Rat testicles LEITZ ORTHOPLAN microscope, PI Apo 40/0.75, 550x
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Title page:
Diffraction patterns of a chessboard-like layer lattice (see also Fig. 6a).
The diffraction patterns are distributed over the entire rear focal plane of the objective. The colourless 0th-order diffraction pattern is in the centre. Round it the diffraction patterns of higher orders are grouped, whose positions are slightly displaced according to their colour. Since longer-wave light is always more strongly diffracted, the red diffraction patterns are displaced outward.
The microscope and its application

The microscope has become a scientific tool that is as versatile as it is indispensable. Not only the natural scientist and the medical practitioner, but also large sections of industry and commerce today make use of methods of microscopic investigations. This extensive application has had the result that the microscope is used not only exclusively by trained microscopists, but increasingly also by technical assistants without any thorough knowledge in the field of microscopy. This booklet has been written to aid and guide the latter category of users.

To keep the description as concise and lucid as possible, the subject has been divided into three sections:

I Theory
II Application
III Instruments

All three sections are self-contained. It is therefore possible to start with the application if only practical information is required. This section has been composed so that the user learns how to operate the microscope correctly, and becomes familiar, step by step, with its application in the classical fields of brightfield, darkground, and phase contrast. If time permits, however, it is suggested that the theoretical relations should also be studied. It will then become obvious that the theory explained can be understood very quickly, and that it is an effective aid to a deeper understanding of the microscope and its application.

The instrument section describes construction and function of a typical LEITZ transmitted-light microscope and the standard accessories of transmitted-light microscopy. As a guide through the LEITZ microscope programme it is meant to assist the user with the choice and composition of the equipment best suited for his purposes.

A special pamphlet 512-99 has been published on the optical systems: "Image-Forming and Illuminating Systems of the Microscope".

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The two-stage image formation of the microscope.

In the literature, terms such as 2-stage magnification and involved optical paths are frequently mentioned when microscopic image formation is explained. These terms, however, convey very little especially in the context of the microscope: the optical system here is very small and compact and the generally deflected optical path of rays is found almost exclusively in the interior of the instrument. Nevertheless, optical models exist which while maintaining close analogy to the microscope, are more instructive. Such models are the slide projector and the enlarger. In the slide projector, too, a magnified image of an object, i.e. the slide, is formed. Here too, as in modern microscopes, a light source with a condenser system is used to form the image.

The first stage of magnification

We shall now try to explain the optical path and the formation of the image in the slide projector and to apply this knowledge to the microscope.

We can think of any slide in terms of a picture composed of screen dots. Let us look at one of these small dots in the object. When it is illuminated by the projector lamp, a cone of rays will originate in it. These rays are deflected by the collecting elements of the lens so that they combine in an image point at a certain distance. We place a transparent projection screen in this point. Naturally, the rays will then not end on the screen, but diverge towards the rear. The decisive fact is this: when we look at Fig. 1, we will realize that the cone of rays originating in the image point is formed by the same rays as that originating in the object point. An eye situated behind the projection screen in the cone of rays, therefore sees the image point on the screen. From the location and separation of the two image points B' A' (Fig. 1) on the projection screen it can be seen that
1) The image is upside-down, and, because of the symmetry of the rays, also wrong-way-round and
2) Magnified.
This part of the illustration corresponds to the first stage of magnification in a microscope. The projection
screen corresponds to the intermediate image plane in the microscope, the slide to the specimen.
If a small groundglass screen or a piece of parchment is introduced in the intermediate image plane, the
image magnified by the objective can be reproduced there, too.

![Diagram of optical paths in slide projector and microscope]

Fig. 1
Comparison of the optical paths in the slide projector and in the microscope.
**Projector:** the optical path in the slide projector starts in the lamp on the left. We see that the
entire surface of the slide AB is transilluminated by the illuminating beam. The objective forms
an image B' A' of the slide on the projection screen. This image is enlarged and inverted.
**Microscope 1st stage:** in the microscope the specimen a b on the object stage corresponds to
the slide AB. Here the objective forms an image of the specimen in the intermediate plane,
which corresponds to the projection screen in the projector set up. The intermediate image b' a'
is also magnified and inverted.
**2nd stage:** the magnified intermediate image is offered to the eye by a magnifier (eyepiece).
This 2nd magnification is called the 2nd stage. The eyelens forms the upright image a' b' on the
retina; it is a well-known fact that it is perceived upside-down by the brain.

**Projector:** To demonstrate the eyepiece magnification, the projected image too, was viewed
through a magnifier. Naturally, this 2nd stage of magnification is not used in practice with the
slide projector.
Furthermore, the illuminating beam is also recognized in the optical path of the projector: an
image of the lamp filament is formed in the projector lens through the slide by the illuminating
optical system. The corresponding situation applies to the microscope except that here the
illuminating optical system, which is rather complicated, has been omitted for reasons of space.
It is described in detail in Fig. 2.

* Obviously this applies to each individual image point, since the image, like the object, is com-
composed of the sum total of all points like a mosaic. Naturally, the image would be produced even
without projection screen, as a so-called aerial image. The eye, however, can discern only indi-
vidual details of it, since most ray cones bypass the eye. The projection screen, whether trans-
parent or opaque, scatters the ray cones into space so that the image can be seen every-
where behind or in front of the screen.
The second stage of magnification
Any photographer who prints his own enlargements knows
that in cases of doubt he inspects the image formed by
the enlarger through a magnifier, for instance to identify
small details or to ascertain whether the image has been
critically focused. We can, of course, do the same with
the slide projector, although in actual practice this will
hardly be necessary. But the principle will become clear:
at the second stage, we look at the image formed by the
objective, and once more magnified by the magnifier.
It is immaterial whether the image is real on a screen or
whether it is aerial.
In the microscope the image of the first magnification stage
is a real aerial one and the microscope eyepiece serves
as a magnifier for the second stage. The eyepiece magni-
ification is largely due to the eyelens of the eyepiece. The
field lens in the bottom of the eyepiece is usually located
below the intermediate image and deflects the rays di-
verging from the objective so that they pass through the
eyelens of the eyepiece. Only then can they be accepted
by the eye.

The involved optical path
We did not yet mention the illuminating system when we
discussed the optical path in the projector. If, however,
the illuminating system were omitted from a projector
it would not be possible either to make optimum use of
the light source or to illuminate the slide uniformly. These
are, however, precisely the essential conditions for a satis-
factory projected image. The light for the illumination is
therefore collected with a condenser system situated
between light source and slide. It has the purpose of
forming an image of the entire luminous area (or the fila-
ment) of the light source in the projector lens. The light
source is thus fully utilized, and the projected image is
illuminated at optimum brightness and uniformly in spite
of the bright and dark zones which are, after all, character-
ristic of the light sources. For the illuminating system has
the effect that every point of the light source trans-
illuminates the entire slide on its own and that in addition
this light is also fully utilized for image formation.
In the optical arrangement “illuminating system/objective”
just described two images interpenetrate: the image of the
slide on the projection screen and that of the light source
in the projector lens. We therefore speak of an involved
optical path. Each of the two groups of conjugated planes
therefore has a function of its own.

Köhler's principle of illumination
The conception of illumination described above is applied
with extraordinary success to microscopy in Köhler’s
principle of illumination. Here, too, almost exactly as in
the projector,
1) an image must be formed of the light source in the
rear focal plane of the objective, and
2) each point of the light source must illuminate the entire
object field on its own.
But whereas in the projector the lens and the size of the slides are unchanged, these conditions must be met in the microscope for a number of objective/eyepiece combinations and object fields of different dimensions. The microscope illuminator must therefore permit the variation of the cross section of the ray bundle both in the rear focal plane of the objective and in the object at will and independently by means of diaphragms.

Let us first look at the illuminating beam, Fig 2a. Directly behind the light source S is the lamp condenser, which is usually combined with the light source into an illuminator. The lamp condenser forms an image of the light source S in the front focal plane S' of the substage condenser. The aperture diaphragm is also situated in this plane. Another image of the light source is formed by sub-stage condenser and objective in the rear focal plane S" of the latter and finally in the exit pupil of the eyepiece S"'. Here is the pupil of the observer's eye. The planes S, S', S", S"' are called optically conjugated, because each is an optical image of the preceding one.

The second system of optically conjugated planes is found in the image-forming beam (Fig. 2b). The field diaphragm L limits the aperture of the lamp condenser. An image L' is formed of this diaphragm in the object plane by the sub-stage condenser. The objective forms in the intermediate image plane a magnified image of the object and of the field diaphragm L", which is once more magnified when viewed through the eyepiece. The third image of the field diaphragm L"' and that of the object are produced on the retina of the eye. We thus have two groups of optically conjugated planes, which succeed each other alternately, i.e. form an involved optical path.

What are the functions of the diaphragms situated in S' and L?
The aperture diaphragm permits the formation of an image of the light source. It thus produces the required ray bundle cross section in the rear focal plane of the objective in the microscope. When the aperture diaphragm...
is adjusted, the ray cones originating in the object points become wider or narrower. Figs. 3a and 3b.

The field diaphragm alters the cross section of the ray bundle in the object plane. It must never be opened more than is necessary to illuminate the object field. Figs. 3c and 3d.

Fig 4, a cross section of the ORTHOPLAN largefield microscope, shows the progress of this optical path in a real microscope.

Fig. 3a and b
When the aperture diaphragm is opened and closed, the cross section of the beam can be varied in the rear focal plane (exit pupil of the objective).
Left: Large condenser aperture for an objective of large aperture (wide open ray cone).
Right: Small condenser aperture for an objective of small aperture (slender ray cone).

Figs. 3c and d
Adjustment of the field diaphragm changes the cross section of the beam in the object plane.
Left: Full cross section of the beam for the lowest-power objective (large illuminated area in the object plane).
Right: Reduced cross section of the beam for a high-power objective (small illuminated area in the object plane).

Fig 4
Optical path in the ORTHOPLAN microscope.
This illustration shows the involved optical path (illumination and image-forming beams) in the ORTHOPLAN microscope. The optical planes and their positions in the microscope are clearly seen here.

S = lamp filament
L = field diaphragm
S' = image of the lamp filament in the plane of the aperture diaphragm
L' = image of the field diaphragm in the object plane
S'' = second image of the filament in the exit pupil of the objective
L'' = second image of the field diaphragm in the field-of-view plane of the eyepiece
S''' = third image of the filament in the exit pupil of the eyepiece
The third image of the field diaphragm is produced together with the microscopic image on the retina of the eye.
Microscopic image formation according to the principle of wave optics

In the preceding chapter the optical path in the microscope was constructed according to geometrical laws. The wave nature of the light was disregarded in this approach. But already the generally known phenomenon that parallel light passing through a narrow aperture does not only proceed in a straight line behind this aperture would suggest diffraction phenomena also in the microscope. The microscopic object, too, after all, consists in principle of very fine structures with minute apertures which can diffract the light.

A little experiment, which any owner of a fine grating can repeat with his microscope will quickly make it clear whether and to what extent diffraction phenomena play a part in microscopic image formation. A stage micrometer, for instance, is suitable as a grating.

The grating is placed on the object stage, critically focused, and the aperture diaphragm closed. When the rear focal plane of the objective is observed after removal of the eyepiece, the following is seen:

The image of the aperture diaphragm appears bright in the centre, and to the left and right a series of dimmer, partly overlapping secondary images with coloured fringes, are seen. Fig. 5. When the grating is removed from the optical path, the secondary images disappear, whereas the central image remains. This central main image is therefore produced according to geometrical laws, whereas the secondary images are the result of the diffraction of the light in the grating. The intensity distribution of the diffracted light and therefore the position of the various diffraction images is caused by interference.

Figs. 5a, 5b
Diffraction on the line grating
Fig. 5a shows the grating on the object stage. The aperture diaphragm has been radically closed. When the rear focal plane is observed after removal of an eyepiece (it is best to use a focusing telescope) a number of diaphragm images will be seen, Fig. 5b. The brightest of them is the 0th order image, and diffraction images of higher orders follow to the left and right at decreasing brightness.

Figs. 6a, b
Diffraction on the layer grating
The diffraction images of the chessboard-like layer grating (Fig. 6a) are, unlike those of the line grating, distributed across an area and extend across the entire rear focal plane. See Fig. 6b and title page. The bright, colourless central image represents the 0th order. Round this central image are grouped the diffraction images, whose positions are slightly displaced according to their colour. Since longer-wave light is always diffracted more strongly the red diffraction images are displaced outwards.
Fig. 7 shows the image formation with regard to diffraction. To avoid confusion, the verticals to the waves, which are identical with the propagation direction of the waves, are drawn instead of the wave front. The plane wave front of the light from the illuminator is incident on the grating. Now each slit in the grating is, according to Huygens, the centre of excitation of an elementary wave (diffraction). These elementary waves in phase reach only the centre of the focal plane of the objective, where they form the main image, which is also called the 0-order maximum. The secondary images, the 1st and 2nd etc. order maxima, follow to the left and to the right. They are produced by the interference of the elementary waves, which arrive there at phase differences of 1, 2, etc. wave lengths. The secondary images display colour fringes because of the different wave lengths in white light. The remaining area of the rear focal plane of the objective remains dark, because there the elementary waves extinguish one another by interference. Elementary waves again originate in the interference maxima, which interfere in the intermediate-image plane to the magnified image of the object, in our case the grating.

Fig. 7
A parallel ray bundle taken from the illuminating ray is incident on a grating. Here bundles 1st, 2nd... order are produced by diffraction. Together with the direct 0th order light they form the microscopic image.
Resolution according to the principle of wave optics

The aperture

The wave-optical approach directly establishes the quantitative relation for the resolving power of an object. Resolving power is the ability of an objective to make two closely spaced points visible as separate entities. A resolving power of 1 \( \mu m \) means that two point-shaped particles at a distance of 1 \( \mu m \) will just be separated.

Fig. 8 shows a section of the grating as well as the 0th and 1st interference maximum. \( d \) is the separation between the slits of the grating. Points A and B are in the same phase of vibration, because the illumination consisted of coherent and parallel light. C and B are also in phase, because \( AC = \lambda \) (wave length). A right angle is situated at C because the wave-front runs vertically to the diffracted bundle. Thus the 1st-order bundle

\[
\sin \alpha = \frac{\lambda}{d}
\]

For the resolution of the grating, the objective must at least accept the diffracted 1st-order bundle, so that an image can be produced through interference in the intermediate-image plane. The aperture of the objective is decisive for this; it is defined by:

Aperture \( A = \sin \alpha \) for dry objectives

\( n \times \sin \alpha \) for immersion objectives (n)

Here \( \alpha \) is the largest angle a ray can form with the optical axis, to be just accepted by the objective. Hence a dry objective resolves a grating in which the separation of the slits is \( d \) if its aperture \( A = \sin \alpha \) is at least \( \lambda /d \).

Expressed as a formula

\[ A = \frac{\lambda}{d} \]

This formula, which also applies to the immersion objectives, represents a good approximation of the resolving power when the illumination is exclusively vertical. Here the value \( d \) can also be interpreted as the separation between two neighbouring object points which can only just be seen discretely.

Fig. 8, 8a

Fig 8 shows a grating with 5 grating slits. Parallel light is incident upon it. Part is propagated in the same direction according to the laws of geometrical optics and is collected in the focal point of the objective. The 1st-order bundles diffracted on the grating are also shown, they are focused on the rear focal plane of the objective. The direction of the 1st-order bundles diffracted on the grating is determined by the triangle ABC, which in Fig. 8a is shown enlarged.
In practice, vertical illumination, i.e. parallel light, is never used. Furthermore, the field aperture is usually smaller than the objective aperture. Here

\[ d = \frac{\lambda}{A_{\text{Obj}} + A_{\text{cond}}} \]

This formula should be used as a rule of thumb. It applies to objects of normal contrast and normal colour contrast sensitivity of the eye. All formulae are also based on perfectly corrected objectives. Obviously, aberrations would adversely affect resolving power.

The numerical aperture, however, is also decisive for the speed and therefore image brightness of an objective, because image brightness changes, in otherwise identical conditions, in proportion to the square of the aperture. If, for instance, the aperture of an objective is stopped down to about 70%, image brightness will be reduced by half. It is true that this increases the depth of field, but at the same time diffraction fringes will surround all image details, reducing the resolving power.
Resolving power as a function of the objective aperture.
A low-power objective with built-in iris diaphragm was used for photography. The aperture diaphragm of the condenser was closed radically.

Left:

a) Iris diaphragm at minimum position, only the 0th order is used for image formation.

b) Diaphragm slightly opened, so that part of the 1st order will be used for image formation.

c) 0th and 1st order enter the objective.

d) When the diaphragm is opened further higher orders will enter the objective.

e) Full aperture of the iris diaphragm.

Right:

The image of the chessboard-like grating is structureless. The irregular structures are caused by dust.

The chessboard-like structure is shown as a ghost image, it is, however, not yet resolved.

The chessboard-like pattern is resolved, the grating constant is recognizable.

The black fields are now sharply outlined. The white patches in the field area are typical image-falsifying diffraction phenomena.

It is now seen that in reality the fields do not touch one another. Only very delicate diffraction fringes are still seen in the fields.

Fig. 6a shows the true aspect of the grating. Here a highly resolving objective was used for photography.
Resolving power as a function of the objective aperture.
A low-power objective with built-in iris diaphragm was used for photography. The aperture diaphragm of the condenser was closed radically.

**Left:**

- **a)** Iris diaphragm at minimum position, only the 0th order is used for image formation.
- **b)** Diaphragm slightly opened, so that part of the 1st order will be used for image formation.
- **c)** 0th and 1st order enter the objective.
- **d)** When the diaphragm is opened further higher orders will enter the objective.
- **e)** Full aperture of the iris diaphragm.

**Right:**

- The image of the chessboard-like grating is structureless. The irregular structures are caused by dust.
- The chessboard-like structure is shown as a ghost image, it is, however, not yet resolved.
- The chessboard-like pattern is resolved, the grating constant is recognizable.
- The black fields are now sharply outlined. The white patches in the field area are typical image-falsifying diffraction phenomena.

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Fig. 6a shows the true aspect of the grating. Here a highly resolving objective was used for photography.
Useful magnification

A) At visual observation
To resolve an object structure, e.g. two points, in the microscope, i.e. to see them as two points, it is not enough to use an objective of suitable numerical aperture. The image of this object structure must be offered to the eye also at a sufficiently large angle. The minimum size of this angle must be somewhat larger than the resolving power of the human eye. We therefore must first determine how far two points may be separated at the reference visual distance of 25 cm to be resolved by the naked eye. With good illumination and sufficiently good contrast, the distance is about 0.15 mm, corresponding to an angle of 2'.

This limiting angle is based on the distance and arrangement of the visual elements on the retina, i.e. it is a physiological parameter. The limit of resolution of the eye and the wave-optically derived resolving power of an objective must now be related. Let us look at two points in the object separated by d. If the two points are just at the limit of the resolving power of the objective, then

\[ d = \frac{\lambda}{2A} \]

This distance must now be magnified so many times until the points appear to the eye at least at a separation of 0.15 mm (corresponding to 2'). Hence

\[ V \cdot \frac{\lambda}{2A} = 0.15 \text{ mm} \quad \text{or} \]
\[ V = \frac{2A \cdot 0.15}{\lambda} \]

For \( \lambda = 550 \text{ nm} = 0.00055 \text{ mm} \)

\[ V = \frac{A \cdot 0.30}{0.00055} \]
\[ V \approx 500 \text{ A} \]

These considerations apply to objects of medium contrast. With high contrast, the two points can still be resolved by correspondingly higher magnification even if they are closer to each other. It must be mentioned here that every point of the object is reproduced as a diffraction disc because of the wave nature of the light. The closer together two points are, the more will the diffraction discs overlap (Figs. 10, 11). With high contrast, obviously the diffraction discs will stand out more clearly from the surrounding field. The eye can then see the constriction at the two points of overlapping better and correspondingly resolve them better.

Experience has shown that the final magnification of such objects can be increased up to 10000 A. At magnifications above 1000 A the images as a rule will only appear unsharp. The range from 500 A to 10000 A is called useful magnification. Lower magnifications than 500 A produce more brilliant images, but the resolving power of the objective is not fully utilized, and the eye can no longer resolve fine details of the size \( d = \frac{\lambda}{2A} \).

With low final magnifications, this can usually be tolerated.

Fig. 10
Two neighbouring diffraction discs at various distances
a) the discs appear clearly separated
b) a separation can no longer be seen with certainty
c) the constriction at the two overlapping points is already so slight that the figure could be caused by a single object particle.
B) At photomicrography

Whereas in visual observation resolution is limited by the arrangement and separation of the visual elements, in the photography the limit of resolution is determined by the silver grains of the photographic emulsion. These silver halide grains have a certain size depending on the gradation of the film. In films of medium speed it is about 2 μm, in fine-grain emulsions less than 1 μm. The exposed film grains build up the photographic picture. During exposure, however, not only those areas that have been directly affected by the light are blackened, but blackening also spreads diffusely within the emulsion owing to light scatter from the individual grains. This diffusion halo is called circle of dispersion. It enters the formula instead of the real grain size.

If the considerations of the preceding chapter are applied to photomicrography and a 1/60mm diameter is assumed as the circle of dispersion of a fine grain film, analogously

\[ \frac{\lambda}{2 \cdot A} = \frac{1}{60} \text{ mm.} \]

where \( M \) is the magnification of the picture on the film, \( \lambda \) the wave length, and \( A \) the aperture.

By transformation and calculation

\[ M = 60A \]

The consequences arising from this formula for the film format in photomicrography will be discussed in the chapter Photomicrography.

Fig. 11

A microscope slide with a vapour-deposited opaque layer was used, which contained submicroscopically small holes. The photomicrographs show two such closely spaced holes at the upper edge. In the picture taken through maximum objective aperture two clearly separated diffraction discs are seen, whereas with minimum aperture the two diffraction discs are so large that they merge into single one, i.e. that they can no longer be resolved. The intermediate photographs were taken through apertures decreasing in steps, at a factor of 1.3x.
Special darkground condensers, so-called mirror condensers, are generally used for darkground investigations. Their most important structural component is a mirror body which deflects the light from the illuminator so that it intercepts the object plane as a shallow, hollow cone, but does not enter the objective. From here onwards let us look at image formation again from the wave-optical aspect. If no object is in the optical path, the light - provided the objective aperture is smaller than the internal limiting aperture of the condenser - bypasses the objective; the field of view is dark, hence the term darkground. But if an object is in the optical path, it will deflect part of the light from the illuminator. Part of the deflected light enters the objective. The direct light, i.e. the 0th order maximum, which is always represented by the geometrical optical path, is excluded from image formation. Thus only the deflected light contributes to the image, Fig. 12. Since for a largely faithful representation of the object the 0th order maximum is an essential condition, the objects are not completely faithfully represented in darkground; only their contours light up. Of gratings, for instance, only the edges are visible. The darkground method is therefore particularly well suited for all kinds of line structures - such as edges, cracks, flagellae, or objects which are hardly, if at all, visible in brightfield because of their lack of contrast. Internal structures are not as well rendered. In the darkground method, too, Köhler’s principle of illumination is used.

Fig. 12a
Wave-optical diffraction in darkground. It is seen that because of the very obliquely incident illumination the 0th order no longer reaches the objective. Only the diffracted light (1st and 2nd order) is used for image formation.

Fig. 12b
In darkground mainly the contours of the specimens light up. Radiolaria (darkground)
Phase contrast microscopy

Up to now we have dealt only with microscopic specimens which, when illuminated with monochromatic light, change the amplitude of the light, so that the individual structural elements appear at different brightness, or in white light differentially absorb the various spectral components, so that the relevant structural elements become visible in different colours. These objects have amplitude structures; they can be investigated in brightfield. In addition, linear amplitude and phase structures can be rendered visible with the darkground method.

But if unstained biological thin sections are investigated in brightfield, the images appear almost empty and structureless. Cells and cell nuclei, for instance, have practically the same transmission within the visible region of the spectrum, so that brightness or colour differences cannot be perceived. Nevertheless an “image” of the object is hidden in the light coming from it. Light waves which have passed through the optically denser cell nucleus are retarded in their phase compared with the waves that have passed through the surrounding field. Neither the human eye, however, nor the photographic emulsion is a suitable detector for these changes. Both record only differences in intensity and wave length, i.e. graduations of brightness and colour, but not wave trains of different phase.

If the microstructure of an object is optically oriented (anisotropic) the polarizing microscope can convert the phase differences of the polarized waves into brightness differences. In optically isotropic specimens, on the other hand, the only way out for a long time was to differentiate the object by staining it. But fixing and staining constitute interference which changes the morphological structure of the object even with the most conservative treatment. With the phase contrast method interference of such kind with the object is avoided. Here, the optical path of the microscope is modified to translate the phase structure of the object into an amplitude image. This method is very sensitive, and phase differences of a few degrees are converted into clearly visible brightness differences.

In the following chapter a model case of a very small amplitude object or phase object is shown to demonstrate the principal differences between the two and how with the aid of the phase contrast method an amplitude image is obtained of a phase object.

Fig. 13
Radiolaria (phase contrast)
Figs. 14a and 14b
Amplitude object in brightfield
In Fig. 14a the illuminating beam A is incident on a very small amplitude object. When it passes the object, the amplitude decreases until the light leaves the object on the right. It continues to proceed as wave B at reduced amplitude.
Fig. 14b shows the image formation in the intermediate image plane according to Abbe’s Theory. The sum of the illuminating light YA and diffracted light YA produces the image of the object YB.

Fig. 15a and b
Phase object in brightfield
In Fig. 15a the illuminating beam is incident on the very small phase object. As it passes the object the light wave is compressed in the optically denser medium of the object and leaves the object on the right (wave B) with a phase displacement when compared with the illuminating beam, which bypasses the object (not drawn here).
Fig. 15b shows the image formation in the intermediate image plane according to Abbe’s Theory. Part of what has been said in 14 b applies here.

Fig. 15c
Phase object in phase contrast
Compared with Fig. 15b, the illuminating ray A was displaced to the right through 90° (1/4 wave length) whereas the diffracted light C remained unchanged. Addition according to Abbe’s Theory produces the new image wave B, which now has a smaller amplitude. When Figs. 14b and 15c – black backgrounds – are compared, the principal similarity of all curves will be seen at first glance. A visible amplitude image has been produced from the invisible phase image.
Amplitude objects

Let us observe a very small object in the otherwise empty field surrounding a microscope specimen. Let the light which passes this empty surrounding field have the amplitude $A$. Let it be the direct 0th-order light and, because of the smallness of the object, let it represent a major proportion of the illuminating light. A small portion of this illuminating light also strikes the object. This object is to absorb light, but leave the phase unaffected. Thus the amplitude of the transmitted light $B$ has become smaller.

Fig. 14a.

According to Abbe's theory, the image of the object is produced by the interference between the direct and the deflected light. Provided that most of the deflected light is used for image formation, the image will be a faithful representation of the object, i.e. the wave trains in the intermediate image plane correspond in their amplitude relation and phase position to those in the object plane.

Fig. 14b.

The deflected light can then be simply constructed from both curves point by point if it is borne in mind that

$$Y_B = Y_A + Y_C,$$

i.e. $Y_C = Y_B - Y_A$

Deflected light = image - 0th order.

The deflected light is phase displaced through $180^\circ$ against the direct light.

Phase objects in phase contrast

If in Fig. 15b the phase of the direct light is displaced to the right through $90^\circ$, the curves $A$ and $C$ are as shown in Fig. 15c. Here, too, the image of the object $B$ is produced by interference of $A$ and $C$; we can construct it according to

$$Y_B = Y_A + Y_C.$$

If Fig. 15c is compared with Fig. 14b, the superficial similarity of all wave trains will become clear at first sight. Here, too, the deflected light is again displaced from the direct light through $180^\circ$. Curve $B$ hence represents again an amplitude image here, which appears dark on a bright background. The phase displacement of the direct light through $90^\circ$ has therefore converted the invisible phase image into a visible amplitude image.

To increase the contrast further, the direct light is also reduced, so that it is almost completely extinguished by the deflected light through interference. The amplitude image will then appear dark against a bright background at high contrast.

Phase objects in brightfield

Fig. 15A shows a very small, transparent object in the empty surrounding field of the microscope. Let us assume that it is optically denser than the surrounding field. The amplitude of the illuminating light is not noticeably changed by this object, but the phase is displaced* relative to the illuminating light that has passed through the surrounding field.

Here, too, the direct light is again mainly represented by the illuminating light that has passed through the surrounding field.

As with the amplitude object we construct the curve of the deflected light from the two curves “direct light” and “image of the object”. This representation shows that the deflected light is phase displaced from the direct light through about $90^\circ$. Fig. 15b.

* In practice these phase displacements amount to a few degrees.
The phase contrast microscope

For the practical execution of the phase contrast method, Zernike suggested that the direct beam should be affected by a phase plate in the rear focal plane of the objective. Today, annular lamellae, so-called phase rings of suitable thickness and absorption, are used for this purpose. These phase rings are placed in the rear focal plane of the objective where the direct light is recombined. The deflected light, on the other hand, is distributed over the entire rear focal plane and is hardly affected by the phase ring. If the phase ring permits the light passing through it to advance by a quarter of the wavelength and reduces it at the same time, "positive phase contrast" is produced when the direct and the deflected light are superimposed in the image plane. The images of objects of higher refractive index than their surroundings appear darker, and objects of lower refractive index brighter than the surrounding field. If the phase ring retards the direct light by a quarter of the wavelength, "negative phase contrast" is produced, with reversed contrast conditions.

An annular phase plate in the objective naturally demands that the light should come from an annular light source. This is achieved by a diaphragm installed in the condenser. The diameter of this diaphragm determines the condenser aperture, which must be roughly half that of the objective. The phase contrast condenser therefore contains several annular diaphragms associated with the objectives; they are arranged on a revolving turret. Fig. 16 shows the optical path of direct and deflected light in the phase contrast microscope between the annular diaphragm of the condenser and the intermediate-image plane.

Because of the annular diaphragm and the absorption of the phase ring, the phase contrast method requires more powerful light sources than the brightfield method.

Please read the text from bottom upwards in the direction of the beam propagation.

As in the amplitude object, illuminating rays and diffracted ray interfere in the intermediate image plane to produce the microscopic image. \( \Delta \phi \) is now 180°, it therefore has the same value as with the amplitude object.

The phase ring located in the focal plane of the objective, allows the annular illuminating bundle to proceed by 90° and at the same time reduces it. The diffracted light is distributed over the entire focal plane; it is practically unaffected by the phase ring.

Light is diffracted on the object. With a phase object, the illuminating wave is displaced in the object plane through \( \Delta \phi = 90^\circ \) compared with the diffracted ray. In the amplitude object \( \Delta \phi = 180^\circ \).

The illuminating ray emerges as a hollow cone from the annular diaphragm of the condenser.
Like phase contrast microscopy, this method is used to reproduce unstained transparent objects at high contrast. An extremely three-dimensional image is produced in which the optical path length differences of the object instead of the geometrical profile become visible. The interference contrast method is useful in the investigation of objects of optical path length differences between $\lambda/10$ and $\lambda$. It thus is the bridge between phase contrast and classical brightfield microscopy.

Principle and function

The LEITZ interference contrast device is based on the principle of two-beam interference. The principle is diagrammatically represented in Fig. 18 for a plane wave, i.e. for parallel light. To explain it, however, the Wollaston prism must first be described.

A Wollaston prism consists of two birefringent prisms cemented together, whose crystallographic axes include a right angle. One axis is in the drawing plane, the other one is vertical to it (Fig. 18a). In addition, both axes are parallel to the entrance and exit face of the double prism. If a light ray enters the Wollaston prism vertically, it is split into two rays vertically polarized to each other. If the point of separation is in the centre of the joint face, i.e. where both prisms are of equal thickness, the rays emerging from the Wollaston prism will be in phase. If the points of separation are laterally displaced, a phase difference between the two part-rays vertically polarized to each other will occur. If the double prism is introduced between two polarizers, a series of straight-line interference fringes can be observed in monochromatic light. In white light, the interference fringes will appear coloured between two crossed polarizers.

After these introductory explanations the principle of the interference contrast method can be better understood (Fig. 18b). Let $O$ be the object through which a plane wave passes. This wave is deformed during its passage through the object. Let the new wave front be $\Sigma$. The Wollaston prism behind the objective $O_b$ splits the wave $\Sigma$ into the two part wave trains $\Sigma_1$ and $\Sigma_2$, which, after passage through the analyser $A$ interfere with each other, so that intensity or colour differences will become visible in the intermediate image plane.

This arrangement requires a very small condenser aperture, which, naturally, reduces the resolving power severely. In practice, therefore, an arrangement is used according to Fig. 18c, in which the condenser aperture is not restricted and which nevertheless provides a homogeneous background:

The natural light coming from the light source is linearly
polarized by the polarizer. From there it reaches the Wollaston prism, which is oriented in the diagonal position to the polarizer. In this position the linearly polarized ray is split into two part-rays polarized vertically to each other and of equal intensity, which diverge from each other. Since the point of divergence is situated in the focal plane of the condenser, the angular split in the Wollaston prism becomes a lateral split in the object space. As a result the part-rays pass the object at two different points where their phases are also differentially affected. The magnitude of the slit is chosen so that it is below the resolving power of the microscope. No visible double image is therefore produced in the microscope. The objective recombines the two part-rays in the rear focal plane. This is where the second Wollaston prism is situated, which recombines the two part-ray-bundles in space. Without this Wollaston prism the part-rays would again diverge behind the point of recombination. They now pass through the analyser and can interfere since they now have a common vibration direction. The interference colour or intensity at any point of the image field depends on the phase difference between the two part-ray-bundles and therefore on the thickness and refractive index of the two object portions. The direction of the beam split appears in the image like the side lighting on a relief.
Light refraction and dispersion

All optical materials exhibit a more or less pronounced dispersion. This is the differential light refraction of the various wave lengths. Light refraction is characterized in the familiar way by the refractive index $n$ with a suffixed index of the relevant wave length. The wave length marked with this index can be obtained from the table below.

<table>
<thead>
<tr>
<th>Spectral lines used</th>
<th>Violat Hg</th>
<th>Blue Hg</th>
<th>Blue Cd</th>
<th>Blue H</th>
<th>Green Hg</th>
<th>Yellow Hg</th>
<th>Red Cd</th>
<th>Red H</th>
<th>Red He</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$h$</td>
<td>$g$</td>
<td>$F'$</td>
<td>$F$</td>
<td>$e$</td>
<td>$d$</td>
<td>$C'$</td>
<td>$C$</td>
<td>$r$</td>
</tr>
<tr>
<td>Element</td>
<td>Hg</td>
<td>Hg</td>
<td>Cd</td>
<td>H</td>
<td>Hg</td>
<td>He</td>
<td>Cd</td>
<td>H</td>
<td>He</td>
</tr>
<tr>
<td>Wave length in nm</td>
<td>404,66</td>
<td>435,84</td>
<td>479,99</td>
<td>486,13</td>
<td>546,07</td>
<td>587,56</td>
<td>643,85</td>
<td>656,27</td>
<td>706,52</td>
</tr>
</tbody>
</table>

The Abbe number $\nu$, which is the numerical indication of dispersion, can be calculated from three refractive indices according to the formula

$$\nu_d = \frac{n_d - 1}{n_F - n_C} \quad \text{and} \quad \nu_e = \frac{n_e - 1}{n_F - n_C}$$
II Application
For shipment or storage the microscope is kept in a microscope cabinet, case, or special container. Before it is taken out, the arrangement of the instrument and its accessories should be noted, so that they can be replaced correctly after use.

Before the microscope is set up, here are a few hints on the conditions of the work room.

The work room should not be too bright, and, if possible, should not receive any direct sunlight. North-facing rooms are preferable.

Laboratories with corrosive fumes are completely unsuitable for the setting up of a microscope. If it is necessary to work with glacial acetic acid etc. in the work room, this should be done under a draught.

If the instrument is transferred from a cold into a warm room, it should be used only after condensation on the lenses has evaporated.

Unfortunately dust is ubiquitous and can adversely affect the image quality or the mechanical functions of the microscope if this is allowed to stand uncovered for prolonged periods. A point should therefore be made at the very outset always to cover the microscope after use and to keep objectives that are not being used in their plastic containers. When objectives are handled, especially when they are screwed onto the revolving nosepiece, care should be taken never to touch the front lenses with the fingers. Even a slight finger mark can lead to a hopelessly flat image.

A rigid, not too high work bench should be chosen for microscopy; it should be large enough to accommodate all the usual accessories. The LEITZ instrument tables are particularly recommended.

To avoid fatigue, the user should work with the microscope only in a sedentary, relaxed position, with the body slightly bent forward. It is recommended to use a vertically adjustable seat.

When a monocular tube is used, the free eye should be kept open. With some practice the user will soon become accustomed to seeing only the image in the microscope and to let the other eye look into “empty space”. It is particularly important to work with relaxed eyes, to avoid the otherwise inevitable eyestrain. Imagine that the image is at infinity. If you want to ascertain whether the eye is really relaxed, make the following tests: hold a mirror in front of the unused eye so that a far distant object is seen. If the eye is accommodated to this object, the other eye must now see the previously critically focused image also critically sharp. Otherwise refocusing is necessary with the fine adjustment.

The correct distance between the eye and the eyepiece is also important. This differs according to the eyepiece. Approach the eyepiece slowly with the eye until the field of views is fully surveyed and delimited. The eyepiece is now in the exit pupil of the eyepiece, where there is maximum constriction of the rays.

With a binocular tube, the correct interpupillary distance must also be set first.

Any visual defects do not normally disturb the microscopist. If he is short- or long-sighted, even if he is moderately astigmatic, he can focus without the corrective spectacles. Different visual defects can be compensated for by means of eyepieces with focusing eyelenses. High-point eyepieces are available for spectacle wearers; they have the advantage that the operator need not take off his spectacles when he uses the microscope.

The following chapters deal with practical work with the microscope.
Microscopy in brightfield

Objects with coloured structures are generally investigated in brightfield. Because of their differential spectral absorption white light makes them appear in different colours. In monochromatic light they only show brightness differences. In theory we have met these specimens as amplitude objects.

Arrangement and function of the illumination

All LEITZ microscopes with field diaphragm are equipped for Köhler's illumination. This comprises: low-voltage illuminator with pre-focus or centring lamp, lamp condenser, swing-out lens, field diaphragm and substage condenser with aperture diaphragm (see also Theory page 8). Illuminators and condensers are usually interchangeable. Since image quality and general image character are decisively affected by the microscope illumination, the illuminating device must be used with great care. This includes above all the operation of the aperture and field diaphragms and the centration of the condenser and, if necessary, of the illuminator.

Fig. 19
Aperture diaphragm fully open. The image of the blood cells appears a little lacking in contrast at full condenser aperture.

Fig. 20
Aperture diaphragm 2/3 open. The picture of the blood cells now has correct contrast.

Fig. 21
Aperture diaphragm closed more than half. At reduced resolution the typical diffraction lines appear round the blood cells.
**Use of the aperture diaphragm**

The aperture diaphragm is part of the condenser. As already explained in the theoretical section, it is used to stop down the image of the light source. It thus directs the required ray bundle cross section to the rear focal plane of the objective.

When the completely open aperture diaphragm is slowly closed and observed after removal of the eyepiece, the image of the diaphragm will at some stage become visible in the rear lens of the objective, provided that the condenser has been correctly adjusted. Now the field aperture equals the objective aperture in diameter. At this diaphragm setting — it is also called full field aperture — however, only those structures will be recognizable which are sufficiently differentiated from their surroundings by absorption differences. The image of such structures is then formed at optimum resolving power. But if the object also includes details that are poor in contrast or differ mainly in the refractive index — such details are in practice almost always present — the following rule should be applied: if at full aperture everything that is visible is sufficiently clearly represented, close the aperture diaphragm gradually to about 2/3 of the full aperture. During this gradual stopping down the less differentiated structures, too, will be clearly rendered. A further stopping down of the diaphragm, i.e. to about 1/2 or smaller still is not advisable. It is true that stopping down increases the contrast, but it should be borne in mind that part of this contrast increase is subjective, because when stopped down the image is darker, thereby simulating an additional increase in contrast. Furthermore, image quality is adversely affected by diffraction fringes if the diaphragm is stopped down excessively.

It has still to be explained how an excess of field aperture, i.e. when the field aperture is larger than the objective aperture, affects the image. In principle this illumination would be an addition of brightfield and darkground illumination. As a result, a brightfield image on which a darkground image is superimposed would be seen. This brightens edges and similar light-scattering structures, and thereby considerably decreases image contrast. We therefore recommend never to use a field aperture larger than the objective aperture. On the other hand, excessive field aperture never produces disturbing stray light, although this is frequently claimed in the literature. We merely have to remember darkground microscopy, in which, after all, an excess of field aperture is always used.

Besides resolving power and contrast, depth of field, too, is affected by the aperture diaphragm. With smaller apertures the image-forming beam becomes progressively narrower and the sharply reproduced layer of the object progressively deeper. Resolving power, however, is thereby reduced, and the function of the aperture that regulates the depth of field should therefore be used only within limits.

**Resolution, contrast, and depth of field** can thus be regulated with the aperture diaphragm. It must, however, never be used to regulate the brightness. For this purpose either the regulating transformer or neutral density filters must be used.
Use of the field diaphragm
The field diaphragm is built into the foot of the stand and designed to enable the microscopist to change the cross section of the illumination in the object plane. With its aid the illuminated field in the object can therefore be narrowed so that it coincides with the field of view of the microscope. This protects the object against unnecessary heating and glare is avoided. The field diaphragm should therefore be opened only far enough so that it just disappears beyond the edge of the field of view of the microscope.

Setting up the specimen
When a new microscope stand is used for the first time, the instructions included with it must be consulted; the operating hints described in this brochure are more general and do not refer to any specific microscope model.
a) Mount the specimen on the object stage.

b) Choose a 10:1 or 16:1 objective for first focusing. These objectives have a sufficiently long working distance so that there is no great risk of damaging the specimen with the front lens.

c) Fully open the aperture and the field diaphragm.

d) If the microscope has a swing-out lens in the foot of the stand, turn this into the beam. It remains in this position during all investigations in brightfield and darkground. Exception: 1:1 objective.

e) Regulate illumination for comfortable brightness. Excessively bright light affects the eye, especially during prolonged observation.

f) Focus the specimen with the coarse and fine adjustment.

g) When a binocular tube S or FSA is used it must be adjusted and, if necessary, corrected to compensate for visual defects before the specimen is focused. Further details are described in the next chapter.
Adjusting the binocular tube S

1) Set the distance between the two eyepiece tubes for your interpupillary distance: the two part images in the microscope must be fully visible at the same time and coincide. Only one circular image is now seen.

2) Set the two rotating eyepiece tubes at the interpupillary distance read off the scale.

3) Refocus the image with the fine adjustment.

4) Refocus the focusing eyepiece until the image is also sharp in the relevant eyepiece tube.

Adjusting the FSA tube with two adjustable eyepieces

1) Set the distance between the two eyepieces used for your interpupillary distance. The two part images in the microscope must be fully visible at the same time and coincide. Only one circular image is now seen.

2) Adjust the eyepieces of both eyepieces until the edges of the diaphragm appear sharp in both eyepieces.

3) Refocus the image with the fine adjustment.
Centring the lamp

Centre the lamp according to the instructions for the microscope in use. Centration can be checked by means of placing the plastic groundglass screen supplied or a sheet of parchment etc. on the dust glass of the microscope and by adjustment of the lamp condenser. The light patch must not move laterally.

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Fig. 27 a - c

Centring the 12v 100W tungsten halogen lamp. Fig. 27.

a  Diaphragm and mirror image are in focus

b  Filament and mirror image have moved into the centre.

c  Filament and mirror image are correctly centred.
Adjusting the condenser
Figs. 28a to f

a) Turn the condenser top in.

b) Move the condenser in to its topmost position.

c) Close the field diaphragm.

d) Focus the field diaphragm with the vertical adjustment of the condenser. The coloured fringe which becomes visible with more highly corrected condensers has no effect on image quality. To correct it completely would be quite uneconomical.
g) Open the aperture diaphragm according to the objective and the object:

1) If the object is very contrasty: field aperture = objective aperture.
2) In all other cases:
   Begin with field aperture = objective aperture. If at this setting all details are sufficiently prominent, close the diaphragm until the structures poor in contrast, too, are well rendered. Generally do not close the aperture beyond 2/3 of that of the objective aperture.

h) If necessary correct illumination by adjusting the lamp condenser. The field of view must be illuminated completely uniformly.

e) Move the image of the field diaphragm into the centre of the field of view with the two centring screws of the condenser.

f) Open the field diaphragm until its rim just disappears beyond the edge of the field of view.
**Change-over to higher magnification**

Once the adjustments described in the previous chapter are mastered and the user has become a little more familiar with the instrument, higher magnifications with dry systems can be attempted. Here a few further fundamental facts are important: the choice of the correct objective/eyepiece combination and the correct coverglass.

**The correct objective-eyepiece combination**

Let us assume a 250x final magnification is required. This can be obtained either with a 10x eyepiece and a 25x objective or with a 25x eyepiece and a 10x objective. In the first case a brilliant image is obtained, in the second image quality will be less satisfactory, because the most delicate details are absent. The reason must be sought in the smaller aperture of the 10x objective. From this, to begin with, the general conclusion can be drawn that at a given final magnification the objective of higher aperture is always to be preferred. In actual practice the rule applies that the final magnification should be roughly within the range between 500x and 1000x the objective aperture. This range is called the useful magnification.

---

**Fig. 29a, b**
Two photomicrographs at 375x magnification with the large-format camera.

On the left with 10/0.25 objective, on the right with 25/0.50 objective. On the left the range of useful magnification has been exceeded.
Example:
A 40/0.65 objective is used. For this the useful magnification is 500x 0.65 to 1000x 0.65 = 325-650x. With a 10x eyepiece, therefore, the middle range of useful magnification is reached. With contrasty specimens even a 16x eyepiece can be used.

It is, however, not necessary to observe these guide values too rigidly. Mostly it is not even advisable and essential to utilize the resolving power of an objective fully. Especially with low and medium powers it is possible without difficulty to work below a final magnification of 500A, i.e. with low-power eyepieces. Sharpness and contrast will then appear noticeably increased. Fine, contrasty details, on the other hand, should be investigated in the upper range at 1000A magnification.

Higher final magnifications are recommended for measurement and counting. It should, however, always be borne in mind with these high supermagnifications that the finest details are no longer faithfully rendered once the limit of resolving power has been exceeded. What the eye sees in this limiting region is usually no longer identical with the real shape.

In the table below the achievable limits of resolution in μm and the range of useful magnification is listed for a number of numerical apertures. The table shows that, for instance, with a dry system of very large apertures (A = 0.95) structures can still be resolved which measure half a wave length (λ = 550nm). The required magnification for this is by no means excessive; its maximum is still below 1000x. If a 63/0.95 objective is used for this purpose, eyepieces from 8x to 16x can therefore be used. With oil immersion objectives the resolving power can be further increased by 1/3.

By comparison of the various lines an estimate can also be made of the importance of the aperture of an objective. An increase of 5 to 10% already increases the resolving power and therefore offers considerably more information about delicate details, a fact with which every practical microscopist is familiar. Illustrations see next page.

<table>
<thead>
<tr>
<th>Numerical aperture A</th>
<th>Resolving power λ/2A (λ = 550 nm)</th>
<th>Lines per mm</th>
<th>Useful magnification 500A to 1000A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04</td>
<td>6.9 μm</td>
<td>145</td>
<td>20 40</td>
</tr>
<tr>
<td>0.12</td>
<td>2.3</td>
<td>436</td>
<td>60 120</td>
</tr>
<tr>
<td>0.25</td>
<td>1.1</td>
<td>910</td>
<td>125 250</td>
</tr>
<tr>
<td>0.50</td>
<td>0.55</td>
<td>1820</td>
<td>250 500</td>
</tr>
<tr>
<td>0.65</td>
<td>0.42</td>
<td>2380</td>
<td>325 650</td>
</tr>
<tr>
<td>0.75</td>
<td>0.37</td>
<td>2730</td>
<td>375 750</td>
</tr>
<tr>
<td>0.95</td>
<td>0.29</td>
<td>3450</td>
<td>475 950</td>
</tr>
<tr>
<td>1.30</td>
<td>0.21</td>
<td>4750</td>
<td>650 1300</td>
</tr>
<tr>
<td>1.40</td>
<td>0.19</td>
<td>5090</td>
<td>700 1400</td>
</tr>
</tbody>
</table>
The correct coverglass with dry systems

Even experienced microscopists never fail to be amazed that sections of the same specimen can produce images of different contrast in identical conditions. It is worthwhile to look at the coverglass in conjunction with this problem. For objectives of apertures above 0.40 the coverglass is included in the optical correction, i.e. it is an indispensable part of the image-forming system. Its rated thickness is 0.17 mm. If the effective thickness diverges from this value, a reduction in contrast may in certain circumstances have to be expected. The larger the objective aperture, the smaller the permissible deviations, as the table shows. This is the theoretical aspect.

This standard, however, applies only provided that the object adheres directly to the underside of the coverglass. But in practice a layer of embedding medium of unknown thickness is found between the object and the coverglass, which effectively increases the thickness of the latter. The coverglass used will therefore generally have to be thinner than 0.17 mm. It is advisable, then, to select coverglasses between 0.15 and 0.16 mm, never to use too much embedding medium, and to weight if necessary the coverglasses during drying. For 5-7 μm paraffin sections, which are probably the most widely used in microscopy, coverglass values of $0.17 \pm 0.00$ mm with the optimum value at 0.155 mm have been calculated.

Figs. 30a to 30c, page 37
The importance of the aperture to resolving power.
All pictures have the same final magnification

Left: aperture 0.40
Centre: aperture 0.65
Right: aperture 0.75
With dry objectives of apertures above 0.75 the required accuracy of the effective coverglass thickness can hardly be achieved any longer. For this reason dry systems of apertures above 0.75 are fitted with correction mounts, which permit the adjustment of the objective to the effective coverglass thickness within a region from 0.12 to 0.22mm.

Objectives of apertures below 0.40 can be used with or without coverglass.

Deviations from the refractive index of the coverglass are not critical with any of the dry objectives.

<table>
<thead>
<tr>
<th>Aperture</th>
<th>Effective coverglass thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>0.60</td>
<td>0.17 ± 0.013</td>
</tr>
<tr>
<td>0.75</td>
<td>0.17 ± 0.004</td>
</tr>
</tbody>
</table>

Figs. 31a to 31c
The importance of the coverglass with dry systems of large aperture. Objective: Pl Apo 63/0.95.

Left: Coverglass too thin; 0.14mm
Centre: Coverglass correct 0.17mm
Right: Coverglass too thick; 0.20mm
Dry objectives without correction mount
1) Turn higher-power objective on the nosepiece into the beam
2) Refocus with fine adjustment
3) Close the field diaphragm to suit the objective
4) Open the aperture diaphragm to suit object and objective
5) Readjust brightness if necessary
Higher-power objectives generally produce images of diminished brightness. The ratio aperture: magnification decides the brightness. This ratio generally decreases as the magnification increases, because the apertures of an objective series do not increase at the same ratio as the magnifications.

Objectives with correction mount
1) Open the aperture diaphragm to suit the object and objective
2) Adjust the field diaphragm to suit the objective
3) Set the correction mount at 0.17.
4) Focus through the section with the fine adjustment in both directions several times and compare the unsharpness of the various images. If the loss of sharpness in one direction is associated with a striking loss of contrast, the correction mount must be adjusted step by step until unsharpness changes uniformly in both directions when the section is focused through. If contrast is reduced when the stage is lowered (raised) the correction mount must be set at a smaller (larger) value.

Fig. 32
Dry system of large aperture in correction mount
Working with the highest magnifications - immersion objectives

Objective systems requiring an immersion fluid instead of air between their front lens and the coverglass are called immersion objectives. When the rays emerge from the coverglass, they are refracted not at all or very little, likewise total reflection on the surface of the coverglasses is eliminated with large angles of aperture. As a result, rays of considerably larger angles of aperture reach the objective; numerical aperture and resolving power of an immersion objective are therefore increased. It is a fundamental rule that numerical apertures above 0.95 can be achieved only with immersion objectives, with the upper limit at about 1.40. For special purposes, however, immersion objectives of smaller aperture and medium magnification are used.

Apart from special applications, oil rather than water immersion objectives are used in microscopy. To utilize the higher performance of these systems fully, the following factors must be considered: the immersion oil, the coverglass, the embedding medium, and the temperature. Optically, immersion oil and coverglass are components of the objective. The immersion oil must have a refractive index $n = 1.518$ exactly at a temperature of $23^\circ C$ and a dispersion of $\Delta = 43^\circ$. In addition, the oil must not resinify. A small bottle of immersion oil which meets all these requirements accompanies all LEITZ microscopes. The user therefore has the guarantee of optimum performance.

Normally coverglasses must be used with oil immersion objectives with only a few exceptions. Deviations from thickness are not very critical, since, after all, a layer of immersion oil acts as compensation above the coverglass. Deviations from the refractive index are more serious. Here the second decimal of 1.522 would already become noticeable with the largest apertures. Unfortunately many manufacturers of coverglasses fail to indicate this value, but here, too, efforts at future standardization should gradually change this situation.

The embedding media recommended are those of similar refractive index as immersion oil, such as Canada Balsam or synthetic resins. Immersion oil should be used sparingly. After the end of the examination it should be wiped off with a dry rag and the residual film of oil removed with a rag soaked in xylene or petrol.

Fig. 33
Leitz immersion oil, non-drying, low primary fluorescence

* See section 1, chapter Refraction and Dispersion of Light
If fresh oil is added to a drop of stale immersion oil, schlieren are formed, which affect the image quality adversely. Strictly, an oil immersion objective with its large aperture also requires a corresponding condenser aperture. If the performance of the system is to be fully utilized, the condenser aperture must in fact be almost the same as that of the objective. For the highly magnifying oil immersion objectives, therefore, an immersion condenser of the same large aperture must be used when the highest demands of image quality are made. The use of such a condenser, however, is appropriate only if oil is introduced between its front lens and the underside of the microscope slide, i.e. if the condenser, too, is immersed. Here, as with immersion on the top of the coverglass, freedom from air bubbles must be ensured. After removal of the eyepiece this can be ascertained in the exit pupil of the objective. Air bubbles are surrounded by black rims. For better control, the auxiliary microscope used with phase contrast microscopy can be inserted in the tube. This will make even the smallest air bubbles visible. If immersion with oil is too cumbersome, either the condenser (never the objective) can be immersed in water, or a dry condenser used. The reduction of condenser aperture and therefore of resolution is not normally very serious in practice. But since water rapidly evaporates, its use is recommended only for short-time work.

Fig. 34
Apply a small droplet of the immersion oil to the coverglass with the pipette.

Fig. 35
For full utilization of the aperture of an immersion condenser the condenser, too, must be immersed.
Working with low magnifications

These are magnifications of objectives of apertures below 0.25. Especially in combination with widefield eyepieces very large object areas—extreme cases of more than 1 cm diameter—can be surveyed with these objectives; naturally these areas must also be fully illuminated by the condenser. For this reason our system condensers have a swing-out top. When it is swung out, only the condenser in the base, with which the fields of all low-power systems down to the 2.5/0.08 objective can be fully illuminated is effective. Here it will be found that the image of the field diaphragm, which was still sharp with the condenser top swung out, is now unsharp. The field diaphragm must therefore first be focused; for this purpose the condenser is lowered. Only now should the aperture be opened to suit the objective. Now Köhler’s principle of illumination is strictly realized down to the Pl 2.5/0.08 objective. The aperture diaphragm loses its function with objectives of 4/0.14 and lower powers, and must therefore be fully opened. Uniform illumination of the image field will be achieved by readjustment of the lamp condenser. The lowest-power Pl 1/0.04 objective can no longer be fully illuminated even with our system condensers. The useful field can in extreme cases be about 28 mm, i.e. almost 3 cm across. For this a special low-power condenser has been designed, which simply replaces the system condenser. The field diaphragm loses its function, it must be fully opened. The aperture is adjusted with the iris diaphragm built into the objective. The hinged lens should be swung out.

With low power objectives it is particularly important to adjust the binocular tube and the focusing eyepieces of the eyepiece precisely.

Fig. 36
Centring system condenser with condenser top swung out. The large condenser lens in the bottom part, used for objectives of small apertures, is clearly visible.

Fig. 37
1/0.04 Plano objective. Can be used only in combination with the low-power condenser.
Darkground microscopy

No rigid guide lines can be established for the use of the darkground microscope. Darkground will be used mainly for objects whose structures are based on changes of the refractive index. The following subjects are therefore suitable for observation:

- extensive objects of regular structure, such as diatoms, radiolaria, etc.
- linear structures such as flagellae, fibres, crystals, bacteria. They will become visible even when their thickness is below the resolving power of the objective.
- point-shaped objects at or below the limit of resolution of the objective. Here, however, only the diffraction disc of the object is seen. The darkground image gives no information about the true shape of such sub-microscopical objects.
- stained objects are usually unsuitable.

The specific range of application of the darkground microscope will be understood better once the way the image is formed is envisaged.

Let us observe, for instance, an object in the microscope, whose refractive index changes abruptly across an edge. Here the light is scattered in various directions by diffraction. The angle of scattering is the wider the greater the change in the refractive index. As already described in the theoretical section, part of the scattered light reaches the objective, whereas no scattered light is emitted by the surroundings. We therefore see the edge brightly illuminated against a dark background. In brightfield this edge would be hardly visible. The situation is similar with the edge of an opaque body in transparent surroundings. Here the optical values change particularly abruptly, and the light is therefore very strongly scattered. Such edges will therefore light up with intense brightness.

Figs. 36a, b
Comparison in brightfield and darkground.
The edges of the object light up brilliantly in darkground.
What is required for darkground microscopy?

All LEITZ microscopes are basically suitable for darkground. All that is required are darkground condensers and suitable objectives, possibly with funnel stops. The darkground condensers available are either the immersion version D 1.20/1.40 for immersion objectives, and high-power dry systems, and the dry version D 0.80/0.95. The first of the two aperture values denotes the internal limiting aperture, the second the external one. The internal limiting aperture is always engraved on the condenser. It indicates the lower aperture limit of the cone of illumination.

Both darkground condensers can be centred, so that the illuminated field coincides with the field of view of the microscope.

The aperture of the objectives should be smaller than the internal limiting aperture of the condenser by about 0.1, since otherwise no darkground will be produced.*

Objectives of too large an aperture can, if their design permits it, be used with funnel stops. It is however, preferable to use special objectives for darkground with built-in iris diaphragm.

A few necessary properties of microscope slides, specimen, and cover glass.

Microscope slide, coverglass, and condenser must be free from scratches, thoroughly clean, and free from dust. Any scratch, any impurity, would lighten the background in an undesirable manner. The front lens of the objective, too, must be absolutely clean.

The microscope slide should have a thickness between 0.9 and 1.1mm. If it is too thick, the rays will be focused within it, so that perfect darkground cannot be obtained.

The coverglass thickness of 0.17mm should be adhered to as accurately as possible with high-power dry systems. With increasing apertures deviations from this value will become increasingly critical.

The specimen must not be too thick, otherwise the contrast in darkground will be reduced and the object brightened excessively. For the same reason it must not be too dense, or contain too many light-diffracting particles.

Illumination

Especially with objectives of higher magnifications, a good darkground image requires a powerful low-voltage illuminator. After all, the central portion of the illuminating ray bundle is cut out from the image of the light source – even with darkground illumination, Köhler’s principle of illumination applies – and only the annular cone outside the internal condenser limiting aperture is effective. This cone alone is decisive for the intensity of illumination of the specimen. Illuminator and condenser must be exactly centred to avoid unilateral illumination.

* Too weak objectives (below 10:1) cover a very large field, which in certain conditions will not be fully illuminated by the condenser.
6) Centre the field diaphragm with the two screws on the condenser. After change-over to higher magnifications the field diaphragm must be recentred, and suitably opened.

**Working with immersion objectives**
1) Place a drop of immersion oil on the coverglass.
2) Turn the oil immersion objective on the revolving nose-piece into the beam.
3) Reduce the aperture by means of a funnel stop or the built-in iris diaphragm.
4) Recentre the field diaphragm.

Specimens to be observed with the darkground condenser D 1.20 must contain an embedding medium between the microscope slide and the coverglass whose refractive index is higher than 1.20. Here, water is satisfactory if the work is of short duration. Freedom from air bubbles should be ensured.

**Working with the dry darkground condenser**
The dry darkground condenser D 0.80, which is simpler to handle, is recommended for darkground investigations with dry objectives of medium power, especially for serial investigations. The various points of setting up and focusing the darkground image apply logically; obviously immersion oil is not used here.

---

**Centring of the light source and darkground condenser**
1) Check centration of the light source.
2) Insert the darkground condenser - in our example D 1.20 - and leave it in its lowest position. Centring screws of the condenser in about middle position.
3) Apply immersion oil to the top of the condenser. Place the darkground specimen in position and raise the condenser with the rack-and-pinion movement, at the same time observing it from the front, until the oil droplet makes contact with the underside of the microscope slide. This becomes evident by a brief flash in the microscope slide.
4) Focus the 10/0.25 objective on the specimen. An unsharp image of the field diaphragm appears in the field of view in the microscope.
5) Focus the field diaphragm with the vertical adjustment of the condenser.

Fig. 40
Optical paths in the immersion darkground and dry condensers
Microscopy in phase contrast

The phase contrast microscope permits the contrasty rendering of transparent objects which do not appreciably affect either the brightness or the colour of the light as it passes through them (phase objects). Structural elements which have differential refractive indices, will therefore be rendered visible. These are mainly living or fixed unstained biological or medical objects. Occasionally the phase contrast microscope is also used for stained specimens lacking in contrast.

A few conditions must be met when the phase contrast microscope is used: phase objects should be thin – 5 μm and thinner – and have no major differences of refraction. In addition, the object details to be observed should be very finely structured.

Influence of the thickness of the object
Let us assume a thick specimen focused in the phase contrast microscope so that its upper layer is sharp. To begin with, as in thick brightfield specimens, the deeper layers of the specimen will appear blurred. But two phenomena specific of the phase contrast method are added to this: the deeper object details produce disturbing phase displacement and, in addition, annular patterns of light scatter because of the annular condenser aperture. The image of the critically focused top layer will then be superimposed by these scattered-light figures. Phase objects, should, therefore, be as thin as possible.

Figs. 41a, b
Comparison between brightfield and phase contrast
Pollen cells, Tradescantia
Phaco, NPL 25/0.65 objective
Magnification 56x.
Fine details will be reproduced only in phase contrast.

Fig. 42
Excessively thick specimen (30 μm)
In phase contrast.
Pine, radial, NPL 16/0.40
Magnification 150x.

Fig. 43
Prominent halation in a phase contrast object.
Yeast, NPL 40/0.65 objective
Magnification 675x.
The influence of extensive object details

The halo effect
A disadvantage of the phase contrast image formation is the halo effect, consisting of halos around dark image details and dark fringes around bright details. It is caused by the practical impossibility of a complete separation between diffracted and direct light.* This halo effect occurs particularly round extensive object details. Thus, for instance the edge of a more highly refractive extensive object detail is surrounded by a bright halo and lined inside by a dark fringe with positive phase contrast; the conditions are reversed with negative phase contrast. The interior field appears at the same intensity as the surroundings. Since the boundaries between bright and dark halo are in most biological objects not identical with the outline of the objects, the phase contrast microscope does not reproduce such objects completely true to size. With measurements, an error of the magnitude of the width of the halo should be allowed for. For exact measurements, an interference microscope is therefore necessary.

Very finely structured object details of an order of magnitude of the lateral resolving power produce images that are largely free from halation.

The influence of the immersion medium
The immersion medium also plays an important part in phase contrast microscopy. Contrast can be considerably influenced by the expert choice of the immersion medium. Physiological media are preferable for unstained biological material, media of a refractive index smaller than that of Canada Balsam should be chosen for permanent specimens. For sealing the coverglasses pure, white vaseline or paraffin is suitable except for permanent specimens.

* The levelling of intensity described in the same chapter is also based on this.

Fig. 44
Photomicrograph of a typical phase object. Unstained thin section through an earthworm. Phaco N10/0.25 objective, magnification 100x.
Linear measurement in the microscope

If a glass disc with a scale (eyepiece micrometer) is placed in the image plane of an eyepiece, the image of the object and the scale division will appear together in the eyepiece. The size of a microscopic object can now be instantly stated relatively in scale units. This measurement, however, offers no information about the true size of the object, even if the length of the scale division in mm is known. For it must be borne in mind that the microscope image is determined by the objective, the tube lens, and the eyepiece, whereas the magnification of the eyepiece micrometer depends solely on the eyepiece, and mostly even only on the eyelens of the eyepiece. It must therefore be determined by calibration what distance in the object in μm or mm is just projected by a given objective on a division of the eyepiece micrometer. This value is called the micrometer value. It is different with each objective/eyepiece combination and naturally also when a different tube lens system is used.

A stage micrometer serves as a precisely defined measuring distance. Stage micrometers are glass plates of the same size as microscope slides, with a scale engraved on them; as a rule 2 mm is divided into 200 intervals, i.e. each division corresponds to 1/100mm.

If the microscope is focused on such a stage micrometer, it is possible to determine quickly how many division lines of the stage micrometer coincide with one division line of the eyepiece micrometer.* This calibration is the more accurate the larger the scale divisions compared with one another. If x parts of the stage micrometer coincide with y parts of the eyepiece micrometer, the micrometer value for the optical combination used = x/y, where x is entered in the measuring unit of the stage micrometer used for the calibration (mostly 1/100mm). It is simplest to use 100,

or, with lower-power objectives, 10 intervals of the eyepiece micrometer for the comparison.

Example Fig. 45 shows the picture of a stage micrometer and eyepiece micrometer in the microscope. The scale in the centre of the field of view (engraving 0-10) is the eyepiece scale, the scale to the right of it the visible part of the stage micrometer. 100 scale divisions of the eyepiece micrometer correspond to 120 scale divisions = 1.220mm of the stage micrometer. One division of the eyepiece micrometer will then correspond to 0.0122mm in the object. Micrometer value = 12.2μm.

* When the stage micrometer is focused it will be noted that, fortunately, the size of the intermediate image remains unchanged, because the image-forming rays are parallel on the object side; as a result, when the image is focused through it becomes unsharp, but its dimensions remain unchanged. Exception: 1/0.04 objective, which is unsuitable for this method of measurement.
**A few practical hints**

The measurements can be carried out monocularly or binocularly. When a binocular tube is used, the interpupillary distance should be kept constant*, since the micrometer value also depends on this (exception: FSA tubes).

The eyepiece used for the measurement must have a graticule and focusing eyepiece for the focusing of the graticule. Our list 512-99 pp. 106-109 offers information on the various graticules. On the binocular tube, the measuring eyepiece should, where applicable, be used with the better eye.

The details to be measured should be moved into the centre of the field of view. Here the distortion error of the objectives is at a minimum; the distortion of the eyepiece does not enter into the measurement.

The resolving power of the objective must be sufficiently high, i.e. the distance between 2 just resolved points must be considerably smaller than the object detail to be measured. The objective suitable for measurement should be chosen from this aspect.

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* On binocular tubes with adjustable eyepiece tubes the interpupillary distance should be carefully set on both tubes.

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**Fig. 46**

Measurement of an object. The object extends across 60 intervals in the eyepiece micrometer. One interval = 12.2μm in the object. 60 intervals = 732μm in the object.
Measurements with the screw micrometer eyepiece.
For the finest measurements under the microscope the screw micrometer eyepiece is used; it has a much higher measuring and reading accuracy than any other type of micrometer eyepiece. A measuring line can be moved across the entire range of the eyepiece scale by means of a micrometer screw on the side of this eyepiece. The drum of the micrometer screw is divided into 100 intervals. A full rotation of the drum moves the measuring line across one interval of the eyepiece scale. Hence one interval of the drum division corresponds to 1/100 of an interval of the eyepiece scale. List 519-17 contains information about the details of the measurement. Because of its precise and robust design the screw micrometer eyepiece stands up to considerable wear and tear in the factory workshop.

Measurements with the mechanical stage or attachable mechanical stage.
LEITZ mechanical stages or attachable mechanical stages are graduated in mm in both axes and have verniers for reading to 1/10mm. With this arrangement, measurements can therefore be carried out in the x and in the y direction, with longer distances also suitable for measurement. If necessary, diagonal values can be determined by calculation. The use of a crosslines eyepiece is advantageous here; the crosslines must be oriented to the axes of the stage.
Photomicrography

Microscope, illumination, and optical system
Generally any modern LEITZ microscope can be used for photomicrography provided it has an interchangeable tube. Stands of modern design have almost always built-in illuminators. For black-and-white photomicrography mains lamps or 6v 5W low voltage lamps are adequate. For photomicrography in colour, the stand must at least have a 6v 15W lamp. Optimum picture quality requires objectives of the best possible correction. LEITZ plan-apochromats should therefore be chosen wherever the resolution of finest structures is essential. Their total image definition, sharpness, contrast, chromatic correction, flattening of the field are fascinating. Where plan-apochromats are too expensive, fluorite systems can also be used. Naturally, conventional achromats or plan-achromats are successfully employed in photomicrographic recording, especially in the black-and-white section.

![Photomicrograph examples](image)

Figs. 48a, b
Effect of curvature of field
Left: Photograph with the 40/0.65 standard achromat
Right: with 40/0.75 plan-apochromat
Here the picture appears sharp from corner to corner.
Magnification 950x
Which camera format?
The question of the correct format for photomicrography can be objectively answered if the following points are considered:

In the theoretical section we derived the minimum useful magnification $M$ for a black-and-white fine grain film at $1/60$mm circle of dispersion $M = 60A$. Let us tabulate the data resulting therefrom for a number of objectives.

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Total magnification for $M = 60A$</th>
<th>Field-of-view index</th>
<th>Object field diameter</th>
<th>Picture diameter = total mag. x Object field diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.3 /0.20</td>
<td>12:1</td>
<td>18</td>
<td>2.86 mm</td>
<td>34 mm</td>
</tr>
<tr>
<td>25 /0.50</td>
<td>30:1</td>
<td>18</td>
<td>0.72 mm</td>
<td>22 mm</td>
</tr>
<tr>
<td>40 /0.75</td>
<td>45:1</td>
<td>18</td>
<td>0.45 mm</td>
<td>20 mm</td>
</tr>
<tr>
<td>40 /0.95</td>
<td>57:1</td>
<td>18</td>
<td>0.45 mm</td>
<td>26 mm</td>
</tr>
<tr>
<td>Öl 100/1.30</td>
<td>78:1</td>
<td>18</td>
<td>0.18 mm</td>
<td>14 mm</td>
</tr>
</tbody>
</table>

In our considerations, we are interested first in the values of the last column. They indicate how large the picture produced on the film of the 35mm camera is at magnification $M = 60A$, with an eyepiece of field-of-view index 18. The derivation of this theoretical magnitude is based on the circle of dispersion on the film being of the same size as the diffraction disc of the objective. Information
losses owing to objective diffraction and film grain are therefore equal. But if magnifications are, as occurs in practice, carried beyond \(M=60\times A\), the film grain will soon cease to have any effect on picture quality. This happens at about the factor 2, i.e. at \(M=120\times A\), and can be compared to the screens in reproduction technology. Whereas the difference between a 20 and a 70 screen is still very prominent, a 160 screen, which does in reality not exist, would no longer yield any further gain in information.

If the values of the last column are compared with the 35mm format, whose diagonal measures 43mm, it will be seen that the format is large enough at field-of-view index 18 to record all the information offered by the objective. In practice a 10x eyepiece and a camera factor 0.32x is used, i.e. a 3x secondary magnification of the intermediate image. The range here already extends from \(M = 75\times A\) to \(180\times A\) and the 35mm format is fully utilized. The situation is similar in 35mm photomicrography with the widefield eyepiece GW 6.3x and field-of-view index 28. If, however, this field-of-view index is to be fully utilized, a larger format is indispensable.

The 35mm format is similarly favourable with highly-resolving colour films. There is the added advantage of low price and economical application. If the colour photograph is to be used for projection or scientific publications at 3-4x secondary magnification in reproduction here, too, 35mm film is recommended. The large format will, however, have to be used if the highest demands of quality must be met or whole-page reproductions obtained.

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Figs. 49a, b
Influence of the emulsion grain on sharpness, shown with the example of a printing screen.
Top: 20 screen, i.e. \(20\times 20 = 400\) dots/sq cm
Bottom: 70 screen, i.e. \(70\times 70 = 4,900\) dots/sq cm
Films, exposure time, and filters

I. Black and white

As a rule, microscope specimens are not specially prepared for recording purposes. It will therefore be often necessary to adapt the exposure material to given conditions.

Black-and-white films will be used when:
- Several prints of a photomicrograph are required,
- Certain object details are to be stressed or suppressed by a change in the illumination through colour filters,
- An increase or reduction of the overall contrast in the photomicrograph becomes necessary.

The black-and-white exposure material is also relatively insensitive to faulty exposure, since its exposure latitude is considerably wider than that of colour material.

Sensitization

Every exposure material is sensitized for a certain region of the spectrum, i.e. the emulsion is more or less sensitive to certain parts of the spectrum. Mainly we distinguish between two different types of emulsion: the orthochromatic and the panchromatic emulsion. Whereas unsensitized photographic material (positive film and lantern plates) extends from ultra-violet to about blue-green (230-500nm), the orthochromatic film is sensitive to up to about 580nm, and the panchromatic film up to about 700nm.

Orthochromatic films

Orthochromatic films will be preferred if the object contains no red, because it is insensitive to red and can therefore be better controlled during development (processing in weak, red darkroom light). This frequently applies, for instance, to phase contrast microscopy as well as to metallography.

Figs. 50a, b

Pictures of the same object detail.
Left: on orthochromatic film.
Right: on panchromatic film.

The red lateral portions have lost all their detail on orthochromatic film, appearing practically black.
On panchromatic film the red portions, too, have good detail...
Panchromatic material
In the majority of cases panchromatic film is used, which, particularly when a green filter is inserted in the beam, yields an approximately correct tone rendering of the colours of the object. This means that grey values of the individual colours approximately match the colour perception of the eye. In addition, it is possible to change the grey-tone reproduction of certain colours most radically by means of colour filters in the illuminating beam when panchromatic film is used (see chapter: Filters).

Thin-film emulsions
Thin-film emulsions are preferred because of their negligible diffusion halation. These usually slow films (8-25 ASA, 10-15 DIN) have very fine grain, produce high contour sharpness, and in addition have a very high contrast factor (gamma) which is desirable particularly in bright-field photomicrography.

Exposure time
Unless the objects move or are sensitive to light, the exposure time in black-and-white photomicrography is not subject to any special requirements. Naturally the exposure time measured should always be adhered to as precisely as possible. Especially films of low sensitivity and steep gradation react more noticeably to over- or under-exposure. If the exposure is too short, details in the dark portions of the specimen are no longer recorded by the film, and a considerable part of the information is lost. If the exposure is too long, the gradation curve flattens out, and contour sharpness is strongly reduced.

Gradation
If we ignore special techniques, the contrast range of the microscopic image must be considered low. This lack of contrast can already be compensated in the negative by the use of films of steep gradation. There is the additional possibility of influencing the gradation of the negative material by variation of the conditions of development (developing time, developer concentration). Longer developing times or stronger concentrations increase, shorter developing times and weaker concentrations decrease the gradation, i.e. the film becomes harder (more contrasty) or softer (flatter).

Filters
Each filter transmits most readily light of its own colour and suppresses most effectively its complementary colour. It is therefore possible to influence the contrast of individual colours within wide limits by means of filters. The green filter occupies a special position in this context; it is part of our equipment.

Green Filter
Achromatic objectives are corrected within the region of the maximum colour sensitivity of the human eye. The correction curves for red, green, and blue have approximately the same shape. Violet, however, is not completely included in the correction, although it is the very colour to which photographic emulsions are particularly sensitive. A green filter is therefore used for photomicrography. This permits only those rays to reach the objective for which it is best corrected.

On panchromatic films, in addition, green filters result in a largely correct tonal rendering of the colours. Blue, because of the higher sensitivity of the film to this spectral region is reproduced relatively dark - blue thus blackens the photographic emulsion more intensely. The green filter reduces the proportion of blue, which becomes lighter in the film. In the positive paper print, such portions will appear darker.
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General filter rule
The following rule applies generally to the positive paper print: stained object structures corresponding to the filter colour are rendered light, the complementary colour dark. The table below gives a survey of the colour rendering to be expected in the positive compared with a photograph without filter when panchromatic material is used.

<table>
<thead>
<tr>
<th>Filter colour</th>
<th>Brighter</th>
<th>Unchanged</th>
<th>Darker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>Red</td>
<td>Orange</td>
<td>Blue, green, yellow</td>
</tr>
<tr>
<td>Orange</td>
<td>Red</td>
<td>Yellow</td>
<td>Blue, green</td>
</tr>
<tr>
<td>Yellow</td>
<td>Green</td>
<td>Blue</td>
<td></td>
</tr>
<tr>
<td>Yellow-green</td>
<td>Green, yellow</td>
<td>Orange</td>
<td>Blue, red</td>
</tr>
<tr>
<td>Green</td>
<td>Green</td>
<td>Yellow</td>
<td>Blue, red, orange</td>
</tr>
<tr>
<td>Blue</td>
<td>Blue</td>
<td>Green</td>
<td>Yellow, red, orange</td>
</tr>
</tbody>
</table>

II Colour

In many cases, the colour photograph will be preferred to the black-and-white picture in photomicrography.

Reversal films, negative films
Colour reversal film, which produces a colour transparency, is the most suitable film for colour photomicrography. When colour negative film is used, it should always be borne in mind that the subsequent positive process requires colour correction with corresponding filters which may lead to varying degrees of colour deviation. It is therefore absolutely essential to make transparencies of the same object, which in the colour processing laboratory serve as originals for the colour paper print. Naturally colour paper prints can also be obtained from transparencies.

Colour temperature
Colour reversal films are available in two versions because of the importance of the colour temperature of the light: as daylight colour film for a colour temperature of 5500°K and as artificial-light film, depending on the manufacturer, for colour temperatures between 3100°K and 3400°K.

A few words about the nature of colour temperature are appropriate here. With a black-body radiator the spectral energy distribution and therefore the colour of the light is a direct function of the temperature T (true temperature measured in °K). For all other radiators the relation between light, colour, and radiator temperature is not so straightforward. But here, too, it is possible to determine, with the aid of the colour triangle, the temperature at which a black-body radiator has the same colour as the radiator observed. This temperature, which deviates from the true temperature, is called the colour temperature T_s. With metallic sources of radiation the colour temperature T_s is always higher than the true temperature.
Exposure time and Schwarzschild effect (reciprocity-law failure)

Colour films are neutrally balanced only for a certain exposure period. The neutral range in artificial-light materials is usually at slower shutter speeds (Agfachrome 50L, 35mm, 1/30sec) with daylight materials at higher shutter speeds (Agfachrome 50S, 35mm, 1/125sec). Greater deviations (factors 10-20x still have no appreciable effect) produce colour shifts because of the Schwarzschild effect. The data supplied by the various manufacturers should be studied.

It is usually possible to adjust the colour temperature of the light source to that of the artificial-light colour film with the aid of regulating transformers. For details consult the table at the right. The lamps must, however, be always in perfect condition. After prolonged use their glass envelope is blackened, which has an adverse effect on the colour temperature of the light.

Neutral-density filters

If the exposure times determined are shorter than the shutter of the camera permits, the light intensity must be reduced by means of neutral-density filters. A change in the current intensity would also change the colour temperature of the light.

Conversion filters

Conversion filters can be used if the colour temperature required for a film cannot be obtained with the microscope lamp. Conversion filters must be used. These change the colour temperature of the light by a certain value. Depending on the filter the colour temperature of daylight films or of artificial-light films can be obtained. The latter is, for instance, necessary if high-pressure xenon lamps are used and the photographs to be taken on artificial-light colour film. The most widely-used conversion filters and their function are listed in the table at the right.

<table>
<thead>
<tr>
<th>Conversion filters</th>
<th>Colour temperature change</th>
<th>from</th>
<th>to</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB6 and CB6</td>
<td>2800° K to 3400° K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB 6</td>
<td>2600° K to 3100° K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB 12</td>
<td>3100° K to 5500° K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB 16,5</td>
<td>2800° K to 5500° K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR 1,5</td>
<td>6000° K to 5500° K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR 12</td>
<td>6000° K to 3100° K</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Microflash

The spectral composition of the microflash is identical with that of daylight. For this reason daylight colour films are used for flash photography.

Fluorescence

The situation is similar for photography in fluorescent light. Here, too, the use of daylight colour films is indicated.
The photomicrograph

Specimens
The most essential conditions for a good photomicrograph are a specimen of corresponding quality, and flawless microscope slides and coverglasses. This should already be considered during the preparation of the material. If sections are used, they should be thin, although they stain less deeply, and are also more difficult to produce. A compromise is often necessary in this situation. The embedding medium must form only a thin film on the object, and must be free from air bubbles.

Setting up the illumination
Special attention must be paid to a careful setting up of the illumination according to Köhler’s principle. Photographic emulsions react considerably more sensitively than the eye to uneven illumination. In colour photomicrography, the colour temperature of the lamp must be adjusted to that of the film before the exposure time is determined and the exposure made.

The vertical adjustment of the condenser, too, is important in colour photomicrography. Every condenser produces colour fringes along the edge of the field diaphragm. The condenser should therefore be vertically adjusted so that the colours along the edge of the diaphragm are balanced, i.e. no colour should be dominant.

Setting up the microscopic image
The structures most important in the photograph should be moved into the centre of the picture area. Optimum utilization of the film format is possible by rotation of the object or of the camera. Now the object must be focused. If plano objectives are used, the image will be sharp throughout the entire photographic format. Four possibilities for setting up and focusing the object exist with the photographic outfits described in part III of this brochure:

1) The groundglass screen
   Its scattering ability caused by the grain of the surface makes it possible to focus exactly across the entire picture format. Fine details may, however, be poorly rendered by the grain of the groundglass screen. At magnifications higher than 200x, the

2) clear glass plate
   is therefore preferred. In combination with a magnifier it is used for the focusing of the aerial image. With the shallow depth of field of the high magnifications, this presents an extremely sensitive possibility of sharpness

Fig. 51
Only flawless thin sections permit optimum utilisation of the microscope.

Fig. 52
Left: uneven illumination
Right: evenly illuminated field of view.
control: first the magnifier is placed on the clear glass plate and then focused on the engraved crosslines. The specimen is now focused with the fine adjustment. To exclude faulty accommodation of the eye, ensure that the microscopic image and that of the crosslines remain mutually stationary during slight lateral movement of the eye above the magnifier. Failing this, correct with the fine adjustment until this condition is established.

3) Focusing telescope or FSA tube.

The focusing telescope or the FSA tube are the two focusing aids in 35mm photomicrography.

a) Focusing telescope

If the micro-attachment includes a focusing telescope, first the double circles in the focusing telescope are focused with the eyepens of the telescope. Now the image is focused with the fine adjustment. Focusing is correct if the double circles and the image appear sharp at the same time.

b) FSA tube

If, for instance, the system camera is used, the image is focused with the FSA tube, which, for this purpose, must be equipped with an adjustable eyepiece with graticule. A specially matched eyepiece is used in the photographic beam.

1) Adjust the eyepens of the eyepiece until the double circles and format outline in the eyepiece are sharp.

2) Focus the image with the fine adjustment. Focusing is correct when the markings and the image appear sharp at the same time.

3) Adjust the eyepens of the second eyepiece until the image appears sharp also to this eye.

Use of the auxiliary telescope

To exclude any faulty accommodation of the eye when the FSA tube is used, it is recommended to use the auxiliary telescope, available for these purposes if very high demands are made of the quality of the photomicrograph and for low powers.

Fig. 53

Axial resolving power as a function of the total magnification for a number of apertures.
1) Hold the telescope above the eyepiece of the microscope and adjust its eyepiece until the two double circles appear sharp.
2) Focus the microscopic image with the fine adjustment until it appears sharp in the telescope at the same time as the markings.

**Depth of field**

The layer of the object of which a sharp image is formed is always relatively thin in the microscope. According to a useful rule of thumb, it is about 25 μm with an objective of aperture 0.25 and final magnification \( V = 100x \), with oil immersion objectives usually only 1 μm and less. The table should be consulted for exact values.

The depth of field can be increased if the condenser aperture is stopped down. This, however, results in a reduction of the resolving power. If the question of depth of field is paramount, the condenser aperture can be reduced by more than 30% although this requires a visual assessment how far the diaphragm can be stopped down at the expense of resolution.

If no resolving power is to be sacrificed, there is only the possibility of choosing lower powers and very fine-grain film.

With 35mm cameras, the 0.32x camera factor basically ensures a depth of field which is adequate in many cases. Care should, however, be taken from the very beginning to restrict the extent in depth of the object sufficiently by suitable preparation. With large apertures, this, too, only helps within limits. Here, maximum resolution of great depth is simply not possible.

**Determination of the magnification**

It is a well known fact that the total magnification of a microscope is

\[
V_{\text{microscope}} = M_{\text{objective}} \times V_{\text{eyepiece}} \times V_{\text{tube factor}}
\]

The image can be intercepted at this magnification at the minimum distance of comfortable vision of 25cm on the groundglass screen of a bellows camera. If the bellows extension deviates from this distance, the microscope magnification must be multiplied by the factor

\[
\text{bellows extension in cm}
\]

\[
\frac{25\text{cm}}{25\text{cm}}
\]

The reproduction scale will then be

\[
M_{\text{bellows camera}} = V_{\text{microscope}} \times \frac{\text{bellows extension in cm}}{25\text{cm}}
\]

The camera factor is engraved on 35mm cameras whose extension is fixed. In our devices it is 0.32x. Hence

\[
M_{35\text{mm camera}} = V_{\text{microscope}} \times 0.32
\]

Generally this simple calculation of the magnification is adequate. If pictures are to be used for size determinations, a second picture should be taken with a stage micrometer. When a bellows camera is used, an eyepiece micrometer can also be photographed simultaneously with the specimen. Another possibility consists in measuring the image of the stage micrometer on the groundglass screen and subsequently transferring the magnification with a black dimension line simply to an unimportant area of the negative. The associated distance is noted with it. For example: —— 100 μm.
### Lamps for photomicrography

<table>
<thead>
<tr>
<th>Type</th>
<th>Operating voltage</th>
<th>Power W</th>
<th>Mean luminous density $\text{cd/m}^2$</th>
<th>Regulation of brightness</th>
<th>Colour temperature $^\circ\text{K}$</th>
<th>Use in photomicrography</th>
</tr>
</thead>
<tbody>
<tr>
<td>15W mains lamp</td>
<td>220</td>
<td>15</td>
<td></td>
<td>None</td>
<td>1800</td>
<td>Black-and-white film</td>
</tr>
<tr>
<td>5W low-voltage lamp</td>
<td>6</td>
<td>5</td>
<td></td>
<td>Transformer</td>
<td>up to 2400</td>
<td>Black-and-white film</td>
</tr>
<tr>
<td>15W low-voltage lamp</td>
<td>6</td>
<td>15</td>
<td></td>
<td>Transformer</td>
<td>up to 2850</td>
<td>Black-and-white and artificial-light colour film with conversion filter</td>
</tr>
<tr>
<td>30W low-voltage lamp</td>
<td>6</td>
<td>30</td>
<td></td>
<td>Transformer</td>
<td>up to 3400</td>
<td>Black-and-white and artificial-light colour film</td>
</tr>
<tr>
<td>60W low-voltage lamp</td>
<td>12</td>
<td>60</td>
<td></td>
<td>Transformer</td>
<td>up to 3400</td>
<td>Black-and-white and artificial-light colour film</td>
</tr>
<tr>
<td>50W tungsten halogen lamp</td>
<td>12</td>
<td>50</td>
<td></td>
<td>Transformer</td>
<td>up to 3400</td>
<td>Black-and-white and artificial-light colour film</td>
</tr>
<tr>
<td>100W tungsten halogen lamp</td>
<td>12</td>
<td>100</td>
<td></td>
<td>Transformer</td>
<td>up to 3400</td>
<td>Black-and-white and artificial-light colour film</td>
</tr>
<tr>
<td>150W xenon lamp</td>
<td>20</td>
<td>150</td>
<td></td>
<td>Neutral density filter</td>
<td>6000</td>
<td>Black-and-white and daylight colour film</td>
</tr>
</tbody>
</table>

Mercury lamps from 50 to 200W for fluorescence only

Black-and-white and daylight colour film

---

**Fig. 55a-e**

Colour temperature as a function of the current load on the various low-voltage lamps.

a) 6v 15W low-voltage lamp
b) 6v 30W low-voltage lamp
c) 12v 60W low-voltage lamp
d) 12v 50W tungsten halogen lamp
e) 12v 100W tungsten halogen lamp
The most frequent faults in microscopy

Before you start using the microscope, always make sure of the following points:

Is the illumination correctly set up?
Lamp centred, field and aperture diaphragms in the correct positions, no filters, groundglass discs in the optical path which do not belong there?
Is the revolving nosepiece engaged?
Is the binocular tube set for your interpupillary distance and are the focusing eyelenses of the eyepieces set correctly?
Is the optical system clean?

1) Uneven illumination
This can be caused by various errors. To begin with, check, whether revolving nosepiece and condenser are fully pushed home, the objective is engaged, any filter holder in the optical path is vignetting the image, the lever actuating the beam splitter of the FSA tube is in the correct position, and the lamp is securely mounted in the socket.
Now lamp centration, and the position of the lamp condenser and that of the hinged lens in the foot of the stand should be checked. The latter must always be in the optical path. Exception: objective 1:1.
If the illumination is still not satisfactory, check the centration and vertical adjustment of the condenser and whether the condenser top is swung in or out, depending on the objective in use.

2) Flat images owing to damaged or dirty objectives
Defective objectives either produce no images at all or the images are flat or move when focused through. It is often the front lens which is damaged, although the spring mount offers a high degree of protection. Such objectives must be returned to the factory or to your local agency. Do-it-yourself repairs usually compound the defect.

Fig. 56
Left: uneven illumination,
Right: evenly illuminated field of view

Fig. 57
Fingermarks on high-quality optical systems render the image hopelessly flat.
Dirty front lenses, however, are far more frequently seen. This should always be the first suspicion when the image lacks contrast. The check is best carried out with a magnifier. Dust should be removed with a soft brush, not only from the front lens, but also from all the other external surfaces of objective lenses. Resistant dirt should be removed with distilled water, xylene, or petrol. Occasionally the eyelenses of the eyepieces, too, should be cleaned. They are often covered by a fine film from the eyelashes.

3) Unsharp patches in the microscopic image
Unsharp patches which remain stationary when the specimen is adjusted are caused by dust etc. on lenses and other optical faces. The precise location can be seen when the eyepiece, condenser, deflecting mirror, lamp condenser, filter, etc. are rotated or moved in turn and the effect this has on the patches. With some experience it is possible to determine where the dust is located. Here, too, cleaning should be carried out with a brush or a piece of soft rag.

4) Flat images with large-aperture dry objectives
When large-aperture dry objectives are used the image sometimes tends to be poor in contrast. Here either the coverglass was omitted, or a coverglass that is too thick or too thin, or too much embedding medium used, or the correction mount - where present on the objective - incorrectly set. The latter is the equivalent of too thick or thin a coverglass. The setting of the field diaphragm, too, should be checked. It should not be opened beyond the edge of the field of view.

5) Flat images or schlieren with oil immersion objectives
In most cases the oil was omitted. Another cause is the addition of fresh oil to an oil immersion that was allowed to stand over night, to continue the observation, disturbing schlieren may form in the oil. Alternatively, the front lens
may be dirty because of failure to remove old residual oil, or the oil may contain air bubbles. Furthermore, suitable immersion oil and coverglasses of about 0.17 mm thickness should be used with immersion objectives of large apertures. Since the refractive index, too, must be the same, the coverglass should not be replaced by other materials. Finally, the room temperature should not differ too greatly from 22 to 23°C. At 15°C or 35°C image quality may already be noticeably impaired.

6) Unnatural contrast owing to wrongly set aperture diaphragm
When objectives are changed the adjustment of the aperture diaphragm is frequently forgotten. The result is an aperture that is too wide or too small. This may produce either too flat or too contrasty images with correspondingly reduced resolution. The correct setting of aperture and field diaphragms should therefore always be ensured. Never adjust brightness with the aperture diaphragm.

7) The specimen cannot be focused
If the specimen cannot be focused at all or only poorly, the microscope slide was invariably placed on the object stage with the coverglass facing downwards. Where specimens are unmarked, this can happen only too easily.

8) Lack of fine detail because useful magnification has been exceeded
Excessive secondary magnification, e.g. with the highest-power eyepieces, may produce empty magnification. The image will then completely lack fine detail. With conventional work with the microscope, the range of useful magnification from 500A to 1000A should therefore always be used. Incidentally: the tube factor, too, enters into the secondary magnification.
9) Extreme unsharpness after objective change
Occasionally objectives may not have been screwed fully into the revolving nosepiece. The result is extreme unsharpness when the objectives are changed. In addition parfocality no longer exists, i.e. an object detail near the centre will no longer appear in the centre after a change of the objective, but its image will be formed somewhere at a distance from it.

10) Floating spots
This is called myodesopsia and is disturbing at high magnifications. It is an entoptic phenomenon, caused by an anatomic condition in the eye, but is localized subjectively in external space. It is caused by minute turbidity of the vitreous body or schlieren in the aqueous humor casting shadows on the retina. Often resting the eyes will help.

11) Unsuitable specimens
Successful work with a microscope often depends on the specimen. Without planning and care during the preparation of the specimen, it is not surprising when microscopic examination proves disappointing. Badly cut, torn, or excessively thick sections, excessive staining, imperfect dehydration will with certainty lead to disappointing results. If, for instance, you want to investigate diatoms enclosed in air in darkground with an immersion condenser, you will have forgotten the total reflection on the glass surface of the microscope slide. You will literally look into empty space. Such examples can be quoted almost indefinitely.
III Instruments
General hints

The instruments section describes the construction and function of a typical LEITZ transmitted-light microscope and the standard accessories. In addition a number of supplementary devices for microscopy are listed: a small selection of special apparatus concludes this chapter. Besides an abundance of technical details the reader will find a comprehensive review of the LEITZ modular system for scientific and technical microscopy. Further information material is available in our brochures, reprints, and LEITZ Technical and Scientific Information.

The LEITZ range of microscope stands

HM-LUX student and teaching microscope, can also be supplied with binocular tube
SM-LUX teaching and routine microscope
DIALUX® laboratory microscope
ORTHOLUX® 2 laboratory and research microscope
ORTHOPLAN® largefield microscope

Fig. 64
Construction of the microscope

No matter how varied the uses to which individual microscopes are put, their most important components are always:
stand with object stage
microscope tube
objective carrier
optical system, consisting of objectives and eyepieces
illumination.
Construction and function of the individual components of a transmitted-light stand are described in the following chapters.

Fig. 65
LEITZ DIALUX 20, Laboratory and Research Microscope
1. PERIPLAN GF eyepieces
2. Binocular tube S
3. Quintuple revolving nosepiece with NPI objectives
4. Slot for light filters
5. Stand
6. Large Mechanical Stage No. 78
7. Knurled knob for the vertical adjustment of the condenser
8. SK achromatic standard condenser
9. Lever for opening and closing the aperture diaphragm
10. Knurled screws for centring the condenser
11. Knobs for the coordinate movement of the object
12. Knurled screw for the upper stop of the condenser
13. Field diaphragm
14. Coarse focusing adjustment
15. Fine focusing adjustment
16. Lamp Housing 102 Z
Microscope tubes

In older stands the microscope tube was the connecting piece between objective and eyepiece. The objectives were joined to the tube either directly or by means of a revolving nosepiece, with the latter a fixed component of the tube. The tube had a precisely defined length, which was called the mechanical tube length. This length, extending from the screw-on base of the objective to the upper rim of the tube, could easily be measured by the user, provided there were no lenses or prisms etc. in the tube. With modern stands it has been found useful to connect the objective carriers no longer rigidly with the tube, since today interchangeability of the tubes and revolving nosepieces independently from one another is preferred. All modern large LEITZ stands offer this freedom of interchangeability of both structural components. In smaller stands usually only the tube is interchangeable.

LEITZ microscope tubes

Most LEITZ stands have interchangeable tubes. They can be rotated through 360°; the tube flange is hard chromium plated, so that the tube remains perfectly adjusted even after years of use.

O tube
This is the simplest tube; it is straight and serves almost exclusively for the attachment of a photographic outfit.

P tube
This is a monocular tube inclined at 45°. It is used either for simple classroom outfits or for very dim objects in fluorescence microscopy.

S tube
Binocular tubes are best for visual observation. The binocular S tube is inclined at 30°. Its prism system divides the light without loss to reach both eyepiece tubes. For compensation of different interpupillary distances of the individual observers – values range from 55 to 75mm – the distance between the two eyepiece tubes can be adjusted.
The necessary compensation of the tube length that has thereby been changed can be carried out on both eyepiece tubes. They are engraved with values from 55 to 75mm.

FSA tube
LEITZ FSA tubes are a combination of binocular tube and photo tube. They can be adjusted for loss-free beam splitting into 20% visual and 80% photographic or 100% visual. In addition they include an automatic tube-length compensator. This ensures that the image is sharp both in the film plane of the camera and for visual observation for any interpupillary distance from 55 to 75mm (this does not apply to bellows cameras) provided that an eyepiece with focusing eyepiece and gatricule is used in the visual observation beam.

Function of the FSA tube
The drawing shows the optical structural elements of the FSA tube. The two eyepieces A and B are shown both at their narrowest and at their widest separation. In the narrowest position let y be the distance between the prisms 1 and 2 and x the distance between the prisms 3 and 4. When the pair of eyepieces is moved into the widest separation the prisms 1 and 2 are moved apart to the distance y' and the set of prisms 1, 2, and 3 lowered in the direction of 4. The new distance between 3 and 4 will then be x'. The distance x' is defined so that the optical path length from prism 4 to the intermediate image plane is identical to that in the narrowest separation. All this applies to any interpupillary distance from 55 to 75mm.

Vario tube
The vario tube is a component which is locked between the stand and the tubes. It permits the continuous variation of the tube factor within the range between 1x and 3.2x. The microscopic image must first be exactly focused with the aid of a gatricule eyepiece; it will then remain sharp within the entire zoom range. The vario tube is particularly recommended for photography or cinematography.
Tube lens systems and revolving nosepieces

Questions of construction or optics often force the designer to deviate from the prescribed mechanical tube length. To maintain compensation with low-power objectives and the image quality at high-power ones, the intermediate image is displaced by means of a tube lens system. The objective is therefore used in a manner always best adapted to its performance and the image displacement carried out only later by means of a highly corrected system, the tube lens system. Depending on the structural advantages or those of image formation tube lens systems of factors 0.8x, 1x, or 1.25x are used. In modern stands the tube lens system is a component of the objective carrier. It is therefore always interchanged whenever the latter is interchanged.

Objective revolving nosepiece with peripheral bearing and internal clickstops.

The revolving nosepiece runs on a peripheral ball bearing 1. It is centred by means of the cylindrical bearing 2, (maintenance-free sintered-metal bearing). Pressure springs 3 apply spring loaded pressure to the bottom part of the nosepiece and push it against the peripheral support through steel balls. The nosepiece is engaged by a catch spring 4 in the notches 5 with great precision.

Fig. 70
Objective revolving nosepiece for the ORTHOPLAN microscope
Object stages

The top of the object stage is vertically adjusted to the optical axis with great precision. The stage can be vertically adjusted with the coarse or the fine adjustment for the focusing of the microscopic image. The tube thereby remains always at the same level. The vertical adjustment runs on ball races. In larger stands the object stage can be interchanged, in medium-sized and small stands the stage is permanently fixed in the factory. It is fitted on the underside with a dovetail guide or a sliding collar for the condenser.

The following types of object stage are available:

Simple oblong object stages
The specimen can be adjusted manually or by means of an attachable mechanical stage. In the simplest case the specimen is immobilized with stage clips.

Mechanical stages
Here the specimen is displaced in the x- or y-direction, in the y-direction by means of the stage top, in the x-direction by means of an object guide built into the stage top. Both coordinates have scales and verniers, so that details of the specimen, once located can always be found again.

Fig. 71

Rectangular object stage
Attachable object stage for same
Large, built-in mechanical stage with graduation and verniers
Sliding stages
The sliding stage consists of a moving and a fixed plate, which are exactly parallel ground to each other. The top plate can be moved in any desired direction. Fine adjustment by hand is also possible with the aid of lubricants of suitable consistency between the two plates.

Rotating stages
Here the specimen is rotated through 360° round an axis running parallel to the optical axis. In large stands the rotation can be read off a scale. So that axis of rotation and optical axis coincide, the objectives can be centred in some revolving nosepieces. The large precision rotating stages for polarized-light microscopy run on ball bearings, and can be arrested at certain angular intervals and clamped in position.
Backlash-free precision stage movement. The diagram shows the almost frictionless movement. It is a combination of ball and steel-needle guides in prismatic basic arrangement. The balls 5 with the hardened steel needle races 6 are visible. Seen from the observer’s point of view the rear guide track 1 is prismatic, the front one 2 plane. Both guide tracks are rigid. The prismatic guide determines the movement, the plane guide the lateral fixation. The guide track 3 is movable and is pressed against the fixed guides 1 and 2 by means of a spring bar 4 via the balls. This ensures constant pressure; the spring arrangement eliminates any backlash.

Fig. 73
Rotating stage running on ball bearings, graduated in degrees, verniers, and clamping device for polarized-light microscopy
Coarse and fine adjustment
The coarse and fine adjustment serves for the focusing of the microscopic image. The drive mechanism acts on the object stage in all new LEITZ stands, so that the level of observation of the eyepiece tube always remains the same. This also avoids additional load of the focusing mechanism when heavy accessories are attached to the tube.

In a good coarse and fine adjustment, play as well as the after effect of the micrometer screw should be extremely slight. In addition the mechanism should be resistant to atmospheric effects and maintenance-free.

The design depends upon the type of stand. Medium-sized and large stands are fitted with coaxial 2-knob controls; coarse and fine adjustment are actuated with separate knobs. In the top-class models a planetary gear has been used for the drive mechanism. Here the fine focusing range is about 40mm.

Smaller stands have a single-knob control both for coarse and fine adjustment. The knob acts as fine adjustment as soon as the rotation direction is reversed. After about 1/3 turn of the knob the end of the fine adjustment is reached. When the knob is rotated further it will again function as coarse adjustment. The illustration on the right shows the function of a planetary gear and of the single-knob control.

In large instruments the drums of the fine adjustment are graduated in 100, in medium-sized stands in 150 intervals. Each interval corresponds to a vertical adjustment of the object stage of about 1 \( \mu \)m and 2 \( \mu \)m respectively.

Fig. 75
Planetary gear for the coarse and fine adjustment.
The dark blue elements are part of the coarse adjustment, the light blue ones of the fine adjustment.
1. The fine adjustment knob
2. Coarse adjustment knob
3. Terminal gear wheel
4. Large worm wheel
5 & 6. pairs of obliquely toothed wheels
7. Casing
The single-knob control has been specially developed for our school and classroom microscopes. In spite of its simple manipulation it ensures perfect focusing even at highest magnifications and is absolutely maintenance-free. Function: the worm screw $S$ is allowed to ride along a short distance on the spindle $A$. Spindle $A$ and drive knob $T$ are rigidly connected. The drive lug $H$ is fixed on the spindle $A$. As soon as this makes contact with one of the stops $K$ the worm screw $S$ will be rotated directly. This motion is transmitted directly to the worm wheel $B$ and from there to the rack $D$ via pinion $C$. The drive knob $T$ therefore acts as coarse adjustment as a result of the direct transmission. If the direction of rotation is reversed, a mechanism consisting of inclined plane and ball moves the worm screw $S$ parallel to the spindle through a very minute distance. This rotates the worm wheel $B$ and the pinion $C$ through a negligible angle and raises or lowers the rack $D$ through a short distance. The drive knob $T$ now acts as fine adjustment. It retains this function as long as the drive lug $H$ moves freely between the two stops $K$.

Fig. 76
Fine adjustment: light blue
The light blue worm $15$ drives the large worm wheel $4$, and this in turn drives the terminal gear wheel $3$ via the planetary wheels $10, 11, 13, 14$. The two gear wheels $10, 11$ move round the internal gear wheel $13, 14$, like two planets.

Coarse adjustment: dark blue
The worm $12$ drives the worm wheel $9$ which in turn drives the terminal gear wheel $3$ via the four gear wheels, $10, 11, 13, 14$. The planetary wheels now act as pure transmission links, similar to the transmission of a lathe.

Fig. 77
Single-knob operation
The illuminators consist of the lamp, the lamp condenser and the illuminating duct in the stand. Depending on their construction, we distinguish between:

1) Lamp attachments
2) Built-in illuminators
   (a) illuminators permanently built into a stand
   (b) interchangeable lamp housings, not restricted to any one stand
3) Lamps on pillar stands

Lamp attachments are mostly placed on the foot of the stand. They have no illuminating guide. The illuminating guide of built-in illuminators is incorporated in the foot of the stand together with the field diaphragm; the associated lamp is either situated outside or inside the stand. This type of illuminator permits the use of Köhler's principle of illumination. For lamp attachments and illuminators permanently built into a stand low-voltage lamps are preferred today; they are mains-operated via a transformer.

All the lamps described here except the HD illuminator are used with a lamp condenser.

---

1) Lamp attachments

15W mains-operated lamp
This centring illuminator is plugged directly into the mains. It is suitable for visual observation in brightfield.

6v 5W low-voltage lamp
This lamp, because of its compact coil, has a high luminous density and is therefore suitable for all visual work in brightfield (and darkground) as well as for black-and-white photomicrography. A conversion filter and a concave mirror are built in.

6v 200mAmp HD Lamp
The 6v 200mA HD lamp combines the function of a condenser with that of a microscope illuminator. It is suitable for observation in brightfield and darkground.

---

Fig. 78 Attachable 6v 5W low-voltage lamp

6v 200mAmp HD lamp
2a) Built-in illuminators

6v 10W low-voltage lamp
This lamp is suitable for visual observation with the SM-LUX microscope in brightfield, darkground and phase contrast, and for photomicrography on black-and-white film. Köhler's principle of illumination can be realized with it. The use of diffusers obviates a need for centration.

6v 15W low-voltage lamp
The 6v 15W illuminator belongs to the standard light sources of the DIALUX microscope. On this stand it can be replaced by lamp housings. The illuminator accepts centrable and precentred lamps. With the latter, the centring facility does not operate. Lamp condenser and heat filter are built into the illuminator.

Uses:
For all visual and photographic work in brightfield, darkground and phase contrast on black-and-white as well as on colour film, with Köhler's principle of illumination. A conversion filter must be used for artificial-light colour film.
2b) Lamp housings not restricted to a certain stand

The lamp housings are designed and executed to fit the new stands and can be interchanged for the built-in illuminators. They are connected either directly or by means of an adapter or mirror housing. Depending on the lamp housing, the most varied lamps can be used. The lamps can be centred, and the lamp condenser adjusted. Filters are inserted in the filter changing device. An interchangeable heat filter is located between the filter changing device and the lamp condenser.

Lamp Housing 50
This is our smallest lamp housing. It is suitable for 12v 50W halogen lamps.
Uses:
Visual and photographic purposes on black-and-white and colour film, micro-projection attachment.

Lamp Housing 100
This is a small but powerful illuminating device for low-voltage filament lamps and tungsten halogen lamps of up to 100W as well as for spectrum lamps. It can be used for visual observation and photomicrography on black-and-white as well as on colour film.

Lamp Housing 100Z
This lamp housing has, in addition, a centring reflector. It is suitable for high-pressure gas discharge lamps of up to 100W and 12v 100W tungsten halogen lamps. Low-voltage lamps can be connected as alternative light sources via a mirror housing.
Uses:
Visual observation and photomicrography on black-and-white as well as on colour film.
Fluorescence microscopy.
Lamp Housing 250
This large lamp housing has a universal centring facility for lamp and reflector. It accepts high-pressure gas discharge lamps of up to 250W. Low-voltage lamps can be connected via a mirror housing as alternative illumination.

Uses:
Visual and photographic purposes.
Photomicrography with daylight-like illumination. Research and routine investigations in fluorescence microscopy, metallography, micro-projection. The illustration top left shows the mirror housing 250/D for transmitted light, which is a minor variant of the mirror housing 250/O for transmitted and incident light. In position 1 the mirror sends the light from the gas discharge lamp, in position 3 that from the filament lamp into the transmitted-light beam.

The illustration on the right shows the mirror housing 250/O with 2 of 4 possible mirror positions. Here the light from the gas discharge lamp is switched to incident light, that from the filament lamp to transmitted light.
Lamp Housing 500
The Lamp Housing 500 is the largest illuminating unit and can be used only with a mirror housing. It has universal centring facilities for the lamp and the reflector and for a wide range of interchangeable filters. All air-cooled gas-discharge lamps of up to 500W can be used in it. Low-voltage lamps can be connected as alternative illuminators via a mirror housing.

Uses:
Visual observation and photomicrography, fluorescence microscopy, metallography, micro-projection. Special lamps are described in the list of the relevant special accessories.

3) Lamps on pillar stands
LEITZ lamps on pillar stands are combinations of our low-voltage lamps and lamp housings with two pillar stands specially developed for them. They are eminently suitable for the solution of general illumination problems in the laboratory, in stereo-microscopy, etc.

Small stand for 6v 15W and 12v 50W lamps
Height of pillar 260mm
Stable foot with favourable position of point of gravity. Single-handed operation for all possibilities of adjustment of the lamp. Lamp self-clamping at any level and rotatable through 360° round the vertical axis.

6v 15W lamp on pillar stand.
This includes the small pillar stand and the 15W low-voltage lamp. The lamp is fitted with an aspherical condenser and a fixed, frosted heat filter. Maximum colour temperature 2850°K.

12v 50W lamp on pillar stand
This consists of the small pillar stand and the Lamp Housing 50, which is equipped with an adjustable, aspherical condenser, permanently centred mirror, and an attachable heat filter. 12v 50W tungsten halogen lamps are used in it. Maximum colour temperature 3400°K.

Fig. 83 Lamp Housing 500 with Mirror Housing 500
Large pillar stand for Lamp Housing 100, 100Z and 250. Robust and large foot, stable pillar. Height 400mm. Lamp rotatable through 360° round the vertical axis.

100W lamp on pillar stand
This includes the large pillar stand and the Lamp Housing 100 or 100Z. 12V 100W tungsten halogen lamps and spectrum lamps can be used in the Lamp Housing 100.

100W tungsten halogen lamps, spectrum lamps, 50 and 100W mercury lamps, as well as 75W xenon lamps can be used in the Lamp Housing 100Z.

250W lamp on pillar stand
This consists of the large pillar stand and the Lamp Housing 250, which is equipped with an adjustable, 2-lens condenser, magazine for 4 filters, and centring mirror. The lamps can be adjusted vertically and horizontally. Gas discharge lamps of up to 250W can be used.

6v 15W lamp on pillar stand 12v 50W lamp on pillar stand 100W lamp on pillar stand 250W lamp on pillar stand Fig. 84
The optical system of the microscope

In the microscope we distinguish between illuminating and image-forming optical systems. The former comprises condensers and incident-light illuminators, light conductors such as lamp condenser, mirror etc., the latter objectives, eyepieces, and tube lens systems; added to this are coverglasses and filters. All these optical elements are described in detail in the brochure "Image-Forming and Illuminating Systems of the Microscopes" 512-99, so that we can be content here to confine ourselves to a brief summary of the most important terms.

Objectives
Generally, objectives are classified according to their state of correction, that for chromatic aberration and curvature of field being the most important. We distinguish between achromats fluorite systems and apochromats.

In addition there are plano-objectives, in which curvature of field has been corrected. Here, too, the classification is based on the correction of the chromatic aberration, so that we speak, for instance, of plan-achromats and plan-apochromats.

Apochromats are objectives in which the intercept distances of two colours, mostly red and blue, have been made identical. They are therefore well corrected within the region of the colour sensitivity maximum of the human eye. In fluorite systems, the secondary spectrum has already been partially eliminated (approximate correction of 3 colours). In apochromats the complete combination of three colours has been achieved.

The state of correction, unless the objectives are achromatic, is engraved on the objective mount, together with the designation "Pl" or "NPl" for plano objectives. The latter are particularly well suited for photomicrography.
The following engravings are also found on the objective mount:
magnification ratio,
aperture,
tube length,
and coverglass thickness.
The engraving of the coverglass thickness may be omitted on low-power systems. Immersion systems have the additional symbol of a black ring.
Example of a complete engraving:
170/0.17
Pl Apo Oel 100/1.32
Where
170 = the tube length in mm
0.17 = the prescribed coverglass thickness in mm
Pl = plano objective
Apo = apochromat
Oel = oil immersion
100 = magnification
1.32 = numerical aperture

These terms are individually explained in our brochure 512-99. Here, however, we must say a word or two about adjustment length. This, with uncovered specimens, is the distance between the screw-on face of the objective and the top of the specimen. With covered specimens, the adjustment length extends to the image raised by the specimen cover. With all modern LEITZ objectives this is 45mm. Earlier objectives still have an adjustment length of 37mm. The longer adjustment length has the advantage that it permits the design also of very weakly magnifying systems which are parfocal on the revolving nosepiece. In addition, the longer adjustment length is required with plano objectives for their additional number of lenses. LEITZ objectives of the same adjustment length can therefore be turned on the revolving nosepiece without appreciable change of the focusing adjustment. It is, however, also possible to use objectives of 37 and 45mm adjustment length on the same revolving nosepiece, provided an adapter is screwed between the objectives of the shorter adjustment length and the nosepiece.

Fig. 87  Design of plan-apochromats and achromats
Pl Apo 40/0.75  Pl Apo Oel 100/1.32  40/0.65  Oel 100/1.30
Eyepieces

The eyepiece is used to view the intermediate image formed by the objective. It functions exactly like a magnifier. The letter x is engraved to denote its magnification, e.g. 10x. We distinguish several types.

PERIPLAN® eyepieces

They compensate astigmatism and lateral chromatic aberration, which become particularly noticeable towards the margin, of the objectives and offer the eye an image which is free from colour fringes throughout the field of view. The eyepieces are suitable for achromats, fluorite systems, and apochromats. The following eyepieces are more useful with flatfield objectives.

GF PERIPLAN widefield eyepieces

They are a further development in the direction of larger fields. At the highest magnifications the angle of view exceeds 50°. They are suitable for all highly corrected systems including plano objectives and naturally also for achromats.

All these eyepieces have the standard diameter of 23.2mm.

GW PERIPLAN widefield eyepieces, diameter 30mm

These eyepieces are specially designed for the ORTHOPLAN largefield microscope. Their diameter is 30mm. The angles of view are very large already at low powers.

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Fig. 68

PERIPLAN wide-field eyepiece GF 23.2mm dia.

PERIPLAN wide-field eyepieces GW 30mm dia.

PERIPLAN high-point eyepiece GW
Highpoint eyepieces
Highpoint eyepieces have a long pupil distance which enables spectacle wearers to use them through their spectacles. The pupil distances are about 20mm. They are of the PERIPLAN series, either GF at 23.2mm or GW at 30mm diameter. The terms field of view, object field, angle of view, etc. are explained in our brochure 512-99.

Microscope slides
Colourless plane glass, of about 1.1mm thickness, is used for microscope slides, which can be bevelled or non-bevelled. The conventional format is 76x26mm. For work in darkground and phase contrast the microscope slides must be absolutely free from scratches. Living objects are investigated in concavely ground microscope slides.

Coverglasses
The coverglass is a component of the image-forming system, which is corrected for 0.17mm coverglass thickness. This thickness must be adhered to with dry systems of apertures of 0.40 and larger the more accurately, the higher the demands on quality and the larger the aperture. Conventional formats: 18x18mm, 24x24mm, and 24x32mm.
Condensers

The condenser has the task both of illuminating the object field through the desired field apertures and of forming an image of the field diaphragm in the plane of the object. For each type of illumination it is generally necessary to use a special condenser, so that in transmitted light we distinguish between condensers for brightfield, dark-ground, phase contrast and fluorescence.

Condensers for brightfield

LEITZ brightfield condensers of the Series 600 consist of a standardized bottom part with condenser lens for low powers, the aperture diaphragm and interchangeable condenser tops of various corrections, intercept distances, and apertures. The condenser top can be swung out for the use of low-power objectives. The condensers have dovetail changers and are vertically adjustable by means of a rack-and-pinion mechanism. They are computed for a field diaphragm built into the stand. We distinguish between dry and immersion condensers. The aperture of the dry condenser is 0.90. This is adequate for all microscopic investigations with dry systems and mostly also with immersion systems. Only in special cases will it become necessary to illuminate the full aperture of an immersion objective.

Concerning optical corrections the demands on condensers are by no means as high as with objectives. With the simplest type, the aspherical condenser, a certain amount of chromatic aberration is tolerated. With an aspherical lens however, sufficient aplanatic correction is achieved.

Achromatic condensers have a correspondingly higher correction. Chromatic aberration is largely eliminated. They are best used with more highly-corrected objectives, such as fluorite systems and apochromats, whose advantages will only then become fully apparent. Aplanatic correction is higher than in the aspherical condensers.

Fig. 89
System condenser with bottom part and six condenser tops

Fig. 90
Cross section through two condenser tops 003 and 002
Condensers of apertures larger than 0.90 are computed as immersion condensers, when apertures of up to 1.40 are reached. Their correction is apochromatic and aplanatic, and a larger number of lenses is therefore involved in their optical design. Because of this complication they are correspondingly expensive; they are used mainly for highly-corrected oil immersion objectives.

For investigation in culture chambers, for very thick specimens, for intravital microscopy and for micro-manipulators condenser tops of long intercept distances are available. Here the image of the field diaphragm is formed at a corresponding distance above the stage top. Distances between 4 and 20mm can be realized.

All these brightfield condensers are system condensers, consisting of a common bottom part and mutually interchangeable tops, so that the desired condenser type can be obtained with a minimum of expense.

Low-power condenser

On LEITZ stands it is used only in combination with the PI 1/0.04 objective. The large field of this objective is not fully illuminated by the conventional condensers. This condenser has no aperture diaphragm, it is built into the objective.

For further condensers please consult brochure 512-99.

Fig. 91

Low-power condenser for the PI 1/0.04 objective
In microscopy, mainly absorption filters or interference filters are used. Absorption filters are dyed or colourless glasses, which transmit certain regions of the spectrum to a higher or lower degree. Transition from transmission to absorption and absorption to transmission is continuous, i.e. there are no filters with abrupt changes. Transitions are very steep with edge filters. But the steep edge faces only towards the short-wave end of the spectrum.

In interference filters the filter action is not based on absorption but on interference of the waves reflected on the interfaces of thin films. The thin films are vapour-deposited on glass or quartz glass in a high vacuum. Here we distinguish between the large groups of interference line filters and interference band filters.

The following types of filter are mainly used in microscopy according to their purposes:
- contrast filters
- heat filters
- UV absorption filters
- conversion filters
- grey filters.

Their transmission is represented by curves. Generally the degree of transmission is entered as a function of the wavelength of the light.

The spectral transmission of this elongated graduated filter extends from the long-wave red across the entire visible spectrum to the short-wave violet; any section can be used as a monochromatic filter. The main field of application is microphotometry.

**Heat filters (selection filters)**

Prolonged heat radiation damages any microscopic specimen. It must therefore be filtered out of the light source. For this partly coated, partly uncoated glass filters are used.

- 2mm KG1 for filament lamps
- Calflex B1/K2 for gas discharge lamps of higher UV content.

**UV-absorbing filters (selection filters)**

A high proportion of UV in the emission spectrum causes damage both to the eye and to the specimen. The following edge filters are used:

- K380 for gas discharge lamps of high UV content
- K420

Filters for fluorescence microscopy and polarized light microscopy: see special lists.
Conversion filters
They are used mainly for photomicrography on daylight colour film and have the task of matching the colour temperature of the filament lamps with that of the colour film.
CB 6 converts from 2800 to 3400°K or 2600° to 3100°K.
CB 12 converts 3400°K into daylight.
CB 16.5 converts 2850°K into daylight.

Grey filters
Grey filters are either glass filters dyed in the mass, reflection filters, or combinations of both. They reduce the light absolutely evenly. Care is recommended with the combination of reflection filters which may cause undesirable reflections.
For purposes of visual observation two grey filters of 5% and 2% transmission are supplied. They are designed for microscopy with gas discharge lamps.
For photomicrography a set of grey filters is available. This makes it possible to reduce the light in steps almost completely at will.
Cameras for 35mm photography, large format, or system cameras for change of formats are used in photomicrography. The smallest format is 35mm (24 x 36mm), the largest 13 x 18cm. Most photomicrographic outfits for 35mm are attachment cameras with a fixed film plane, the devices for large formats are either attachment cameras or bellows cameras, in which the film plane can be varied.

Micro-attachment for the LEICA
In this type, a LEICA is used as the camera. It is adapted to the microscope via a micro-photo attachment. Here the microscope eyepiece forms an image of the object, to begin with at infinity; an objective built into the micro-photo attachment is responsible for a further image on the film plane. The exposure meter is pushed into the measuring tube on the right. The specimen is focused in the telescope of the micro-photo attachment, and exposed with the camera shutter. The built-in anti-vibration damper ensures that the pictures are always sharp.

Fig. 92
Micro-attachment with vibration-damper for the LEICA
System cameras for changing the format
This type of camera is an expandable system from the 35mm to the large 9x12cm or 4x5in format. The central component is the shutter part with shutter and measuring tube for the exposure meter. The camera bodies for the various formats are placed on the shutter part and locked onto it.

The optical design corresponds largely to that of the device described in the preceding chapter, although the system camera has no focusing telescope. Here the microscope eyepiece in the photographic beam is exactly matched for the intermediate image plane. The camera can therefore be used only in conjunction with an FSA tube. But the user has the advantage of being able to take his photographs without having to shift the eye from one observation tube to another.

The COMBIPHOT® automatic system camera is available for automatic recording.

Fig. 93 System camera

Fig. 94 COMBIPHOT® Automatic system camera with LEICA attached, on the DIALUX microscope
ORTHOMAT W microscope camera
The ORTHOMAT W is a fully automatic largefield microscope camera with zoom optical system from 6.3x to 10x for the 35mm format. Depending on the type of stand, field-of-view indices of up to 22 can be photographically utilized. The exposure range of the device is practically unlimited.
Large-format cameras with bellows
Many cameras for the 9x12cm and 13x18cm large formats are equipped with bellows. They offer the possibility of continuous variation of the magnification. An image of the object is formed by the eyepiece directly on the film plane or on the groundglass screen. Focusing and exposure measurement are very precise on the groundglass screen, and the negative format can be put to optimum use because the groundglass screen frame is rotatable.
Because of their relatively heavy weight, large-format cameras are used either with a photographic stand (ARISTOPHOT) or on a special rail to be fitted to the microscope. When the ARISTOPHOT and a macro-apparatus is used they are also suitable for macrophotography.
For automatic large-format photomicrography the 4x5in (9x12cm) large format camera with fully automatic exposure control is available. Here all functions proceed automatically as soon as the shutter is opened.

Exposure meter
For the determination of the exposure time, a special exposure meter for photomicrography is used with all these cameras unless they are automatic. The measuring eye, connected to the measuring mechanism by a cable, permits, to name only two examples, the determination of the exposure time on the groundglass screen of the bellows camera or in the measuring tube of the system camera. This makes detail measurement of high accuracy possible.

Fig. 96 LEITZ MICROSIIX-L exposure meter
Devices for tracing, discussion and projection

Tracing
Tracing devices for microscopy can be divided into two types: instruments which function according to the a) projection principle b) coincident-image principle

For projection tracing the microscopic image is projected onto a tracing area by means of a mirror mounted above the eyepiece. Powerful light sources are therefore necessary, and the workroom must sometimes be dimmed. The projected image itself is very contrasty.

For tracing according to the coincident-image principle the LEITZ tracing device or a tracing attachment can be used. In both cases the microscopic image and that of the tracing sheet are projected on top of each other by means of mirrors and beam-splitting prisms. When looking into the eyepiece the user sees both images and the tracing pencil simultaneously and all he has to do is trace the contours. The contrast is rather soft. Normal light sources built into the stand are completely adequate.

The tracing device offers variable magnification of the image to be traced and the advantage of monocular or binocular tubes; with the tracing attachment, on the other hand, the magnification of the image to be traced is fixed and observation restricted to the monocular tube.

Fig. 97
Tracing device on the DIALUX microscope
Coincident-image method
Discussion
Four discussion devices have been developed which allow two or several observers simultaneous observation of the microscopic image.
In the LEITZ discussion device a bridge with two tubes is placed on the stand. The observers see the image as they are accustomed to when looking into the microscope. They can also photograph it instantly. In each case the normal microscope lamps are adequate.
For the demonstration of the microscopic image to a small number of persons the microprojection attachment is suitable. Here the image appears on a groundglass screen, but requires more powerful light sources.

LEITZ Tele-PROMAR projection microscope
The LEITZ Tele-PROMAR projection microscope serves for the direct projection of images of microscope specimens in transmitted light.
Plano objectives of up to the highest magnification (oil immersion) permit brilliant and contrasty reproduction of the microscopic structure in the original colour of the specimen.
Together with the 2.5x or 4x largefield projection eyepieces screen images of up to 3.5m diameter can be obtained at projection distances of up to about 15m. Reproduction ratio on the projection screen up to 1500:1 with full utilization of the resolving power of the microscope objectives. Independently of the projection distance, scale marks can be projected on to the screen image for measuring purposes and for the demonstration of the real dimensional relations. A pointer which can be moved within the entire projection area facilitates the location of features of interest.
Microscope stand, lamp housing, power unit and mirror housing are permanently mounted on a baseplate. The straight monocular tube can be removed and accepts the projection eyepiece and prism. The quintuple revolving nosepiece is permanently mounted on the tube carrier. The microscope stand has a single-knob control on both sides for the coarse and fine focusing of the microscope image. The focusing mechanism acts on the object stage, which has a scanning area of 160x138mm. Four condensers are correlated to the various object magnifications for optimum illumination of the object field; they are mounted on the permanently centred condenser turret. The correct combination of condenser, objective, and a scale mark chosen according to the magnification is indicated by coloured light marks and can therefore be obtained without effort even in a darkened room.

Fig. 98 Tele-PROMAR for microprojection and television


LEITZ Neo-PROMAR projection microscope
The LEITZ Neo-PROMAR serves for the projection of microscopic specimens in transmitted light. Its magnification range can be set in four stages. Maximum reproduction scale 3,000:1 with full utilization of the resolving power of the microscope objectives. For projection distances of up to about 9m the 2.5x projection eyepiece is recommended. Screen image diameters of up to 2m can be obtained with it. With the 4x projection eyepiece, the same image size is obtained at a projection distance of about 5.7m. The specimens are protected by means of a cold-light interference deflecting mirror. The 250W tungsten halogen lamp ensures bright and contrasty projection. For demonstrations in daylight to a small number of persons, a projection attachment with a 155mm dia. groundglass screen can be used instead of the projection eyepiece and prism.

Fig. 99
MPV 2 microscope photometer,
Microscope photometer/spectral photometer with:
interchangeable and variable measuring diaphragms,
reflection of the measuring diaphragm image into the field
of view
applicability of all microscopic methods,
automatic measurement.
LEITZ T.A.S.*
Texture Analysis System
The T.A.S. is an optical electronic device for automatic quantitative image analysis in microscopy and macroscopy. The image to be measured is recorded by a television camera, the image element selected for measurement is visible on a monitor screen. An electronic evaluation and analysis centre evaluates the image signals according to the modern methods of mathematical morphology. The measured values can be displayed digitally, printed out, and instantly fed into any electronic computer.

DIAVERT inverted transmitted-light microscope
This instrument serves for the microscopic investigation of cultures, fluids, sediments etc. in the usual laboratory vessels. The magnifications provided in the low-power range correspond to the relevant conditions of examination: the wall thicknesses of the vessels have no effect here. With micro chambers of 0.17mm wall thickness the microscope can be used up to the highest magnifications. The possibility of extension corresponds to that of a modern microscope. Brightfield, darkground, phase contrast, interference contrast, fluorescence, and orientating polarized light can be used. The worker can attach a photomicrographic, cinematographic, or a television unit.

Special micro-manipulators are in preparation. For examinations of living cells and tissues an air-conditioning box with remote controlled motor drive for the object stage is supplied.
TS largefield stereo-microscope

For the dissection and investigation of objects in incident or in transmitted light at various magnifications.
Free working distances from 140 to 30mm.
The magnification is changed by means of a 3-stage magnification changer on which two of the paired objectives can be exchanged at will. A paired 1:1 objective is fixed. The following pairs of objectives and eyepieces are available:

Paired objectives: 1.61, 2.51, 4.01, 6.31, and 10.0:1.
Paired eyepieces: W6.3x, W8x, W10x, W16xM, W25x, and W32x.

This makes magnifications from 6.3 to 320x possible.
The stereo tube can be attached to special stands. Pillar stand for the scanning of large objects, stand with mechanical 60x60mm adjustment, stand with table clamp.
Filter Comparison  Six black-and-white photomicrographs of a histological section.

1) Without filter. The colours are rendered in their equivalent grey values. Their reproduction depends solely on the sensitization of the film.

2) Red filter. The compact red portions including the red cell nuclei appear much lightened throughout. Blue, on the other hand, is rendered very dark.

3) Orange filter. The red portions are no longer as light; blue, on the other hand, is still rendered very dark.

4) Yellow filter. Red is rendered more and more prominently. The blue portions are in very good contrast.

5) Green filter. The rendering of the red portions is now at its best. Blue is lightened and has optimum contrast to red.

6) Blue filter. Here the blue structures are completely suppressed. They are practically indistinguishable from red.
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Work room for microscopy 27
Leaf primordium, cross section, pinus silvestris
LEITZ ORTHOPLAN Microscope
Pl Apo 16/0.40, magnification 200x
108
Axillary bud, cross section, pinus silvestris,
LEITZ ORTHOPLAN microscope
Pl Apo 25/0.65, magnification 300x

Stem node, corn, cross section
LEITZ ORTHOPLAN microscope
Pl Apo 6.3/0.20, magnification 110x