Microscopy from the very beginning

F. K. Moellring

CARL ZEISS · Oberkochen/Wuertt.

Microscopy from the very beginning

by
Dr. Friedrich K. Möllring
Dipl.-Biol.

member of scientific staff,
Microscopy Laboratory

CARL ZEISS, Oberkochen/West Germany

Microscopy – from the very beginning

1 st Step:
Microscopy in a nutshell

2 nd Step:
Microscopy for the serious worker

Annexes

Preface

Whereas instruction manuals deal with the operation
of one particular microscope model,
this brochure is intended to furnish general information
on microscopy, that is, facts which apply equally to all types
and even makes of microscope.
The booklet serves three purposes:

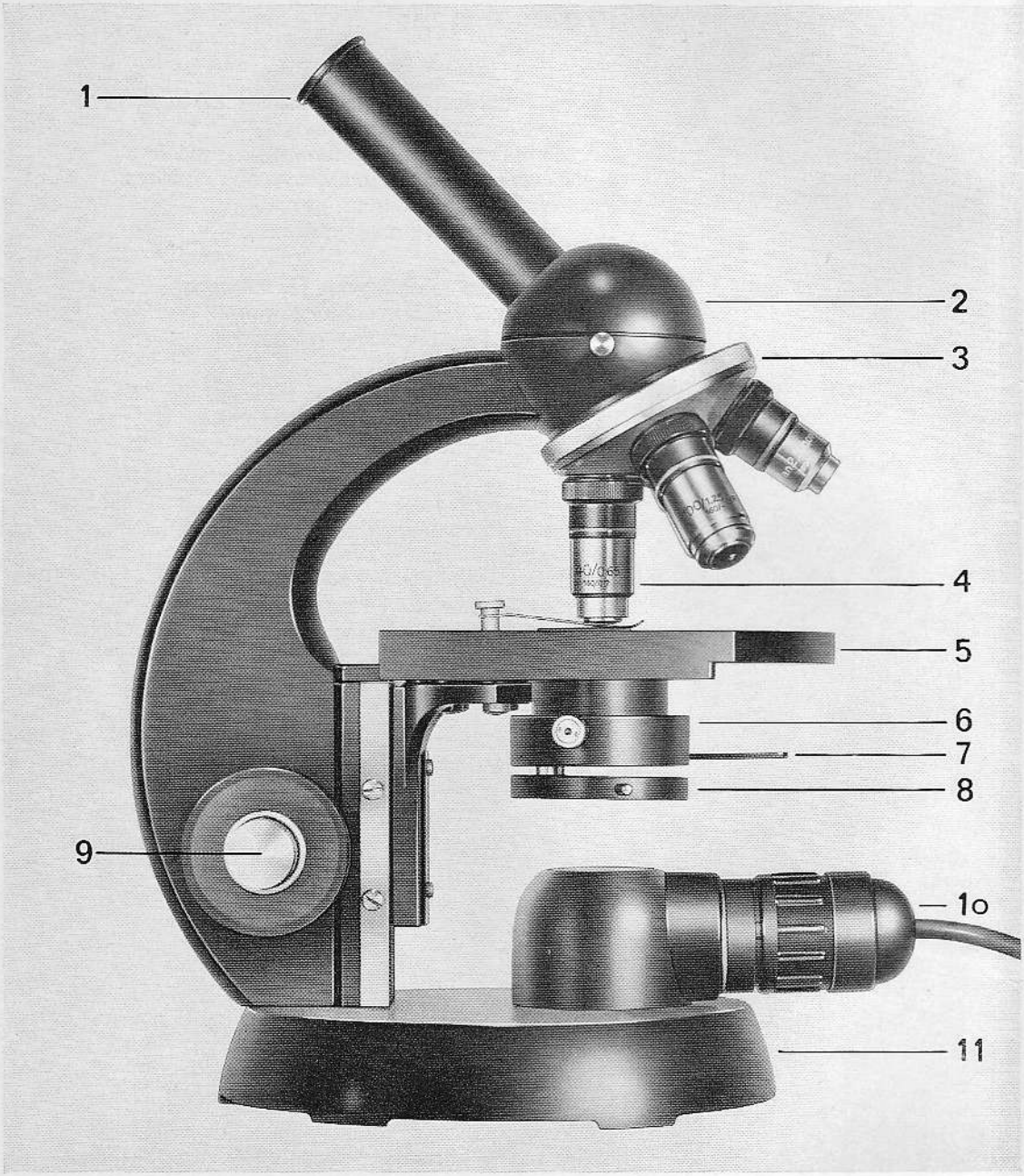
1) It is intended to be a guide for the beginner,
with the aid of which microscope work
should be a pleasure from the outset.

2) It will briefly recapitulate the most essential facts
about microscopy for the benefit of those
who do not use the microscope regularly.

3) It is also for the use of the experienced microscopist
who wants to introduce others to the field of microscopy.
He may find one or the other of the hints
contained in the booklet helpful for teaching.
It should be remembered that we take many things
for granted, and all too quickly forget the difficulties
we once had when we made our first acquaintance
with microscopy.

We wish you all success and are sure you will enjoy working
with your ZEISS microscope.

CARL ZEISS, Microscopy Department


1
2
3
4
5
6
7
8
9
10
11

1. This is a microscope

1) Eyepiece, slipped into upper end of tube.
The engraved value, e. g., 12.5×, indicates the magnification of the eyepiece; multiplied by the magnification of the objective, this gives the total magnification of the microscope.

2) Tube (here an inclined tube for monocular observation). The tube is interchangeable.
When the screw collar is loosened and the tube pressed against a spring in it, it can be lifted out.

3) Nosepiece for quick exchange of objectives.

4) Objective designation 40/0.65
160/0.17 means:
objective magnification 40×, numerical aperture 0.65 (see explanation on page 37), computed for mechanical tube length of 160 mm and 0.17 mm cover-glass thickness.

5) Specimen stage with spring clips.

6) Condenser (correct position is always just below the upper stop).
Condenser front lens swinging out for illumination of larger object fields.

7) Iris lever (condenser or aperture diaphragm).

8) Swing-out filter holder for filters of 32 mm diameter.

9) Fine adjustment knob. On STANDARD KF Microscopes this allows unlimited rotation; on other microscopes its rotation is limited by two stops (index beside the knob, see Fig. 2).
Coarse adjustment knob. With all Zeiss microscopes, rotation in the direction of the arrow brings objective and specimen closer together (the same applies to the fine adjustment).

10) Attachable simple substage illuminator for direct connection to the mains (which can be exchanged for mirror) with rotary switch and ground glass.

11) Base with three-point support. On the underside, tapped holes for screwing down in cabinet as well as two holes for mounting the connecting brackets of separate illuminators.

Fig. 1
STANDARD KF Microscope

2. And now a bit of practice: we focus a specimen

You have received your ZEISS microscope and unpacked it according to the recommendations contained in the pertinent instruction manual. If you should be using a microscope for the first time, we recommend that you start by making yourself familiar with its most essential components, such as objective, eyepiece, condenser, coarse and fine adjustments, using Fig. 1 as a guide.

Screwing in the objectives

Start by screwing the objectives into the nosepiece, working from low magnifications upwards. Please avoid touching glass surfaces, particularly the front lenses of the objectives. If you touch them inadvertently, remove the fingerprint immediately with the aid of a soft linen rag (see also page 35).

We need specimens

Our first acquaintance with a microscope may end on a rather disappointing note, because we may not have realized the fact that a normal microscope cannot simply be used for magnifying any desired object — such as a fly — (for which there are ZEISS stereomicroscopes), but that the object we want to examine should always be more or less transparent and as flat as possible. The wing of a fly is a good example. These requirements therefore usually necessitate the so-called preparation of specimens, about which details can be gathered from introductory books on microtechnique, see the section on literature, page 59. For the purpose of the present description we must assume that suitable specimens — of which, incidentally, a wide choice is offered by companies specializing in teaching aids — are available. Our specimens are usually mounted on a so-called slide, a piece of glass about 26×76 mm in size and 1 mm thick, which is covered by an extremely thin cover glass (various sizes; thickness as close as possible to 0.17 mm). — Be sure that the cover glass is always on top, facing the objective.

Workplace

The microscope stands on a table so that you can look into the eyepiece from a convenient posture. Microscopes without a built-in illuminator are best set up in front of a lamp with a large, uniformly emitting surface. (Work with special microscope illuminators is discussed on page 45).

Height of condenser, scanning objective

The condenser is in place and turned or pushed up all the way. The condenser front lens is swung in. The eyepiece will be inserted later. At the beginning of an examination, we always use a low-power objective of about 6.3 or 10×, because it permits coverage of a wider field and is thus best suited for scanning. In addition, its depth of field is greater than that offered by objectives of higher power, and it is thus easier to find the plane of best definition.

What is the magnification?

The power of an objective is indicated by its initial magnification. This is the first one of the values engraved on every objective (see page 28). The total magnification of the microscope is determined by multiplying the magnification of the objective with that of the eyepiece, which is engraved on the latter. A 10× objective used in conjunction with a 12.5× eyepiece thus yields a total magnification of 125×.

Caution — in spite of built-in slide protection!

The specimen has been fixed on the stage, and we now cautiously move specimen and objective closer together by the coarse adjustment, until they are only a few millimeters apart (observe from the side!).
In ZEISS microscopes, a stop and the spring mount of the objectives prevent ordinary specimens (1 mm slide, 0.17 mm cover glass) from being damaged by the objective. Only a few special-purpose objectives are exceptions.

The objective is "illuminated"

Before inserting the eyepiece of lowest power into the tube, we open the iris diaphragm of the condenser and turn the illuminating mirror until the back lens of the objective, which is visible at the lower end of the tube, is evenly illuminated. When the attachable simple substage illuminator for direct connection to the mains shown in Fig. 1 is used, this operation is not required. After the eyepiece has been inserted, we

close the condenser iris about halfway, look into the eyepiece and increase the distance between the objective and the specimen by the coarse adjustment until details of the specimen can be recognized, even if they are still blurred.

Focusing

Exact focusing is now achieved with the aid of the fine adjustment. In the case of specimens of very low contrast, finding the focal plane may occasionally be somewhat difficult; it is facilitated if during operation of the coarse adjustment the specimen is slowly displaced on the stage, because moving structures can be recognized more readily. Closing the condenser iris further may also be helpful. When the area to be examined is in the center of the field of view, the objective of next higher magnification may be moved into position, for which only the fine control is needed for focusing (parfocal objectives, see page 29).

Do not stop down too far!

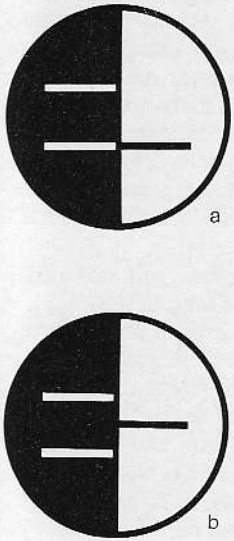
The adjustment of the condenser is a particularly frequent source of error. The condenser is correctly adjusted when it is in its highest position or directly below this and when its iris has been closed only far enough to obtain just sufficient contrast. The beginner usually stops the condenser down far too much. Never use the condenser iris to dim the image! Other means, e. g., gray filters, must be used for this purpose. Lowering the condenser "to increase contrast", which is popular with many microscopists, only has the same effect as further closing the condenser iris and is therefore inappropriate.
If with objectives of very low power the illuminated field should be too small, swing out the front lens of the condenser and open its diaphragm fully.

Always keep a hand on the fine adjustment!

The depth of field in the specimen corresponding to a certain setting of the fine adjustment is extraordinarily shallow (more pronounced with high-power objectives than with lower powers, more with high-quality optics than when these are less good). This is the reason why in microscopic work the fine adjustment is continuously moved up and down in order to cover the entire depth of the specimen.

When the fine adjustment "fails"

Most microscopes (except the STANDARD KF) have a limited fine adjustment range, and it may happen that the upper or lower stop is reached during observation. A glance at the side of the rack and pinion will confirm this (Fig. 2 a). In this case, rotate the fine adjustment knob until the index line lies roughly in between the two others (Fig. 2 b). Then refocus with the coarse adjustment, and you will again have sufficient upper and lower margin for fine adjustment.



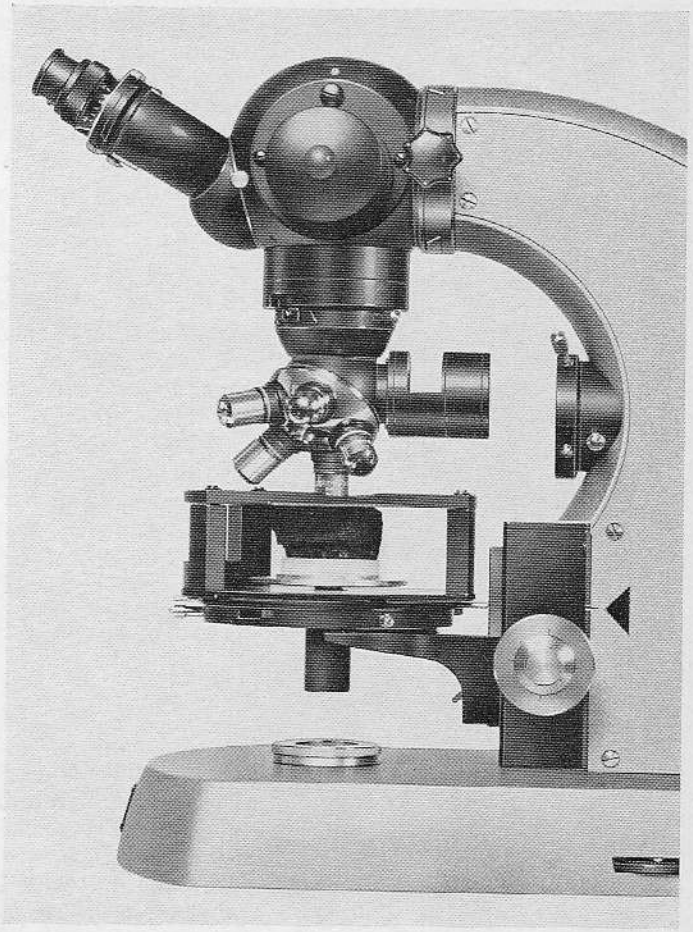


Fig. 2
Marks for fine adjustment,
here on STANDARD UNIVERSAL

Two important points:

When using optical instruments, such as a microscope or a telescope, two things are of great importance and should be kept in mind:
1) the eye should be relaxed when looking into the eyepiece, and
2) the eye should be kept at a certain distance from the eyepiece.

. . . relaxed accommodation

Item 1:
Beginners often make the mistake of focusing the microscope as if the magnified image they want to view were located very close to the eye, as close as a postage stamp which we want to study in detail, for instance. This near setting of the eye (= accommodation) is, however, extremely tiring if maintained for a longer time. We therefore recommend working with perfectly relaxed eyes. At the beginning, this is done more easily if we imagine that we are viewing the image as if it were at infinity. Thus do not look "into the microscope" but "through the microscope".
If you are not quite certain as to whether you are really working with relaxed eyes, make the following test:
First focus the specimen. Then hold a mirror in front of your second eye, which is not looking into the microscope, so that you can look out of the window or at an object at the other end of the room. When you have focused this eye at the far object, the other eye must at the same time see the microscopic image with maximum definition. If this is the case, you are sure that you can work in an absolutely "relaxed" state. Always remember that the microscope can be refocused with the aid of the fine adjustment and can thus relieve your eye of accommodation. It is characteristic of the experienced microscopist that he keeps playing with the fine adjustment. In this way his eyes will never get tired, or actually hurt.

. . . and good "contact" of the pupils

The second important point is the correct distance of the eye from the eyepiece. Should the eye be brought as close to the eyepiece as possible — as with a magnifier? Generally not, because the correct eye point, as this distance is called, varies according to the eyepiece used. The best procedure is to move in from a few inches' distance until the field of

view is widest and sharply defined. In this case, all the light rays emerging from the microscope find their way into the eye. As is evident from Fig. 3, this is possible only if the eye pupil is brought very exactly to the point of constringence of the microscope's light rays, which has therefore been named the "exit pupil of the microscope". When you have acquired a certain amount of practice in the use of a microscope, you will automatically find and keep the right distance from the eyepiece.

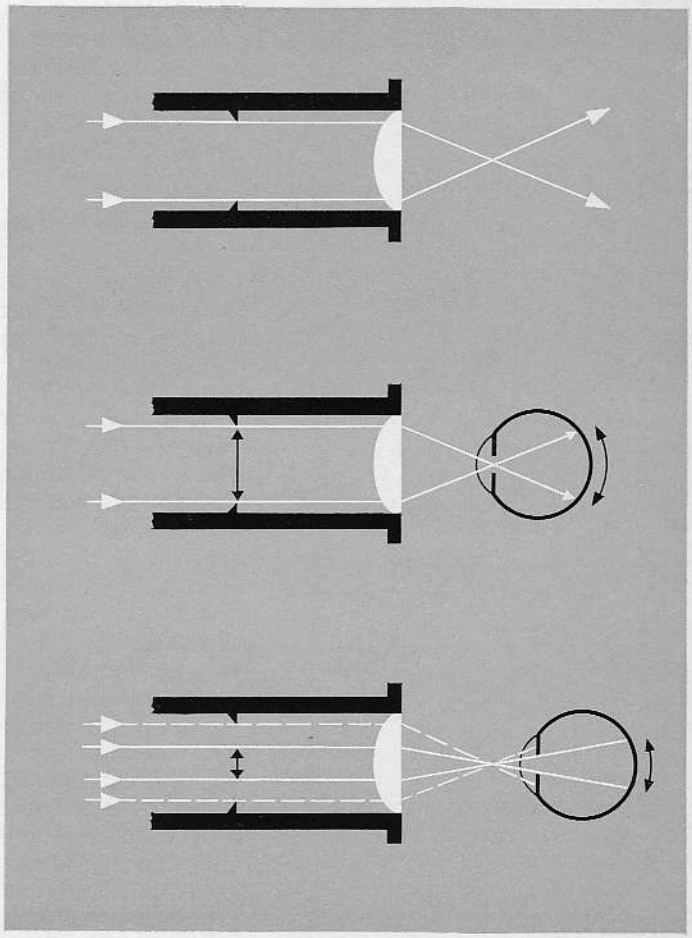


Fig. 3
Correct distance of eye from eyepiece.
Top: Location of microscope's exit pupil
Center: Exit and eye pupils in the same plane
Bottom: Eye too far away.
Eye pupil reduces the field.

Let us now pause to consider where you may still encounter difficulties in your first acquaintance with the microscope.

The image is laterally reversed and upside down

You have, of course, noticed that the microscopic image is upside down. If you displace the specimen to one side, the image moves in the opposite direction. You will get accustomed to this so quickly that you will soon forget the fact completely.

You cannot obtain a sharp image

It may happen that you cannot get a sharp image of the specimen in spite of everything you do. In this case, the fine adjustment has either reached a stop (remedy see page 9), or the objective touches the specimen before a sharp image is obtained. Then the cover glass is either far too thick, or you have inadvertently placed the specimen wrong side up on the stage. Whatever the cause may be, the spring mount of your ZEISS objective prevents damage to the specimen.

Oval field of view?

It is not only the beginner who suddenly finds that the field of view is not round. In this case, either the objective has not fully engaged its click-stop position or the filter holder below the condenser or in front of the illuminator is not swung in fully to the stop.

"Mouches volantes"

Another phenomenon may at first be inexplicable to the beginner: Specimens with a wide, clear background may show strange, irregular patches, shaped rather like worms, which remain stationary when the eye is fixed, but which start moving when the viewing direction is changed. These are the shadows of inhomogeneities in the aqueous humor of the eye, which become visible whenever the exit pupil of the microscope becomes very small: if the condenser iris is closed down too far or if a high-power eyepiece is used. (Incidentally, the same shadows can be seen if you look at a uniform sky. However, the sky must be bright, because only then do the eye pupils close far enough.)

Oblique illumination

A strange effect may occur, when the image in the microscope adopts a certain relief, appearing almost to have a third dimension, and focusing seems to "shift" the image. This always happens if you have "oblique illumination". A glance into the tube with the eyepiece removed will show

you that the back lens of the objective is not uniformly illuminated. This can be corrected by readjusting the mirror or the condenser.

Keep both eyes open!

If you are not lucky enough to have a binocular tube from the very start (so that you can look into the microscope with both eyes), observation with only one eye will at first be somewhat difficult. We therefore advise everybody who throws more than just an occasional glance into the microscope to keep his second eye open from the very start. The image seen by the second eye, which is naturally superimposed on the microscopic image, will be found disturbing only in the beginning and will later be subconsciously suppressed. In order not to harm the unused eye by this form of "disconnection", the eyes should be used alternately.

An accessory: the eye screen

Closing one eye for a longer period of time is very tiring. If you cannot get accustomed to leaving the second eye open and if the acquisition of a binocular tube is not possible, a cardboard screen may be slipped onto the tube in such a manner that the second eye is covered. Some people make their own "blind" cup from an ordinary eyepiece eyecup (Fig. 4, not commercially available).

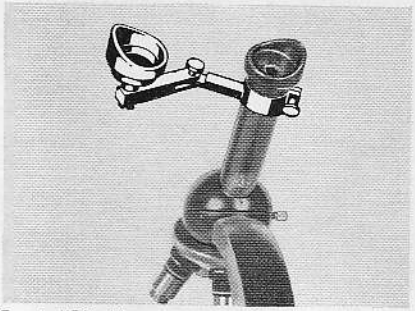


Fig. 4: "Blind" cup

Any ZEISS microscope can easily be converted for binocular observation, since this only means changing the tube.

With or without eyeglasses?

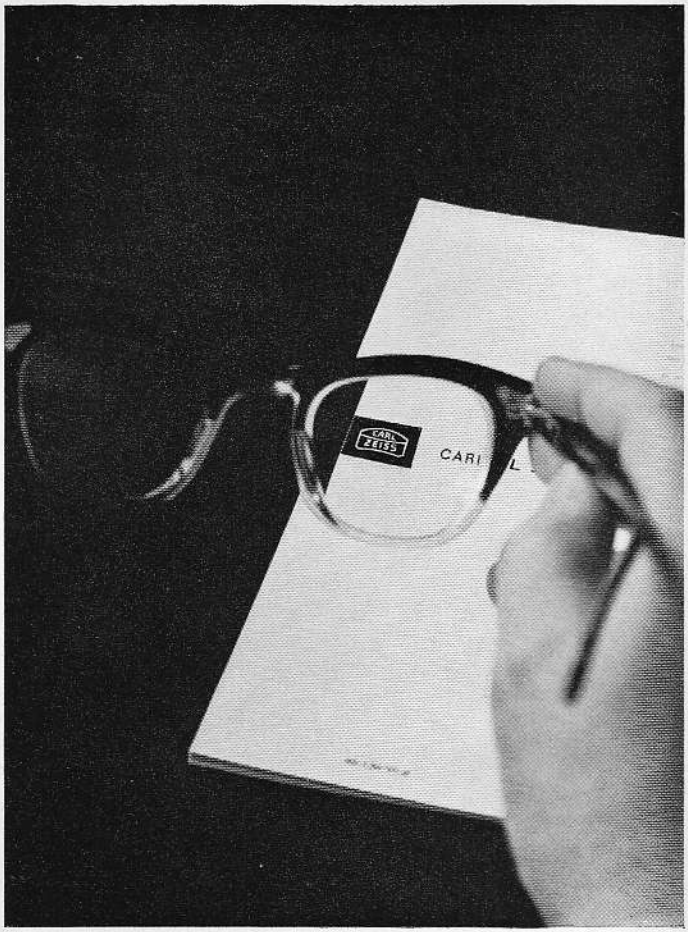
Here also the question arises how eyeglass wearers should work. The best solution is certainly to keep the glasses on and to use special eyepieces for eyeglass wearers. Rubber guards avoid scratching of the eyeglasses.

An eyeglass test

If this is not possible, you should first of all find out whether your eyeglasses have spherical or toric lenses. This can be determined very easily. Hold your glasses in front of you at arm's length: the lenses will either reduce or magnify an object on the table. Then rotate the glasses about the center of the lens through which you are looking. If the shape of the object does not change as the glasses are rotated, the lens

has only spherical power; however, if length and width of the object change during rotation, the glasses have toric lenses (Fig. 5). In the latter case you will have to keep the glasses on or have auxiliary lenses made from your eyeglass prescription, which are then slipped onto the eyepieces (ensure correct orientation!). If your glasses have only spherical lenses, you can remove them to work with the microscope and no loss of sharpness will be observed. In this case the visual defect is compensated by the adjustment of the microscope.

Fig. 5
Eyeglass test.
If length and width of an object change during rotation of the glasses, the lenses are toric, and the glasses must be kept on while working with the microscope.



3. A brief glance behind the scenes

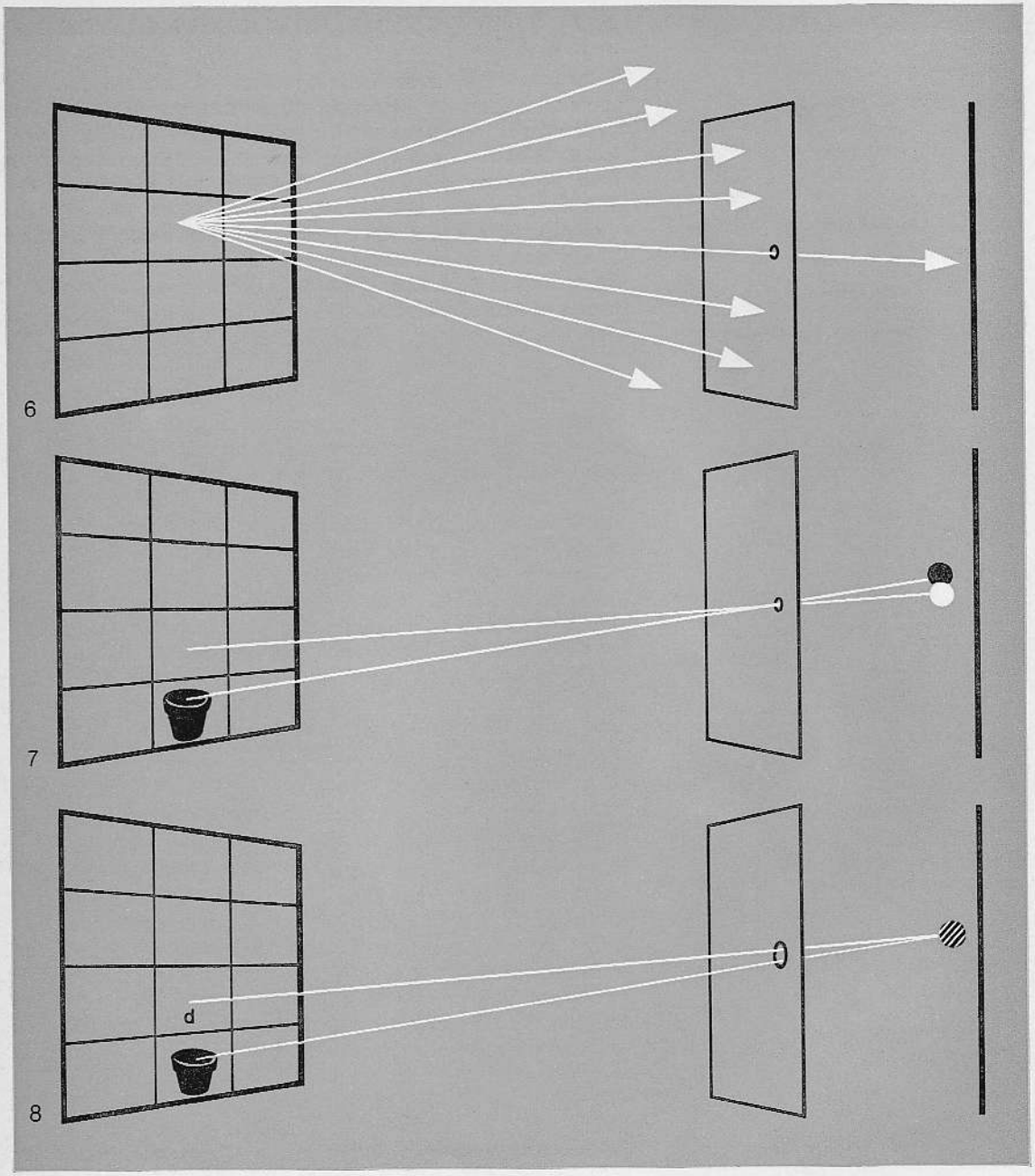
After you have focused the specimens in accordance with the explanations in the preceding section, you will know that your microscope **does** work. However, you will only derive full satisfaction from it if you know at least something about **how** it works. It is natural that this "glance behind the scenes" can only be a brief one, and users who wish to study the theory of the microscope in more detail are referred to the literature indicated on page 59.
As an optical instrument, the microscope forms a magnified image of a small object.

How is an image formed?

We must first of all understand the formation of an image, for which we shall choose a very primitive example. If you are standing in a dark corridor and the keyhole of a door is of appropriate size, you can clearly see a picture of the window in that room projected on the opposite wall of the corridor (pin-hole camera). You immediately recognize what is happening: every point of the window emits light rays in all directions. While most of them end on the walls or at the door, a few of them pass through the keyhole (Fig. 6). Consequently, a bright part of the window produces a bright patch on the wall, and a dark part produces a dark patch. And because all light paths can just as well be followed in the opposite direction, we may also say that every point on the wall of the corridor (=unit of area) "sees" a more or less bright spot of the window through the keyhole (Fig. 7).

Pin-hole camera: brightness and sharpness are incompatible

The drawback of such an image is above all its low brightness, which would immediately be increased if the hole were larger. So let's make it larger!
Through the bigger hole, a unit of wall area now "sees" not only the dark flower pot, but also the bright window pane beside it (Fig. 8). As a result, this unit is now neither dark nor bright, but gray — the dividing line between the pot and the pane is now blurred in the image on the wall. In other words, the two points at a distance d are no longer "resolved", i. e., they can no longer be recognized separately. Naturally, the bigger the hole the more blurred is the image. This is due to the fact that in a pin-hole camera the **rectilinear propagation of light** does not make a point correspond to another


6
7
d
8

point, but to an area which increases in size, the bigger the opening is (Fig. 9).

A lens is the solution

A lens has the extremely valuable property of refracting the light, thus counteracting the principle of the rectilinear propagation of light (Fig. 10). In an ideal case it therefore becomes possible to combine both requirements, viz., bright image due to large aperture and imaging of a point as a true point (the latter with certain restrictions; see page 37).

Fig. 6
Fig. 7
Fig. 8

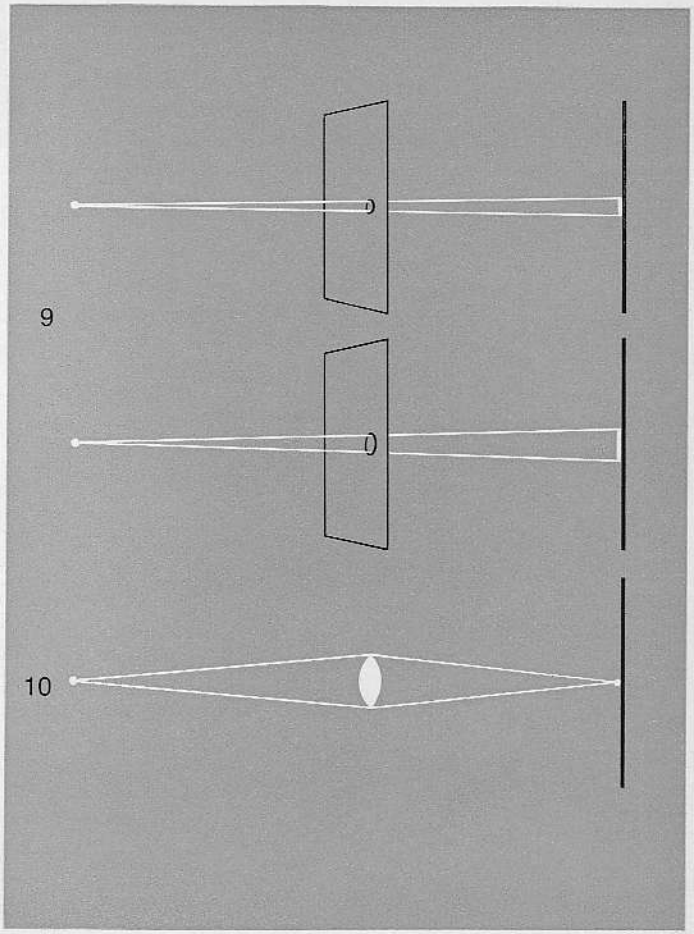


Fig. 9
Fig. 10

There is only one plane of best definition

As an example, let us consider a camera taking a picture of a plant. The light travels from any point on the plant in all directions (Fig. 11). A part of the light rays is intercepted by the objective lens, refracted and recombined in the film plane. If behind the plant there is, for example, a window, then the rays emanating from there do not recombine exactly in the film plane but in front of it, and on the film a circle of diameter l is imaged instead of the point: the window is out of focus (Fig. 12). "Focusing" can be achieved only by a relative displacement between lens and film plane. This shows that in an image-forming system a given object plane is always correlated to a definite plane of sharp imagery.

How big will the image be?

Apart from the location of the image plane, however, the object-image ratio is likewise fixed. This ratio is normally exactly identical with the relationship between object distance (object-lens distance) and image distance (lens-image distance) (Fig. 13). The formula would thus read: $\frac{A}{a} = \frac{B}{b}$. Since the image distance b in the camera is smaller, usually much smaller than the object distance a, this optical instrument of course furnishes reduced pictures.

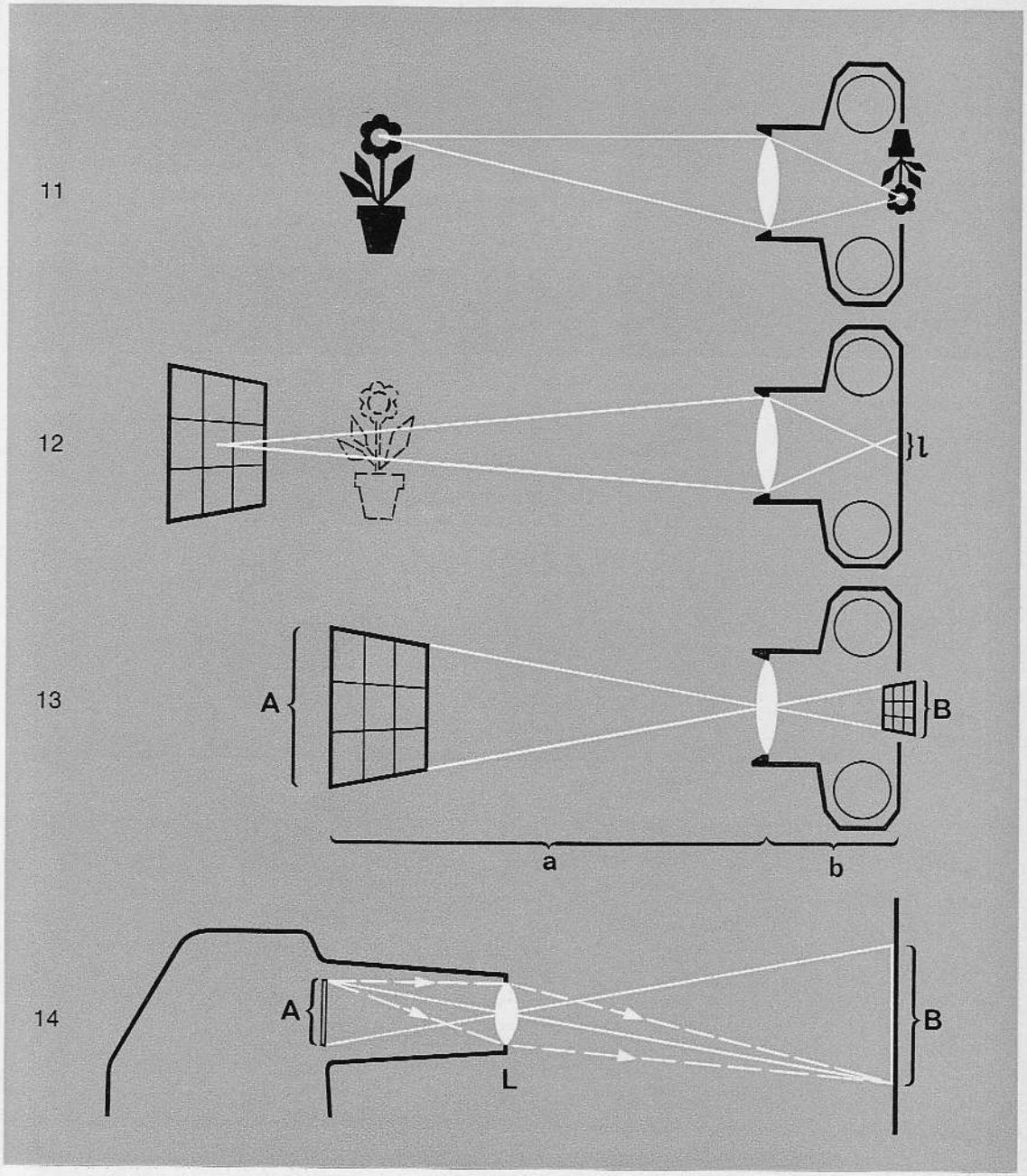
We build a model microscope

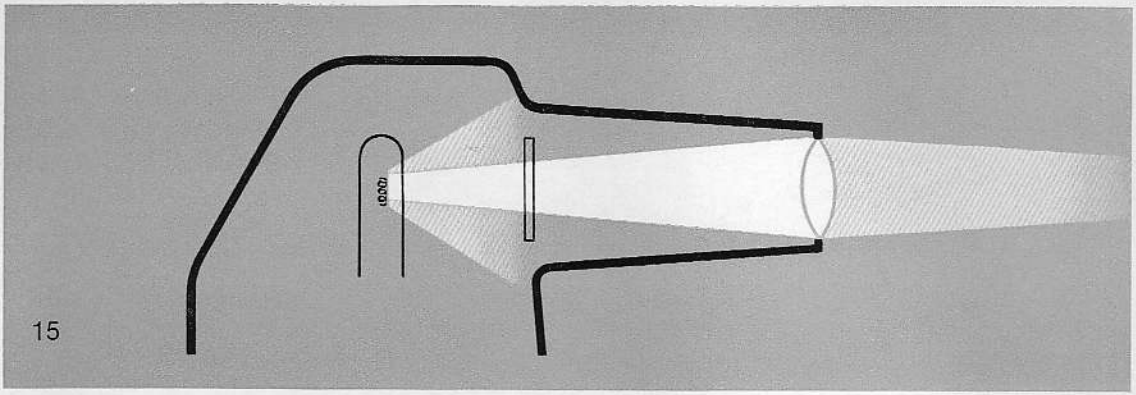
We now know enough to try and build a model microscope from the components we have learnt to understand — a concrete model if possible, or otherwise an "imaginary" one.

Illumination and first image-forming system

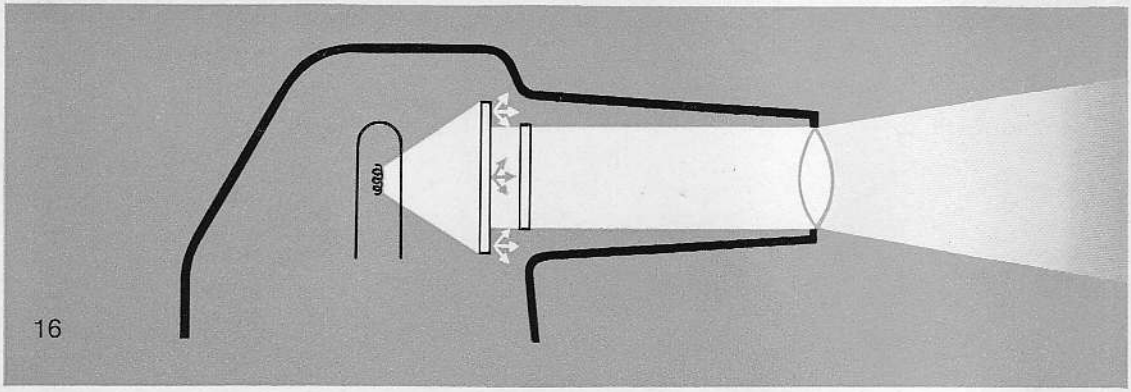
The microscope is to magnify; therefore the camera giving reduced images is not suitable as a basis for our model microscope. We are looking for an optical instrument which provides enlarged images, i. e., in which the image is much further away from the lens than the object. Such an instrument is found in the familiar slide projector (Fig. 14; A = object, the slide; B = screen image; L = projection lens). Since in this case the object, the slide, does not emit light itself, it must be illuminated from behind. Let us therefore place a lamp behind it (Fig. 15). However, the slide is much larger than the lamp filament and the lens diameter, so that only the light rays passing through the center of the slide

Figs. 11, 12, 13 and 14, right

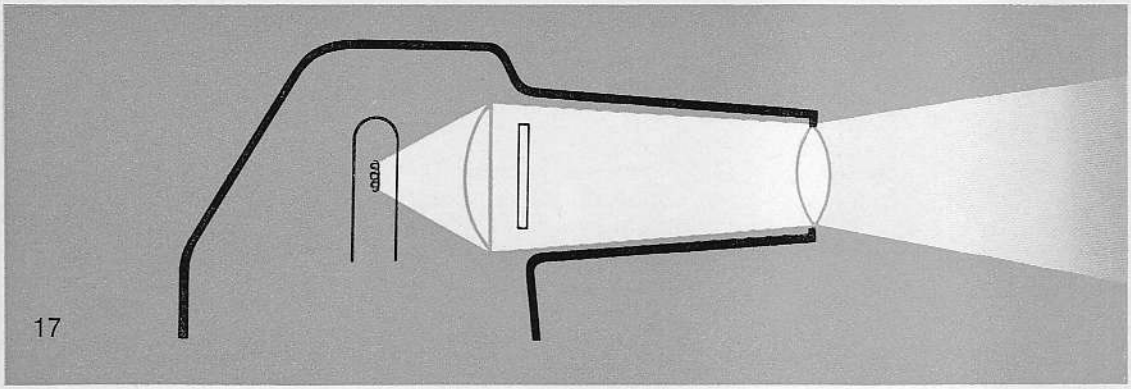

11
12
13
14
A
B
a
b
l
A
L
B



15



16



17

reach the lens. Result: the field is not fully illuminated, the marginal zones remain dark. — This can be remedied by a light source which is at least as large as the slide. An illuminated ground-glass screen is suitable for this purpose. The light striking this screen is deflected in all directions (Fig. 16). Such a screen also allows the light rays transmitted by the peripheral parts of the slide to enter the lens. However, this is rather uneconomical because the major part of the light rays is deflected in directions in which we do not need any light. A better solution is a lens (here called condenser) which directs all incident rays exclusively to the point where they are required: to the projection lens (Fig. 17). We thus have the fact that in the first place the light source is imaged by the condenser in the projection lens, while in the second place an image of the slide is formed by the projection lens on the screen: a so-called interlocking light path such as we find in the microscope with the so-called Köhler method of illumination (see page 45).

The second image-forming system

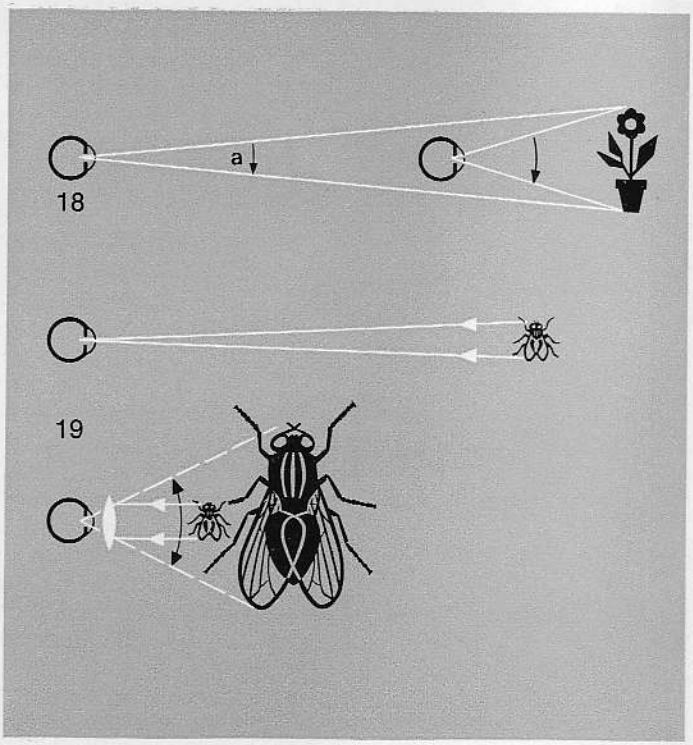
Let us now turn to the enlarged image on the projection screen. How big we see this image depends quite decisively on the distance from which we view it. If we move halfway up to the screen, we see the image exactly twice as large as before, i. e., with 2× "magnification".

If we draw a line, as shown in Fig. 18, from one edge of the picture to the eye of the observer and from there back to the other edge, an angle (α) is subtended, which is the angle of view. The magnification is determined solely by the magnitude of this angle. For normal slide projection we shall not move too close to the screen, because the unsharpness of the image (e. g., due to grain) would be disturbing. For our experiments we therefore use a microscopic specimen instead of a slide, for example a flee, which, contrary to the slide, reveals ever new details even under considerable magnification. In the interest of high magnification we may, of course, move up as close as possible to the image, but eventually our own shadow will interfere. We therefore look at the picture from the back of the transparent paper screen. The fact that the picture is thus laterally reversed need not worry us. The next step: we exchange the paper screen for a ground-glass screen and, in addition, use a magnifier for viewing the picture. A magnifier can increase the angle of view and thus the magnification considerably. An object

Fig. 15
Fig. 16
Fig. 17

(Fig. 19) viewed once with the naked eye from a "reading distance" of 10 ins. and then through a magnifier is seen under considerably higher magnification due to the refraction of rays. But the magnifier also magnifies the texture of the ground glass so that it becomes disturbing (Figs. 20 and 20 a), and we therefore investigate what happens if we simply remove the ground glass. The image will still exist in the same plane in the form of a so-called aerial image. Through our magnifier this aerial image will, however, be visible only in the direction of the illuminated projection lens and around it there will be darkness, because the rays miss the magnifier. This is illustrated in Fig. 21. In this case, the remedy consists once more in the use of a lens in exactly the plane in which the image is formed. Just as a magnifier placed directly on writing does not magnify the letters, a lens located exactly or almost exactly in the image plane has no effect on image

Fig. 18
Fig. 19



sharpness, but contributes to brightening the marginal zones of the field because the rays passing these zones are transmitted into the eye (Fig. 22). These "field lenses", as they are called, are therefore better than ground glasses, and in addition they do not introduce troublesome grain into the image. (In other words, we have here a case which is very similar to the one mentioned above, where we had a lens placed as a condenser between lamp and slide — likewise as a ground-glass substitute).

The efficiency of our model microscope is proved by the illustrations on the following page. These are not genuine microscopic images but images actually produced by a 2×2 projector and two spectacle lenses. One spectacle lens was used as a field lens, the other as an eyelens; the eye was represented by a reflex camera with its lens focused at infinity.

Fig. 20
Fig. 21
Fig. 22
(See the corresponding illustrations on the following page)

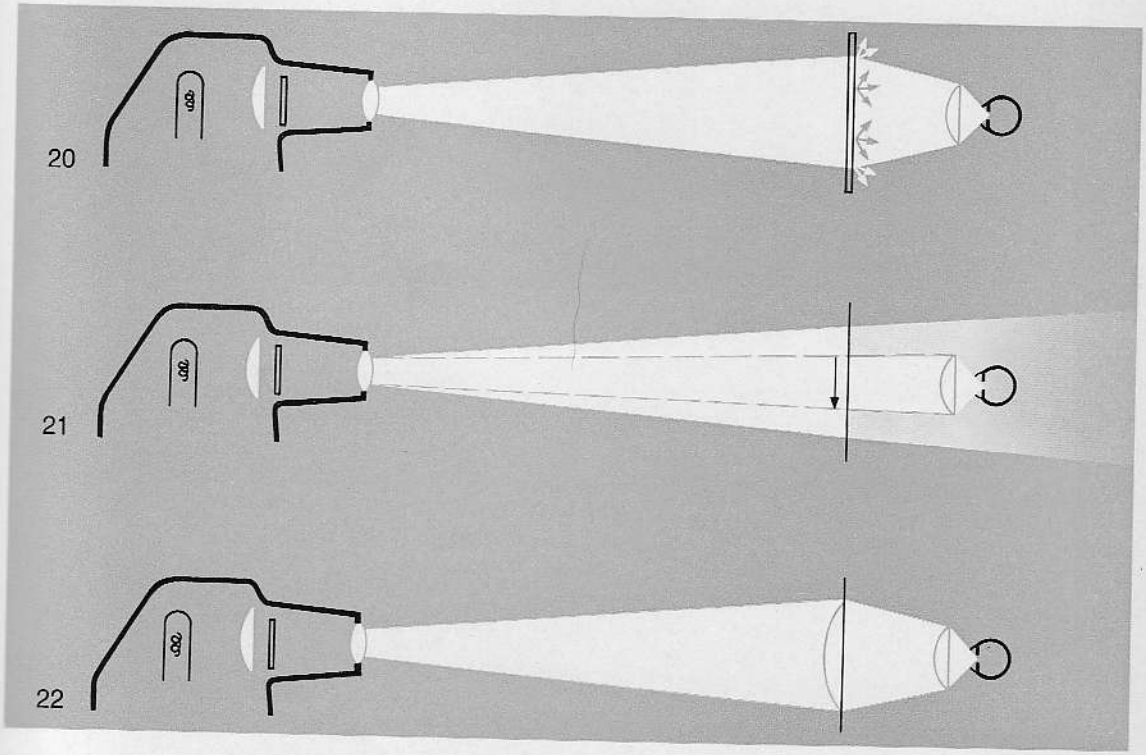


Fig. 20 a
This is what the intermediate image looks like when projected on to a ground-glass screen and observed through a magnifier (Fig. 20)

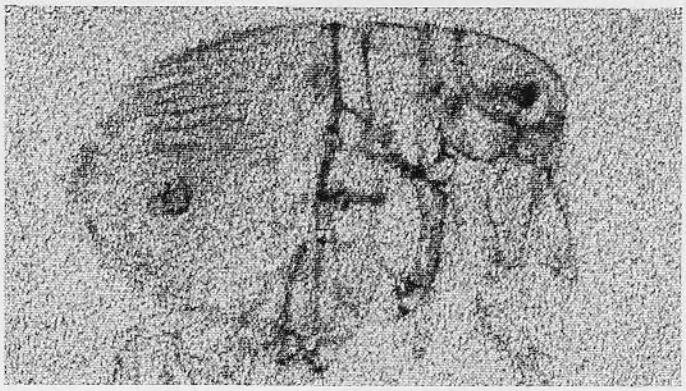


Fig. 21 a
When the ground-glass screen has been removed (Fig. 21) the graininess disappears but strong vignetting is present.

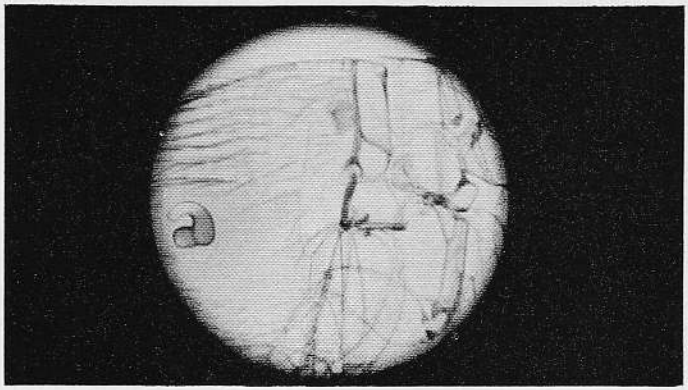
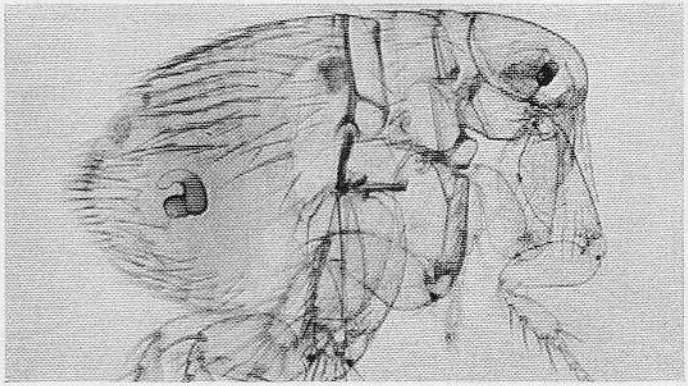


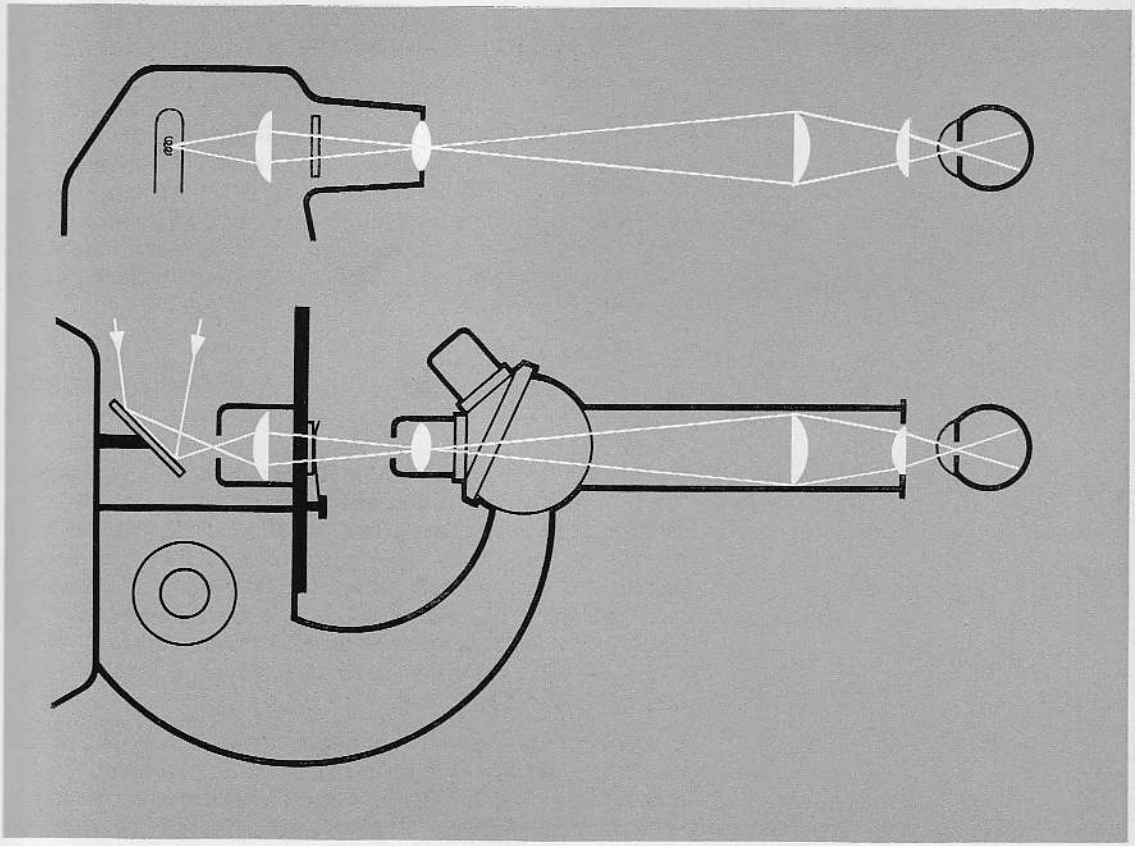
Fig. 22 a
Optimal results are produced only when using a field lens (as in Fig. 22).



From the model to the real microscope

Very few changes are required for the step from this projector with magnifier-viewing to the microscope. Thus, the image distance of several feet is too awkward. We should also like to have both optical systems, viz., the one causing the first magnification (the objective) and the one magnifying this image a second time, combined in one instrument. The entire setup can be kept much smaller by appropriate selection of the lenses, and especially of an objective of very short focal length. We then need only draw the outlines of a microscope around this light path and keep in mind that the path of rays is usually "folded" for greater convenience — and we have a "real" microscope (Fig. 23).

Fig. 23



The principle in a nutshell

The principle of the microscope may thus be summarized as follows:
The microscope objective first forms in the tube a magnified aerial image (intermediate image) of the specimen illuminated from below by the lamp and the condenser. However, this image is not intercepted, but viewed under magnification through a magnifier (called eyepiece).

The design of the light path

In the foregoing paragraphs we have tried to explain the basic facts of the microscope as clearly as possible; in doing so, however, we would give no exact description. In the following, the reader who is more familiar with optical subjects will find instructions for the design of a microscope light path. In principle, this is really quite easy.

First image-forming system

Fig. 24 shows the objective Ob with the two foci F 1 and F 2. The specimen of size A B is located slightly outside the focal length. From A two rays are traced: one parallel to the optical axis through the focus F 2, and the other through the lens center, which is therefore not refracted. The intersection of both rays gives the image of A, A′; in the same manner, B′ results as the image of B. The image A′ B′ thus obtained is enlarged, real (i. e., it can be intercepted) and reversed. This corresponds to the path of rays in the microscope up to the first aerial image.

. . . and second magnification

In another case (Fig. 25) we arrange a lens Ok in such a manner in relation to an object B′ A′ that the latter is shifted slightly off the focal point in the direction of the lens. The design of the path of rays is analogous: After being refracted, the parallel ray passes through the focal point, while the ray through the lens center is not deflected. Still, the two rays do not intersect; only their extensions to the rear have a point of intersection at B″ or A″. The image B″ A″ is likewise enlarged, but erect and virtual, i. e., it cannot be intercepted because the light rays do not really intersect at the points B″ and A″. — This is the light path of a magnifier and thus also of a microscope eyepiece.

If we connect these two light paths in such a manner that the image formed by Ob is at the same time the object for Oc, then we have the two-stage image formation of a compound microscope (Fig. 26).

Geometrical optics, wave optics

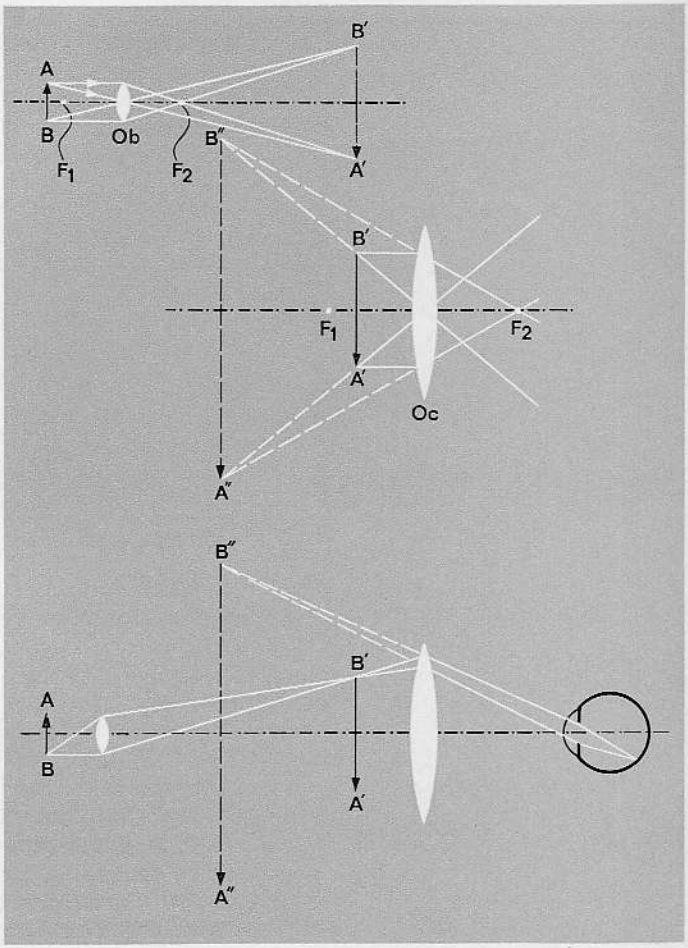
We must confess that with the geometric optical light path discussed up to now we have investigated only one aspect of image formation in the microscope. Another is the wave-optical aspect which is, however, beyond the scope of this booklet. A concise description, in which this second aspect is also taken into account, will be found, for example, in "How to use a Microscope" by W. G. Hartley, American edition edited by John J. Lee and Bernard Friedmann, The Natural History Press, Garden City, New York 1964.

Light path in the microscope

Fig. 24

Fig. 25

Fig. 26



By courtesy of R. Barer, 1953

4. Practical hints

After a brief excursion into the theory of the microscope there still remain a few facts the understanding of which is of importance for practical work.

Objective
Engraving and other characteristics

The objective is engraved with a value (Fig. 27) which indicates far more than initial magnification alone. Let us repeat the example on page 5:

40/0.65
160/0.17

means: initial magnification 40× (multiplied by eyepiece magnification = total magnification of microscope); numerical aperture 0.65 (for explanation, see page 37); for use with microscopes with a mechanical tube length of 160 mm (distance between objective flange and eyepiece seating face) and with a cover glass of 0.17 ± 0.01 mm thickness.

Fig. 27

Instead of /0.17 the engraving may also read /— = insensitive to larger deviations of the cover-glass thickness; /0 = corrected for "zero cover-glass thickness", i. e., for uncovered specimens.
Objectives without any further engraving are the widely used achromats. Other engraved abbreviations frequently found are:

Plan = Planachromat, i. e., flat-field achromat.
Neofluar = high-aperture objective (see page 37) with improved color rendition, high contrast, but without flat field.
Planapo = Planapochromat; objective of optimum color correction, maximum aperture and flat field.

Ultrafluar = special objective for ultraviolet microscopy, corrected for wavelengths between 230 and 700 nm.

Epiplan = flat-field objective for vertical bright-field illumination.
Epiplan HD = flat-field objective for vertical bright-field and dark-field illumination (in conjunction with concentric reflecting or refracting condenser).
POL = strain-free objective for polarized-light microscopy.
Ph 2 = phase-contrast objective for diaphragm 2 of the phase-contrast condensers.
m. I. = objective with iris diaphragm, also suited for transmitted dark-field illumination.

Oel	immersion objectives for	oil	(refractive index 1.515) black ring
W		water	(refractive index 1.333) white ring
Glyz		glycerin	(refractive index 1.455) orange ring
Methylenjodid		methylene iodide	(refractive index 1.740) yellow ring

Korr. = high-aperture objective with correction collar to allow for different cover-glass thicknesses (see page 43).
In addition, colored rings near the knurled ring facilitate recognition of the different magnifications: black — 1×, brown — 2.5×, red — 4×, orange — 6.3×, yellow — 10×, light green — 16×, dark green — 25×, light blue — 40×, dark blue — 63×, white — 100×. (The color rings referring to the immersion medium, however, are close to the objective front lens; see table above.)

Parfocal objectives . . .

Formerly, changing objectives was rather inconvenient, because the tube had to be lifted before turning the nosepiece, and every objective had to be focused separately. Today, the objectives are "parfocal". This means that they are optically and mechancially designed so that the distance between the specimen and the aerial image is always constant. Slight refocusing with the aid of the fine adjustment is therefore sufficient to restore critical sharpness of the image after changing from one objective to another, while the coarse adjustment need no longer be operated. This feature is particularly valuable when working with oil immersion, in which case the depth of focus is so small that focusing without parfocalized objectives usually presents considerable difficulty. (Only the especially long Planachromat 1 objective is not parfocal).

. . . and their prerequisites

It should, however, be noted that a few conditions must be satisfied to maintain the parfocal setting of the objectives: in the first place, only eyepieces engraved "CARL ZEISS" must be used (other eyepieces are designed for a different location of the aerial image); secondly, the observer must have normal vision (or the visual defect must be corrected by eyeglasses or a focusing eyelens); and finally, correct tube length is essential (with binocular tubes, identical values must be set at the eyepiece tubes and the wheel between the eyepieces. See page 32.).

Slide protection

Normal medium to high-power objectives have a relatively short working distance and may knock against the specimen unless special care is taken. In order to prevent damage to the specimen or the front lens of the objective, these objectives therefore have a built-in spring so that the objective as a whole will yield to pressure. Some objectives, particularly those for oil immersion, can be locked in the retracted position by clockwise rotation. This is to avoid soiling of the specimen with oil when the nosepiece is turned.

A very important hint regarding high dry objectives will be found on page 39 (correction collar).

Eyepieces

A single type for all objectives

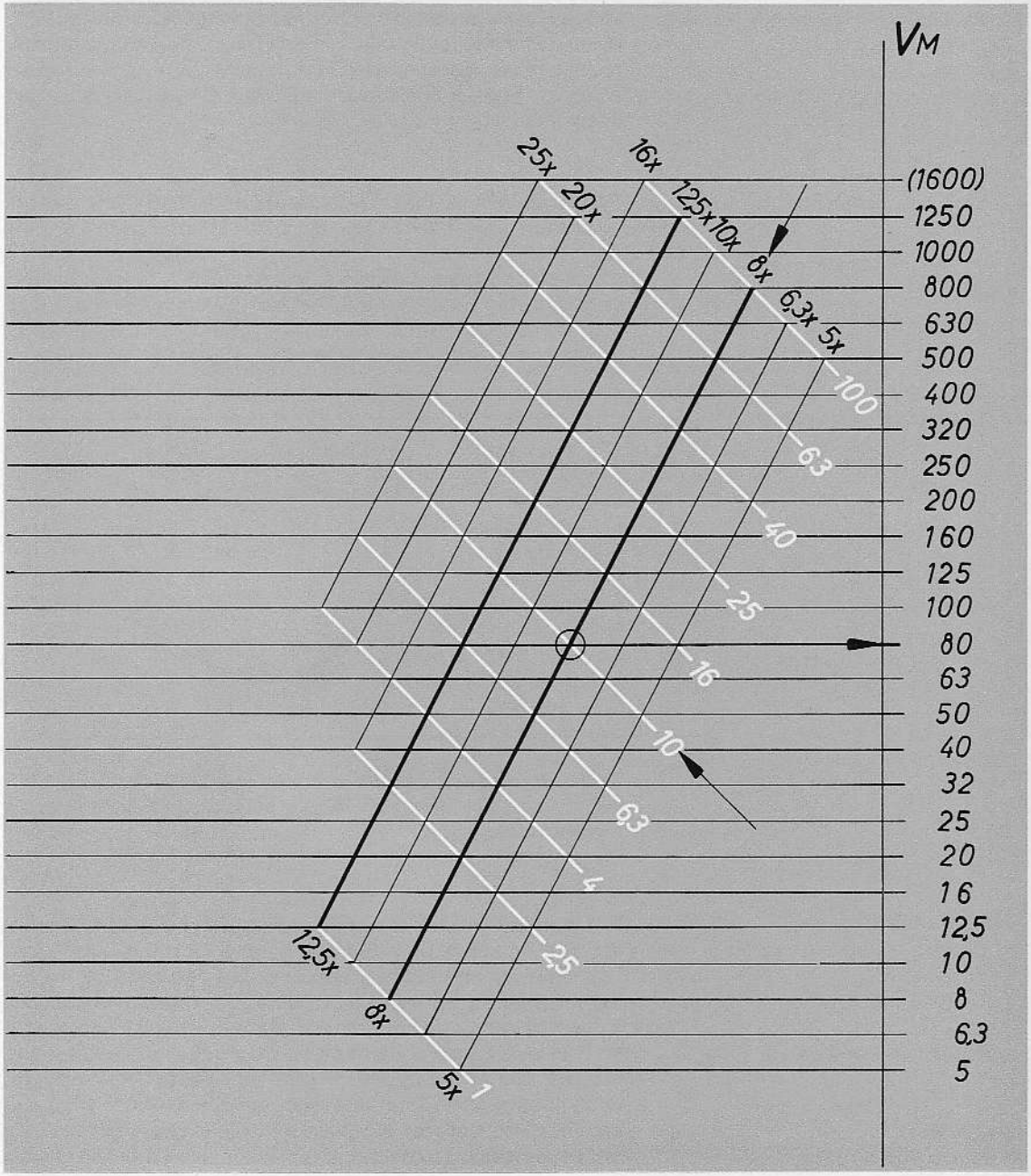
While a few years ago it was necessary to use different types of eyepiece for low-power and high-power objectives (Huygens and compensating eyepieces), ZEISS microscope objectives are now generally used with compensating oculars. Consequently, eyepieces need no longer be exchanged when changing over from one objective to another. In spite of this, two classes of eyepiece are still being manufactured. While the simple C-type eyepieces are primarily used in conjunction with achromatic objectives, the Kpl (compensating flat-field) eyepieces are intended for the other objectives of higher correction, but may, of course, also be combined with achromats.

Eyepieces for spectacle wearers

The most widely used eyepiece magnifications are 8× and 12.5×. Fig. 28 shows a list of all resultant magnifications which can be obtained with our microscope objectives and eyepieces.

Particular mention should be made of the eyepieces specially designed for spectacle wearers. As was mentioned already on page 10, the exit pupil of the microscope and the eye pupil must coincide if the entire field of view is to be covered. With normal eyepieces, the distance between the edge of the eyepiece and the exit pupil (eye point) is so short that eyeglasses cannot be kept on if the two pupils are to coincide. The high eye point of the special eyepieces for eyeglass wearers makes this possible even when the eyeglasses are not removed. The rubber guards supplied with these eyepieces prevent scratching of the spectacle lenses. — If these

Fig. 28, right
Table of microscope magnifications.
Objective scales: white
Eyepiece scales: black
Example shown:
10× objective, 8× eyepiece =
80× total magnification


VM
(1600)
1250
1000
800
630
500
400
320
250
200
160
125
100
80
63
50
40
32
25
20
16
12,5
10
8
6,3
5
25x
20x
16x
12,5x
10x
8x
6,3x
5x
12,5x
8x
5x
100
63
40
25
16
10
63
4
25
1

special types of eyepiece are used by observers not wearing glasses, care must be taken to keep the eyes at an adequate distance from the eyepieces. If necessary, finding the correct distance can be facilitated by slipping eyecups onto the eyepieces.

Field-of-view number

Apart from its initial magnification, an eyepiece is characterized by its field-of-view number. With the aid of this number it is easy to calculate the diameter of the field covered in the specimen plane, because this is (in millimeters) equal to the field-of-view number divided by the initial magnification of the objective. The field-of-view numbers of the most frequently used eyepiece types are given below:

C-eyepiece	5 ×	20
C-eyepiece	6.3×	18
C-eyepiece	8 ×	16
Kpl-eyepiece	8 ×	18
C-eyepiece	10 ×	16
Kpl-eyepiece	10 ×	16
C-eyepiece	12.5×	12.5
Kpl-eyepiece	12.5×	12.5
Kpl-wide-angle eyepiece	12.5×	18
Kpl-eyepiece	16 ×	10
Kpl-eyepiece	25 ×	6.3

Example: a 10× objective in combination with a 12.5× eyepiece permits a field of 1.25 mm diameter to be covered in the specimen plane.

Binocular tube
Adjustment

The microscope can easily be equipped for binocular observation by exchanging the ordinary tube for a binocular tube. The eyepiece sleeves of this tube can be displaced in relation to each other on a slide to allow for adaptation to the individual interpupillary distance of the observer. If you know your interpupillary distance, you may set this value on the wheel in the center by drawing the tubes apart. If you do not know this value, look into the eyepieces and vary the distance between the tubes until the fields of view seen by both eyes coincide exactly. The value indicated on the central

wheel must then also be set on each of the eyepiece sleeves. This restores the correct mechanical tube length which has been changed by the interpupillary distance setting, at the same time ensuring that the objectives are parfocal (see page 29).

Eyepiece focusing

If you should have the impression that your visual acuity is different in the two eyepieces, adjust the eyepiece sleeve on the poor side until both eyes see the same, sharply defined picture.

Condenser
Height adjustment

Because of its great importance we want to repeat once more that the correct position of the condenser is always at its upper stop; it is lowered **slightly** only for the Köhler method of illumination (see page 45).

Illuminating large fields

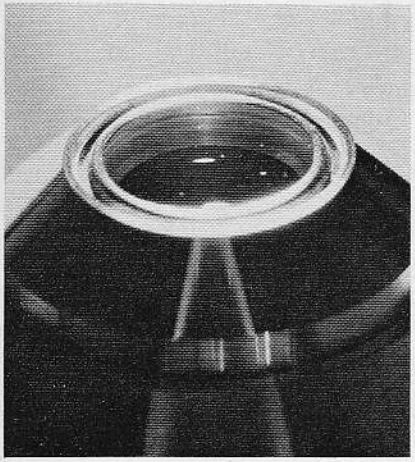
The requirements made of a condenser are partly contradictory, because it is not only the large object fields of the low-power objectives which have to be illuminated, but also the pupils of high-power systems of high aperture. Some condensers are therefore equipped with a swing-out front lens: as soon as a low-power objective reproduces more than the condenser illuminates, the condenser iris is opened fully and the front lens swung out. Condensers without a swing-out lens usually allow the front lens to be unscrewed; if even this is impossible, then it is necessary to change the condenser.

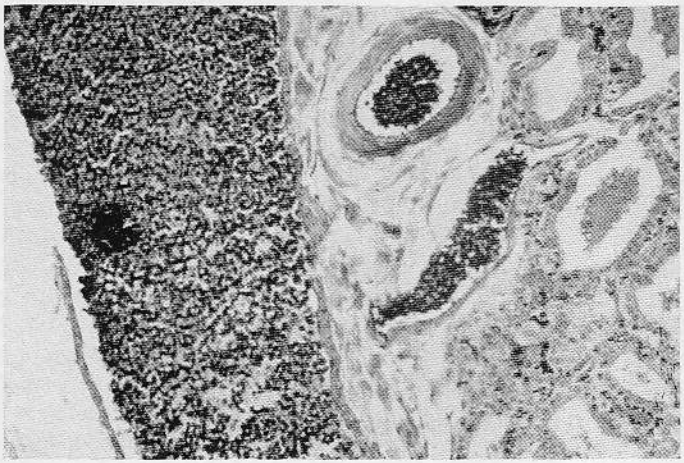
Oblique illumination
with Ph-condenser

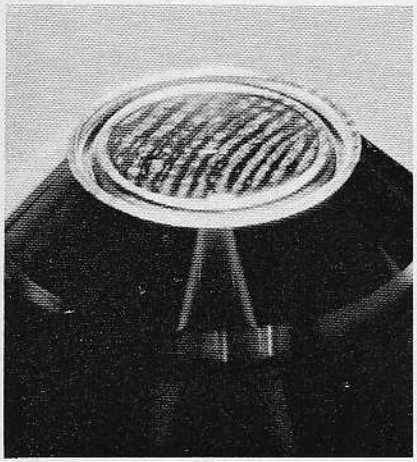
For phase-contrast illumination a special condenser is used, the design and operation of which are described in the pertinent instruction manual. These phase condensers are also suited for so-called "oblique illumination", and in some cases for dark-field illumination. After the microscope has been adjusted under bright-field illumination, turn the turret plate from its click-stop position "I" (iris, i. e., bright field) to the left or right, and you will immediately notice the three-dimensional effect which is typical of oblique illumination. Shortly before the field of view is completely obscured as you keep turning the plate, one-sided dark-field illumination is obtained.

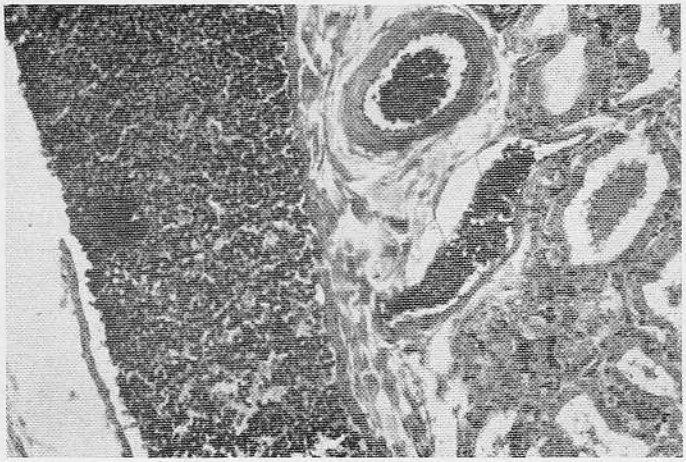
Normal, i. e., dark-field illumination from all sides can be obtained with low and medium-power objectives by choosing annular diaphragms of the phase-contrast condenser which, due to their size, remain outside the objective aperture; this can easily be checked by looking into the tube after having removed the eyepiece.

For further details on special dark-field condensers, reference is made to the brochure G 40—165.









5. Care and cleaning of the microscope

An instrument which has to satisfy the most exacting requirements regarding mechanical and optical precision naturally demands a certain amount of care.

Enemy No. 1: dust

Dust is practically everywhere and may become a source of trouble wherever — as in the microscope — mechanical parts are moving in precision guides. When the tracks are dirty they need washing and regreasing — which should preferably be done by the manufacturer.
Dust on optical elements will degrade the image quality to a greater or lesser extent. It is true that all surfaces exposed to dust are also easily accessible for cleaning, but no glass surface is improved by "cleaning", so that the best advice is always to avoid exposure of the microscope to dust by covering it up with a hood when not in use or by keeping it in a cabinet. Special care should be taken to ensure that the tubes of the microscope are always closed either by an eyepiece or a dust plug.

Dirty objective front lens

While dust particles on the eyepiece will only give rise to patches in the image — which not every observer finds disturbing — a dirty objective front lens may hopelessly reduce the sharpness of the image, or at least its contrast. Due to the fact that it is close to the specimen and possibly to the immersion oil of the coverglass, but particularly to the hand operating the nosepiece, the front lens of the objective is in special danger of getting soiled. Even the lightest fingerprint may have grave consequences (Figs. 29, 30). Before starting important work it is advisable — particularly if the microscope is not used by one observer alone — to unscrew every objective and check it carefully with the aid of a magnifier. Dirt is easily recognized if the objective is held so that the image of a light source is reflected from its plane surface. In the case of objectives which have a concave front lens, a different approach is indicated, viz., to examine the surface of the front lens from the screw-thread side. The remaining lens elements can also be examined easily and any faults (cracks, "starting" of the cement) detected without difficulty.

Fig. 29
A clean objective front lens is the first prerequisite for brilliant images (top).
Fig. 30
Even a fingerprint on the front lens will ruin the contrast (bottom).

Hints on trouble-shooting

Should structures be found in the image which are suspected of being extraneous to the specimen, the fault may be traced as follows:

If the trouble can be eliminated by slight adjustment of the condenser, the cause must be sought in the bulb of the lamp, the lamp condenser or the filter in front of it. However, if adjustment of the condenser does not produce any result, the next step is to turn the focusing adjustment, which should eliminate all faults due to soiling of the condenser front lens or the specimen. If this does not lead anywhere, slightly turn first the objective and then the eyepiece, and you will immediately notice in which case the foreign body follows the rotation.

Dust particles are most clearly seen when the aperture stop has been fully closed, because in this case the depth of focus is at its greatest.

Cleaning, but how?

In almost all cases it will be sufficient to clean the outer lens faces with the aid of a grease-free brush (if necessary, wash in ether first) or with a frequently washed, absolutely dust-free linen rag and distilled water, produced easily by breathing upon the surface to be cleaned.

If an organic solvent cannot be dispensed with, it is advisable to use **very little** ether or benzine instead of water, but never alcohol which might destroy the cement between lens elements.

Ether is usually preferred because it evaporates most quickly and any harmful effect is thus less likely. Finally, residues are always removed with water as described above.

Should compressed air be available for cleaning, be sure to use a filter of cotton wool.

Humid climate

If the air in the work room continuously has a relative humidity of more than 60 %, certain precautions should be taken to avoid fungus growth on the optical elements. Do not keep microscopes under plastic covers, do not store them in cabinets, but ensure good ventilation, if necessary with the aid of a fan. In a particularly humid climate it is advisable to keep optical parts in perfectly airproof containers provided with a dessicant or in which, for instance, a lamp and a fan circulate air of 40—50 ° C (100—120 ° F). — Our agents have special instructions and will be pleased to assist you.

6. Numerical aperture, useful magnification and limit of resolution

The grain in the slide . . .

To understand this question better, let us now think again of the projector and the projected slide described in section 3. At first we observe the landscape on the screen from far behind the projector. In this case, magnification is still low, because the angle of view is quite small. As we move closer to the screen, magnification increases and we begin to recognize details which had originally escaped our attention. But finally a point is reached where higher magnification (= moving closer) does not give better results. Instead of single leaves on the trees we then only see the structure of the color film, an accumulation of dye particles, the so-called grain. By further reducing the viewing distance we could, of course, further increase magnification, but this would be so-called "empty magnification", i. e., magnification which does not reveal any new detail.

. . . is equivalent to a "diffraction grain" in the aerial image

In the microscope, the situation is the same. As we have seen, the projected image corresponds to the aerial image formed by the microscope objective in the tube. Just as with the projected slide, there is also a limit for the projected aerial image, where "useful magnification" ends and "empty magnification" begins. It is true that the microscopic image will not exhibit any actual grain structure, but here also we may speak of "grain", because there is no optical image in which an object point is really reproduced as a point (i. e., with no measurable diameter). Due to the phenomena of diffraction, an object point is always transformed into a small disk in the image. It is these so-called "Airy disks" which form the "image grain". This type of grain can be found in any optical image if the magnification is high enough. It is obvious that image details will no longer be clearly recognized as soon as they reach the size of the Airy disk — just as we were unable to recognize the leaves in the slide when they were as small as the dye grain.

Another example: Let us examine a mosaic from a long distance (i. e., with low magnification). As we move closer, we recognize more details. As soon as we begin to recognize individual elements of the mosaic, however, we pass the limit of "useful magnification". In this case, the elements of the mosaic correspond to the Airy disks making up the aerial image.

Beware of pseudo structures!

If we view a slide or a mosaic with higher than useful magnification, no harm is actually done, because we know very well that the above mentioned grain structure does not belong to the object, for instance the landscape. However, in microscopy the situation is different: so-called empty magnification may show structures in the image which do not exist in the specimen. But since we do not know the specimen, there is a danger of erroneous conclusions. And in fact there are examples of things being discussed in scientific literature which did not exist in reality but eventually revealed themselves to be a kind of "image grain", i. e., the result of diffraction phenomena.

What is the highest power we should use?

It follows from the above remarks that we should know the performance limit of the microscope objectives we use, i. e., the point at which their useful magnification ends. This is indicated by a value engraved on every objective and called "numerical aperture". The tremendous importance of this characteristic will immediately be recognized if we say that the numerical aperture is the deciding factor when determining the size of the Airy disks in the microscopic image, i. e., the size of the "screen". There is a definite relationship between the numerical aperture and the size of the Airy disk. For the moment, you need only know the following:
The total magnification of the microscope (i. e., the magnification of the objective multiplied by that of the eyepiece multiplied by any tube or OPTOVAR factor) should not exceed one thousand times the numerical aperture.
Microscopists who have particularly good visual acuity should even use five hundred times the numerical aperture as a limit. Exceptions to this rule may be allowed in order to facilitate measuring and counting work.

Numerical aperture

We still owe you an explanation of the term "numerical aperture". Fig. 31 gives a diagrammatic view of an objective front lens and specimen. The angle u' subtended by the optical axis and the outermost rays still covered by the objective is a measure of the aperture of the objective; it is the aperture angle. However, the magnitude of this angle is not indicated in degrees but in the form of a sine value, that is a numerical value. This explains the origin of the term "numerical aperture". It is the sine of the half aperture angle multiplied by the refractive index n of the medium filling the space between the cover glass and the front lens.
Numerical aperture $= n \cdot \sin u'$

Since air has a refractive index of 1, n may be neglected when dry objectives are used. Immersion oil, which in the case of an oil immersion objective fills the space between the cover glass and the front lens, has a refractive index of 1.515, and it is obvious that such an objective makes it possible to achieve a considerable gain in numerical aperture.

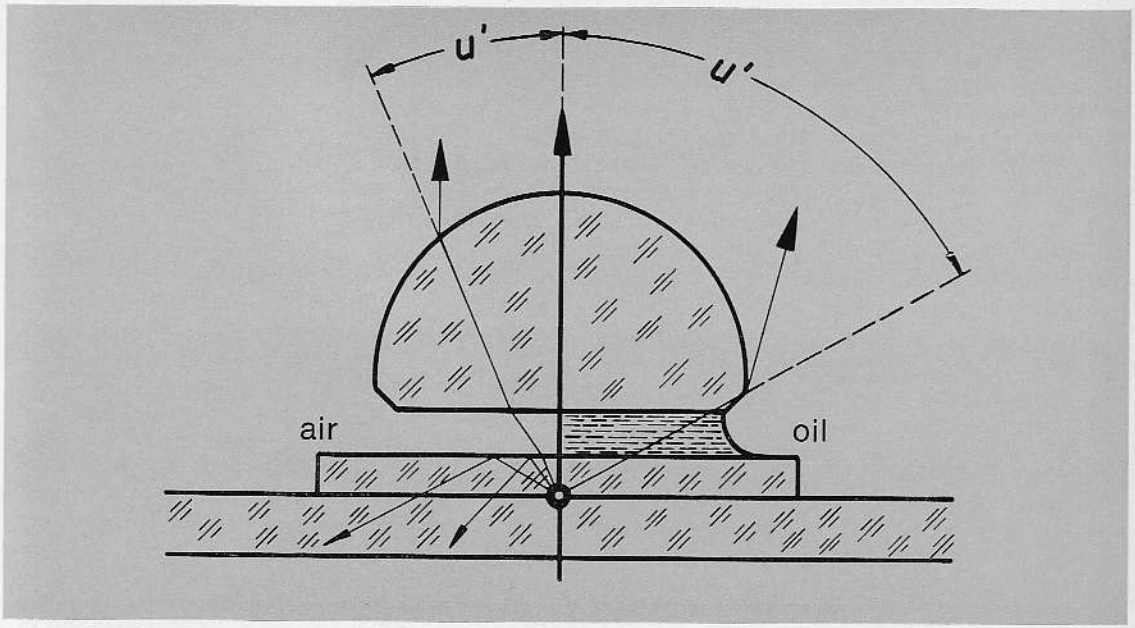


Fig. 31
shows the gain in aperture by oil immersion (right)
as compared with a dry objective (left)

What is the resolving limit?

With the aid of the numerical aperture we can also compute the limit of resolution or maximum resolving power. By this we understand the smallest separation — designated d — which two structural elements, e. g., two adjacent flagella, may have in order to be imaged as two separate elements instead of one.

And how is it determined?

If n. a. $_{obj}$ and n. a. $_{cond}$ are the numerical apertures of objective and condenser and λ is the wavelength of the light used for observation (in 1/1000 mm or microns), then the smallest resolvable separation between two object points (likewise in microns) is

$$d = \frac{\lambda}{\text{n. a.}_{obj} + \text{n. a.}_{cond}}$$

Example: Using a green filter, the wavelength is 0.55 μ; assuming the objective used to have an aperture of 1.25, and the condenser one of 0.9; then d = 0.25 μ, i. e., the fourth part of a thousandth of a millimeter. — However, structural elements which are just resolved are **not** reproduced with full fidelity.

7. Oil immersion

What is oil immersion?

Oil immersion objectives are objectives which are used with immersion oil (instead of air) between objective front lens and cover glass. As was explained in the previous section, this method leads to a gain in numerical aperture. For the same image scale, an oil immersion objective is always superior to a comparable dry objective as regards correction. There are, of course, cases which do not permit the application of the oil immersion technique. 100× objectives are always oil immersion objectives.

Important:
correct application of the right oil

The user should, if possible, employ only the type of immersion oil supplied by the objective manufacturer, because not only the refractive index must have a certain value, but also the dispersion of the oil. The immersion oil supplied by ZEISS is synthetic, non-fluorescing and non-resining oil with a refractive index of 1.515 and a dispersion of 43.96. The oiler supplied by us with every immersion objective is designed to facilitate application of the oil to the cover glass without air bubbles: turn oiler upside down, wait until the air has risen and apply a little oil. The objective may be moved into position as usual. In this case, excessive oil and, possibly, bubbles are pushed aside due to the short working distance. In addition, objectives with a plane front lens can be pushed upward in their spring mount, locked by clockwise rotation and lowered vertically into the oil only after the nosepiece has been turned and engaged the notch. Personal experience will teach you which of these two methods is more convenient.

Immersion objectives mostly have a short working distance

If focusing the specimen with oil immersion should prove impossible, remember the previously mentioned possibility that the cover glass may be too thick for the short working distance available or that the space between the specimen and the cover glass, which is filled with mounting medium, may be too wide, which comes to the same thing.

Some condensers also require immersion

If your condenser has a numerical aperture higher than 0.9, it is advisable also to immerse the condenser (if not with oil, at least with water) for use in combination with an oil immersion objective. For this purpose, a drop of oil is applied to the underside of the specimen slide and another one to the condenser front lens. This will effectively reduce the danger of bubbles forming when the condenser is immersed. — Whether both oil films are actually free from bubbles can easily be determined by looking into the objective from the top after having removed the eyepiece. The centering telescope of the phase-contrast equipment simplifies this quite considerably.

Although ZEISS immersion oil is of the non-resining type, it should be removed at the end of the day by means of a clean rag of the kind used for cleaning optical elements.

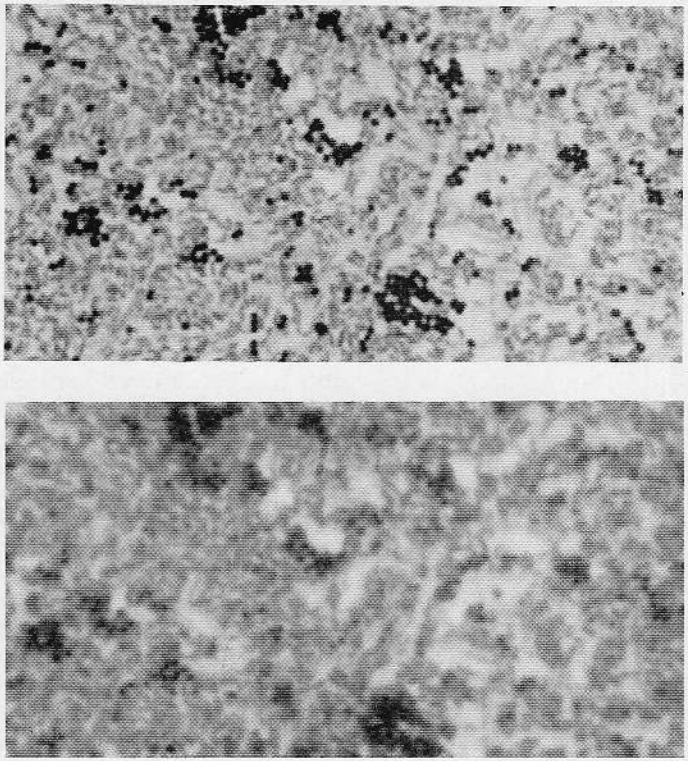


Fig. 32
Planachromat 63/0.90 with correction collar
Top: carefully adjusted for cover-glass thickness
Bottom: not adjusted
Coli bacteria and staphylococci (Gram's stain)

8. The question of cover glasses

The cover glass —
the first "lens" of the objective

Knowing the high precision required for the production of microscope objectives which are to give first-rate images, we should not be surprised that the cover glass, which is located between the specimen and the objective front lens, may not be just any cover glass. We should not forget that simple glass plates, that is plane plates, also have an optical power and that the optical power of the cover glass was taken into account in the computation and design of the objective. With normal microscope objectives, the cover glass is part of the image-forming system. It is a "lens element" (though with infinite radii of curvature), which is located outside the objective mount but still forms a part of the optical system. — The optimum thickness of a cover glass is 0.17 mm.

The higher the aperture,
the more accurate the cover glass!

Strictly speaking, this applies only to objectives whose numerical aperture exceeds a certain value. With objectives having an aperture not exceeding 0.3, specimens may be examined both uncovered or with cover glasses as thick as a millimeter, and the spherical aberration will not be noticeable. With apertures between 0.3 and 0.7, the cover-glass thickness should not deviate from the nominal value by more than 0.03 mm. With higher apertures, even a deviation of 0.01 mm will considerably impair the quality of the image.

Objectives with a correction collar
are for careful users only

In order not to be restricted to specified cover-glass thicknesses when using dry objectives of particularly high aperture, objectives were designed in which the optimum cover-glass thickness is not a fixed 0.17 mm, but can be varied within a range of 0.12 to 0.22 mm (objectives with correction collar). If the cover-glass thickness of the specimen to be examined is unknown, first set the correction collar at 0.17 mm and find a high-contrast area in the specimen. Then try whether the contrast is improved if the correction collar is set for greater or lesser cover-glass thickness. This also requires refocusing. It is obvious that in the hands of inexperienced users, who usually leave the correction collar at one end of its range of adjustment, these objectives may give very disappointing results (Fig. 32).

Oil immersion is less critical

The situation is different with oil immersion objectives, because then a medium of high refractive index, namely oil, is in front of the first lens. In this case, the thickness of the cover glass is not very critical. Still, it should be remembered that immersions are today no longer "homogeneous" enough (i. e., glass and oil of identical refractive index) to allow the cover glass to be dispensed with as a matter of course. When the cover glass is omitted, for very critical work a correction is required either by increasing the mechanical tube length or by using a special immersion oil (for more details, see, for instance, Michel, Vienna 1964, page 176).

How to obtain good cover glasses?

For practical work, it is thus necessary always to use cover glasses which are exactly 0.17 mm thick, for specimens which are to give optimum images even when examined with the aid of high dry objectives. Micrometers, so-called cover glass gages, are available for measuring. Appropriate covers may thus either be selected by the microscopist himself, or others used which have been specially selected by the manufacturer.

It should be mentioned that in those cases where the cover glass is not in direct contact with the specimen, the intermediate layer of mounting medium has, of course, the same effect as additional thickness of cover. It is therefore advisable to weigh down the covers of specimens during drying.

9. Microscope illumination for more exacting requirements

Two important requirements: object field and pupil must be bright

For less critical work, the microscope mirror may be illuminated either by daylight — preferably diffused light, such as given by a white cloud — or an ordinary desk lamp with inside frosted bulb. More convenient are the attachable simple substage illuminators for direct connection to the mains for teaching microscopes (see Fig. 1). In all these cases optimum conditions will have to be determined by trial and error. Two things are important: the field of view should be evenly illuminated and — when the condenser iris is open and the condenser in its uppermost position — the back lens of the objective should be filled with light as completely as possible. This latter condition is checked after the eyepiece has been taken out of its tube.

Köhler illumination

For microscopic research work, photomicrography, microprojection, etc., precise control of the light path should already start before the light reaches the specimen, i. e., at the light source. Prof. August Köhler first used this exact control of the light path in the illuminating beam of the microscope, and the method is today called "Köhler illumination" (Journal of the Royal Micr. Soc.).

Requirements

Use of the Köhler method depends on the availability of a microscope illuminator with a lamp condenser and an iris diaphragm in front of it (Fig. 33). The lamps used in such illuminators usually have a small, concentrated filament and operate on 6 or 12 volts; they are therefore called low-voltage lamps. Contrary to normal bulbs for electric lighting it is not their wattage which is decisive for their efficiency, but their luminance.
The low-voltage illuminator incorporated in the base of larger microscopes is, of course, also suited for applying Köhler's rules of illumination.

. . . and procedure

The procedure for applying this method of illumination is as follows (Fig. 34):

Fig. 33
Low-voltage microscope illuminator with iris diaphragm, on stand

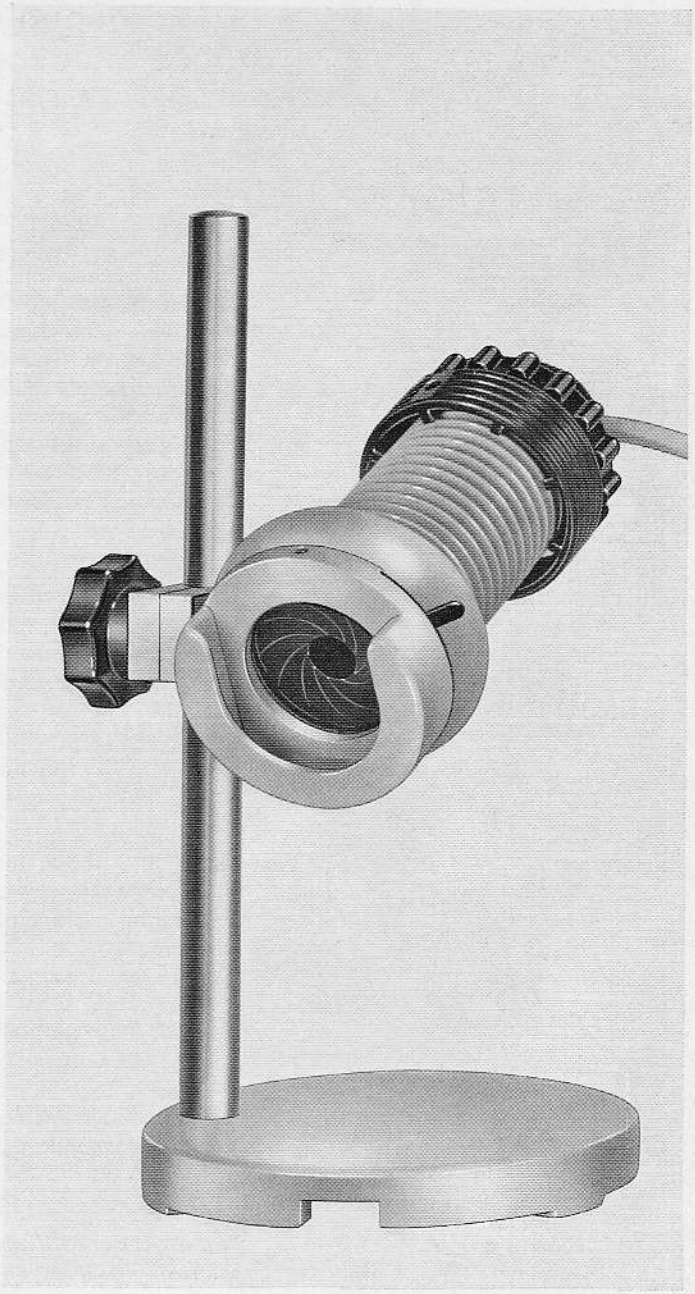
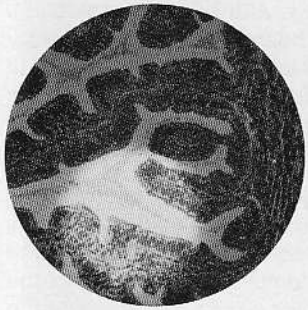
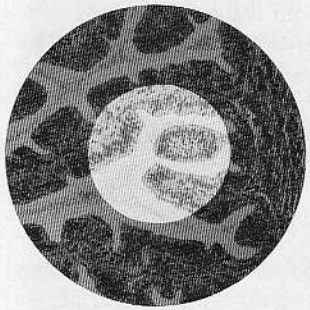


Fig. 34, right
A lamp diaphragm, blurred
B lamp diaphragm, focused
C lamp diaphragm, centered
D lamp diaphragm, open

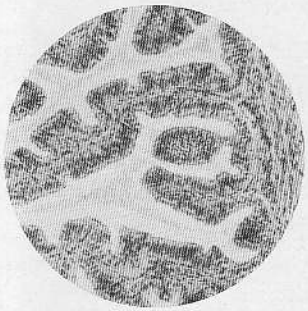


A

B



C



D

1. Align microscope illuminator with plane mirror of the microscope and adjust it so that a small, bright spot of light is projected onto the mirror or an image of the filament formed (this adjustment is facilitated if a sheet of paper is held over the surface of the mirror).

2. Rack up the condenser to its top and take the eyepiece out of the tube. Keep adjusting the mirror until the back lens of the objective is filled with light. Dimming the light by a neutral density filter would be advisable. Insert the eyepiece and focus the specimen with a 10× or 16× objective.

3. Close the iris diaphragm of the illuminator (field stop) almost completely and move its (unsharp) image on the specimen into the center of the field of view by rotating and tilting the mirror.

4. Focus the edge of this diaphragm image by slightly lowering the condenser. Both the specimen and the field stop are now sharply defined. Then open the field stop until the entire field of view is just clear.

5. Slightly readjust the lamp socket or the lamp condenser, until the field of view is evenly illuminated.

6. At first open the condenser iris (aperture stop) completely (image very bright) and close it only far enough to eliminate glare in the most important image elements and to make them appear with satisfactory contrast. With stained specimens this is the case even at a relatively wide aperture, while fresh specimens require slightly more stopping down. Stopping down further than is absolutely necessary (for example, to reduce the brightness of the image) is one of the biggest mistakes a microscopist can make, since it entails a loss of resolving power. — If the intensity of the light is too high, reduce the lamp voltage or insert gray or green filters into the filter holder.

7. If with low-power objectives only part of the field is illuminated, swing out the condenser front lens and open the condenser iris fully. Contrast is then controlled with the aid of the lamp diaphragm.

In the case of microscopes with a built-in illuminator, step 1 and partly also step 2 are omitted. Under 3, centering is not achieved with the aid of a mirror, but by the centering screws of the condenser. Please consult the operating instructions supplied with the microscope.

The path of rays . . .

Let us now examine the path of rays with Köhler illumination. It consists of two superimposed beams. For greater clarity we shall show both beams separately and only on one side (in the case of the pupil beam, for instance, only the rays originating at one end of the filament).

. . . for the windows

Fig. 35 shows the path of the rays originating and meeting in the planes which are in sharp focus together with the specimen, i. e., the so-called windows. The rays emanating from the field diaphragm of the lamp are combined in the specimen by the condenser (in other words, an image of the field diaphragm is formed on the specimen). From there, the two images are projected onto the field of view diaphragm of the eyepiece (aerial image). When the adjustment is correct, the images of the field and field of view diaphragms appear sharp, both are centered, i. e., they are concentric, and they are of identical size.

. . . for the aperture stops

Fig. 35a shows the rays which combine at the aperture diaphragm planes. Closing down these diaphragms would affect the diameter of the previously mentioned exit pupil of the microscope above the eyepiece. Due to refraction by the condenser, the rays emanating from the source meet in the aperture diaphragm of the condenser and are then imaged by condenser and objective in the exit pupil of the objective, whence they are transmitted by the eyepiece to the exit pupil of the microscope. At all these points, an image of the light source L is formed.

The advantages of Köhler illumination

What advantages does the Köhler method offer?

1. In spite of small filaments, the entire area of the lamp diaphragm has the same luminance as the filament, so that only a low power input is required for the lamp.

2. Due to the adjustment of the lamp diaphragm, the area illuminated in the specimen corresponds to the one which is imaged in accordance with the field stop of the eyepiece. The specimen is thus not subjected to unnecessary irradiation (examination of living organisms!), and contrast-reducing stray light from specimen areas outside the field of view is eliminated.

3. The aperture and thus contrast as well as depth of focus are controlled by the condenser iris.

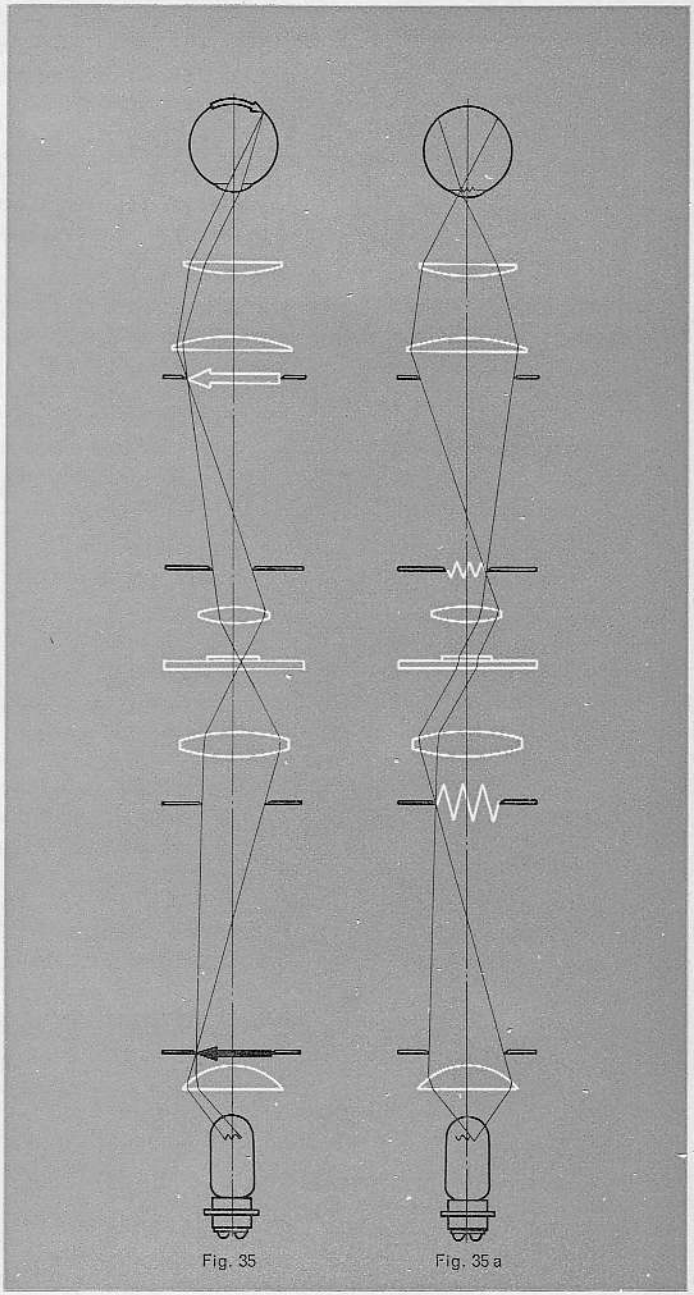


Fig. 35 Fig. 35 a

4. Viewed from the specimen, the light source is at infinity (parallel rays in the aperture ray path, Fig. 35a). Any irregularity in the source is therefore least effective in the specimen plane.

5. Since the field stop is just fully filled with light due to the adjustment of the lamp diaphragm, no light which could give rise to disturbing flare reaches the inner walls of the tube. As a result, particularly brilliant images are obtained.

Possible difficulties

When adjusting the equipment for Köhler illumination, it may happen that a certain granularity is noted which is superimposed on the specimen over the entire field of view. This is the texture of a ground-glass screen or an etched collector lens which are intended to cover up the irregularity of the source. If this plane should accidentally be in sharp focus as well, it is only necessary to alter the height of the condenser slightly.

With high-aperture condensers sharp imaging of the lamp diaphragm is frequently possible only if the specimen slide is not thicker than 1 mm.

Why auxiliary lenses?

In microscopes with built-in illuminator the field diaphragm is sometimes located very close to the condenser. When imaging this diaphragm on the specimen in accordance with Köhler's rules, the condenser has to be lowered to such an extent that a considerable loss of aperture ensues. This is avoided by a so-called auxiliary lens which is supplied together with the microscope and is located directly below the condenser. Its function is illustrated in Fig. 36. If a separate high-performance illuminator is used, the auxiliary lens should be swung out or, better still, replaced with the longer-focus "auxiliary lens II".

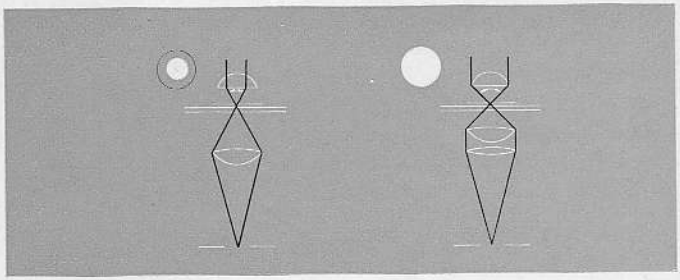


Fig. 36
Effect of auxiliary lenses.
With Köhler illumination and built-in illuminator, lowering the condenser would lead to an excessive loss of aperture (left). This is avoided by the use of auxiliary lenses (right).

10. Length measurements under the microscope

Equipment

Measurement of microscopic objects is relatively simple, but depends on the availability of the following accessories: measuring eyepiece, eyepiece micrometer and stage micrometer (Fig. 37).

A round glass disk with a scale — the micrometer disk — can be inserted in the measuring eyepiece, and the focusing eyelens ensures that even observers with defective eyesight can see a sharp image of the scale. — The stage micrometer is a scale graduated in units of 1/100 mm on a conventional specimen slide.

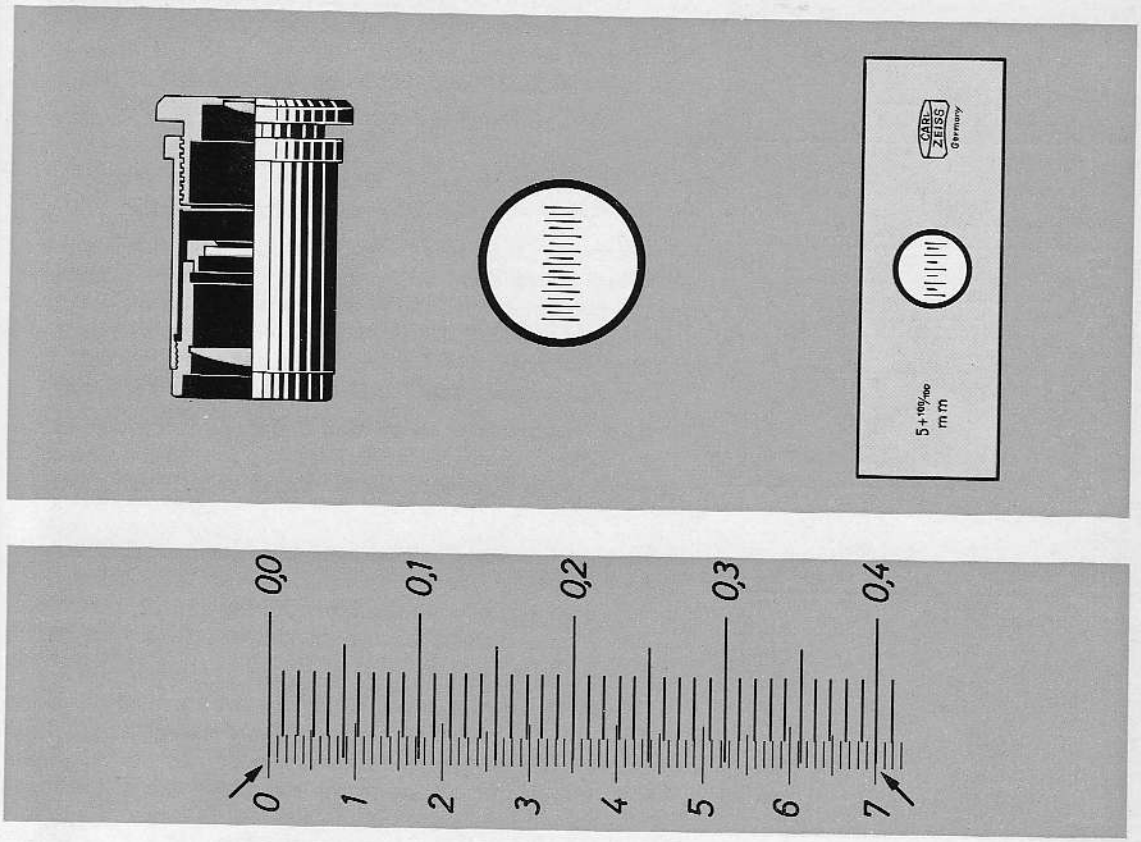


Fig. 37, top
Eyepiece with focusing eyelens, eyepiece micrometer, stage micrometer
Fig. 38, bottom
Determination of micrometer value

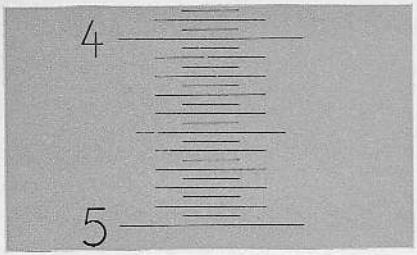
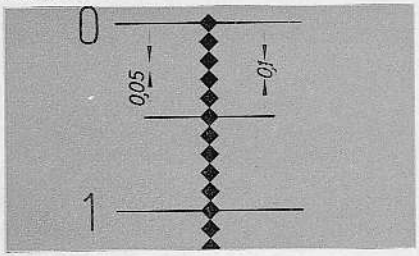
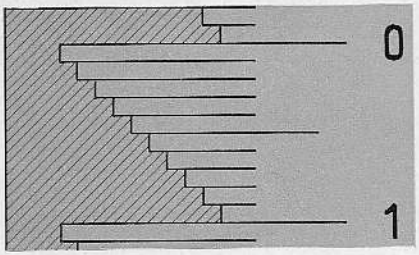


Fig. 39 Eyepiece micrometers

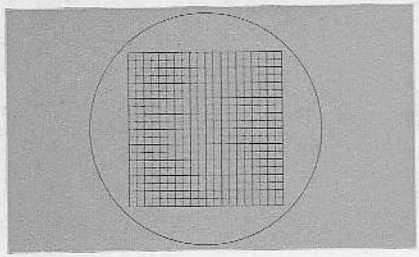
Contrast micrometer



Scale contrast micrometer



Net micrometer



The procedure is as follows:

1. Position the round eyepiece micrometer in the measuring eyepiece. For this purpose unscrew the lower part of the eyepiece, remove the ring above the eyepiece diaphragm and replace it after having inserted the micrometer disk (scale upwards). Then screw the eyepiece together again.

2. Focus the eyelens in such a manner that the scale appears with maximum sharpness; then insert the eyepiece into the tube.

3. Place the stage micrometer on the stage and focus the microscope on the scale. Both scales will then appear sharply defined. By turning the eyepiece, they are laid parallel to each other.

4. Find out how many divisions of the eyepiece micrometer correspond to a certain distance on the stage micrometer and calculate the length which corresponds to one division of the eyepiece micrometer.
Example: (Fig. 38) 80 divisions correspond to 1.35 mm

$$\text{one division} = \frac{1.35}{80} = 0.0169 \text{ mm or } 16.9 \text{ microns.}$$

5. The "micrometer value" thus determined applies only for the objective with which the calibration was made.

Subsequently, it is only necessary to multiply the number of divisions covered by an interesting detail in the specimen by the micrometer value in order to determine the length of this detail in the object plane. (For serial measurements, the micrometer value need only be set once on the slide rule, whereupon the different lengths can simply be read off.)

Since the micrometer value also depends on the mechanical tube length, it is advisable to keep an eye on the setting of the eyepiece sleeves of the binocular tube which must not be altered.

If the measurements extend over a longer period of time or if the specimens themselves exhibit a fine linear texture, we recommend that a contrast, line-contrast or net micrometer disk be used instead of the normal eyepiece micrometer (Fig. 39 a).

A measuring eyepiece can, of course, be used in the same manner as any ordinary eyepiece if the micrometer disk is taken out. In this case, an observer who has normal vision should set the zero mark on the eyelens scale at the black instead of the red line.

11. The camera on the microscope

If necessary, it can be simple

The simplest way of taking photomicrographs is to mount a camera with its lens set at infinity as closely above the microscope eyepiece as possible and to open the camera stop fully. If the microscope was first focused with relaxed accommodation, then the photomicrograph will be sharp, but usually vignetted. This is due to the fact that in combination with a camera lens of normal focal length (50 mm for 24×36 mm, 75 to 80 mm for 6×6 cm, 105 mm for 6×9 cm) only wide-angle microscope eyepieces cover the entire field of view. — Any doubts regarding critical focus can be eliminated if a single-lens reflex camera is employed (Fig. 40).

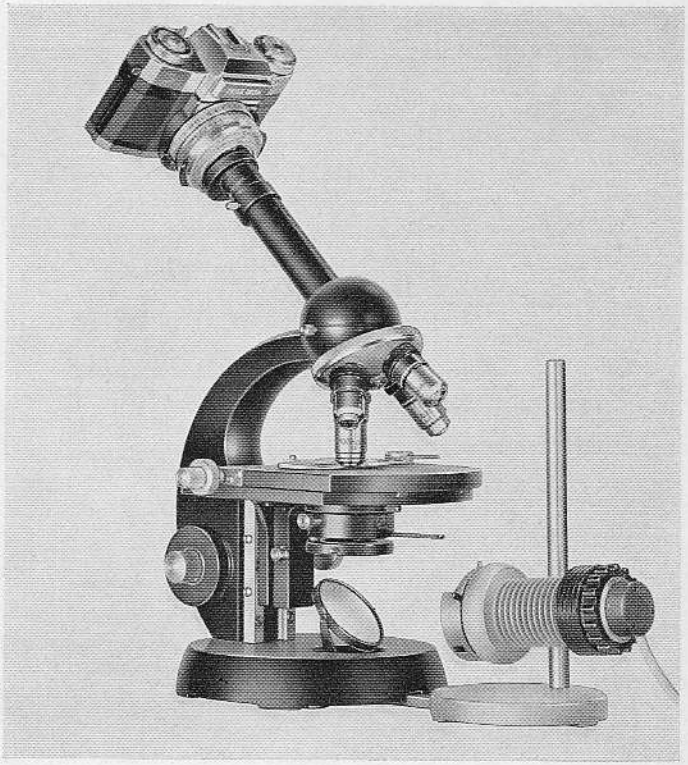
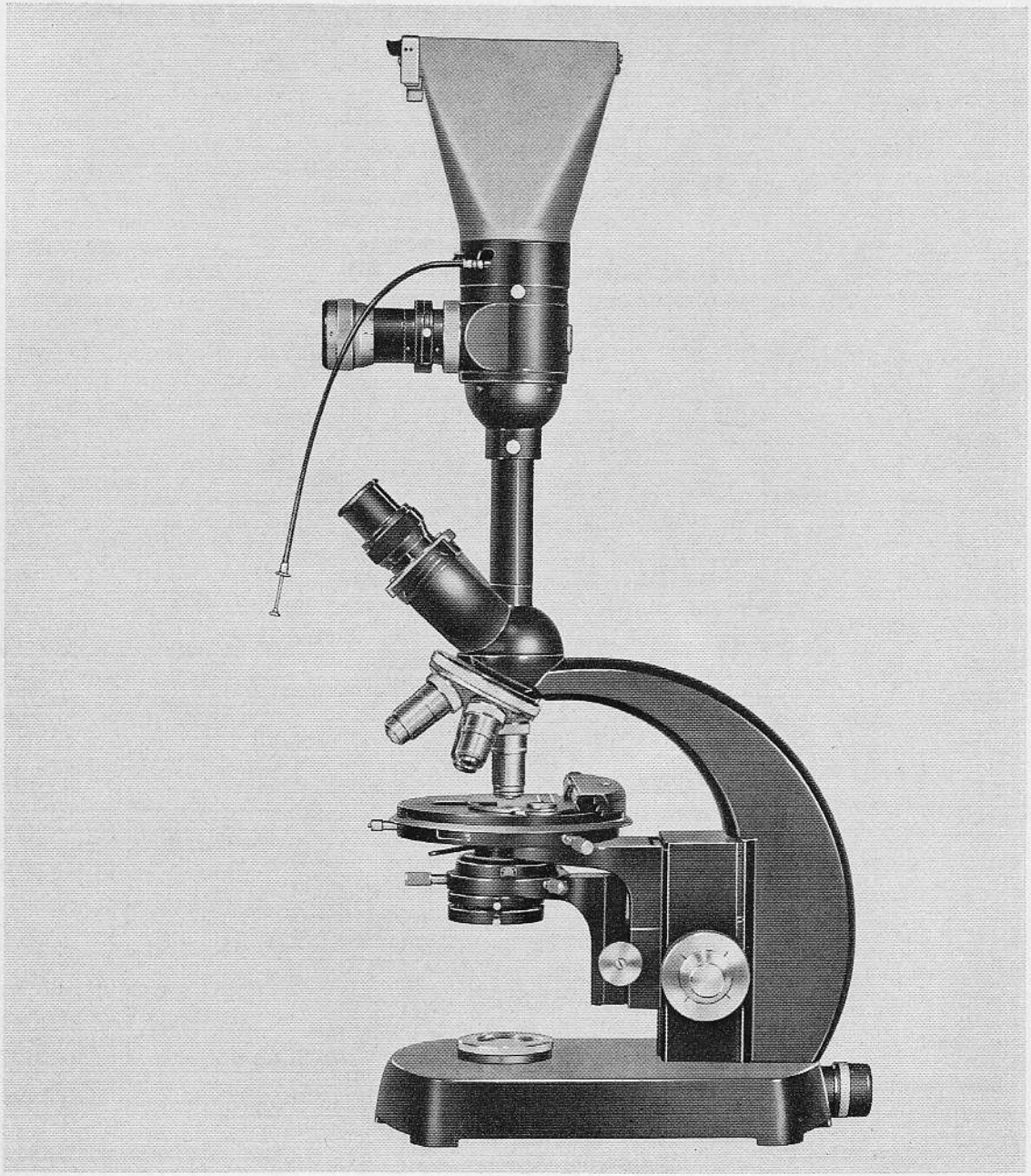


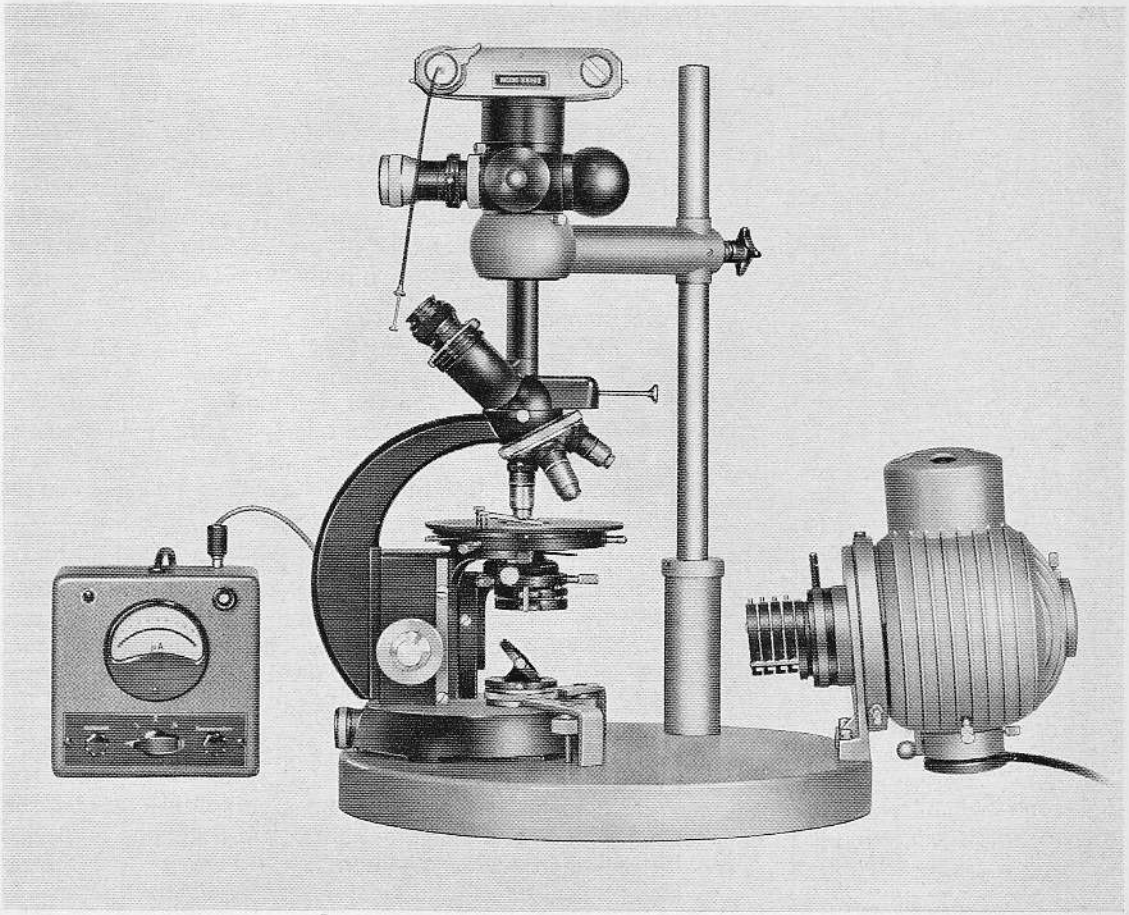
Fig. 40
Photomicrography with a reflex camera



Focusing beam splitters are ideal

Since a portion of the light is lost by the time it reaches the camera lens, the focusing elements of normal reflex cameras (ground-glass screen, split-image rangefinder, microprism focusing ring, etc.) are not very convenient. Considerably more accurate and brighter are special focusing beam splitters with cross hairs for focusing on the aerial image. With cameras with focal plane shutter such as Contarex, Contax, etc., it is advisable to remove the lens and attach the camera body with a special adapter to a so-called attachment camera which is available either without (Fig. 41 a) or with photoelectric exposure device (Fig. 41 b).

Fig. 41 a, left
ZEISS attachment camera I
without exposure device
Fig. 41 b, bottom
ZEISS attachment camera II
with exposure device



What image scale is obtained with a photomicrographic camera?

The image scale on the film differs by a certain factor from the magnification given by the microscope during visual work. This factor is found by dividing the focal length of the camera lens (in millimeters) by 250 mm. (Example: A Contaflex camera as shown in Fig. 40 has a focal length of 50 mm. Consequently, the factor is 1/5. Objective magnification × eyepiece magnification × 1/5 thus gives the image scale on the 35 mm negative.) The special ZEISS attachment camera shown in Fig. 41 has camera factors dependent on the individual film format.

Exposure time

Determination of the correct exposure for photomicrography is very convenient if special photoelectric exposure devices are used (Fig. 41 b).

Another simple and accurate method is direct measurement of the finder image of a reflex camera, preferably by means of a photoelectric exposure meter which has a small acceptance angle and measuring surface (e. g., the ZEISS IKON "IKOPHOT CD"). Not even calibration photos are required, if, for instance, the following procedure is used:

Point camera with lens set at f/2.8, for example at a bright wall. Take a meter reading of the wall, with, say, f/2.8—1/50 sec. Then take a reading with the meter pointing at the ground glass or the finder image and read the f-stop corresponding to 1/50 sec, e. g., f/1.4. When taking the photomicrographs, all readings should then be taken with the meter pointing at the finder image; the exposure time is then the value corresponding to f/1.4.

If working conditions are always identical (objective, eyepiece, position of condenser iris, lamp, film speed), then exposure measurements are not required. A few test exposures may be made, after which the optimum exposure time determined can always be used.

High-contrast film or energetic developer!

With normal development, high-contrast film should be employed, because contrast in photomicrographs is usually lower than in outdoor photography. — Films of "particularly high resolving power" are not necessary, because especially

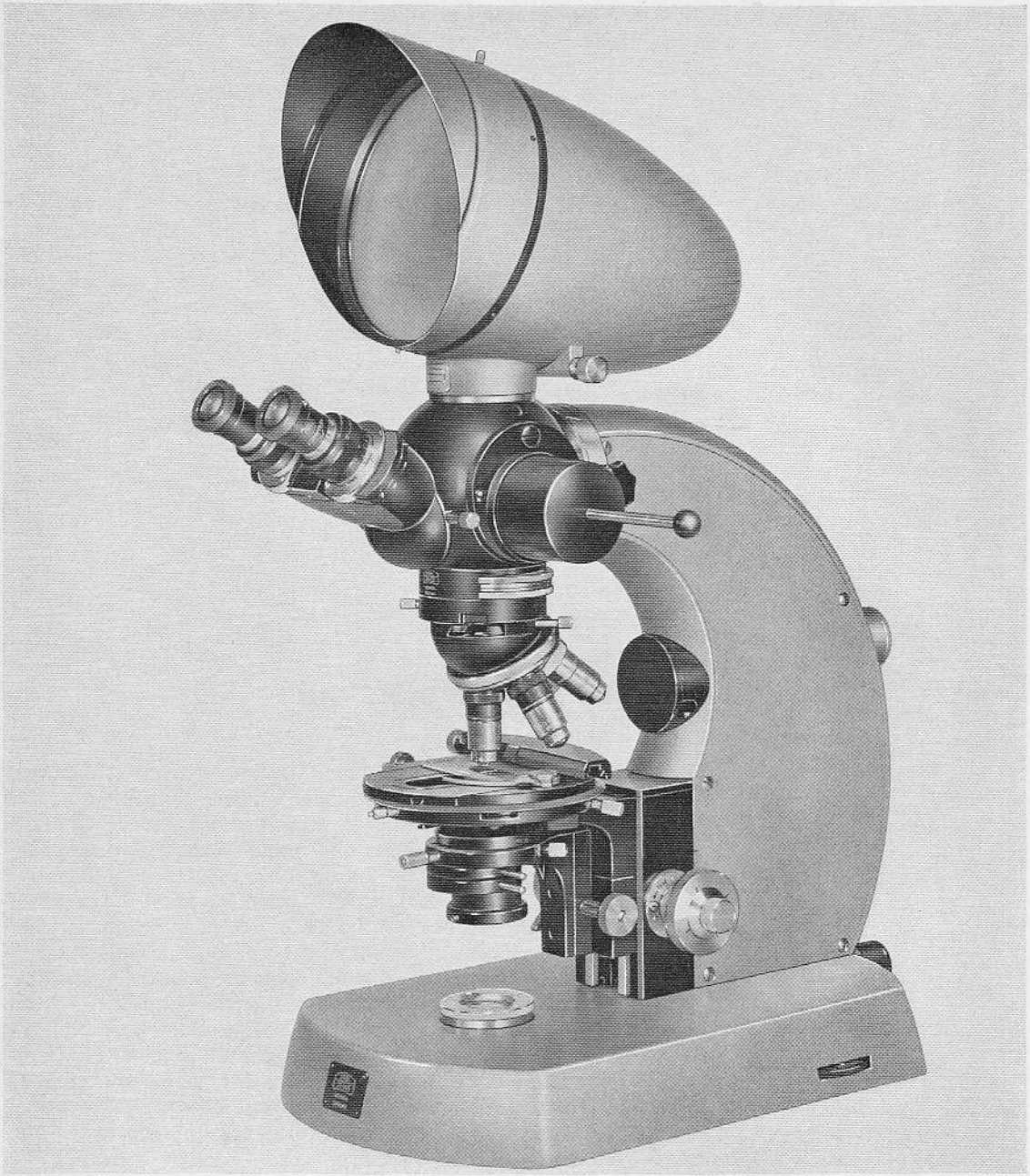
in high-power work the resolution of even the least suitable films is still higher than that which is physically possible in the microscope. The simplest and one of the best methods is to use ordinary all-round film and to develop it in a paper developer.

When is 35 mm too small?

It has been found in practical work that negative sizes larger than 24×36 mm are of advantage only if small-scale photomicrographs, and particularly photomacrographs, predominate. In addition, larger sizes should be used if color photos have to be taken for printing purposes. ZEISS supplies special photomicrographic attachment cameras for $2^1/_4$″×$3^1/_4$″ (6×9 cm), $2^1/_4$″×$2^1/_4$″ (6×6 cm), $3^1/_4$″×$4^1/_4$″ (9×12 cm) and 4″×5″ (Polaroid).

12. Micro-projection

Micro-projection is a valuable technique if several people want to observe a microscopic image simultaneously. For smaller groups a viewing screen is employed, which is simply exchanged for the observation tube and which offers an image scale of 10× the objective magnification (Fig. 42). A less expensive solution is to project the microscopic image onto a white surface with the aid of a deviating prism attached to the eyepiece. When the observer stands beside the eyepiece, the magnification is the same as in the microscope. If the viewing distance is halved, magnification is doubled, etc. Due to the frequently large image diameters and the relatively low efficiency of projection in reflected light, light sources of higher luminance are then required, with which above all the special equipment for micro-projection is provided. For further details, reference is made to the corresponding ZEISS literature.



Annex

Bibliography

For more comprehensive information on the field of microscopy we give below the titles of a number of works.

"Using the Microscope" by A. L. E. Barron
Champman & Hall, London, 1965

"Microscopy" by W. G. Hartley
English Universities Press Ltd., London, 1962
American edition edited by John J. Lee and Bernard Friedman
The Natural History Press, Garden City, New York, 1964

"Lecture notes on the use of the microscope by R. Barer
Blackwell Scientific Publications, Oxford, 1953
and F. A. Davies Co., Philadelphia, Pa.

"An Introduction to the Principles of the Microscope
and its Application to the Practice of Photomicrography"
by R. Schenk and G. Kistler
Chapman & Hall, London, 1962

"Photomicrography" by R. M. Allen
D. Van Nostrand Co., Inc. Princeton, New Jersey, 1958

"Analytical Cytology"
Edited by R. C. Mellors
2nd Edition, 1959, McGraw Hill, London

"Progress in Microscopy" by M. Francon
Pergamon Press, London, 1961

"Handbook of Chemical Microscopy"
by E. M. Chamot and C. W. Mason
John Wiley and Sons, Inc., New York, N. Y., 1958

Comprehensive works in German and French:

"Die Grundlagen der Theorie des Mikroskops" by K. Michel
2nd edition; Wissensch. Verlagsgesellsch., Stuttgart, 1964

"Die Mikrophotographie" by K. Michel
2nd edition; Springer, Vienna, 1964

"Traité de Microscopie"
by A. Policard, M. Bessius and M. Locquin
Masson et Cie, Paris, 1957

Fig. 42
STANDARD UNIVERSAL Microscope
with viewing screen

ZEISS literature

The Microscopy Department has distributed a whole series of catalogs which gives a good idea of almost the entire line of equipment supplied by them; the most important of these are:

Routine and Research Microscopes	40—120
STANDARD KF Microscopes	40—130
STANDARD UNIVERSAL M Microscope for Vertical Illumination	40—143
Phase Contrast and Interference Contrast	40—160
Fluorescence Microscopes	40—215
Inverted Microscope	40—250
Microscope Illuminators and Light Filters	40—340
Instruments for the Photographic, Graphic and Projective Reproduction of Microscopic Specimens	40—400
Photomicroscope	40—430
ULTRAPHOT II	40—452
Research Metallograph ULTRAPHOT II M	40—453
Instruments for Polarizing Microscopy	40—550
Interference Microscopy with Transmitted Light	40—560
Epi-Microscope	40—655
STANDARD Metallurgical Microscope	40—660
Stereomicroscopes	40—700

ZEISS reprints

Reprints of particularly interesting articles are likewise available. However, the stock of such material is limited, and very often the articles were reprinted only in the language in which they originally appeared.

On request, schools, institutes, etc., may receive an instructional diagram in the form of roller posters, showing the cross section of a microscope and the corresponding light path (Cat. No. 40—020).

Additional information

The staff of our guest laboratory at the Oberkochen main factory as well as our subsidiaries or representatives all over the world are entirely at your service for consultation on your particular problems.

May we also call your attention to the fact that anyone who understands German and is interested in photomicrography may participate in a five-day introductory course which is held every year in March or April at Göttingen. For applications, please write to our ZEISS agent or directly to the Microscopy Department, CARL ZEISS, 7082 Oberkochen-Wuertt. — West Germany.

Delivery programme for ZEISS Microscopes and Supplementary Attachments

Routine and Research Microscopes
Microscopes with built-in automatic camera
Attachment cameras with mechanical and automatic shutter for photomicrography
Micro-cine cameras
Polarizing microscopes and accessories
Equipment for interference microscopy with transmitted light
Phase-contrast microscopes
Fluorescence microscopes and fluorescence illuminators
Special microscopes for metallographic examinations
Epi-Microscopes, Epi-Technoscopes
Microscopes for depth measurements
(microscopes for measuring the depth of etching wells)
Different types of Stereomicroscopes
Microscopes for plankton and tissue examinations
Cytopherometer for electrophoretic examinations of cells
Universal Micro-Spectrophotometer UMSP I
Microscope Photometer MPM
Micro-projection devices
Microscope illuminators and light filters
Tubes for connecting the microscope to the TV camera
Drawing instruments for microscopes, heating stages and other accessories
Diffraction apparatus for microscopic demonstrations

ZEISS and the art of microscopy

The origin of your ZEISS microscope
lies in the workshops of the firm of CARL ZEISS. In 1846, the university mechanic of that name established a workshop for optical mechanics at Jena, which gained world renown after Dr. Ernst Abbe had joined him as scientific collaborator in 1866. Ernst Abbe, who was also professor of mathematical physics at Jena University, laid the scientific basis for the entire field of microscopy. After the death of Carl Zeiss he set up the "CARL ZEISS Foundation" (1889), transferred his entire fortune to it and put into practice social ideas which at that time were sensational (e. g., pension rights and profit sharing). Ever since, ZEISS has been world famous for top-quality optics for use in the fields of microscopy, photography, astronomy, etc. After World War II, the firm of CARL ZEISS was dismantled and expropriated. The Management and about 100 leading personnel of the company began to build a new ZEISS factory at Oberkochen (Wuerttemberg/ West Germany), where they were gradually joined by many specialists from the old works. In the new production program, which ranges from spectacle lenses to planetaria and from magnifiers to electron microscopes, light microscopes are commercially the most important item. They are being built in an effort to carry on the tradition of a great name.

ZEISS makes microscope history

The ZEISS works have made decisive contributions to the development of the modern microscope. From the long list of innovations introduced by this company, we have selected those points which may be of particular interest to you.

1872

For the first time in the history of the microscope objectives were offered which had not been made simply by "trying it out", but on the basis of mathematical and optical research. The CARL ZEISS list No. 19 (1872) opens with the sentence:

"The microscope systems below are exclusively based on theoretical computations carried out by Professor Abbe at Jena."

At the same time, the big Abbe illuminator appeared for the first time "combined in all its parts exclusively on the basis of theoretical considerations".

1886 In the ZEISS works, Ernst Abbe succeeded in making microscope objectives of unusually high correction, which he called Apochromats. At the same time, the corresponding compensating eyepieces were introduced.

1893 Professor A. Köhler, who joined ZEISS in 1900, published the method of illumination later called after him.

1896 ZEISS built the first Greenough stereomicroscope.

1904 Professor Köhler published his work on the ultraviolet microscope.

1911 Following a suggestion by Köhler, ZEISS introduced parfocalized microscope objectives.

1933 ZEISS revolutionized the construction of microscopes by designing the L stand (not tiltable, stage always horizontal, low controls).

1935 For military reasons, the anti-reflection coating of lens surfaces ("blooming") invented by ZEISS had to be classified as secret.

1936 ZEISS built a first prototype of the phase-contrast microscope based on suggestions by the later Nobel Prize winner, Professor Zernike.

1938 After prolonged development work, ZEISS were able to offer the first flat-field objectives (Planachromats).

1943 The microscopy laboratory of the ZEISS works produced the first motion picture on cell division using the phase-contrast microscope (Dr. K. Michel). With this examination method, cell research entered a new phase.

1944 ZEISS built the first stereomicroscope with magnification changer (eliminating exchange of objectives, offering constant working distance).

1950 The ZEISS STANDARD Microscope introduced coaxial controls and spring-loaded objectives (specimen protection) into microscope construction.

1950 ZEISS obtained a patent for a magnification changer designated as OPTOVAR.

1955 The first photomicrographic device with fully automatic exposure control was sold (ZEISS Photomicroscope).

1959 With their Ultrafluars, ZEISS succeeded in making refracting objectives suitable for both ultraviolet and visible light (important for microspectrophotometry and cancer research).

G 40—100—e

Printed in Germany

S III/66 PUoo