diaphragm is closed as the condenser aperture becomes smaller than that of the objective; at the same time the depth of field increases. On the other hand, the original resolution corresponding to full condenser aperture is largely maintained for detail visible only due to its differential refractive index, even when the iris diaphragm is closed.

Thus, the aperture diaphragm should be used only to achieve the best possible image quality, not in order to regulate the image brightness.

This procedure should be most thoroughly studied, because it is the only correct method of using the microscope. The often-recommended way of chiefly working with a small stop or oblique illumination through an eccentric iris diaphragm is ill-advised, and is justified only with periodic structures.

Since the upper focal plane of the condensers at their correct height lies near the object plane, a feature at a relatively large distance from the microscope, such as a window frame, may be reproduced in the object plane if daylight is used as light source. If the microscope or the mirror cannot be adjusted so that the disturbing feature is removed from the field of view, the insertion of a groundglass screen is recommended. Also, a slight vertical adjustment of the condenser or a switchover from the plane to the concave mirror may be an effective remedy.

With objectives of outstanding correction, marginal, annular illumination can be used for making visible and resolving certain detail. For this purpose a central stop is inserted in the illuminating attachment; on looking down the tube after the removal of

the eyepiece, the observer should see a ring-shaped beam of light taking up approximately the outer 1/4 or 1/3 of the diameter of the rear lens of the objective. Any advantage of this method will, however, be realized with particularly well corrected objectives only.

## The Lateral Resolving Power

A given final magnification in a microscope can be produced either with an objective of low initial mangnification and a powerful eyepiece, or, conversely, with an objective of high initial magnification and a weak eyepiece. Both images will appear basically different; the second will show considerably better detail than the first.

The reason for this is the generally higher aperture of the powerful compared with the low-power objective, because the extent to which fine detail can be recognized depends on the size of the objective aperture used.

The ability of an objective to render two closely adjacent object points as separate visual elements is called its **resolving power;** we speak of **lateral** resolving power when the points concerned lie in a plane vertical to the optical axis. The value $\delta$ of the resolving power is defined as the minimum distance between two points seen as separate features. Thus, a resolving power of 1 $\mu$m means that two point-shaped particles separated by a distance of 1 $\mu$m can still be seen as two different parts, while at a distance of, say, 0.8 $\mu$m, they would appear as a single feature.

This will become immediately clear if the following facts are borne in mind: –

Even an objective of perfect spherical and chromatic correction does not produce an ideal image: – it does not reproduce a point in the object as a point in the image; every object point in the focusing plane of the microscope has as its image a tiny conjugate light disc, called a diffraction disc. This phenomenon is based on the wave nature of light. If we imagine this diffraction disc projected back into the object, i. e. if we think of its dimensions in terms of dimensions of the object detail, its diameter at full condenser aperture will be $d = 1.22\lambda/N.A.$, where $\lambda$ represents the wave length of light, N. A. the numerical aperture of the objective, and 1.22 a theoretical factor. The diffraction disc is surrounded by darker diffraction rings which are, however, less bright, visible only in dark field illumination, and can normally be disregarded. The size of this apparent diffraction disc, measured in the object plane, is obviously decisive for the resolving power.

If two object points are situated so that their diffraction discs just touch each other, they can certainly be observed as two distinct features. The two object points can be considered separate

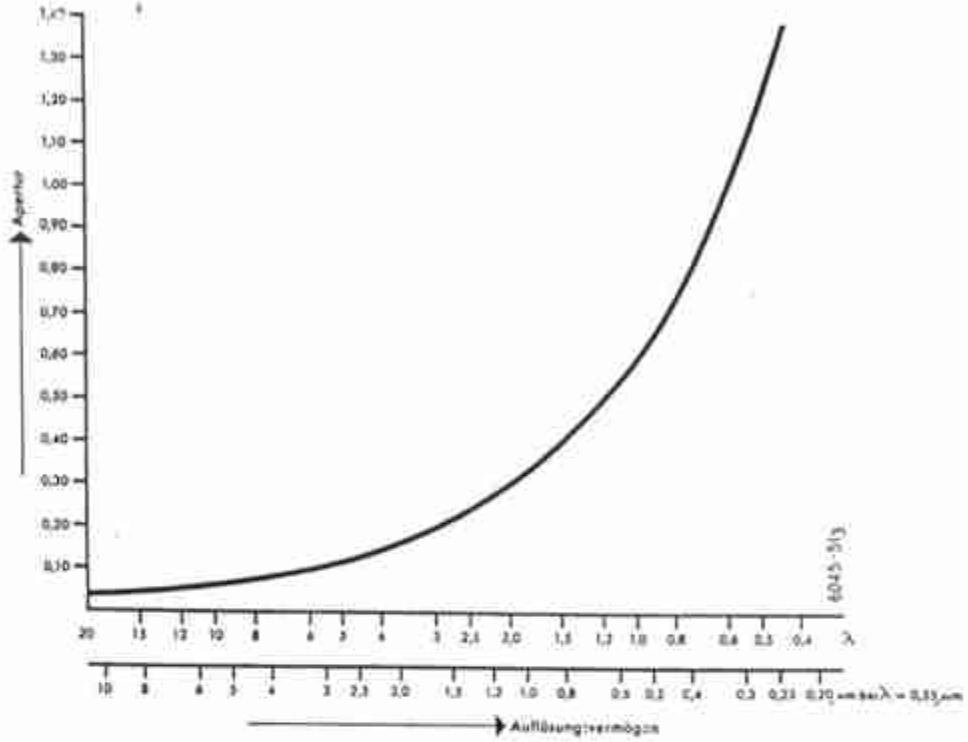



Fig. 22 Resolving power in $\lambda$ and for green light ($\lambda$ = 550mμ) depending on the aperture

points even if their diffraction discs are partly superposed and interfere with each other. However, the ability to differentiate also depends on the ability of our eyes to recognize differences in form and brightness. After all this has been taken into account, the resolving power can be defined as follows after Berek: –

$$\delta = \varkappa \frac{\lambda}{\text{N. A.}}$$

where $\varkappa$ is a factor incorporating these conditions; usually it can be taken to be much smaller than 1. From this it follows that with a given kind of light ($\lambda$) the resolving power can be increased only by increasing the aperture.

The remarks about the reproduction of a point logically apply to the reproduction of a line, except that here the diffraction disc is replaced by a diffraction band.

Fig. 22 gives the approximate values of the resolving power in light wave lengths and $\mu$ (for $\lambda$ 0.55 $\mu$) depending on the aperture.

It is seen that the resolving power obtainable with a microscope using a high-power oil immersion objective will optimally reach about ½ wave length. Since the ratio of violet and red light wave lengths is approximately 1 : 2, the resolving power can be roughly doubled within the visible range of the spectrum simply by changing from long-wave, red light, to short-wave, blue light.

The optimum performance of an objective will be fully realized only if its state of correction is as near perfect as possible. This condition must be met all the more strictly the wider the cones of rays used for the illumination.

In a special borderline case, preferred particularly by E. Abbe when he developed his theory, the question about the limits of

performance of a microscope is answered in a different way. If an object of a periodic structure, such as a grating, is illuminated by a narrow beam of light of a very small aperture (almost parallel light) it can be shown that a periodically recurring structural feature in the object will just appear in the image if its value

$d = \frac{\lambda}{N.A.}$ with straight, and

$d = \frac{\lambda}{2\,N.A.}$ with extremely oblique illumination.

If we revert to the question outlined at the beginning of this chapter, we will appreciate that the realization of a desired magnification is not the only factor in the reproduction of microscopical detail; as a first step, the aperture of the objective must be chosen so that a certain resolving power is ensured. The eyepiece magnification must then be selected so that all the detail resolved by the aperture of the objective is presented to the eye at comfortable dimensions, i. e. at a sufficiently large angle of view. This is the case when the eyepiece magnification $M_{ey}$ is chosen

$$\text{between } 500\ \frac{N.A.}{M_{ob}} \text{ and } 1000\ \frac{N.A.}{M_{ob}}$$

or, in other words, when the final magnification of the microscope lies between

500 and 1000 x the value of the N. A.

of the objective used. This range represents the so-called "useful magnification". Final values appreciably exceeding this limit will result in "empty" magnification, while final magnifications below the lower limit do not make full use of the potentialities of the optics. This rule applies also to photomicrography and microprojection; the operative value is always the final magnification of the microscope image.

Thus, the 40/0.65 objective is best used with eyepieces from 8x to 16x in order to obtain magnifications between 320x and 630x, approximately between 500 x 0.65 and 1000 x 0.65. It would be pointless to take, say, the 25x eyepiece here in order to see "more" – the 1000x magnification achieved with it would not resolve any additional detail of the preparation; it could, however, make the reading of measurements considerably easier.

Nor must the magnification factor of any tube lens in the microscope be overlooked in the consideration of the final magnification, as we have shown on p. 11. In such cases obviously a correspondingly lower eyepiece magnification must be used. In our present example this means that with the inclined monocular tube with a tube lens factor of 1.25x, the highest useful eyepiece magnification is now 12.5x.

## The Axial Resolving Power
(Depth of Field)

In the last chapter we discussed the limits of the resolution of structural details close to each other in a plane vertical to the optical axis; we must now deal with the question of how far structural details situated at various levels can be clearly recognized as lying in different planes (axial resolving power). This will become acute when they require individual focusing by means of the micrometer screw.

According to the ideal laws of geometrical optics only **one** plane passing through the object should be reproduced sharp at a given focusing adjustment. Since microscope objectives of normal design, particularly the high-power ones, are never completely free from field curvature, the focusing plane is replaced by a curved surface. However, the wave nature of light and physiological factors which have a bearing on lateral resolving power also affect the question of depth of field. As a result, a small extent in depth instead of a plane will appear uniformly sharp at a given focusing position. This extent in depth T, in contrast with the lateral resolving power, depends not only on the numerical aperture of the objective used, but also on the final magnification of the microscope. These relationships (for full condenser apertures) are shown in the graphs opposite (fig. 23).

If, for instance, differences of 4 $\mu$m in depth must be measured in an object, the value T = 4 $\mu$m is found on the vertical scale of the diagram, and a horizontal projected to the right. All points of intersection of the aperture curves with this straight line, and all points of the aperture curves lying above it will produce the desired or even better resolution. Let us assume the 25/0.50 objective is used. A vertical is drawn from the point of intersection of the projected horizontal and the aperture curve 0.50, when the value 400 will be found as the required final magnification of the microscope. Since the primary magnification of the objective is 25 : 1, at least a 16x eyepiece must be used in order to obtain the necessary final magnification. The diagram shows that in order to achieve high axial resolution (small value of T) both high apertures **and** high final magnifications are called for; *in such cases the highest-power eyepieces should be used regardless of the fact that the upper limit of useful magnification, i. e. of the lateral resolving power (see p. 29), may be considerably exceeded.*

The diagram will satisfy most needs. The following formula, established by M. Berek after thorough investigations, is used for the calculation of the depth of field T in special cases: –

$$T = n_e \left| \frac{4\lambda}{A^2} \varkappa_a + \frac{S}{AV} \omega \right|$$

The first element inside the brackets allows for the wave nature of light, the second refers to the capacity of the human eye. $n_e$ is the refractive index in the specimen (in medical and biological objects normally an average of 1.45). $\lambda$, as usual, stands for the wave length of light, for which generally the value of 0.00055mm can be inserted. A is the numerical aperture of the objective, V the total magnification of the microscope referred to the minimum distance of comfortable vision S = 250mm (see pp. 10 and 11). The constants $\varkappa a = 1/_8$ and $\omega = 0.00136$ are derived from experiments. For evaluations, $\lambda$ and S must be expressed in the same units of length.

If the refractive index of an object differs from the value $n_e = 1.45$ assumed in the diagram, the correct values for T are obtained by multiplying the tabulated values by $n'_e \div 1.45$, where $n'_e$ is the refractive index of the object. Thus, $n'_e = 1.515$ for an object embedded in oil, hence $n'_e \div n_e = 1.515 \div 1{,}45 = 1.04$. A depth-of-field range of 16 $\mu$ as it may be found, for instance, on the aperture line 0.25 for a final magnification of 250x (due to, say, the 10/0.25 objective and 25x eyepiece), will increase to 16 $\mu$ x 1.04 = 17 $\mu$ in an oil-embedded specimen. For a specimen in air, on the other hand, it would be reduced to 16 $\mu \cdot (1 \div 1.45)$ = 16 $\mu \cdot 0.69$ = 11 $\mu$.

When low final magnifications are used in the microscope, the depth of field T can appear considerably enlarged by the ability of the eye to adjust itself to near and far distances (accommodation), by the value T' to be added to T: –

$$T' = n_e \frac{S^2}{V^2} \cdot \left( \frac{1}{S_1} - \frac{1}{S_2} \right)$$

where $S_1$ is the lower, $S_2$ the upper limit of accommodation. For the normal eye they are approximately 250mm and $\infty$ respectively.

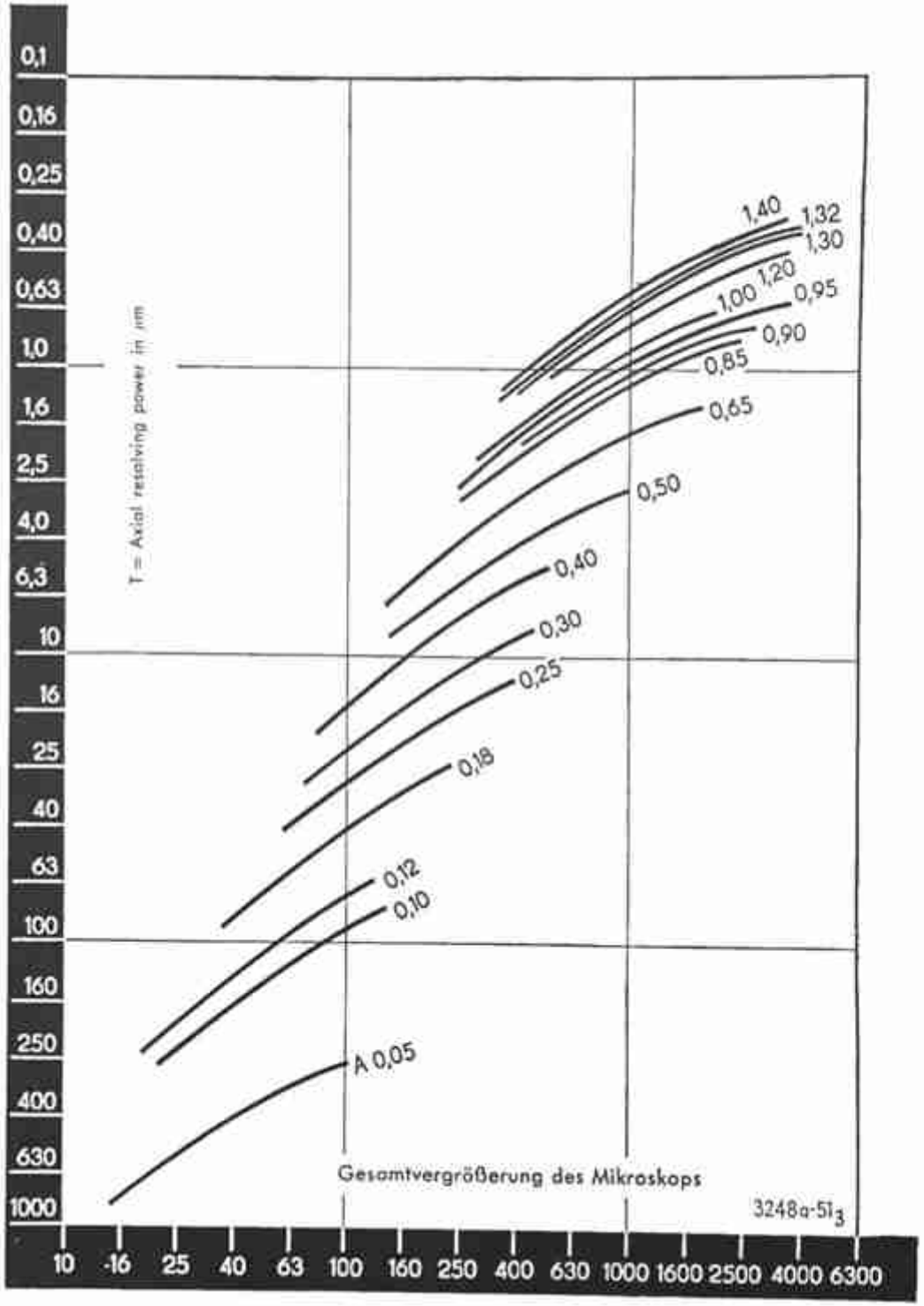



Fig. 23
Axial resolving power in $m_\mu$ for biological specimen n = 1.45 (mean value) and $\lambda = 555\,m_\mu$.

For this limiting value and $n_s = 1.45$ the following table gives the values of T' in $\mu$ for some magnifications. They must be added to the values taken from the diagram or calculated with the formula given on p. 32: –

| V | 10 | 16 | 25 | 40 | 63 | 100 | 160 | 250 | 400 | 630 | 1000 | 1600 | 2500 | 4000 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T' in $\mu$ | 3600 | 1420 | 580 | 230 | 91 | 36 | 14 | 5.8 | 2.3 | 0.91 | 0.36 | 0.14 | 0.06 | 0.02 |

Photomicrography differs from visual observation by normally recording, if possible, the entire depth of the object to be sharp in one negative, i. e. the depth of field must be adjusted. The diagram shows that such a case may call for the use of a lower-power objective in combination with a higher-power eyepiece for a desired final magnification, although this will be at the expense of lateral resolution. Conversely the diagrammatic representation also shows the limiting thickness of an object in which sharpness differences are to be avoided with a given objective/eyepiece combination.

Finally, the diagram also indicates the demands which the micrometer screw of the microscope must meet. The movements of the micrometer screw corresponding to the measured values for T are obtained by multiplying the numbers found by $n \div 1.45$; here, n represents the refractive index of the medium between cover glass and the front lens of the objective.

It is realized that with the highest-power objectives the play of the micrometer screw must be less than the wave length of light to make accurate focusing possible, and that any movement of the micrometer screw after the completion of the adjustment must also remain below this order so that the setting will stay unchanged for prolonged periods; this is essential particularly in photomicrography. The LEITZ micrometer screws mounted in ball bearings meet both requirements most admirably irrespective of whether the tube is used normally or carries extra weight (cf. p. 13).

## Measuring with the Microscope

A monocular tube in combination with a micrometer eyepiece is most suitable for **measuring distances** in an object. The micrometer eyepiece has a micrometer with a scale division (normally 10mm = 100 parts) mounted in the plane of its diaphragm.

The "micrometer value" of the objective used must be known before the measurements are carried out. This is the distance in the object plane produced by the objective equivalent to one interval in the eyepiece micrometer scale. Since in binocular tubes this value changes with the individual interpupillary distance of the observer, calibration and measurements must take place at the same interpupillary-distance setting.

The micrometer values are found as follows: – The image of a scale division of a micrometer in the object plane (stage micrometer) is compared with a division in the eyepiece micrometer. If x parts of the stage micrometer scale correspond with y parts of the eyepiece micrometer scale, the micrometer value of the objective is x : y, where x is expressed in the units of the stage micrometer used in the calibration (normally 0.01mm).

This is illustrated by the following example: –

11 parts of the stage micrometer scale correspond with 29 parts of the eyepiece micrometer scale. The micrometer value will be 11/100mm ÷ 29 = 0.11mm ÷ 29 = 0.0038mm or 3.8 $\mu$m (1 $\mu$ = 0.001mm).

The accuracy of the micrometer value obtained increase with the number of the scale divisions compared. It is simplest to compare 100 divisions (with low-power objectives 10 divisions) of the eyepiece micrometer scale with the division of the stage micrometer. The micrometer value can thus be read off directly by shifting the decimal point (see fig. 24).

The following examples may serve for the determination of a measurement: – A scale of Hipparchia Janira, measured with the 40/0.65 objective, has a length of 49 and a width of 18 scale divisions of the eyepiece micrometer. By calibration, let the micrometer value of the 40/0.65 objective at 37 $\mu$m be 0.0037mm. Thus,

length of the scale 49 x 3.7 $\mu$m = 181 $\mu$m = 0.181mm
width of the scale 18 x 3.7 $\mu$m = 67 $\mu$m = 0.067mm

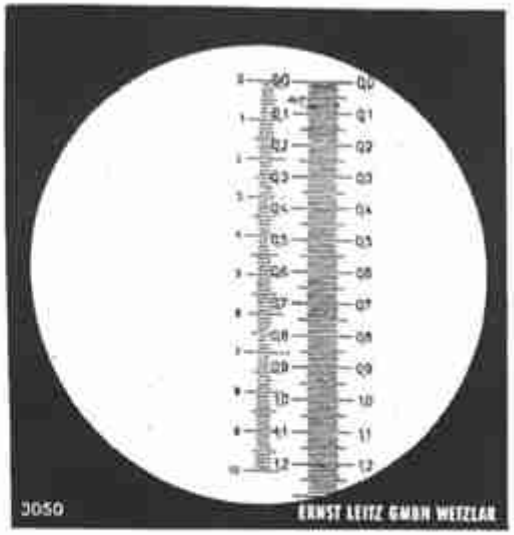



Fig. 24

Determination of the micrometer value. The scale in the centre of the field of view (division 0 to 10) is the fixed scale of the eyepiece, the scale to the right is the visible portion of the image of the stage micrometer. 100 divisions of the eyepiece micrometer correspond to 122 divisions of the stage micrometer (1.220mm), 1 division of the eyepiece micrometer corresponding to 0.0122mm in the specimen.
Micrometer value: – 12.2 $\mu$

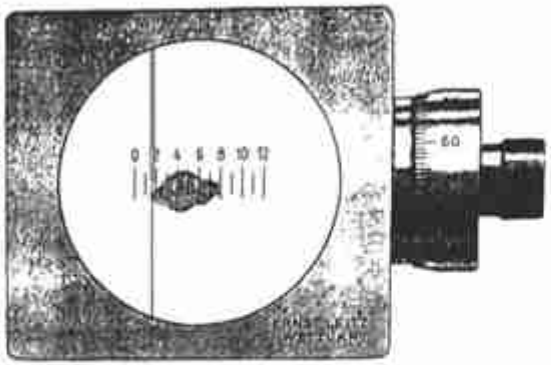


Fig. 25 Diagram of the screw micrometer eyepiece with scale divisions in the field of view. Let the first reading be 8.00 drum intervals, the second reading in the present case is 1.67 = 167 drum intervals. Hence, the image of the object extends between 800–167 = 633 drum intervals. The true size of this object particle will be derived from

$$\frac{633 \times 0.004\text{mm}}{\text{Objective magnification} \times \text{tube factor}}$$

A specimen of Pleurosigma angulatum measures

15 divisions of the eyepiece micrometer scale with the 10/0.25 objective
61 divisions of the eyepiece micrometer scale with the 40/0.65 objective
94 divisions of the eyepiece micrometer scale with the 63/0.85 objective.

Thus, the length of this specimen will be found with sufficient accuracy from the three measurements and determination of the corresponding micrometer values: –

15 x 15 $\mu$m = 225 $\mu$m = 0.225mm
61 x 3.7 $\mu$m = 226 $\mu$m = 0.226mm
94 x 2.4 $\mu$m = 226 $\mu$m = 0.226mm.

If a monocular drawtube T is used on earlier microscopes it is possible to obtain whole numbers for micrometer values which are easier to calculate, by varying the tube length. However, this results in a certain reduction of image quality which must be accepted, particularly with high-power objectives (see p. 16).

The screw-micrometer eyepiece is used for the most exact measurements with the microscope; it has far a higher measuring and reading accuracy. By turning the micrometer screw at the side of this eyepiece a measuring line can be moved across the entire range of the eyepiece scale. The drum of the micrometer screw is graduated in 100 divisions. One full turn of the drum moves the measuring line across one division of the eyepiece scale. Hence, one division of the drum scale corresponds to one hundredth part of a division of the eyepiece scale. When a screw micrometer eyepiece is used on a stand mentioned in this booklet or on a similar stand, 1 drum interval = 0.004mm. The object size is found by multiplying the number of drum intervals through which the movable line in the eyepiece had to travel from one edge of the image to the opposite edge (in Fig. 25: 633 intervals) by 0.004 and dividing the product by the objective magnification x tube factor. Due to its high precision and robustness the screw-micrometer eyepiece will stand up to heavy demands during technical measurements in workshop and tool room.

## Care and Maintenance of the Microscope

For protection against dust the microscope should always be returned to its case or suitably covered after use (fig. 26). From time to time the stand should be cleaned with a piece of linen or chamois leather; spirit must never be used for this purpose as it attacks varnish. Kerosene, on the other hand, is extremely well suited for cleaning varnished parts.

Light patches on the object stage caused by kerosene can be removed with neat's foot oil.

Work with acids (particularly acetic acid) or corrosive substances requires special care, since these easily spoil the appearance of the instrument and may attack the metal parts and lenses.

The optical components of the microscope must be kept meticulously clean. Dust on glass surfaces is removed with a fine, dry camel hair brush, blowing gently across the glass surface as the brush is applied. For hardened dirt an often-washed linen rag or piece of chamois leather moistened with a little water is suitable. If this is not successful, kerosene or xylene should be used.

During work with chemical reagents, their contact with the objectives must be strictly avoided; the objectives must be cleaned at once after any accident. They must never be dismantled for cleaning.

If any damage is discovered inside an objective, it should be sent to our works for repair.

Particular care is required during the cleaning of anti-reflex coated optics. The outside surfaces of eyepieces and objectives are coated with films of approximately the same hardness as that of glass. They are cleaned as carefully as uncoated glass surfaces. However, very soft films are sometimes used to coat the interior surfaces of objectives and eyepieces; these should be cleaned by very gently blowing on them, and brushing with a camel hair brush, never by wiping. For the same reason it is not recommended to clean the inside of eyepieces.

Oil immersion objectives should be cleaned immediately after use to prevent the oil from drying or becoming tacky. It should be removed with a piece of blotting paper or a linen rag, and the front lens wiped clean with a piece of soft chamois leather. If necessary a little xylene, but never spirit or alcohol, should be used.

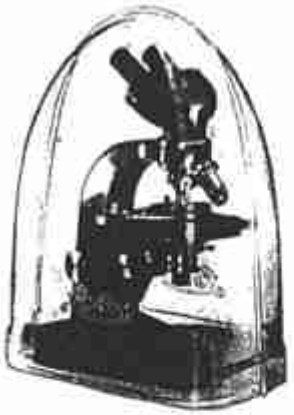


Fig. 26
A plastic hood is recommended for the protection of the microscope on the laboratory bench. Plastic hoods are available in various sizes to suit the various bench microscopes.

Fig. 27
Combination bottle for immersion work (oil and xylene).

The combined immersion oil- and xylene bottle (fig. 26) is recommended for work with oil immersion objectives. The bottle proper is filled with xylene, the brown insert with some immersion oil. The glass rod is used for applying the immersion oil to the specimen and the objective.

The high performance of a LEITZ microscope is ensured for many years of use by proper handling. However, should the examination or repair of a damaged instrument become necessary, this should be entrusted either to our works at Wetzlar or to one of our official agencies.

The screw micrometer eyepiece, eyepiece micrometer, and other measuring devices are described in special leaflets which we shall be pleased to send on request.

In the interests of reliable results it is advisable to carry out all measurements near the centre of the image, since the accuracy of readings taken at the periphery of the field of view may be somewhat impaired by residual distortion.

The simplest method of **measuring depth** in the object, i. e. measurements along the optical axis with the microscope would at first glance appear to be the focusing of the points to be measured successively with the fine adjustment, and the reading of the axial displacement of the objective off the scale divisions. However, this procedure has a number of faults which under certain conditions can lead to completely wrong results.

It must above all be borne in mind that the objective fine adjustment is designed on principles different from those of a high-precision gauge on account of its different purpose. If the fine adjustment is to be used for such measurements, only approximate values can be expected. Really precise readings can be obtained only with the aid of a reliable dial gauge suitably mounted on the microscope, indicating the displacement of the objective.

However, the extent of the displacement is not normally a direct measurement of the vertical distance between two points. This would be the case only if the same optical medium filled both the space between front lens and cover glass and that of the object itself. With aqueous preparations, for instance, a water immersion objective would have to be used in order to meet this condition; an oil immersion objective with specimens embedded in oil.

If this demand is not met the measured value must be multiplied by $n_o \div n$ in order to obtain the real distance in depth in the specimen. $n_o$ is the refractive index **in the specimen** while n represents the index of the medium between the front lens and the cover glass. Thus, the refractive indices of the embedding medium and the immersion fluid must be known or be measurable. For dry specimens $n_o = 1$, with oil embedding $n_o = 1.515$, and with infusions or many aqueous or moist medical and histological specimens $n_o$ can be assumed to be 1.33.

The following examples show the effect of this factor on the final result.

With a dry objective a difference in depth of 0.06mm is found between two points in an aqueous specimen. In this case, the approximate refractive index of water 1.33 can be inserted for $n_o$, while $n = 1$, since the medium between the cover glass and the front lens is air. Thus, the real distance is $0.06 \times (1.33 \div 1) = 0.08$mm.

If, again with an aqueous specimen, a distance in depth of 0.1mm were found with a water immersion objective, this would also be the real distance, because 1.33 must be inserted both for n and $n_o$. Thus, $n_o \div n = 1$, which leaves the measured value unchanged.

Analogously for a specimen embedded in oil to be viewed with a water immersion objective, $n_o = 1.515$, $n = 1.33$, so that the distance measured must be multiplied by $1.515 \div 1.33 = 1.14$.

Finally, the **depth of field** of the objective used has a considerable influence on the accuracy of such measurements. This term is discussed in more detail in a separate chapter on p. 32 because its extreme importance extends to other contexts of microscopy. The conclusions drawn there also apply here, in other words: – The accuracy at which points at various levels in the specimen can be **focused** increase with the aperture of the objective and the final microscope magnification chosen. With low final magnifications the inherently low focusing accuracy can be considerably further reduced by the adoption of the human eye (see pp. 32, 34).

The use of comparatively high condenser apertures is recommended for such measurements. Also, depth measurements should be carried out only on points in the immediate vicinity of the centre of the field.

These indications show that some experience and very careful determination and evalution of differences in depth are necessary in order to obtain reliable results.

# Index

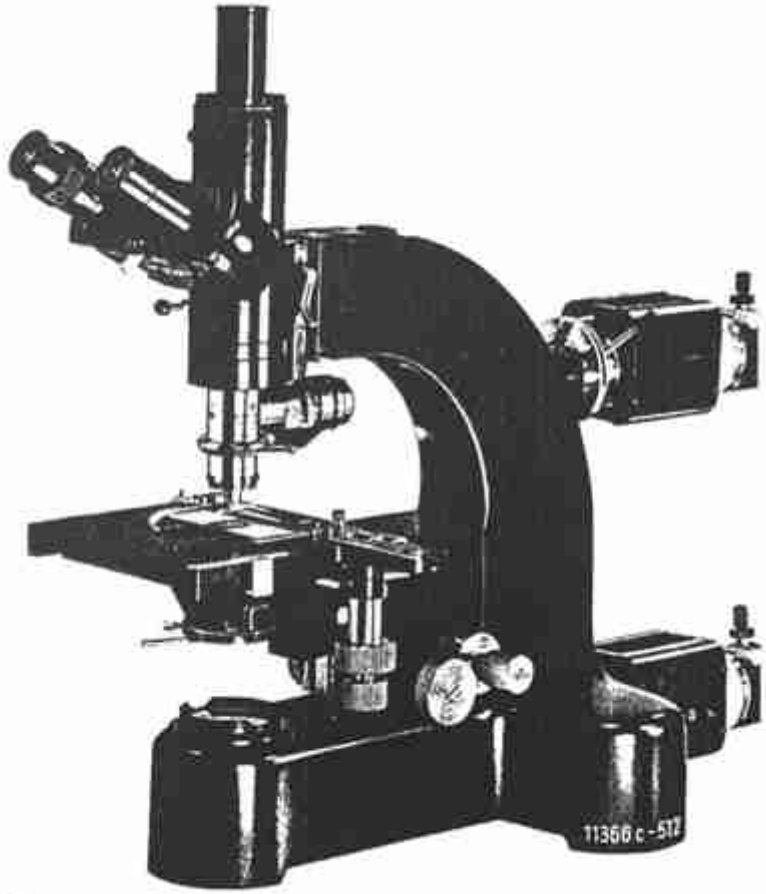



Fig. 1c Large ORTHOLUX research microscope with built-in sources for incident and transmitted light

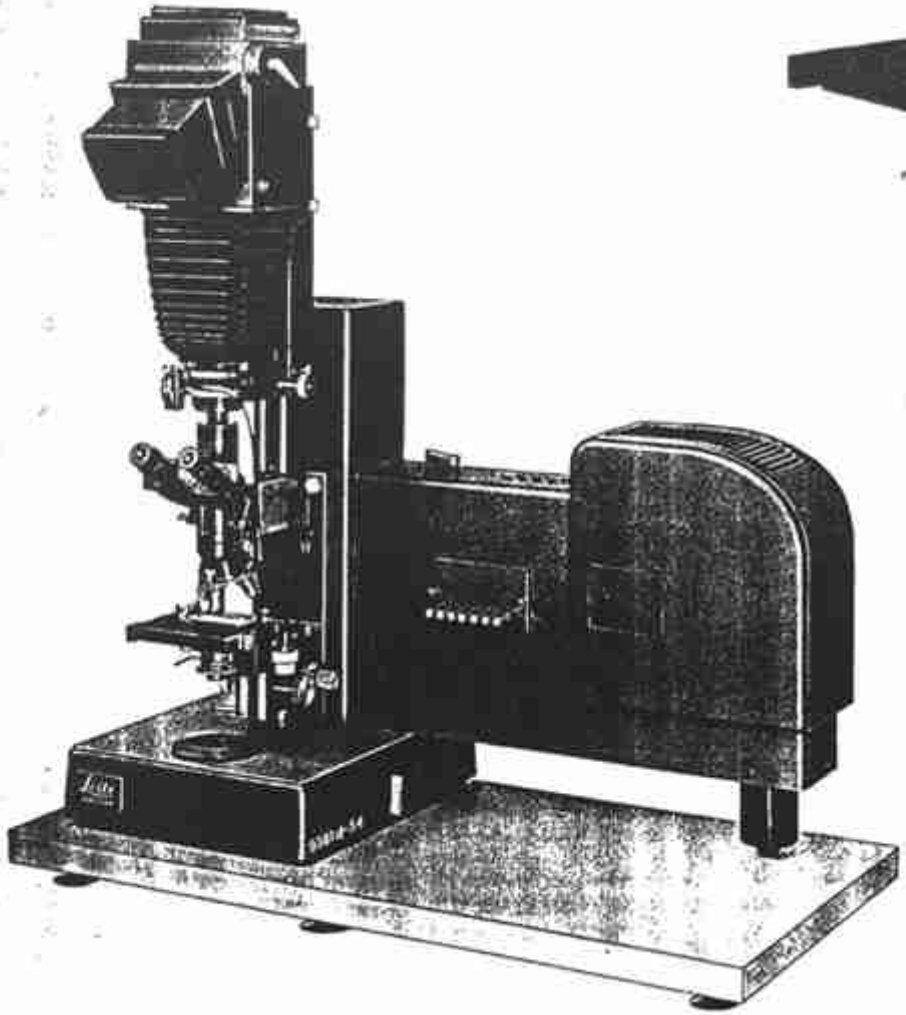


Fig. 1d PANPHOT camera microscope with combined low-voltage and high-intensity light sources

Shown below and opposite are microscopes of characteristic design.

All have a rigid stand to which the various types of tube can be firmly locked; the microscope image is focused by raising or lowering the object stage. Thus, the load on the stand, which may vary considerably due to the greatly different weights of the tubes and camera attachments does not affect the focusing motion.

The microscopes illustrated below differ in their sizes and in their provisions for using equipment for the various methods of examination and for photomicrography.

The SM stand is a relatively simple, extremely sturdy routine microscope. It is popular for classroom work, and fitted with the well-known single-knob control for coarse and fine focusing. Lamp attachments can be used instead of the mirror for daylight (fig. 1a). The larger LABORLUX® is a laboratory microscope with a built-in low-voltage lamp for transmitted light. It has an interchangeable objective revolving nosepiece, which can be replaced by the ULTROPAK® incident-light illuminator with built-in light source (fig. 1b).

The ORTHOLUX® research microscope with light sources for transmitted, incident, and mixed light represents a step further; more-over, all parts are interchangeable, which is an essential feature in a research microscope. This stand has separate fine- and coarse focusing drives (fig. 1c).

The PANPHOT® camera microscope is a combination of a research microscope of the ORTHOLUX standard, a camera for photomicrography and macrophotography, and an interchangeable lamp attachment in which a low-voltage light source is organically combined with a high-power source such as a mercury or xenon high-pressure lamp within a single unit (fig. 1d).

® = Registered Trade Mark

Fig. 1a SM Microscope
for classroom and routine work

Fig. 1b LABORLUX Laboratory microscope
with built-in transmitted-light source

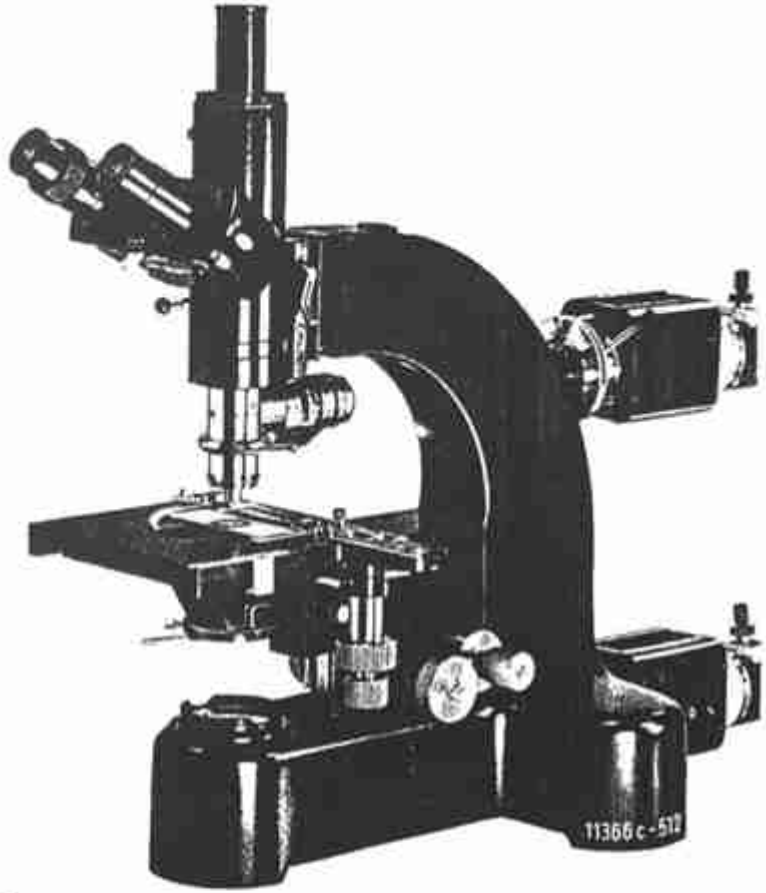



Fig. 1c Large ORTHOLUX research microscope with built-in sources for incident and transmitted light

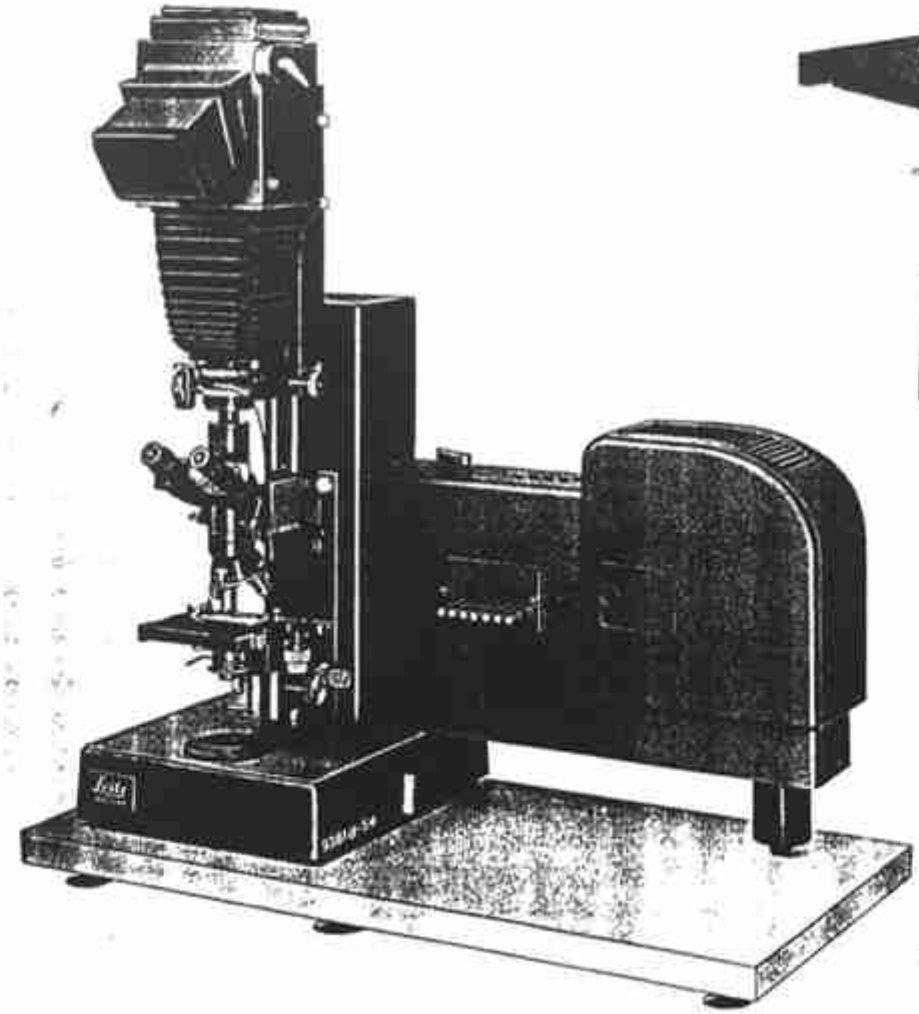


Fig. 1d PANPHOT camera microscope with combined low-voltage and high-intensity light sources