Handbook of incident light microscopy

West Germany

1. Qualitative microscopy

Handbook of incident light microscopy

Dipl.-Min. W. Cebulla

Table of contents

D. S. Kurlyšov

		Page
Incident light specimens	Opaque materials	4
	Amplitude and phase specimens	4
	Reflectance and diffuse reflectance effects	5
	Preparation of polished specimens	5
	Influence of preparation methods on the micro structure of specimens	5
	Treatment of polished specimens after preparation	7
Incident light microscope	Mechanical design	8
	Objectives	10
	Reflectors	11
	Microscope stages	13
	Geometric optics	13
	Köhler illumination	15
	Two different planes in the incident light microscope	16
	Wave optics	16
	Useful magnification	18
	Contrast as a function of the illuminating aperture	19
Examination methods of incident light microscopy	Chemical etching	20
	Evaporated interference layers	21
	Contrasting in a gasion reaction chamber	22
	Darkfield examinations	23
	Differential interference contrast	26
	Polarizing microscopy	31
	Immersion objectives	36
	Antiflex method	39
Photomicrography	General information	41
	Film material	42
	Color photomicrography	46
	Resolving power of photographic emulsions	48
Photomicrographic equipment	Photomicrographic camera	50
	Image scale	54
	Tessovar photomacrographic zoom system	55
	Photomicroscope III	58
	Ultraphot III b	61
	Axiomat NAC and IAC	62
Literature		66

Incident light specimens

Opaque materials

Incident light microscopes are used to examine primarily opaque specimens. A material is considered opaque by microscopists if as thin section or polished thin section of about 25 μm it is non-transparent in the visible spectral range from 450 to 650 nm. Metals are specimens of this kind (Fig. 1). Specimens which are transparent as thin sections but opaque in thicknesses of a few millimeters, such as coal, wood, slag, rock, plastics or bones, are also examined with incident light microscopes. Findings obtained with a transmitted light microscope are supplemented by information derived from examinations with an incident light microscope and vice versa.

Amplitude and phase specimens

Opaque and transparent microscopic specimens have pronounced amplitude or phase characteristics. The individual components of amplitude specimens differ in the amount of light absorption, i. e. the eye sees different features of an object in different shades of gray or even in different colors.

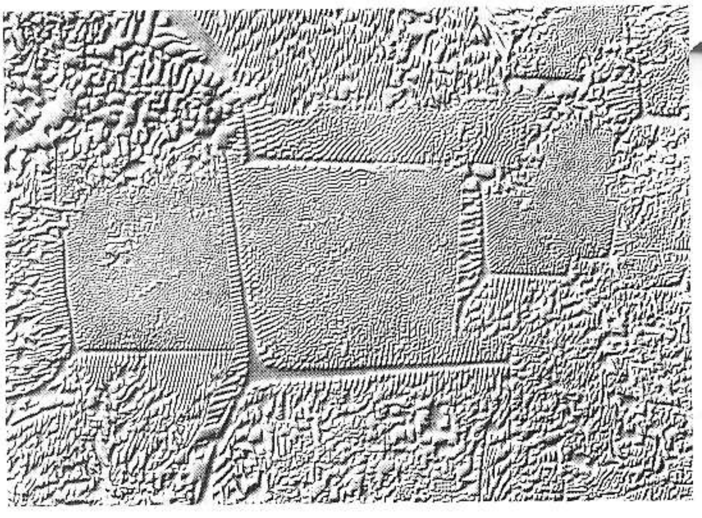


Fig. 1
Magnesium zink alloy MgZn 30
Magnification 200x
Differential interference contrast

Phase specimens differ only in the refractive indices of the individual components, but the eye is incapable of recognizing such differences.
In practice there are no pure amplitude or phase specimens; either the one or the other property is predominant. Opaque specimens are considered phase specimens if the reflection differences between the individual components are below about 10 %. Refractive index and reflectance of an object component have a specific relationship to which Beer's law applies (see the chapter on reflection photometry).

Reflectance and diffuse reflectance effects

Whether the surface of a specimen reflects or reflects diffusely depends on its micro structure or morphology. Reflectance and diffuse reflectance of a specimen are the ratio of reflected to incident luminous flux and are always smaller than 1, i. e. less luminous flux is reflected than was incident.

Reflectance includes reflection. Reflectance is actually a diffuse reflection and depends on the angle the specimen surface elements make with the microscope axis (unequal 90°). Reflection occurs if the surface elements of the object components are oriented perpendicularly to the microscope axis, which means that the reflection angles are merely a function of the microscope's illuminating aperture.

Preparation of polished specimens (lit. 1)

Preparation is a matter of experience, because each specimen type requires a specific technique to prepare a polished section. There are no general rules. A polished specimen for microscopic examination in incident light is generally prepared in the following way: a piece about walnut size is cut off the specimen with a diamond saw and mounted in synthetic resin. The specimen surface is then ground and polished.
The choice of the grinding and polishing media depends on the type of specimen, and that of the substrates on the media and the hardness composition of the specimen.
A specimen should never be dry-ground or polished; the fluid added to the grinding medium depends on the abrasive and the microscopic specimen.
A grinding machine is usually applied for coarse and fine grinding, also for part of the polishing, while the fine polishing, that is the surface finish, is done by hand. Coarse grinding with an abrasive of decreasing grain size reduces the depth of roughness of the specimen surface

to about 10 μm; fine grinding to about 1 μm, and fine polishing to about 0.1 μm; by the surface finish it is at least reduced to far less than 0.1 μm.
The most universal grinding and polishing media are Al_2O_3 and diamond pastes with grain sizes for the entire grinding and polishing processes. Steel and glass plates are generally used as grinding substrates, steel plates in particular for coarse grinding. For the polishing process synthetic fiber cloths are used as substrates for diamond pastes, and woollen tissues for Al_2O_3 (alumina).
Specularly reflecting surfaces for incident light examination can also be produced with a microtome with diamond knife. The Vickers hardness of the material should be less than 200 kp/mm^2. Compared with conventional grinding and polishing methods, a specularly reflecting surface prepared with a microtome has the following advantages: no smudge on the specimen surface, no relief if the object phases are of different hardnesses, homogeneously plane marginal and central areas of the object (no vignetting of the microscopic image), and time-saving preparation of polished specimens.

Influence of preparation methods on the micro structure of specimens (lit. 2)

The structure of the specimen surface is more or less strongly influenced by the mechanical grinding and polishing processes. This influence is lessened the longer the grinding and polishing, the harder the removing material, and the sharper the edges of the tool (best results are obtained with natural diamonds), the lower the temperature of the specimen surface, i. e. the lower the grinding pressure and the wetter the processing.

Changes are liable to occur in the surface structure of substances with low recrystallization temperature. The surface structure of specimens is changed by mechanical polishing methods due to plastic flow, a cold-working process resulting in surface smoothing. A smudge layer on top of the deformed layer, the so-called Beilby layer, contains crystal debris in the order of magnitude of 0.5 to 1 nm, mixed, for instance, with oxides.
After polishing the surface of the examined material shows roughnesses between some 10 nm and some 100 nm. Absorption and reflection processes take place in depths of a few 10 nm.
When preparing a polished specimen with a microtome, consider that the thinner the chip cut off with the diamond

knife, the flatter the angle the knife makes with the specimen surface, and the slower the cutting, the smaller the deformation of the surface structure, and the more changes of the structure restricted to the chip thickness.

Treatment of polished specimens after preparation

Depending on the material the phases of a polished specimen are more or less sensitive to water (rust) or oxygen (oxidation) of the ambient air, and should therefore be stored in lockable plastic containers (sensitive surface on cotton). In humid, hot climate an exsiccator is recommended for storage.
Clean polished surfaces with a clean cotton wad after wiping off dust with a clean brush. Never touch polished surfaces.

Quite a few specimens, e.g. iron-carbon alloys or nonferrous metals, must be examined microscopically immediately after preparation, because they change chemically when exposed to air.

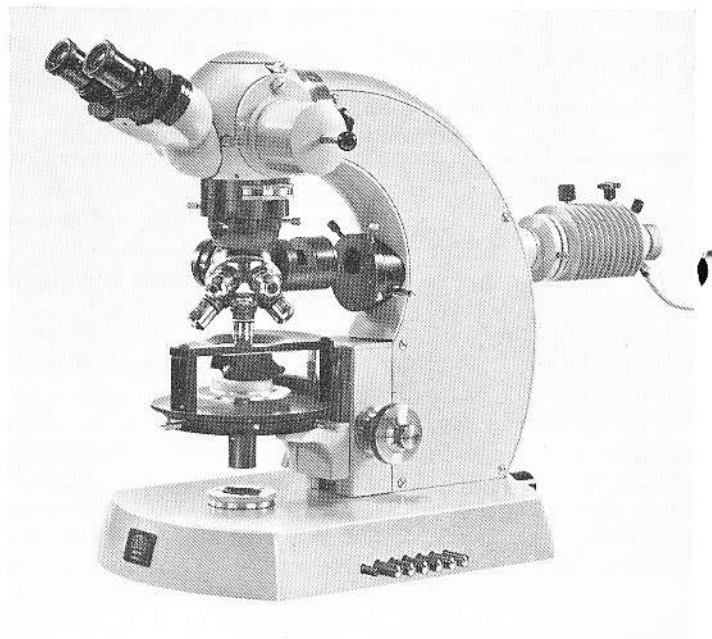


Fig. 2
Universal M incident light microscope

Mechanical design (lit. 3)

If a microscope is used for incident and transmitted light examinations microscope base, stand, tube head, tube and eyepieces can be used for both types of observation. The light source is mounted at the height of the vertical illuminator to offer more intensity for incident light microscopy. A halogen filament lamp is sufficient for most incident light examinations. Fig. 2 shows the Universal M incident light microscope, Fig. 3 the beam path of this microscope.
The illuminator is an essential component of an optical microscope for incident light examinations. It is composed of the light source, the incident light aperture diaphragm insert, lamp field stop, reflector, and the incident light objective. The aperture diaphragm insert and the incident light objective have the functions of the vertical illuminator (Fig. 4).

Fig. 3
Beam path in the Universal M microscope

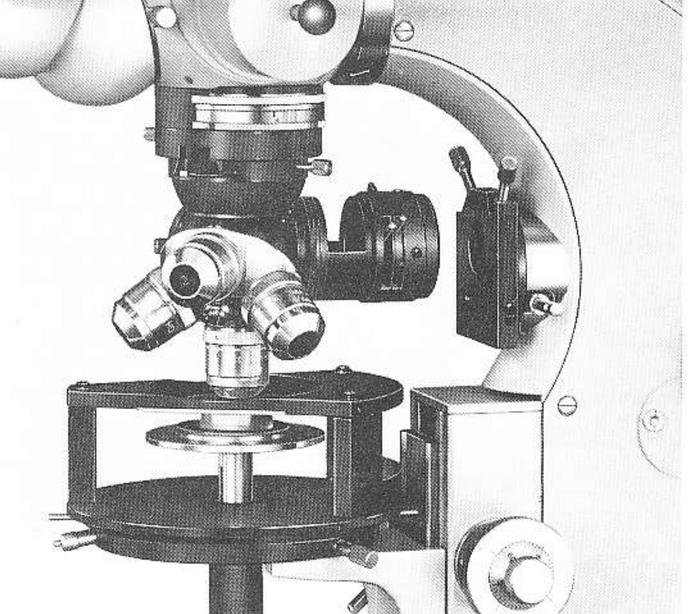


Fig. 4
Vertical illuminator III C

Fig. 3
Beam path in the Universal M microscope

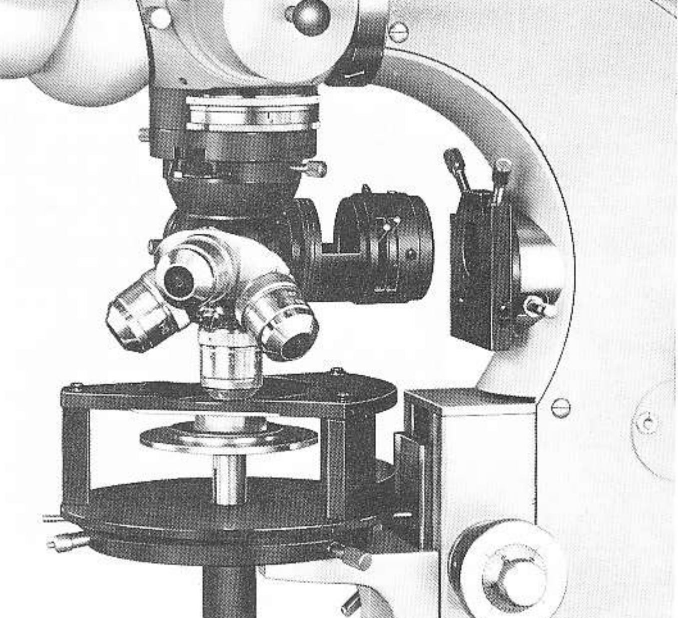


Fig. 4
Vertical illuminator III C

Objectives (lit. 4; 5)

The objectives are termed Epiplan where Epi stands for incident light examination and plan for the flatfield reproduction of plane specimen surfaces in the microscopic image. Due to the chromatic correction of Epiplan objectives object structures observed in white light show only minor color fringes, which is achieved by a combination of glass and fluorite lens elements.

If lens surfaces (glass/air or glass/optical cement) are coated, i. e. transparent substances evaporated, disturbing reflections within the optical system are considerably reduced (A. Smakula of Carl Zeiss developed this technique in 1935).

An uncoated lens surface reflects about 4–5% of incident light. A single coating of the surface reduces the amount of reflected light to 1–2%, a triple coating to a few tenths of a percent.

The more layers of different transparent substances having thicknesses of fractions of a wavelength are deposited on a glass surface, the smaller the amount of reflected light, and the more wavelengths are covered by the anti-reflection coating.

The anti-reflection coating of element and cement surfaces of incident light objectives is important if the reflection of opaque objects is extremely low, i. e. $< 5\%$ with brown coal, slags, etc.

Because of the metallographic standard magnifications (50–100–200–500–1000x) Epiplan magnifications differ from those of transmitted light objectives (Table 1).

Table 1
Epiplan objectives

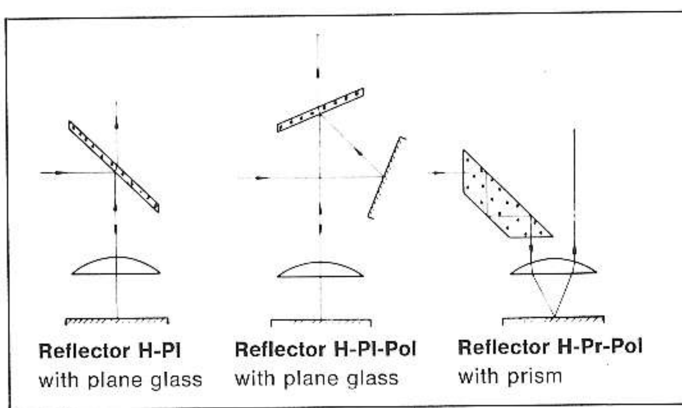
Epiplan objective	Working distance (mm)
4/0.1	9.0
8/0.2	7.2
16/0.35	2.8
40/0.85	0.23
80/0.95	0.09

Two figures are engraved on each objective, the first is the magnification, the second the numerical aperture. The structural resolving power of the microscope depends essentially on the second.
Besides Epiplan objectives there are special objectives, e. g. for examinations with immersion media, for polarizing microscopy, darkfield microscopy or long working distances (Epiplan LD) and, for large object fields, Luminar objectives which are described later.

Reflectors

The reflector is a vital component of the vertical illuminator. It deflects the beams coming from the light source through 90° to the specimen surface (Fig. 5).

Fig. 5
Reflectors H-Pl, H-Pl-Pol, H-Pr-Pol



The reflectors for incident light examinations are listed in the following Table 2.

Table 2
Reflectors

Type of reflector	Principal use
H-Pl	brightfield, differential interference contrast
H-Pl-Pol	polarized light
H-Pr-Pol	polarized light
D	darkfield
Fl	epi fluorescence excitation

The reflectors D and Fl are for special applications; only the first three reflector types are therefore compared in the table below.

Table 3	**H-Pl** w/plane glass	**H-Pl-Pol** w/mirror and plane glass	**H-Pr-Pol** triply totally reflecting prism
Application (examination method)	brightfield	brightfield	brightfield
	differential interference contrast		
Photomicrography	suitable	suitable	suitable w/restrictions (vignetting)
Image brightness	high losses in intensity due to double partial reflection		low light loss
Illumination direction	almost perpendicular dependent on illuminating aperture		oblique
Illuminating aperture	fully utilized		to half the objective aperture
Resolving power of objective	fully utilized		one half utilized perpendicular to prism edge
Influence on linearly polarized light	depolarization	almost none	no depolarization
Reflections due to reflector	unavoidable		almost none

Light is reflected in the plane glass reflector H-Pl (brightfield plane glass) by a glass plate a few tenths of a millimeter thick. It is partially mirror-coated on the light source side and has an anti-reflection coating on the image side. In spite of this double images are unavoidable in the marginal zone of the weakly reflecting components if the reflection differences in the object are large and no polarizer is used.

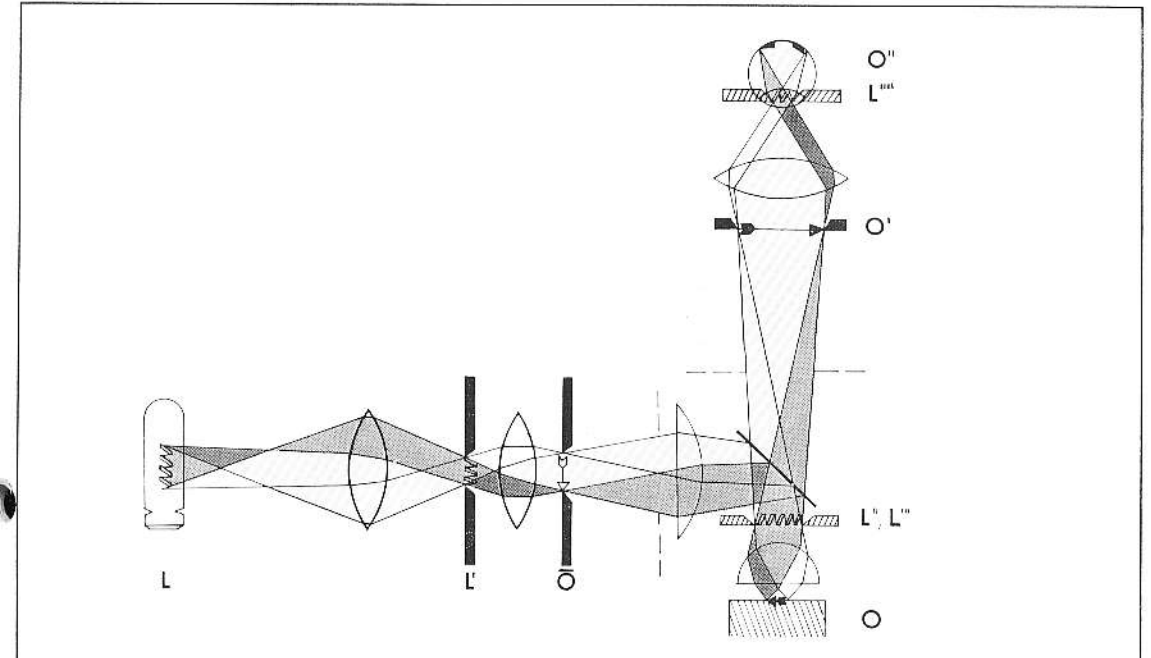
In the H-Pl-Pol reflector the light is at first reflected by a surface mirror then by a plane glass; with reflecting angles of the central beam of 22.5° in both cases.

The reflector H-Pr-Pol comprises a triply totally reflecting prism (trapezoidal prism).

Microscope stages

All types of microscope stages can be used for incident light microscopy. Specimens are fixed with a levelling press on a metal slide coated with plastiline, and their polished surfaces aligned plane parallel with the metal slide. Previous alignment of the specimen surface with metal slide, plastiline and levelling press is superfluous if an autolevelling stage is used. The specimen is then fixed below the proper microscope stage surface, and the polished surface is automatically aligned perpendicular to the microscope axis (Fig. 4).

Fig. 6
Beam paths in the incident light microscope



Geometric optics (lit. 6)

The beam path in the incident light microscope is bound by a number of diaphragms. Images of specimen and light source are produced in certain diaphragm planes (Fig. 6).
The image-forming beam path referred to the specimen plane and the illuminating beam path referred to the light source contain fixed or iris diaphragms.

In the image-forming beam path an image of the specimen O is formed by the objective in the plane O′ and subsequently enlarged by the eyepiece in the plane O″ (retina of the eye). Because of straylight only that part of the specimen surface in O may be illuminated by a diaphragm limiting the luminous field in $\bar{O}$, which in O′ corresponds to the size of the field of view.
In the illuminating beam path the lamp condenser optics form an image of the light source in the plane of the illuminating aperture diaphragm L′. The aperture in L′ corresponds to the entrance pupil of the microscope. Optically, the incident light microscope begins at this point. The diaphragm in plane L′ controls the illumination in plane L″; it is the entrance pupil of the objective. An image of light source L is produced in L″.
Reflected by the specimen surface the light source image L″ is imaged back in plane L″ by the objective functioning as condenser. Due to the specimen structure (diffraction) the light source image is changed.
The resolving power of the incident light microscope is also influenced by the aperture of the iris diaphragm in the plane L′ and the fixed diaphragm in L″ or L‴.
The light source image L‴ is relayed to the plane of the eye's pupil by the eyepiece, where it appears as light source image L⁗.
The above-mentioned conditions of geometrical optics in the incident light microscope prevail with Köhler illumination (Carl Zeiss, 1893) which alone provides proper brightfield illumination.

Köhler illumination

Opaque absorption specimens can be examined in incident light brightfield after the following adjustments (Fig. 7):

1. Close lamp field and aperture stops.

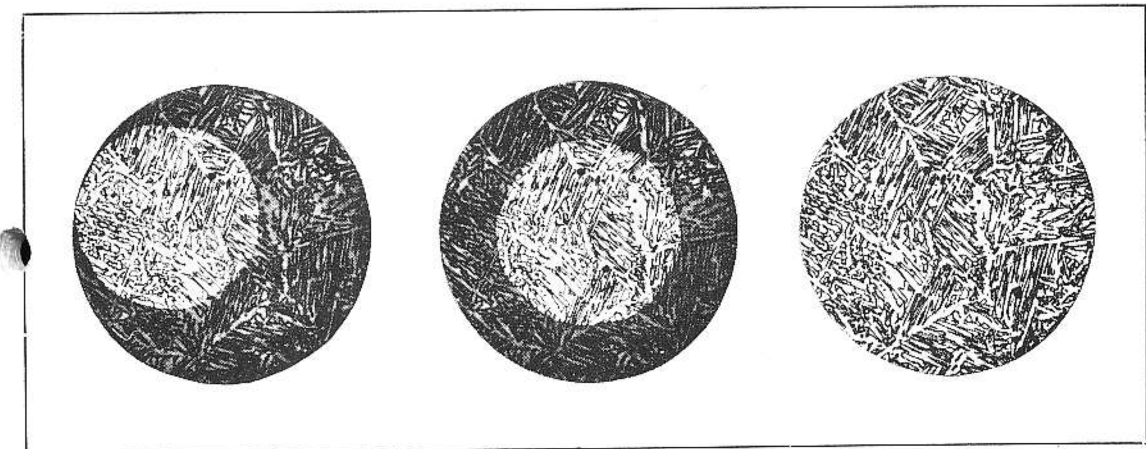
2. Adjust specimen vertically until a sharp image of the lamp field stop appears; the specimen structures are also visible.

3. Center lamp field stop and open it as far as the edge of the field of view to avoid excessive straylight.

4. Observe the objective pupil with a centering telescope:
a) open aperture stop completely to check uniform illumination of the objective aperture by the light source; if specimen areas reflect differently, use the specurlarly reflecting side of an interference color filter as specimen at this point.
b) close the aperture stop about 1/5 of the objective pupil and center it.

5. The aperture stop is finally adjusted during observation of the specimen structures. This adjustment is a compromise between resolution and contrast enhancement of the specimen structures in the microscopic image.

Fig. 7
Adjusting the lamp field stop



Two different planes in the incident light microscope

Once Köhler illumination is adjusted in the incident light microscope, let us find the planes conjugated to specimen or light source:
a) The specimen planes $\bar{O}$, O' and O''. If the lamp field stop is half closed it is in fact imaged in the specimen plane. If the eyepiece is replaced by a ground glass funnel 10 mm below the tube rim, an image of the specimen in O and of the lamp field stop in $\bar{O}$ is covered in the plane of the real intermediate image O'. With a Galilean telescope above the eyepiece, it is shown that the images of the planes $\bar{O}$, O and O' are at infinity.
If the eye lens or a camera lens is provided above the eyepiece, images of these planes are imaged in the plane O''.
b) A structureless specimen, e. g. a surface mirror is recommended to demonstrate the light source planes, i. e. imaging of L in L', L'', L''' and L''''.
The eyepiece is replaced by a centering telescope for direct observation of the plane L'' or L'''. The image of the light source L can be observed if the diaphragm in the plane L' is opened and the ground glass in the lamp condenser swung out. If the aperture stop in plane L' is half closed its image is produced in plane L'' or L'''. With a high-power magnifier on the eyepiece the plane L'''' can be directly observed, which is the plane of the microscope's exit pupil or the Ramsden circle. L'''' covers images of the planes L''' or L'', L' and L.

Wave optics
(lit. 7)

The resolving power of an optical system determines the smallest distance between two structural elements which is resolved by the objective, expressed by the numerical aperture. The Epiplan 40x incident light objective, for instance, has a numerical aperture of 0.85 (40/0.85).
According to E. Abbe's theory of diffraction, the wave fronts coming from the light source are diffracted by the specimen structure. The diffraction image is produced in the image-side focal plane of the objective (L''') and is called primary microscopic image.

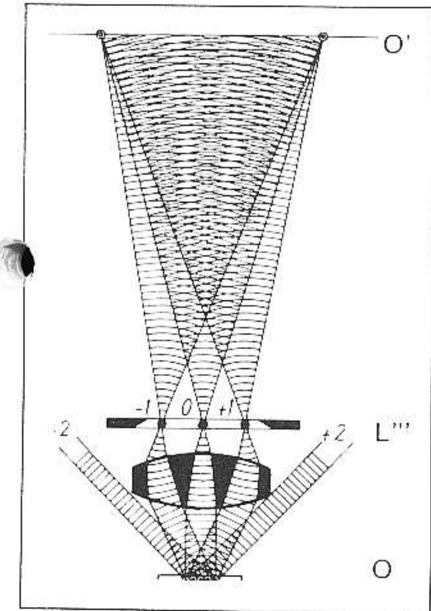


Fig. 8
Diffraction and interference in the microscope

Interference between planes L''' and O' results in the secondary microscopic image in O', which is the real microscopic intermediate image subsequently enlarged by the eyepiece (Fig. 8).

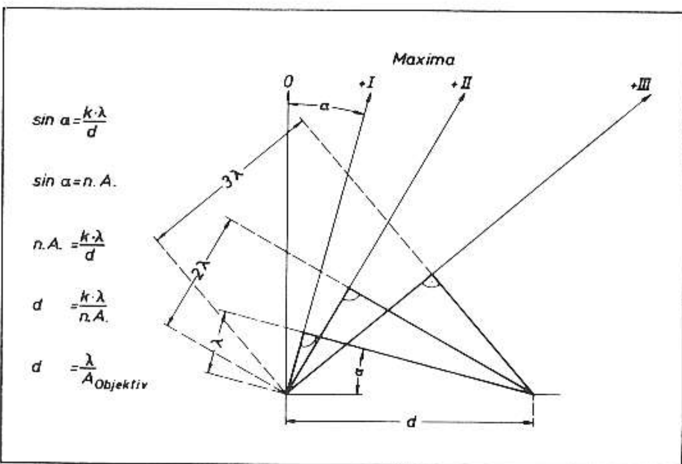
The diffraction processes cause diffraction maxima in the image-side focal plane of the objective. The more diffraction phenomena are covered in the objective focal plane (L'''), the more realistic and sharper the structures of the image.

Resolved specimen structures become visible only with an absorption specimen. The number of diffraction phenomena in the objective focal plane depends on the distance between the structuring elements (d), the wavelength (λ) and the sine of half the objective aperture angle, expressed by the numerical aperture (Fig. 9).

The resolving power of an incident light microscope also depends on the illuminating aperture in the image-side focal plane of the objective (L''), which is a function of the size of the aperture stop in plane L'. The higher the illuminating aperture, the more diffraction maxima in the focal plane of the objective (L'''), which increases the information content of the image.

Fig. 10 shows a grating as microscopic test specimen taken at different objective apertures; magnification 260x. The grating period d, i. e. the spacing of the periodic structure is 0.016 mm.

Fig. 9
Resolving power of the microscope



The ratio of bright to dark lines is about 1:3. The aperture of a is insufficient for resolution of the grating. In b resolution is achieved due to an increased aperture. Further increase in numerical aperture in c results in more resolved detail than in b.

Useful magnification (lit. 8)

It is about 500 to 1000x the numerical aperture of the objective. If the total magnification of the microscopic specimen is at the lower limit, the resolving power of the objective is not fully utilized. If the upper limit is exceeded, we enter into empty magnification. Structures not resolved by the objective cannot be subsequently resolved by the eyepiece or the camera factor.

Fig. 10
Resolution as a function of the numerical aperture

a

b

c

The smallest distance between two structuring elements (d) which can be made visible with an incident light microscope is about 0.25 μm. This resolution can be achieved only with a numerical aperture of the objective of 1.25. Since already the numerical aperture 1.0 would thereoretically correspond to half an aperture angle of 90°, the information content of the primary microscopic image in the plane L‴ can only be increased by a medium between specimen and front lens of the objective with a refractive index higher than 1.0 ($n_{air} = 1.0$). With oil immersion objectives the aperture can be increased by using a special paraffin oil with a refractive index of $n_{oil} = 1.515$. The mathematical formula of the numerical aperture is thus:
$NA = n_{Im} \cdot \sin \alpha$

Contrast as a function of the illuminating aperture (lit. 9)

The theoretical maximum resolution is achieved if the illuminating aperture (plane L″) reaches the value of the imaging aperture (plane L‴). In practice however, this would bring about low-contrast, blurred microscopic images. It is best to choose an illuminating aperture which is more or less smaller than the objective aperture, depending on the reflection conditions in the specimen. This applies in particular to specimens which are more of the phase than of the absorption type.

The higher the illuminating aperture, the higher the percentage of the high-intensity, yet non-interfering wave fronts coming from the zero diffraction maximum in the plane L‴. These "empty" wave fronts do not contribute to the formation of the secondary microscopic image in plane O′ (real intermediate image) but bloom it, i. e. the specimen image is blurred and of low contrast.
To adjust the microscopic image the resolution of specimen structures and contrast are adjusted with the aperture diaphragm, which means a compromise regarding the illuminating aperture.

Observation in incident light brightfield

Amplitude (absorption) specimens are examined in incident light brightfield. Natural absorption specimens are distinguished from phase specimens which are converted into absorption specimens by certain manipulations.
Such manipulations to enhance the contrast of structures are: chemical treatment of the polished specimen (etching) or coating of the surface with transparent, thin, highly refractive substances.
Such absorption specimens are then examined in incident light brightfield.

Chemical etching (lit. 10)

These are electrochemical processes having reduction oxidation character. In contact with chemical agents metals by emitting electrons tend more or less strongly to change to the ionized (electrically positive) state.
In incident light brightfield specifically etched structures of the polished specimen become visible due to shadow effects caused by the formation of reliefs, reflections by cover layers or reflectance effects brought about by etch pits.
Misinterpretations of the structure are likely if the etching is not corretly made. For a specific etching, a freshly prepared, correctly ground and polished specimen must be available.
Besides dip etching in chemical agents the following etching techniques are applied: electrolytic or anodic etching (specimen as anode in an electrolyte), cathodic etching (bombarding the specimen with high-energy ions in vacuum), thermic etching in normal atmosphere or thermic etching in vacuum or inert gas.
The chemical etching process damages structural elements of the polished specimen. Small crystallites are lost. There are specimens which after the etching display only the crystal boundaries but are not differentiated according to the specimen phases.

Fig. 11
Normal nickel cast anode, etched; etch pits
Differential interference contrast
Magnification 500x

Evaporated interference layers (lit. 11)

Damage to the specimen and inadequate phase differentiation are avoided by evaporated interference layers. This purely physical method uses a transparent, thin, highly refractive substance corresponding to the examined specimen phases, which is evaporated on to the polished specimen. TiO_2, ZnSe or ZnTe are such substances. Multiple reflections and interference processes within these evaporated films increase the contrast of single specimen phases so that the structure can be examined in incident light brightfield. The following parameters are important for the contrast enhancement of object phases with evaporated interference films: the refractive index of the specimen phase, the refractive index and the thickness of the evaporated film, and the wavelength of the light used for microscopy.

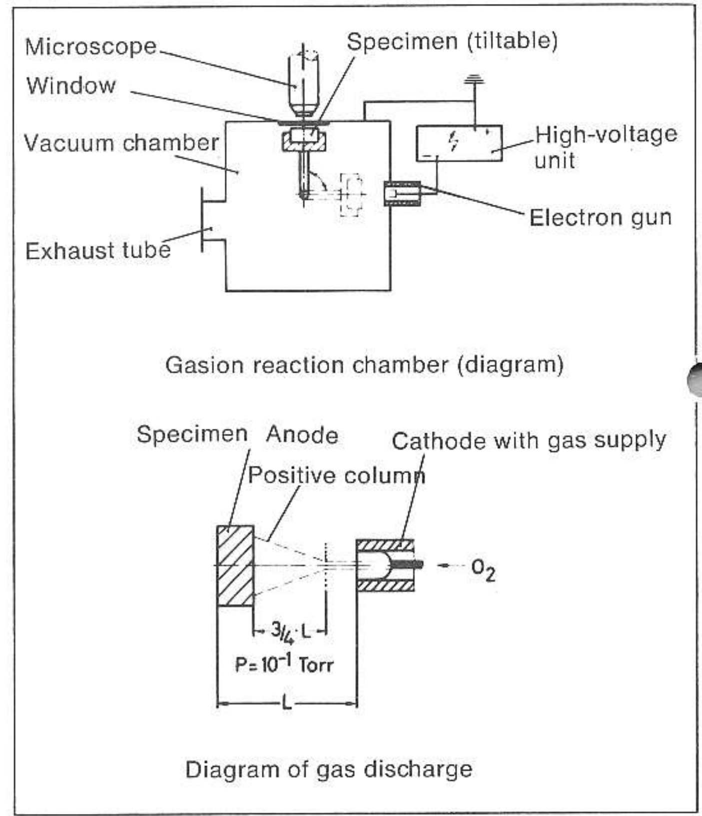
For incident light examinations of this kind not only white light should be used but also monochromatic, different wavelengths. A continuous interference filter is recommended for examinations using different wavelengths; this accessory for the incident light microscope allows continuously variable wavelength adjustment between 450 and 650 nm.

Contrast enhacement in a gasion reaction chamber (lit. 12)

Specific contrast enhancement of single phases, e. g. of a polished metal surface, is achieved in a gasion reaction chamber. This method uses a residual gas ionized by electron irradiation; the specimen surface is subjected to this gas in a vacuum chamber at 10^{-1} to 10^{-2} Torr (Fig.12). With oxygen as residual gas, the different specimen phases form oxide layers which in the incident light microscope are colored due to absorption and interference of the light.

Contrast enhancement is influenced by the type of residual gas, the degree of the vacuum, the density of the electron current coming from the cathode, the amount of discharge voltage between cathode and specimen used as anode, and the temperature of the specimen surface.

Fig. 12
Gasion reaction chamber



If this method is applied to achieve a specific contrast enhancement instead of only a general material contrast, no carbon polymerization films (contamination) should deposit on the specimen surface and no material be removed by electron bombardment. Contamination can be avoided by heating up the specimen, reducing the current density and using oxygen ions in the chamber. Removal of material can be reduced by decreasing the discharge voltage.
If the residual gas in the vacuum chamber is, for instance, argon, the argon ions will only remove material, i. e. a general material contrast is achieved but not an unambiguous specific contrast enhancement.
The gasion reaction method can also be used for non-metallic specimens for general material contrasting. Whether single specimen phases of a non-metallic specimen can be specifically contrasted must be checked in each case using different gas ions in the vacuum chamber.
The following accessories are required for an incident light microscope for this contrasting method: objective with long working distance (Epiplan LD), a vacuum chamber which can be mounted on the microscope stage, where the polished specimen surface can be directed towards the light or electron beam path, a cathode in the vacuum chamber as electron gun for the ionization of the residual gas, a high-voltage unit to produce an electron current between cathode and specimen used as anode and a two-stage mechanical vacuum pump (10^{-1} to 10^{-2} Torr).

Incident light darkfield examinations (lit. 13)

Darkfield illumination lends itself especially for straylight-producing specimens. It reveals fissures, pores and grain boundaries, and in particular the structures of semi-opaque specimens (Fig. 13). Oxidation products have often characteristic intrinsic colors. The quality of a polished specimen can be checked in incident light darkfield; on dark background scratches appear as bright lines.

In incident light darkfield illumination there are only wave fronts in the field of view which have reached the objective due to stray reflection by structuring elements. Wave fronts reflected by surface elements perpendicular to the microscope axis do not reach the field of view; these specimen areas remain dark.

Fig. 13
Sphalerite (ZnS) in incident light darkfield
Magnification 45x

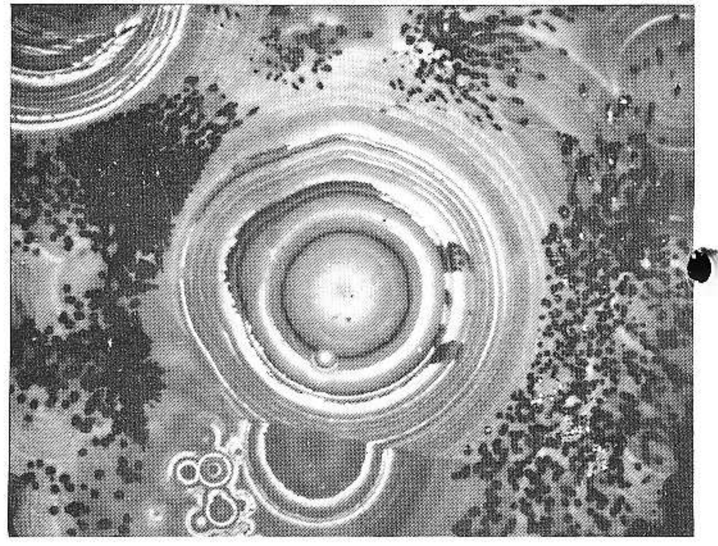


Image contrast may be high in darkfield illumination. Highest contrast, i. e. 1.0, is achieved if the background of the microscopic image is black and the structural image (e. g. grain boundaries) white. The image contrast is defined as

$$K = \frac{E_{max} - E_{min}}{E_{max}}$$

where E is the luminance of the different specimen areas. Even with a contrast of 0.2 the human eye recognizes luminance differences of the microscopic image.
The path of the illuminating wave fronts in the incident light microscope for darkfield examinations is defined by the special technical design of reflector and objective (Fig. 14).

The darkfield reflector (D) deflects only the outer beams of the illuminating wave fronts in the illuminator 90° rotated to the objective, while the central area is blocked out by a diaphragm in the reflector.

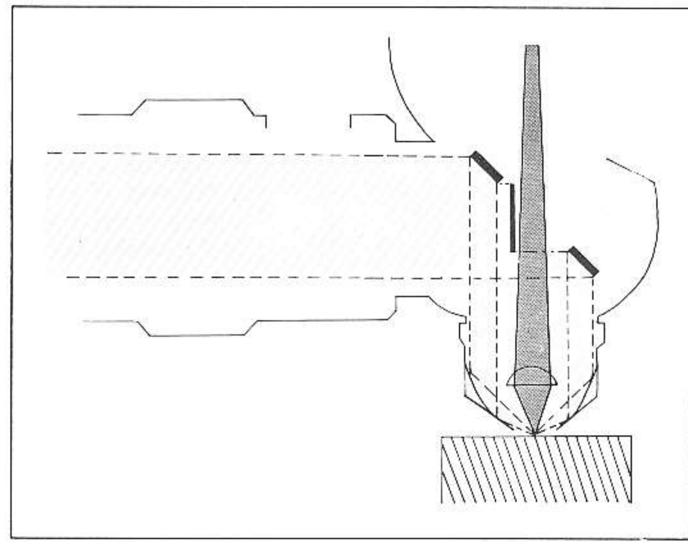


Fig. 14
Beam path in incident light darkfield

Via a concave mirror in the outer part of the Epiplan HD objective the illuminating wave fronts are relayed to the specimen surface, producing polydimensional darkfield illumination. The optical elements of the objective do not contribute to specimen illumination. Only the wave fronts diffusely reflected by the specimen surface (reflectance) reach the objective and contribute more or less strongly to the adjustment of the darkfield image. The Epiplan HD objectives are for incident light darkfield and brightfield examinations (Table 4).

Table 4
Objectives for brightfield and darkfield examinations

Epiplan HD	Working distance (mm)
4/0.1	2
8/0.2	2
16/0.35	2
40/0.85	0.23
40/0.6 LD	2
80/0.95	0.09
100/1.25 oil	0.25

The lamp field stop must be completely opened for darkfield examinations. The position of the aperture stop depends on the contrast of the microscopic image.

Differential interference contrast (DIC) (lit. 14)

The differential interference contrast method helps to qualitatively enhance specimen gradients within a wide contrasting range from some wavelengths to a few Angstrom units.

With this method surfaces can be examined which are
1. mechanically and chemically untreated
2. polished (Fig. 15)
3. polished and etched

The following accessories are required for differential interference contrast in incident light microscopy:
1. Polarizer
2. Analyzer
3. Epiplan Pol objectives
4. Interference contrast system (one for each objective)

The interference contrast system is screwed to the Epiplan Pol objective instead of the centering change ring (Figs. 16/17).

The interference contrast system contains the Nomarski modification of a Wollaston prism; the crystallographic optic axis in the upper part of the prism is oriented obliquely to the microscope axis. (Fig. 18). This shifts the interference plane from the center of the original Wollaston prism to the focal plane of the objective. Interference fringes in the field of view are avoided and uniform contrasting achieved.

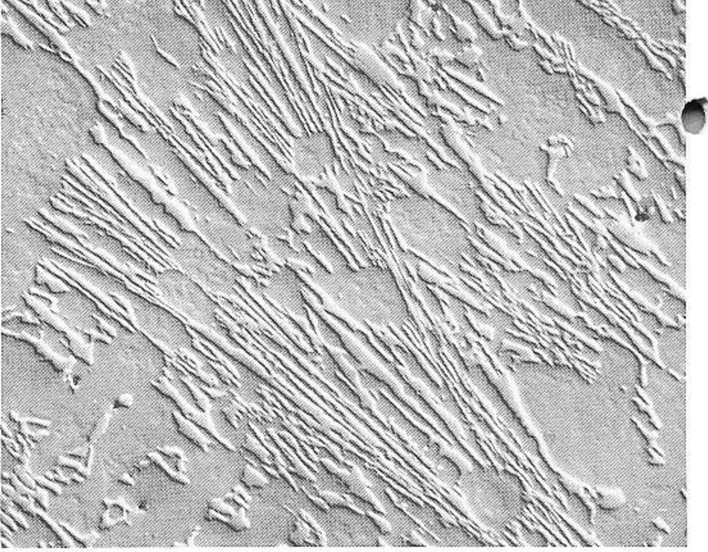


Fig. 15
Polished, unetched surface of a chill casting (Cr 18 Ti 1 Mo 0.5)
Differential interference contrast
Magnification 75x

Fig. 18
Difference between Wollaston and Nomarski prism

Wollaston prism | Nomarski prism

Interference plane prism

Objective focal plane | Objective focal plane

Prism interference plane

Directions of optic axes in the quartz prisms

Directions of linearly polarized light

Fig. 19
Beam path in incident light differential interference contrast

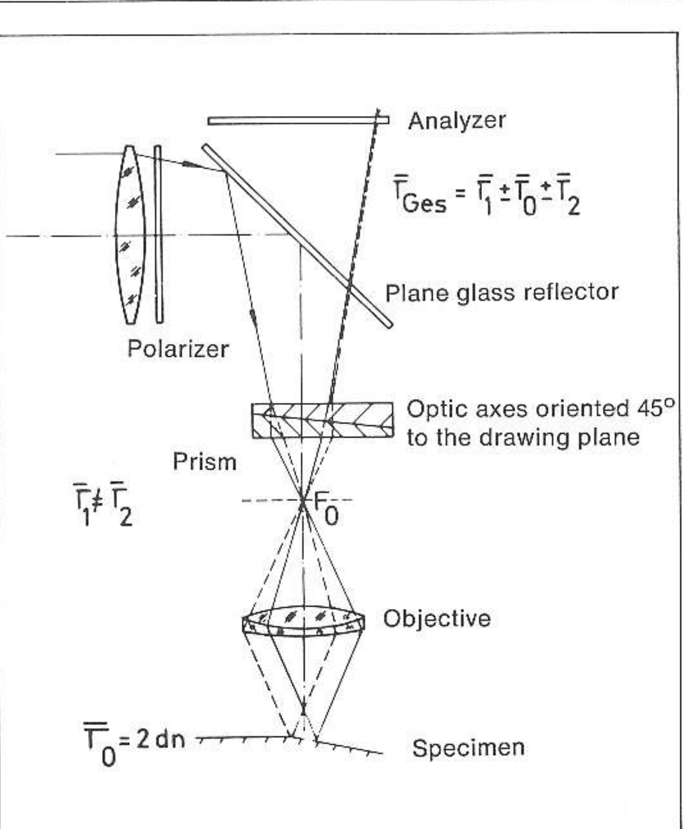


Fig. 16
Epiplan Pol objective on centering change ring

Fig. 17
Interference contrast system for Epiplan objective 100/1.25 oil

In high-power objectives there is no space to mount the Wollaston prism in the image-side focal plane of the objective.
Fig. 19 shows the beam path between polarizer and crossed analyzer in an incident light microscope equipped for differential interference contrast.
The linearly polarized (East-West) wave fronts coming from the polarizer are split into two partial waves in the modified Wollaston prism. The shear of these waves is below the resolving power of the objective used, which avoids double images of specimen structures.
The path difference of the sheared wave fronts is Γ_1, i. e. when leaving the prism one of the two waves is ahead of the other one by a certain nanometer amount (1 nm = 10^{-6} mm). Following reflection by the specimen the path difference Γ_1 has changed due to geometric height differences of the specimen surface and different phase jumps as a function of different refractive indices of the specimen phases hit by the sheared wave fronts. This change in path difference Γ_1 is referred to as Γ_0 in Fig. 19.
Following reflection by the specimen surface the sheared wave fronts have a path difference Γ_2 in the prism, where they are recombined without interfering with each other because they are still linearly polarized perpendicular to each other.

The path difference before the analyzer is expressed by the formula:
$\Gamma_{total} = \Gamma_1 \pm \Gamma_0 \pm \Gamma_2$
Only sheared wave fronts of $\Gamma_{total} = (2k + 1)\ \lambda/2$, with $k = 0, 1, 2$, etc. pass the analyzer. The interfering sheared wave fronts as a function of the wavelengths leave the analyzer with different intensity which is expressed by the Fresnel formula:

$$I/I_0 = \sin^2 \frac{\pi}{\lambda_0} \cdot \Gamma_{total}$$

where I_0 is the intensity after the polarizer without consideration of the reflectance of the specimen phases.

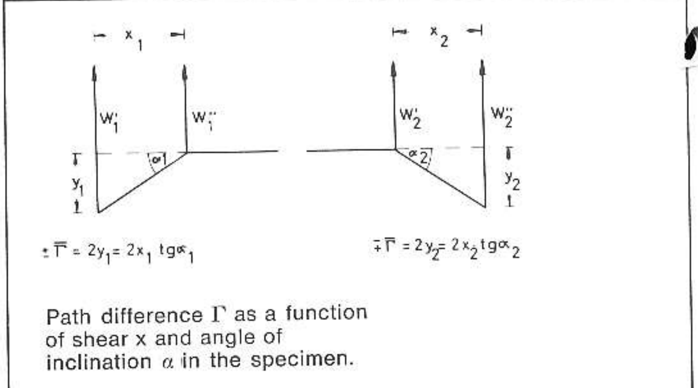
If the modified Wollaston prism is shifted laterally within a certain range, the ratio of Γ_1 to Γ_2 changes, i. e. if the prism is symmetrical to the microscope axis, $\Gamma_1 = \Gamma_2$. Thus the intensity conditions in the field of view are only a function of Γ_0 bound to geometric height differences and phase jumps.
Except for the physical path differences in the specimen plane (phase jumps), the object path difference is with this interference method a function of the shear x below the resolution limit and the increasing gradient tg α of the corresponding quite extended surface element in the specimen plane. The following formula for one wave front pair can be derived from Fig. 20: $\Gamma = 2y = 2x \text{ tg } \alpha$, where Γ is the path difference between the waves W′ and W″. It follows for a complete extended surface element with constant decreasing gradient that $\Gamma_0 = 2\Delta_x \text{ tg } \alpha$.
Consequently the path difference $\Gamma_{obj.}$ influences the image contrast as a function of the gradient according to the site coordinate, i. e. the object contrast depends on the path differences of the sheared wave fronts at the gradient of a surface element in the specimen plane.
Because of these processes the method was named differential interference contrast (DIC). Image contrast as a function of the specimen gradient produces in the microscopic image different intensities of opposite object sides. Fig. 20 shows the retardation of wave W′ relative to wave W″, while in the opposite specimen gradient W″ is retarded relative to W′.
Setting in the equation $\Gamma_{total} = \Gamma_1 \pm \Gamma_0 \mp \Gamma_2$ the expression $\Gamma_1 \pm \Gamma_2$ produced in the modified Wollaston prism Γ_u, i. e. as the path difference defining the intensity of the image background, yields for one specimen gradient $\Gamma_0 \pm \Gamma_u$ and for the other $\Gamma_0 \mp \Gamma_u$. According to the above-mentioned Fresnel formula

$$\left[I/I_0 = \sin^2 \frac{\pi}{\lambda_0} \cdot \Gamma_{total} \right]$$

the intensities are different for opposite specimen gradients with an asymmetrical position of the modified Wollaston prism.
In the microscopic image these intensity differences cause relief effects resembling unilateral oblique illumination.

Contrast effects occur in the microscopic image only where specimen structures are aligned perpendicularly to the direction of the shear in the modified Wollaston prism. A rotating microscope stage must therefore be used especially for texturized specimens (unilaterally aligned structure).

Fig. 20



Path difference Γ as a function of shear x and angle of inclination α in the specimen.

DIC adjustment

1. Use an isotropic, specularly reflecting specimen (surface mirror).
2. Adjust Köhler illumination.
3. Detach objective and interference contrast system.
4. Set analyzer to zero and turn polarizer until minimum intensity is achieved in the field of view; the polarizer must be oriented East-West.
5. Bring interference contrast system without objective into the beam path and remove eyepiece from tube: the interference fringes must run diagonally through the objective pupil, and the zero interference fringe must be black.
6. If this is not the case, loosen the prism mount with a screwdriver, turn it by a certain amount and fix it again.
7. Screw the objective to the interference contrast system and insert eyepiece in tube.
8. Provide the specimen.
9. Vary contrast by turning the mount of the interference contrast system. Re-adjust the aperture diaphragm according to the desired contrast.
10. The contrast can be further varied by a rotating microscope stage.

Polarizing microscopy (lit. 15)

The following accessories are required:
1. Polarizer and analyzer
2. Plane glass Pol reflector
3. Epiplan Pol objectives
4. Rotating microscope stage
5. 60 or 100 W filament lamp

Polarizer and analyzer should be rotatable for exact alignment. The transmission direction of the polarizer must be oriented perpendicular to the reflection plane of the reflector (Fig. 21).
The East-West oscillating linearly polarized wave fronts emitted from the polarizer are directed to the specimen surface by the reflector. An isotropic phase will reflect the wave front without affecting polarization. These wave fronts will not pass the second polarizing filter, the analyzer, if its transmission direction is perpendicular to the first polarizing filter, the polarizer. Isotropic specimens remain dark between two crossed polarizing filters, while anisotropic objects will be four times bright and four times dark alternately as the stage is rotated through 360° (Fig. 22).

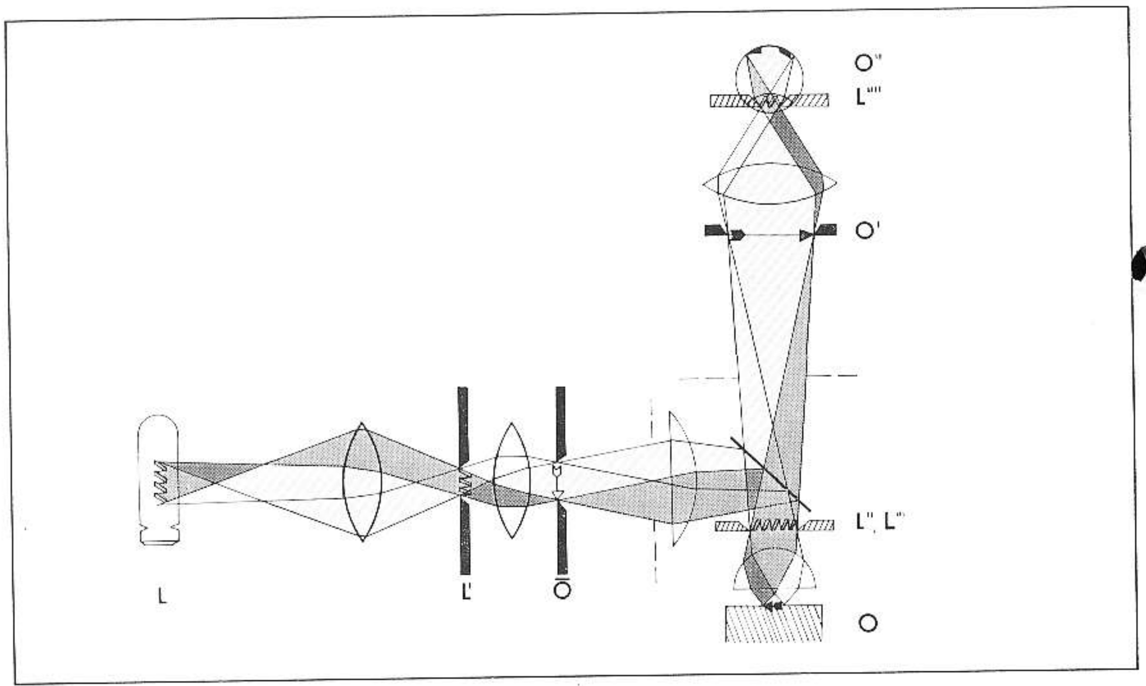


Fig. 21
Beam path in the incident light polarizing microscope.
Dotted lines:
light source side = polarizer
image side = analyzer

An anisotropic object will be dark if the structural primary reflection directions of the specimen are oriented parallel to the oscillation, i. e. transmission directions of polarizer and analyzer. In the so-called bright or diagonal position the primary reflection directions of the specimen are at 45° to the crossed polarizing filters. Each wave incident on the specimen is thus split up into the reflection components R_1 and R_2 (Fig. 23). Following reflection by the specimen phase these two components form in the most general case an elliptical oscillation (Fig. 24).

Fig. 22
Anisotropic object between crossed polarizing filters; decomposition lamellae of titanium magnetite.
Magnification 150x

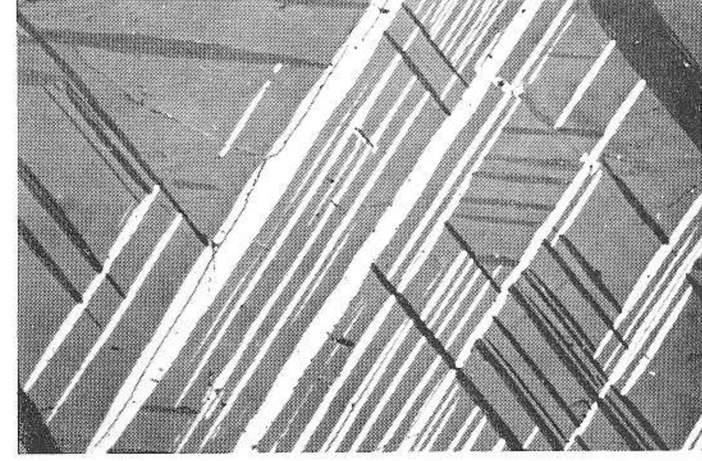
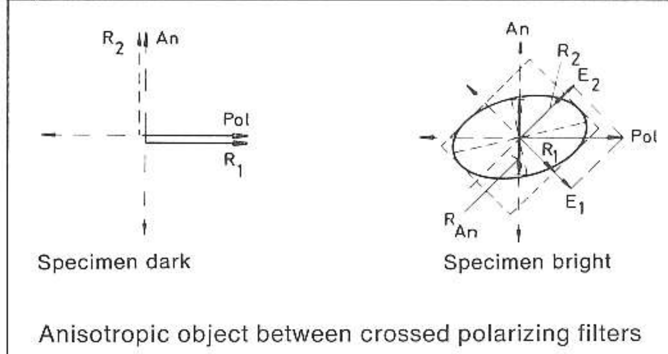


Fig. 23



Anisotropic object between crossed polarizing filters

There are two reasons for the elliptic character of reflecting light:

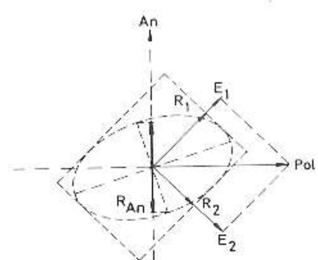
1. The different refractive values of the specimen for the two primary reflection directions cause different phase jumps of the reflected components R_1 and R_2 in the specimen surface, and
2. The two wave components are subject to different absorption in the specimen.

Due to the analyzer two reflected components can interfere with each other. The anisotropic phase appears bright or colored.
If anisotropic specimens are observed with the polarizer alone, grains of anisotropic specimen phases of different orientation may be of different brightness and color, a phenomenon which is called reflection pleochroism.
Polarizing effects can be observed only if the object phase has an anisotropic internal or surface structure. There are the following reasons for anisotropy effects:

1. the object phase itself is anisotropic
2. anisotropic top layers, e.g. oxides
3. the specimen causes the magneto-optical Kerr effect, or
4. deep etching of the specimen surface.

Magnetic structures of object phases can be made visible between two crossed polarizing filters. This is the magneto-optical Kerr effect, i.e. the incident linearly polarized waves are magnetically influenced by the specimen. The reflected wave fronts no longer vibrate perpendicularly to the transmission direction of the analyzer, which means that they partly pass it energetically.
Deep etched isotropic specimens are bright between two crossed polarizing filters. In polarized state the linearly polarized light is influenced at the oblique surfaces of the etching pits along the internal structure of the object phase, so that it no longer vibrates linearly perpendicular to the transmission direction of the analyzer.

Fig. 24



Elliptically polarized light reflected by the anisotropic specimen
E light flux irradiated from the polarizer: $E_1 = E_2$
R light flux reflected by the anisotropic specimen: $R_1 > R_2$
R_{An} may pass through the analyzer

Part of the energy may thus pass through the analyzer. For polarizing microscopy electrochemical polishing methods are superior to mechanical ones. Purely mechanical polishing often causes surface irregularities (relief), smears and top layers. These mechanical preparation errors may influence anisotropy effects.

Adjustment of a polarizing microscope:

1. Adjust Köhler illumination with the plane glass Pol reflector H-Pl. Remove the analyzer from the beam path.

2. Cross polarizer and analyzer.

a) Set analyzer to zero with the fine and coarse scale.

b) Turn the polarizer until a strongly reflecting isotropic specimen phase – or better a surface mirror – appears black.

3. Focus to a strongly anisotropic feature. During stage rotation the feature must be four times dark and four times bright alternately or colored, and thus must re-appear every time the stage is exactly 90° rotated.

4. If during a 360° rotation of the stage the object will only be two times bright, the wave fronts emitted from the polarizer are not oriented perpendicular to the reflection plane of the reflector.

5. In this case turn polarizer and analyzer synchronously by small degrees until the phenomena described under item 3. above are visible. Check the cross position of polarizer and analyzer from time to time with a surface mirror.

Immersion objectives (lit. 16)

Besides dry objectives immersion objectives are used in incident light microscopy for low magnifications. Paraffin oil (n ~ 1.5) is used as medium between specimen surface and objective front lens. Table 6 lists the incident light immersion objectives for low and medium magnifications.

Table 6
Incident light immersion objectives for low and medium magnifications

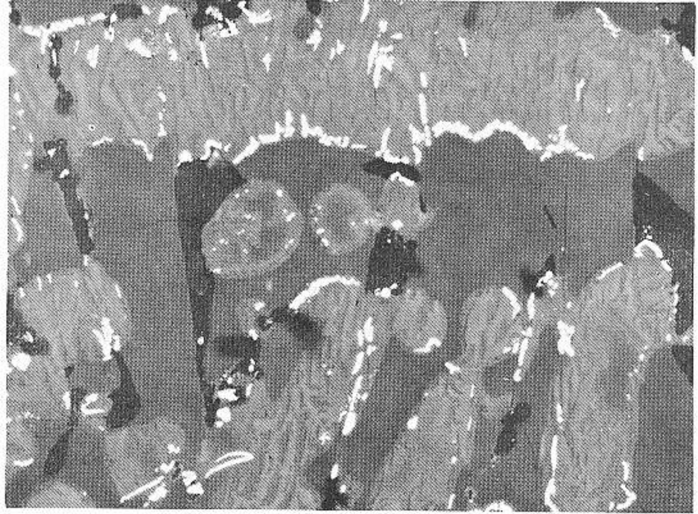
Type	Magnification	Immersion medium	Working distance (mm)
Epiplan	4/0.1	oil	0.3
Epiplan	8/0.2	oil	0.3
Epi-Achromat	16/0.4	oil	0.85
Epi-Achromat	40/0.85	oil	0.5

In incident light microscopy immersion objectives change the image contrast (Figs. 25/26), especially of phases with low absorption coefficients.
The higher the deviation of the refractive index of an object phase from that of the medium between specimen and objective, the more noticeable the surface gloss. This suppresses reflectance effects from the internal structure and the image will lack structural detail. This applies, for instance, to an increase in carbonization of pit coal, i. e. the higher the carbonization of the coal, the higher the carbon content, and the higher its refractive index.

An immersion medium like paraffin oil reduces the jump in refractive index between specimen and immersion medium and the surface gloss; the result is a better image of the specimen structure. In coal microscopy paraffin oil may be replaced by glycerine as immersion medium, which with water is more easily removed from the polished coal surface than paraffin.
Specimen phases with low absorption coefficients and low gloss effect due to an immersion medium show internal reflections whose intensity and color are characteristic of the corresponding phase. These internal reflections occur, for instance, at grain boundaries, cleavage surfaces and inclusions.

Fig. 25
Slag rich in phosphorus,
Epiplan 40/0.65

Fig. 26
Slag rich in phosphorus,
Epi-Achromat 4/0.65



Specimen phases with high absorption coefficients and considerable changes of the refractive index as a function of the wavelength change the reflection color pronouncedly when using dry instead of immersion objectives.
This is due to the λ dispersion curve for air which intersects that of the specimen at another wavelength than the λ dispersion curve of the immersion medium.

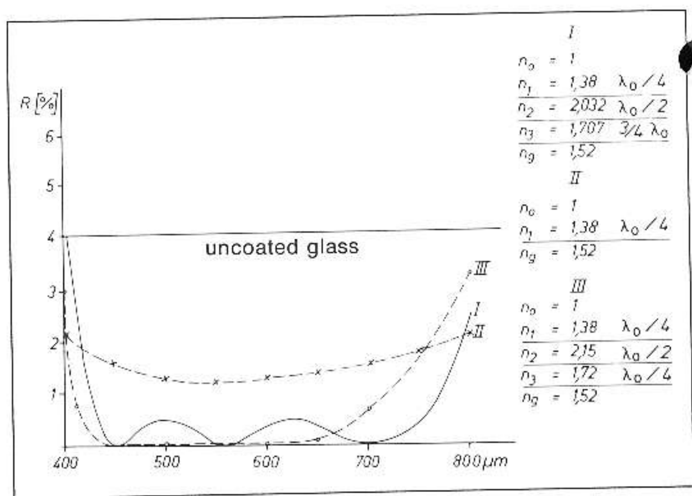
If an object phase has in addition a pronounced spectrally-dependent bireflection (reflection as a function of the direction), immersion objectives increase the contrast still further.

An immersion objective increases the image contrast because it reduces multiple reflections between front lens and objective surface more than a dry system. The T (transparent) coating of each lens surface at all events increases the image contrast.

Another advantage of immersion objectives is that they suppress scratches and reliefs in the image more than dry systems, which reveals fine specimen structures.

Immersion objectives do not increase the resolving power at low magnifications. 4/0.1 and 8/0.2 objectives were originally dry systems which are provided with immersion caps. With low-power objectives this reduces the working distance and thus provides for plane-parallel immersion layers.

Fig. 27
Decrease in reflection due to triple layers



4. Antiflex method (lit. 17)

With low-power incident light objectives and a reflectance far below 0.1 ($< 10\%$), there is the danger of blurred or invisible structures in the microscopic image, because of residual reflections of lens surfaces superimposed on the field of view, in spite of multiple anti-reflection coatings on the lens element surfaces (Fig. 27).

If the structures of low-carbonized (geologically young) brown coal, slags, ceramics or plastics are to be studied, these disturbing lens reflections are eliminated with the Antiflex sysem.

The system comprises two crossed polarizing filters and a rotating birefringent crystal plate in the front part of the objective (Fig. 28). The linearly polarized wave coming from the polarizer is split up into two components by the crystal plate. These two components are reflected by the specimen, pass the crystal plate again and strike the analyzer. In the crystal plate in the objective front part the two wave fronts have a path difference of two times $\lambda/4$ (180° phase shift). Due to the analyzer they form a linearly polarized wave parallel to the transmission direction of the analyzer. The wave fronts emitted from the polarizer and reflected by the glass-to-air surfaces of the optical system are blocked out by the analyzer. They do not pass the crystal plate in the objective front part and thus oscillate perpendicular to the transmission direction of the analyzer.

This results in an unblurred microscopic image of the specimen structure.

The following items are required for the Antiflex method:
Simple, swing-out polarizer
Simple analyzer
Alternatively objectives Epiplan 4/0.1 Pol, 8/02. Pol,
LD Epiplan 16/0.3 Pol or LD Epiplan 40/0.6 Pol
Antiflex cap oil for Epiplan Pol 4/8/LD 16
Antiflex cap oil for Epiplan Pol LD 40
Immersion objectives increase the antiflex effect.
The immersion medium is paraffin oil.

Use of the Antiflex system:

1. Without objective cross analyzer and polarizer using a strongly reflecting isotropic specimen (surface mirror).

2. Screw antiflex cap to objective and with the objective focus on the specimen.

3. Turn antiflex cap until maximum brightness of the specimen surface is achieved; secure cap with counterring.

The Antiflex method cannot be used for the examination of bireflecting object phases because the birefringent crystal plate in the cap would influence the polarizing effects of the object.

Bireflecting specimens can be investigated with the antiflex cap if the antiflex effect is eliminated by setting the birefringent crystal plate in dark position relative to polarizer and analyzer. This is achieved if an isotropic object phase is dark after rotation of the birefringent crystal plate in the antiflex cap.

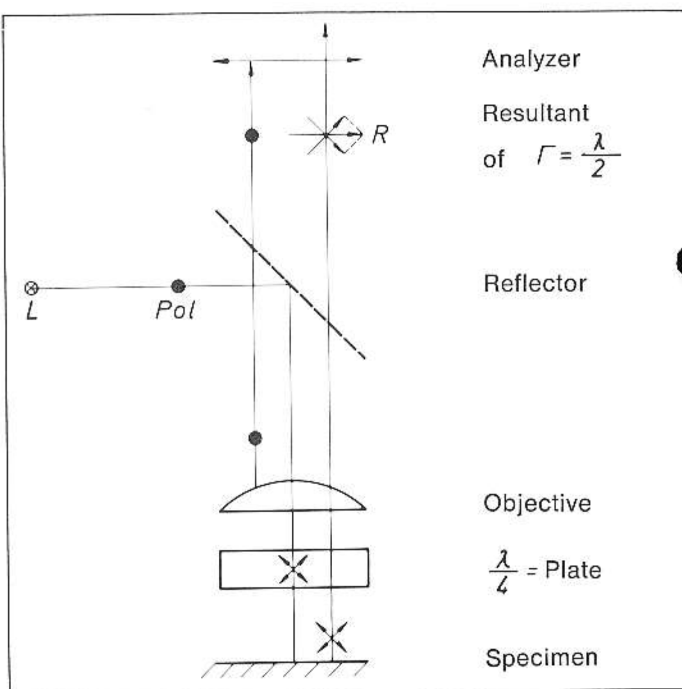


Fig. 28
Antiflex system

General information
(lit. 18)

Photomicrography of structures is of great importance, because the photomicrograph is a reliable record of the investigated specimen.
In materials testing, for instance, photomicrographic recording is indispensable.
There is an ample selection of photomicrographic equipment; which is used depends on the requirements. Photomicrographic equipment is either mounted on the microscope (photomicrographic cameras, Fig. 29) or built in (Fig. 30).

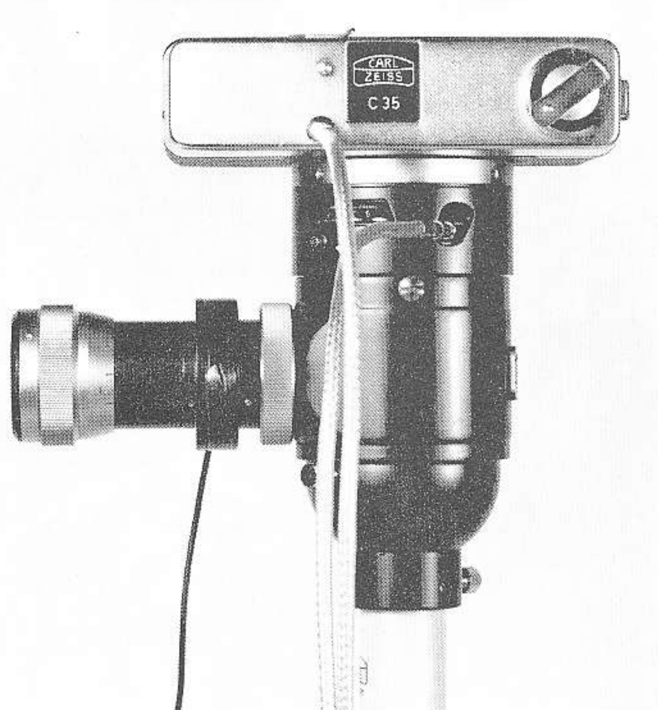


Fig. 29
Photomicrographic camera with mechanical shutter

Both camera types are provided for 35 mm or large format (9 x 12 cm or 4 x 5") negative material or transparency film. Whether the miniature or large format is photographed directly depends on several factors. The positive will contain the more information the larger the original negative or positive slide format. The 9 x 12 cm format has more "storage capacity" while the 35 mm format must be subsequently enlarged which reduces the information content compared with the original microscopic image.

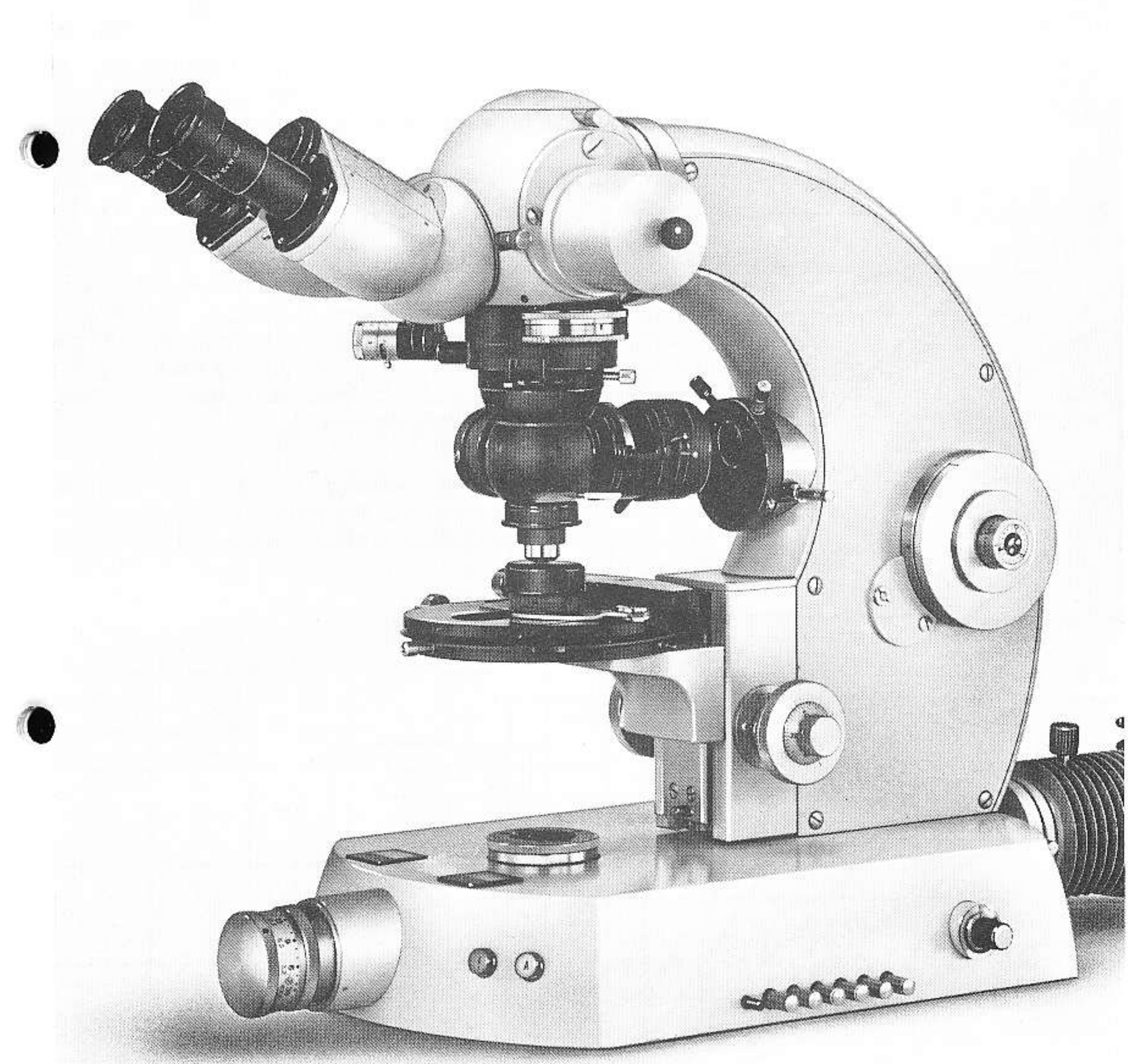
Film material

The following properties of the emulsions should be considered when choosing the film material:

1. Gradation
2. Sensitivity
3. Resolving power
4. Color temperature in case of color reversal films
5. Schwarzschild effect

Age and storage conditions of the film material are also important.

Fig. 30
Photomicroscope III



The gradation of an emulsion determines the intensity range the material can faithfully reproduce. The gradient gamma is given by the sensitometric curve shown in Fig. 31.

The density or absorbance ($S = \log\frac{1}{T}$) of an emulsion is a function of the exposure ($E \cdot t$) which in turn depends on the intensity (E) and the time of influence (t). The value of gamma results from the slope of the characteristic curve (tg α).

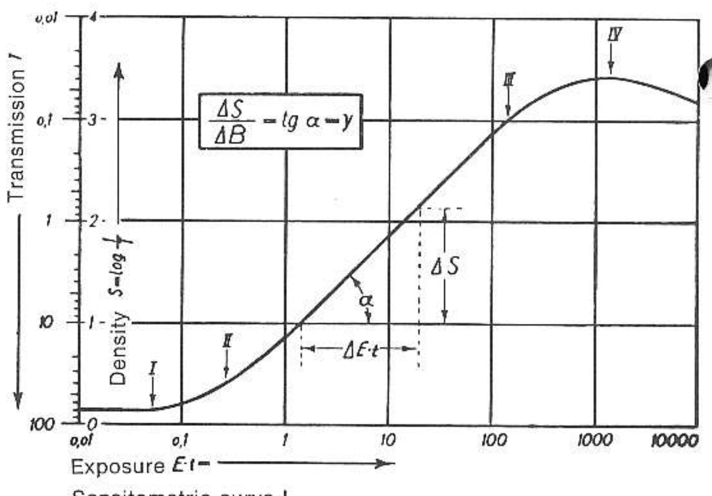
Regarding the gradient there are soft (gamma $<$ 1), normal (gamma = 1) and hard (gamma $>$ 1) emulsions. Fig. 32 shows how brightness differences are reproduced by an emulsion.

Gradation is not only a function of the emulsion, but also of temperature and development time (Fig. 33).

The choice of the gradation of the film material depends on the specimen. It is always better, though, to have a "soft" negative containing a wealth of information and to produce "hard" paper prints of this negative.

As a rule, the gradient (gamma) decreases as the film speed (DIN or ASA) increases; the emulsions are softer.
The sensitivity of emulsions is given in DIN or ASA values.

Fig. 31



Sensitometric curve I

Three DIN or two ASA values correspond to double sensitivity, 12 DIN = 12 ASA, 15 DIN = 25 ASA, 18 DIN = 50 ASA, etc. The DIN or ASA values are determined sensitometrically by the manufacturer.

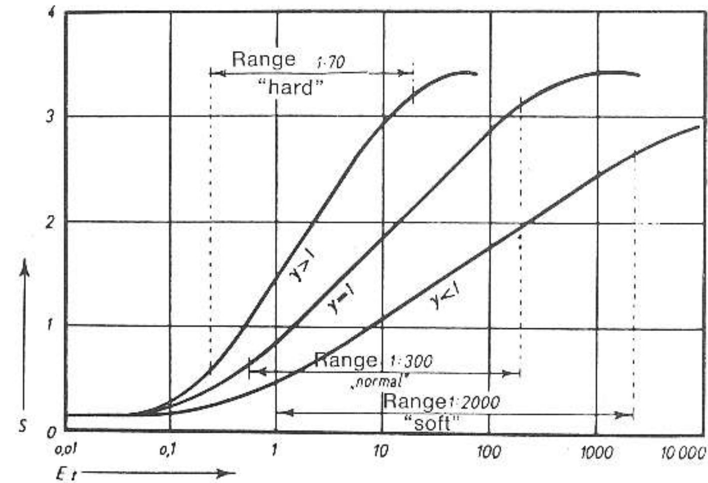


Fig. 32
Density curve, gradation

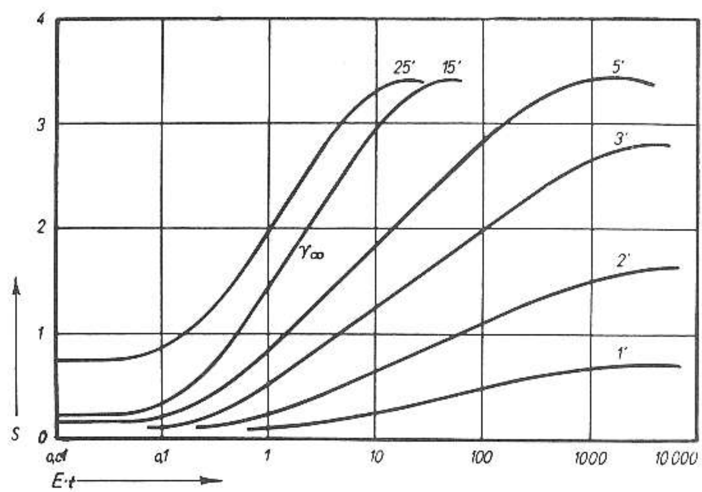


Fig. 33
Density curve, function of the development time

Emulsions are sensitized for specific spectral ranges. Black-and-white film material is available with orthochromatic and panchromatic emulsions (Fig. 34).
Panchromatic emulsions are sensitized for the entire spectral range, while orthochromatic emulsions have no response in the red visible spectral range. The density of a photographic emulsion is also dependent on the spectral emission of the light source, which is important especially for color transparency films.

Color photomicrography

Especially important for photomicrography on color transparency film are:
1. The color temperature of light source and film
2. The Schwarzschild effect
The color temperature of the light source must correspond to that of the film. If this is not the case, conversion filters are required, which are available from microscope and film manufacturers. A daylight color transparency film, for instance, is sensitized for a color temperature of 5500° K (Kelvin) and intended for daylight exposure. Color transparency films for artificial light are provided for light of slightly more than 3000° K.
If, for instance, the color temperature of the light source is 3200° K and the film sensitized for daylight, the procedure is as follows: at first the so-called deca-mired (DM) values of light source and color transparency film are calculated. One mired (micro reciprocal degree) corresponds to the reciprocal value of one millionth of a degree. 100 000/5500 yields 18 DM and 100 000/3200 yields 31 DM. The difference of 31 and 18 is 13 DM, i. e. the color temperature of the light source of 3200° K must be transformed to the color temperature of the daylight film of 5500° K with conversion filters of 13 DM B (blue).

Under-exposures and false representation of the object color are possible on color transparency film if the Schwarzschild effect is neglected. Fig. 35 explains why the density is not only dependent on the product of illuminance and illumination time (t) but also on short-time exposure with high or long-time exposure with low illuminance (E).
The E curve has a steeper slope than the t curve. The ratio of the two gamma values (t/E) is a term p which in the Bunsen-Roscoe law of photochemistry appears as exponent: $E_1 \div E_2 = (t_2 \div t_1)^p$ and is termed Schwarzschild exponent according to the astronomer Schwarzschild. Its value lies generally between 0.95 and 0.65.

In practice this means: if intensity and color of an object are correctly reproduced on film at an exposure time of 1″, under-exposure and color falsification would occur at one tenth of the illuminance and 10″ exposure time. As a rule, correct exposure can be achieved by reducing the illuminance by the factor 10, if the exposure is not made with the factor 10 but 2 x 10 (Table 7).

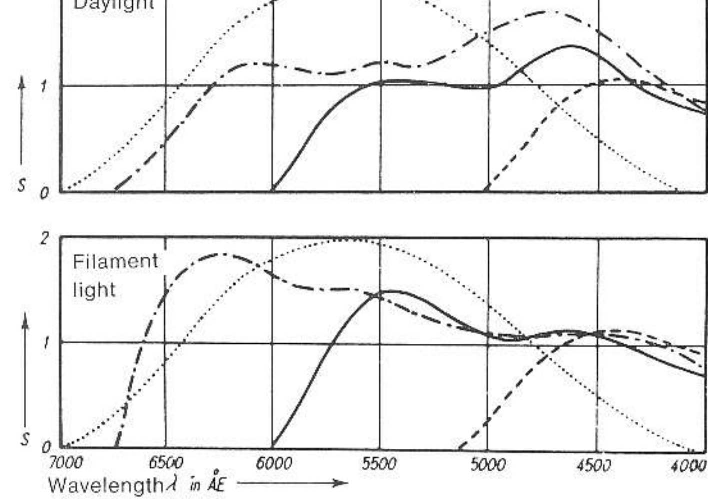


Fig. 34
Spectral sensitivity of photographic emulsions
. . . . . . sensitivity of the eye
- - - - - orthochromatic film
— · — · panchromatic film

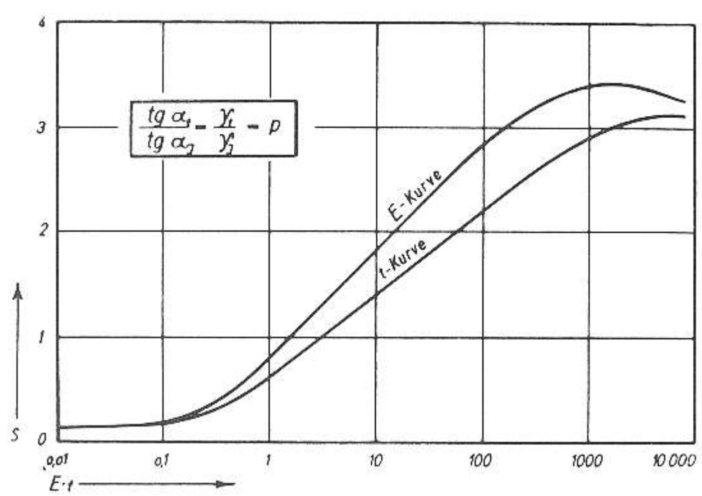


Fig. 35
Sensitometric curve; intensity and time curve

Table 7
Elimination of the
Schwarzschild effect

Exposure time longer than calibrated	**Extension factors**
2.5 x – 7 x	1.5 x
7 x – 20 x	2 x
20 x – 50 x	3 x
50 x – 100 x	4 x

The corrective factors for the exposure time listed in Table 7 are experience values. When using color transparency material, make three test exposures, one at an exposure time corrected according to Table 7, one at half this exposure time and one at double this exposure time.

Resolving power of photographic emulsions

The resolving power is another property of photographic emulsions. It is important for retaining microscopically resolved object structures. The resolving power of a photographic emulsion is given in lines per millimeter, and is for commercial photographic emulsions of medium sensitivity about 100 lines/mm.
As proved by a practical example, a film of 100 lines/mm resolving power is adequate to retain the object structures resolved by the microscope.

With an objective Epiplan 40/0.85, an 8x eyepiece, and a camera factor 0.5 (35 mm film), the total magnification on the photographic emulsion will be 160x. The Epiplan 40/0.85 objective resolves object details of about 0.4 μm spacing.

$$d = \frac{\lambda}{NA_{objective} + NA_{illumination}}$$

$$d = \frac{0.550}{0.85 + 0.65} = \frac{0.550}{1.5} = 0.4\ \mu m$$

This distance of about 0.4 μm in the object plane is enlarged in the plane of the 35 mm film to about 60 μm: 0.4 x 40 x 8 x 0.5 (line spacing in the object about 0.4 μm, Epiplan 40/0.85 objective, 8x eyepiece and camera factor 0.5). With 35 mm film having a resolving power of 100 lines/mm object structures in the microscopic image of 10 μm can be resolved, which means that this film covers structures six times finer than resolved by the microscope. Commercial film materials have adequate resolving power for the documentation of microscopic structures.

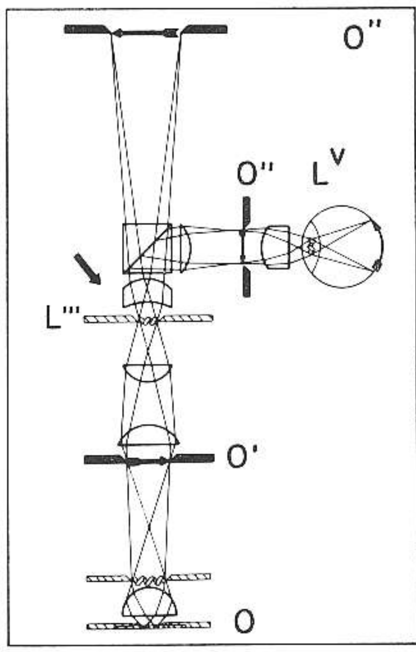
Lit. 19

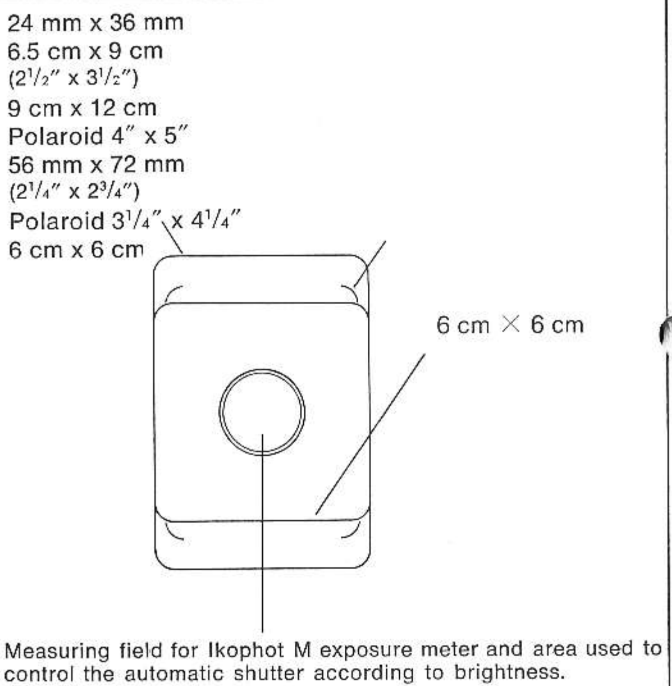
The following microscope attachments or microscopes can be used for photomicrographic documentation:

1. Photomicrographic cameras
2. Photomicroscope III
3. IM 35 and ICM 405
4. Ultraphot III b
5. Axiomat IAC and NAC
6. Tessovar

Photomicrographic camera

Simple or automatic models are available. In both types an objective (oblique arrow in Fig. 36) in the basic unit relays the microscopic image lying at infinity behind the eyepiece to the film plane and into the focusing eyepiece.





Measuring field for Ikophot M exposure meter and area used to control the automatic shutter according to brightness.

Fig. 36
Beam path in the photomicrographic camera

Fig. 37
Reticle in the focusing eyepiece for the photomicrographic camera

A reticle in the focusing eyepiece indicates the object area which is photographed (Fig. 37).
Before focusing the objective, the focusing eyepiece is focused on the double circle in the center of the reticle. If the microscope is adjusted to the specimen plane, this guarantees that the image of the microscopic specimen is also in focus in the film plane.
The exposure time is also measured in the double circle in the simple photomicrographic camera with the Ikophot M (Fig. 38), or with the automatic photomicrographic camera.

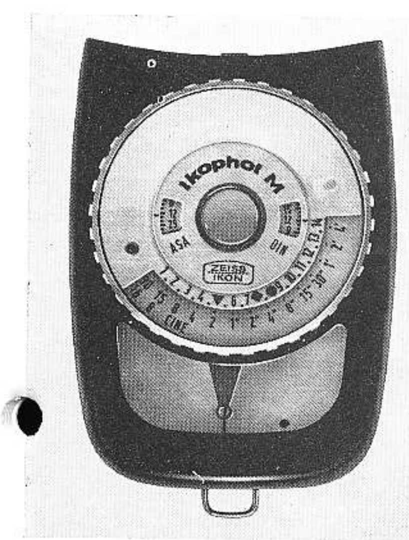


Fig. 38
Ikophot M exposure meter

With the Ikophot M the exposure time is determined as follows:

1. Set film speed on DIN or ASA scale of Ikophot M exposure meter.
2. Bring light sensor in the slide of the focusing eyepiece in the beam path.
3. Push red knob of exposure meter and release it if the pointer stops.
4. With the outer ring bring the red pointer to coincidence with the measuring pointer.
5. The red symbols indicate the exposure times for different camera formats:

Triangle for 35 mm
Square for 65 x 90 mm
Circle for 90 x 120 mm

With the simple photomicrographic camera exposure time setting and 35 mm film transport can be made manually; with the CS-matic shutter and corresponding control (Figs. 39/40) they are automatic.

Automatic exposure:

1. Set CS-matic shutter to Automatic.
2. Set film speed (DIN or ASA) with the outer ring of the control unit.
3. With the inner ring adjust the photographic format.
4. Bring the light sensor in the slide of the focusing eyepiece in the beam path; if the image brightness in the focusing eyepiece is dazzling, there is the danger of exceeding the range where the shutter controls the exposure time reliably. Light intensity can be reduced with a neutral density filter.
5. Cock shutter; after exposure the film is automatically advanced.

The following camera attachments are most frequently used in the above-mentioned photomicrographic camera system:

C 35 for 35 mm film
CP 100 for Polaroid film packs 3¼ x 4¼″
C 912 for 9 x 12 cm sheet film
CP 545 for 4 x 5″ Polaroid film

The information content is greater on 9 x 12 cm film than on 35 mm film, but the 35 mm format is preferred to the large-size format if a great number of photomicrographs is taken in rapid sequence. Polaroid film is recommended wherever paper prints must be instantly available. However, contrary to Polaroid film material, normal negative and positive film materials can be influenced by darkroom processing.

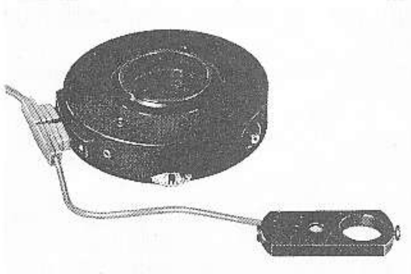


Fig. 39
CS-matic shutter

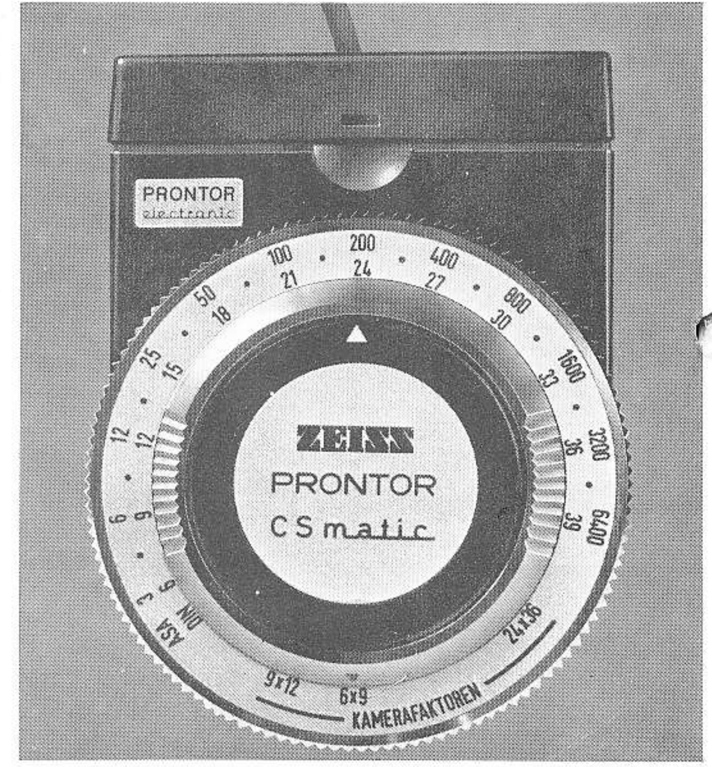


Fig. 40
Control for CS-matic shutter

Fig. 41
Image scales of microscope and photomicrographic camera

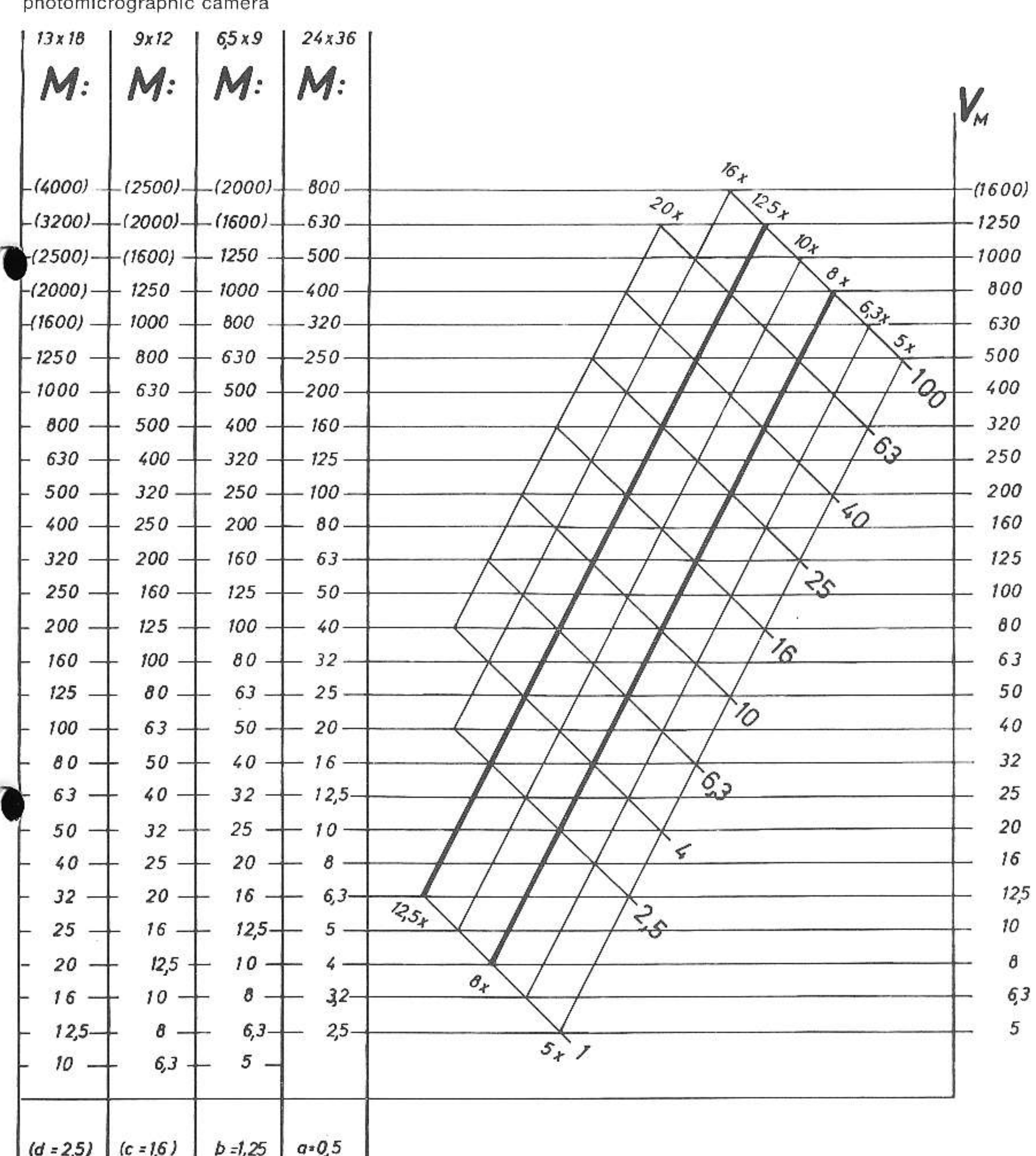


Image scale

The image scale is of great importance for photomicrographic documentation. Fig. 41 shows the image scales of different objectives, eyepieces and photomicrographic cameras.
The image scale of a photomicrograph is the ratio of image size to object size. For exact determinations the specimen is replaced by a micrometer on the microscope stage, and the enlarged image of the micrometer is measured on the camera ground glass or on the film.
For the compound microscope the image scale on the fi[illegible] can be approximately determined from the objective magnification x eyepiece magnification x camera factor. The camera factor of the 35 mm attachment and of commercial 35 mm cameras is 0.5.
The 6.5 x 9 cm attachments (Rollex cassettes or 6 x 6 reflex cameras) have the camera factor 1.25, and the Polaroid film pack attachment and the 9 x 12 cm (4 x 5″) attachment the camera factor 1.6.
Problem: take a specimen with 35 mm attachment (factor 0.5), objective 40 and Kpl eyepiece 12.5x. The image scale is approx. (0.5 x 40 x 12.5 = 250) 250 : 1.

Fig. 41 shows in the right-hand margin the total magnification of the image when viewed through the microscope. The difference between the individual steps is 1.25. In the left-hand margin these steps are given magnified by the factors of the camera attachments for 35 mm and sheet film. These data are for image scales of the negative or subsequent enlargement to the 9 x 12 cm and 13 x 18 cm formats. At these steps the guide lines for magnifications or objectives (from the lower right to the upper le[illegible] intersect those of the eyepiece magnifications. The thicker lines are the guide lines of the most frequently used eyepieces 8x and 12.5x.

Microscope magnification should only be increased so far that all object structures resolved by the objective can still be made visible to the eye. The range of useful magnification lies between 500x and 1000x the numerical aperture of the objective used. Higher magnifications are put into brackets in Fig. 41 in order to demonstrate that they do not make visible additional object details (empty magnifications).

Tessovar

The Tessovar is a specialized photomicrographic instrument (Fig. 42).
This photomacrographic zoom system has a magnification range between 0.4 and 40x. Photomacrography is possible of object fields between 55 x 82 mm and 1.7 x 2.6 mm. The instrument is primarily used for incident light darkfield photomacrography.
Photomicrographic cameras can be used in conjunction with the Tessovar, especially the 35 mm or the Polaroid camera attachment CP 100. Fig. 43 is the incident light darkfield photomicrograph of a bullet taken with the Tessovar with automatic 35 mm photomicrographic camera.
The Tessovar is an optical system offering an optimum compromise between depth of field which is particularly important for photomacrography and resolving power. Changing the magnification with the zoom system automatically controls adjustment of the aperture diaphragm which controls depth of field and resolution of the optical system. This ensures that the above-mentioned magnifications are obtained at optimum depth of field and optimum resolution (Table 8).

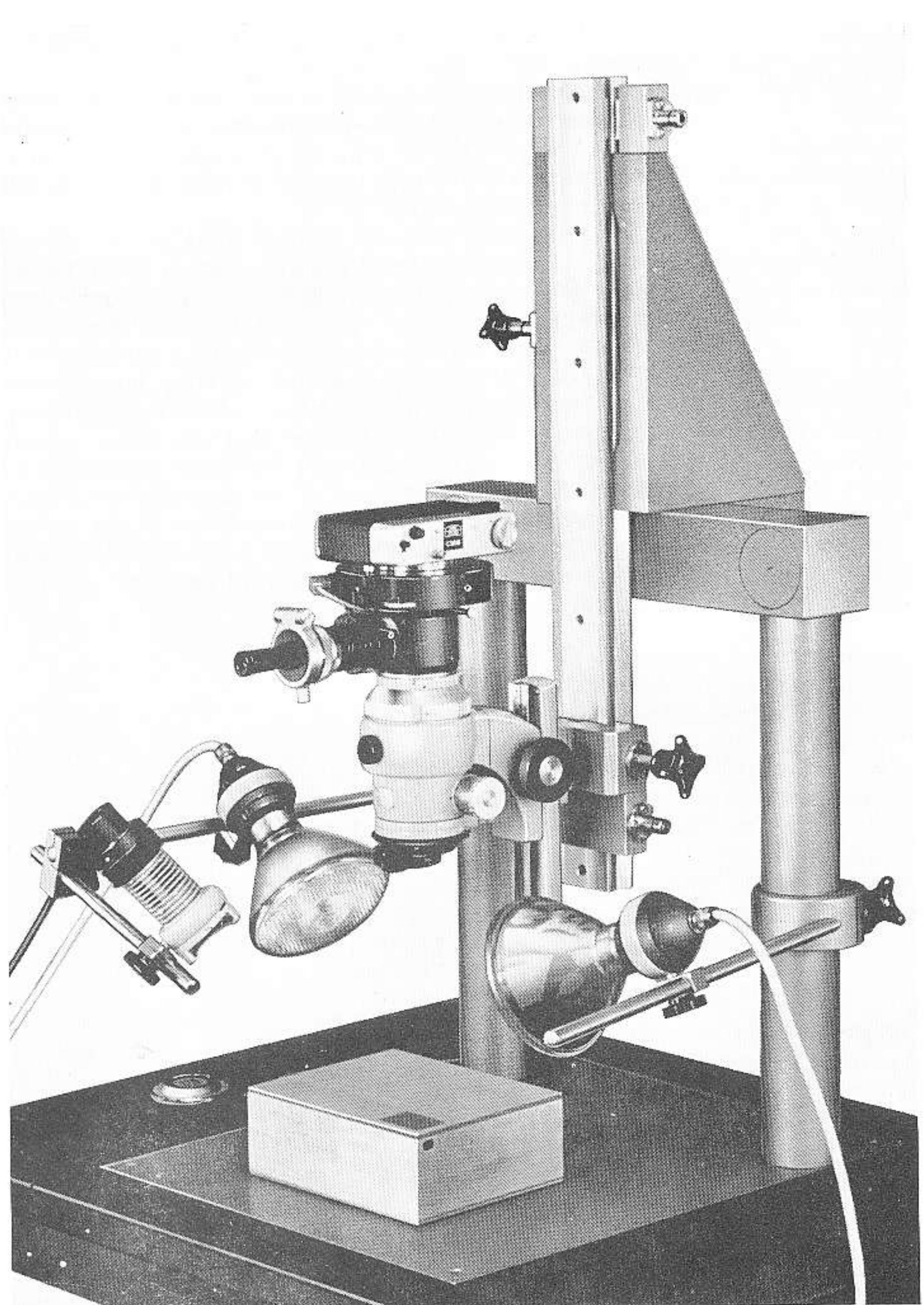


Fig. 42
Tessovar photomacrographic zoom system

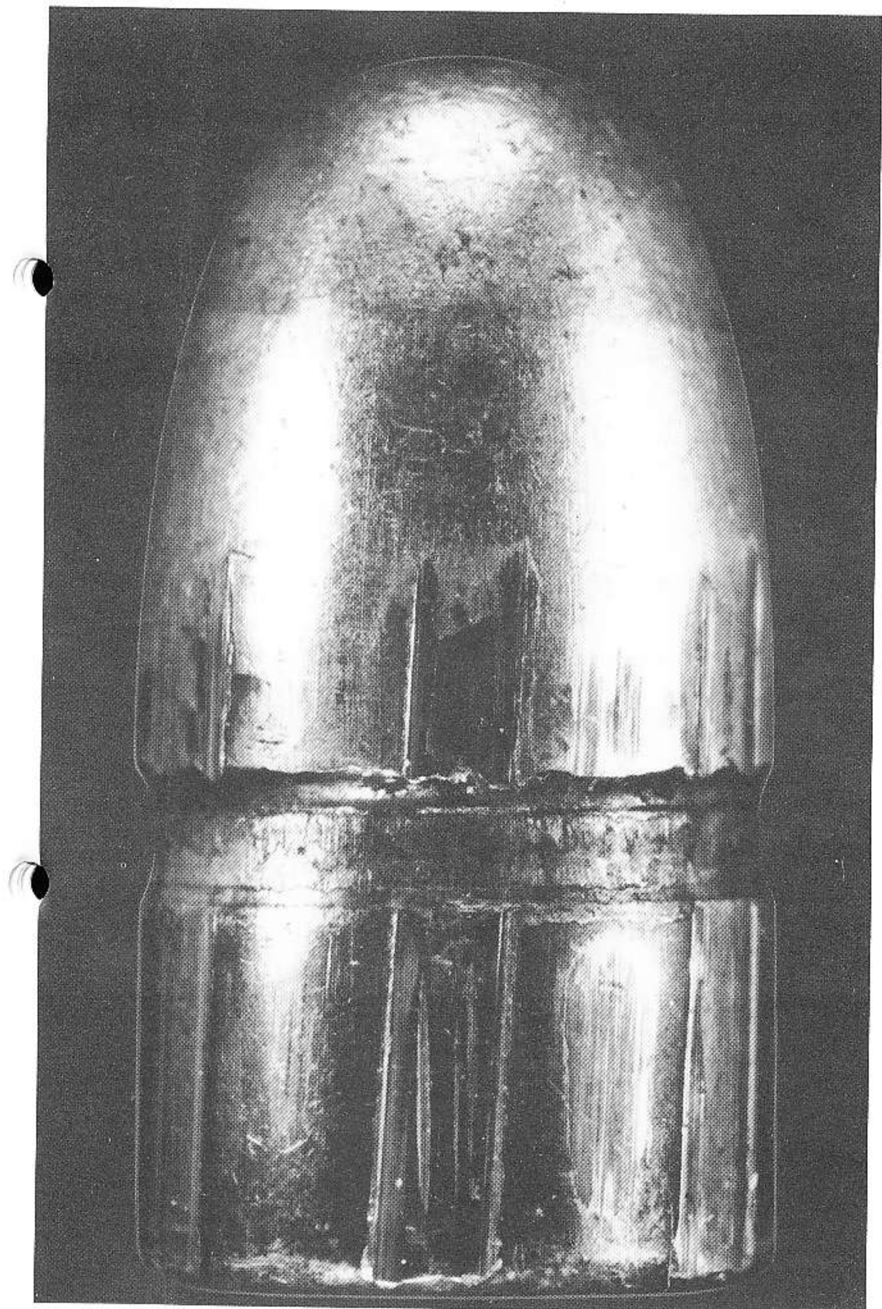


Fig. 43
Bullet photographed
with Tessovar
in incident light
darkfield
Magnification 2.5x,
subsequently
enlarged 7.5x

Table 8
Tessovar photomacrographic zoom system

Image scales

Turret position Free working distance	(black) 320 mm							(red) 150 mm				
35 mm camera attachment (engraved values) Magnification factor 1	0.4	0.5	0.63	0.8	1	1.25	1.6	0.8	1	1.25	1.6	2
Camera attachments C 120 and CR 120 Magnification factor 2.5	1	1.25	1.6	2	2.5	3.2	4	2	2.5	3.1	4	5
CP 100 camera attachment (Polaroid) C 912 (9 x 12; 4 x 5″) Magnification factor 3.2	1.25	1.6	2	2.5	3.2	4	5	2.5	3.2	4	5	6.4

Aperture ratio – effective stop

Aperture with stop 1		0.007	0.0075	0.0085	0.01	0.011	0.0125	0.014	0.014	0.015	0.017	0.019	0.022
35 mm camera attachment	stop 1	1 : 30		1 : 36		1 : 45		1 : 64	1 : 30		1 : 36		1 : 45
	2	42		50		64		90	42		50		64
	4	60		72		90		125	60		72		90
Camera attachment C 120 CR 120	stop 1	1 : 75		1 : 90		1 : 112		1 : 160	1 : 75		1 : 90		1 : 112
	2	105		125		150		225	105		125		150
	4	150		180		225		320	150		180		225
Camera attachments CP 100 (Polaroid) C 912 (9 x 12; 4 x 5″)	stop 1	1 : 90		1 : 112		1 : 150		1 : 180	1 : 90		1 : 112		1 : 150
	2	125		150		225		280	125		150		225
	4	180		225		300		400	180		225		300
Depth of field (mm)	stop 1	10.2		6.9		4.1		2.6	2.6		1.7		1
	2	20.4		13.8		8.2		5.2	5.2		3.4		2
	4	40.8		27.6		16.4		10.4	10.4		6.8		4

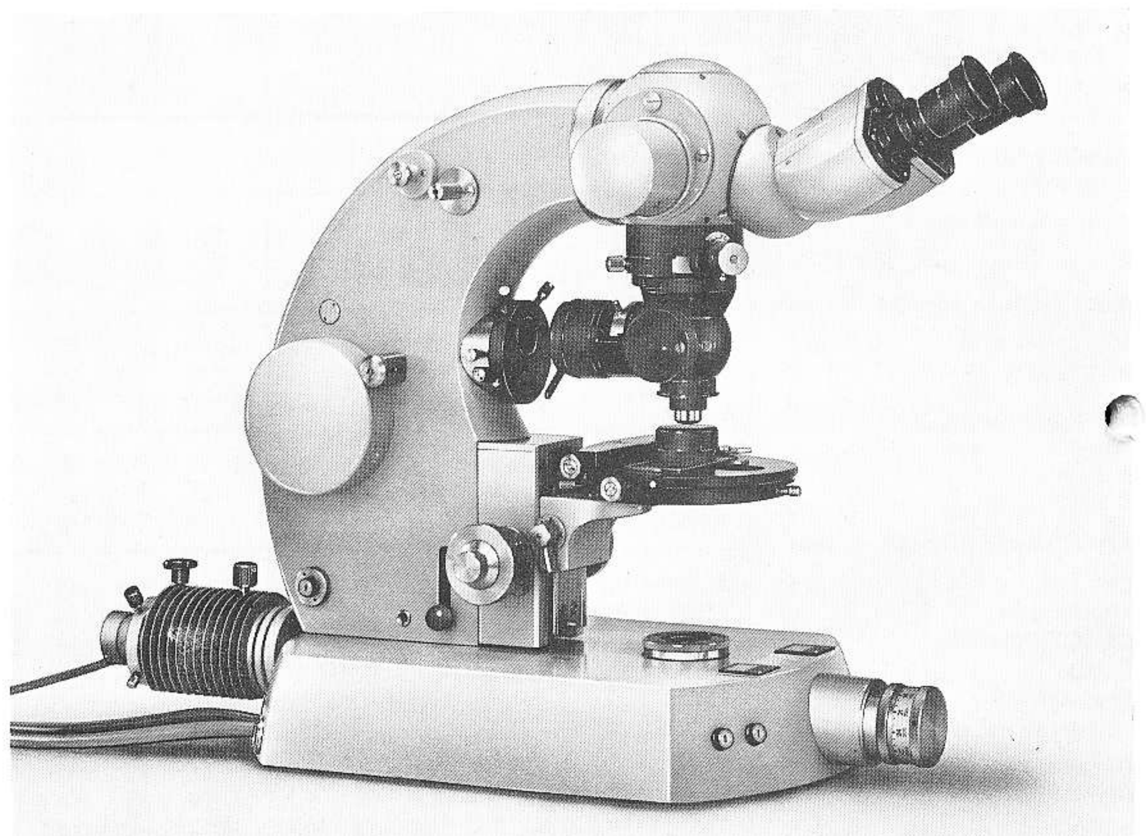
			(yellow) 75 mm							(light green) 36 mm						
2	2.5	3.2	1.6	2	2.5	3.2	4	5	6.4	3.2	4	5	6.4	8	10	12.8
5	6.3	8	4	5	6.3	8	10	12.5	16	8	10	12.5	16	20	25	32
6.4	8	10	5	6.4	8	10	12.8	16	20	10	12.8	16	20	25.6	32	40
0.022	0.025	0.028	0.028	0.031	0.034	0.039	0.044	0.050	0.056	0.056	0.054	0.068	0.068	0.088	0.100	0.110

: 45 64 90	1 : 64 90 125	1 : 30 42 60	1 : 36 50 72	1 : 45 64 90	1 : 64 90 125	1 : 30 42 60	1 : 36 50 72	1 : 45 64 90	1 : 64 90 125
: 112 150 225	1 : 160 225 320	1 : 75 105 150	1 : 90 125 180	1 : 112 150 225	1 : 160 225 320	1 : 75 105 150	1 : 90 125 180	1 : 112 150 225	1 : 160 225 320
: 150 225 300	1 : 180 280 400	1 : 90 125 180	1 : 112 150 225	1 : 150 225 300	1 : 180 280 400	1 : 90 125 180	1 : 112 150 225	1 : 150 225 300	1 : 180 280 400
1 2 4	0.64 1.28 2.56	0.64 1.28 2.56	0.43 0.86 1.72	0.26 0.52 1.04	0.16 0.32 0.64	0.16 0.32 0.64	0.11 0.22 0.44	0.06 0.12 0.24	0.04 0.08 0.16

Photomicroscope III

The Photomicroscope (Fig. 44) is a fully automatic photomicrographic system. Only the DIN or ASA number of the 35 mm film must be set; pushbutton-operated the automatic system opens the shutter, controls the exposure, closes the shutter and advances the film. Image scales between 10 : 1 and 630 : 1 are obtainable on 35 mm film. They result by multiplying the initial magnification of the microscope objective by the intermediate magnification of the Optovar magnification changer and the magnification of the camera projection lens with the factor 3.2.

Fig. 44
Photomicroscope III



In photographic position (beam path see diagram Fig. 45) a reticle is seen through the eyepiece, which indicates the object field of the microscopic image which is photographed. The microscopic image is focused with the reticle, and is used to check for which area of the object the exposure is measured. The exposure time is determined either by integral measurement of $^2/_3$ of the image format or as spot measurement in the center of the 35 mm format.
A 12 V 60 W filament lamp is recommended for incident light work with the Photomicroscope instead of the 6 V 15 W filament lamp which is sufficient for normal transmitted light work.

Fig. 45
Beam path in Photomicroscope III

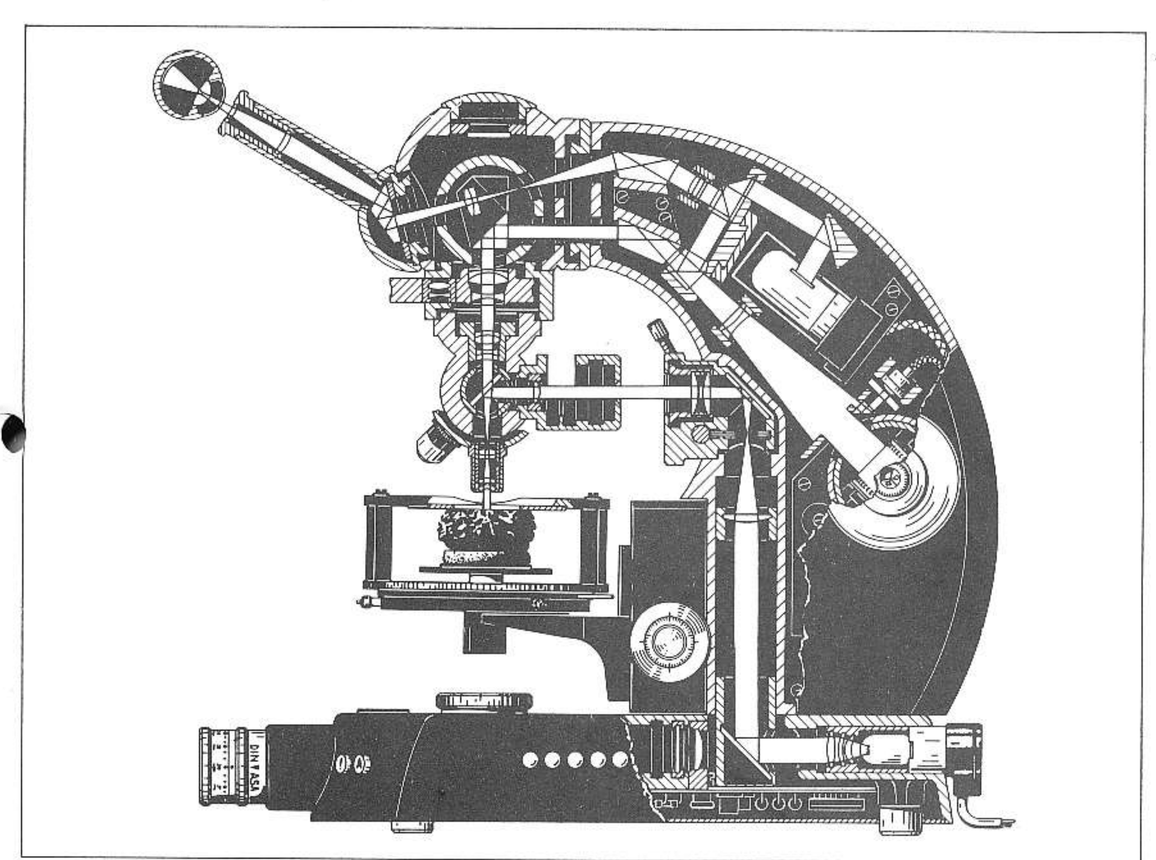


Fig. 46
Ultraphot III b camera microscope

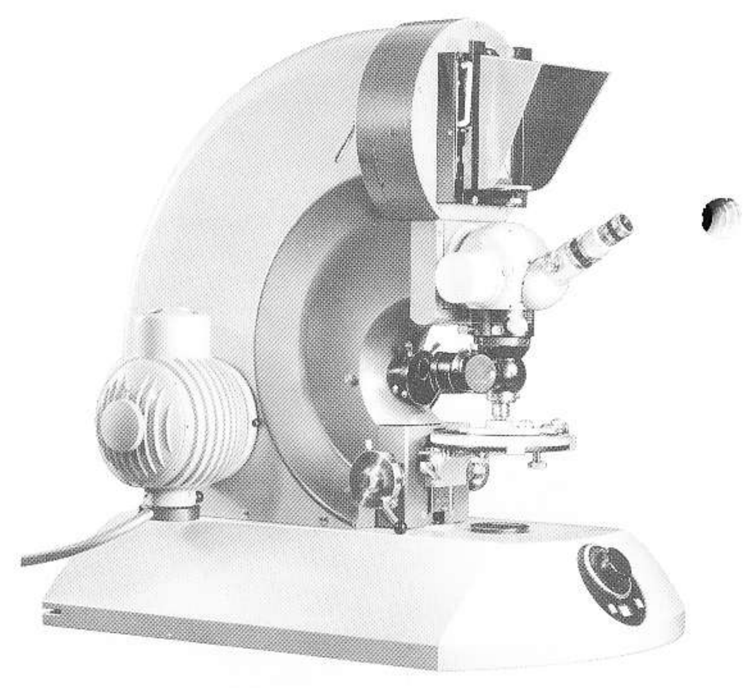
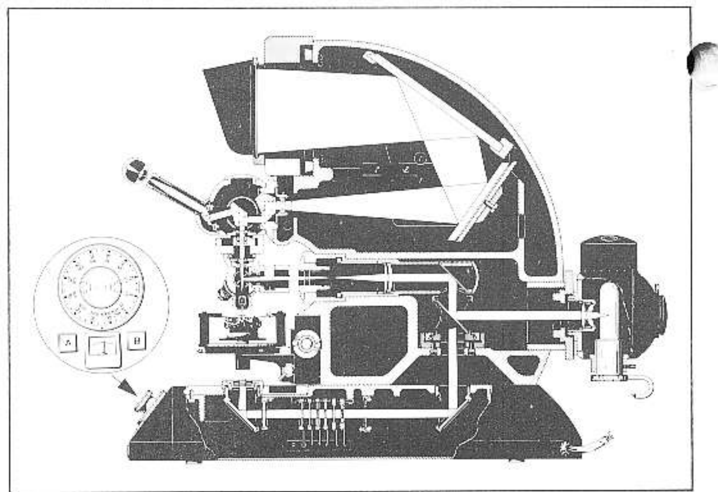


Fig. 47
Beam path in Ultraphot III b.
Switchover from incident to transmitted light illumination is possible.



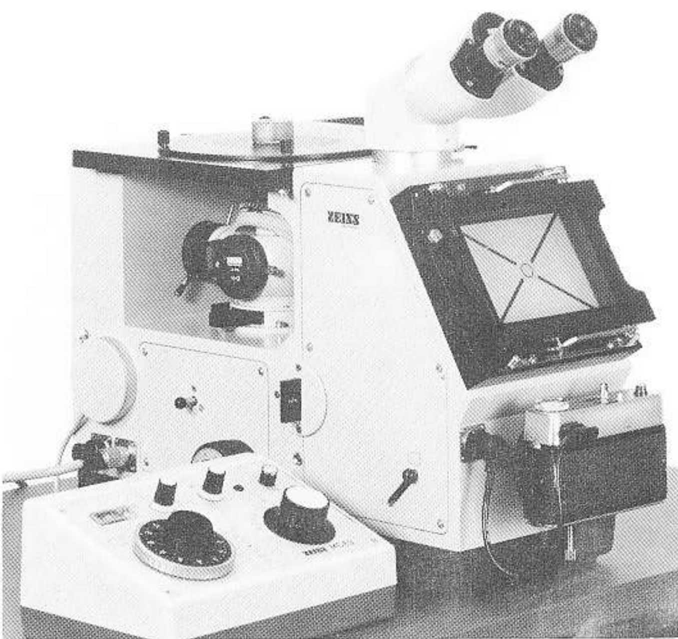
Ultraphot III b

Ultraphot III b (Fig. 46) is another specialized microscope for photomicrography. The compound microscope can be equipped with an automatic 35 mm camera or with an automatic camera for the 9 x 12 cm (4 x 5″) format. The 35 mm format lends itself for image scales between 10 : 1 and the feasible maximum of optical microscopes. When changing the objectives and the camera length, image scales of 32 : 1 and more are obtainable on the 9 x 12 cm format (Fig. 47).

Ultraphot III b can also be used as simple incident light microscope in that the real microscopic intermediate image is projected on 9 x 12 cm sheet film by means of the variable camera length using special photomicrographic Luminar objectives.

Luminar objectives are recommended for image scales of 6.8 : 1 to about 50 : 1 for incident light darkfield and incident light brightfield photomicrography. The image quality of these objectives is superior to that of a compound microscope (objective and eyepiece) for the abovementioned image scale range.

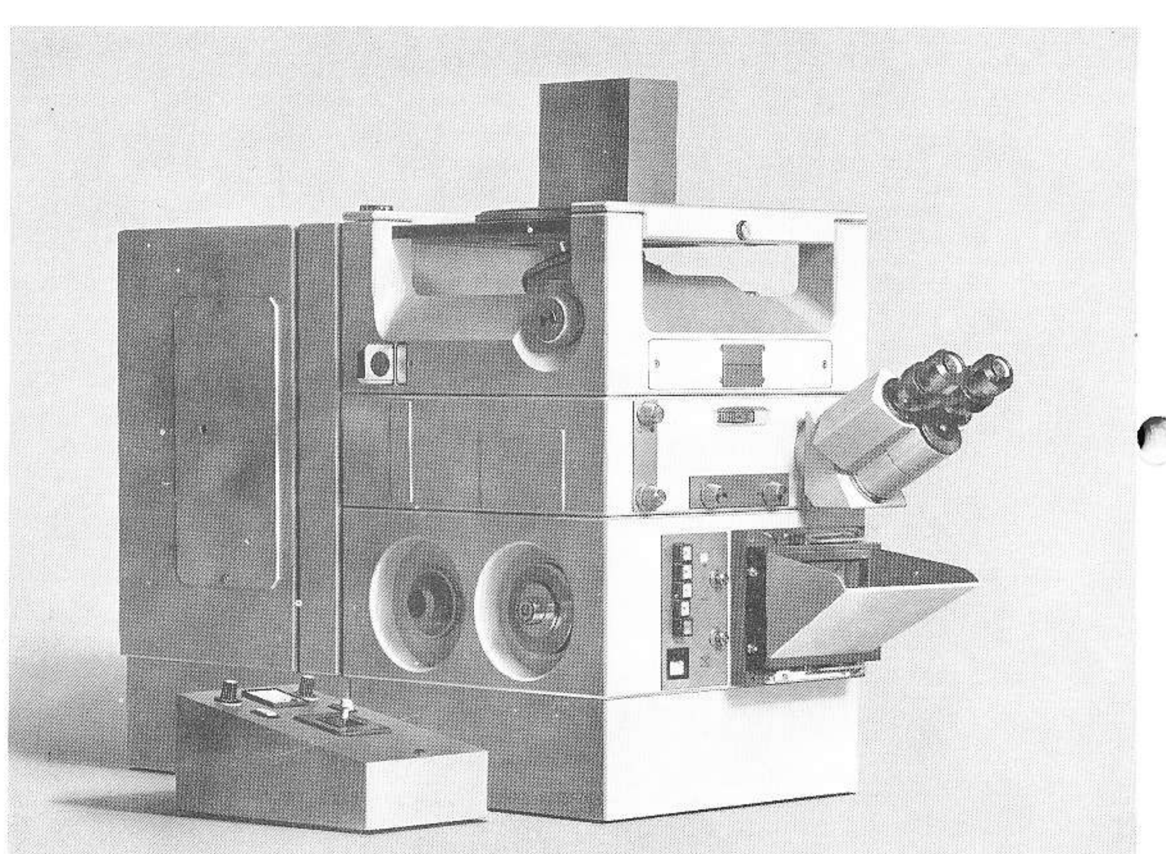
Fig. 48
ICM 405 inverted camera microscope



Zeiss Axiomat

The Zeiss Axiomat evolved from a completely new mechanical and optical design concept. There are two Axiomat versions for incident light examinations including documentation: Zeiss Axiomat IAC (Fig. 49) and Zeiss Axiomat NAC (Fig. 50).
These microscopes are of outstanding mechanical stability, because the central mechanical axis of the microscope coincides with the optical axis.
Fig. 51 shows the individual modules of the Zeiss Axiomat IAC, the inverted Axiomat version for incident light microscopy with camera.

Fig. 49
Zeiss Axiomat IAC



In the IAC version of the Zeiss Axiomat the object is placed above the objective, an arrangement corresponding to an instrument built according to the Le Chatelier principle.
A zoom system in the observation module has magnification factors between 0.8 and 3.2.
The optically feasible total magnification of the Zeiss Axiomat through the binocular tube lies between 10x and 1600x. The automatic 35 mm and large format cameras are included in the camera module.

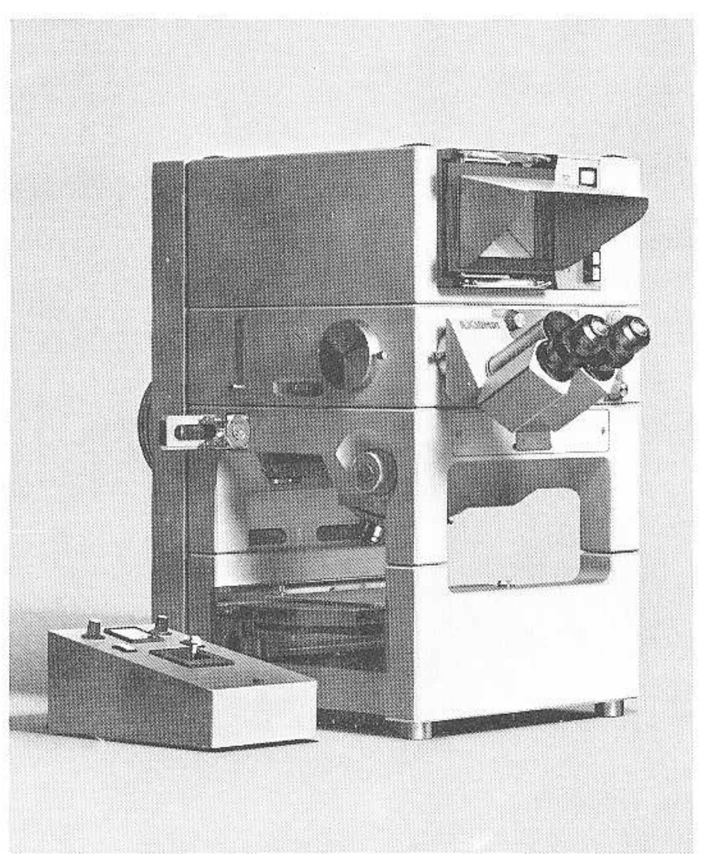


Fig. 50
Zeiss Axiomat NAC

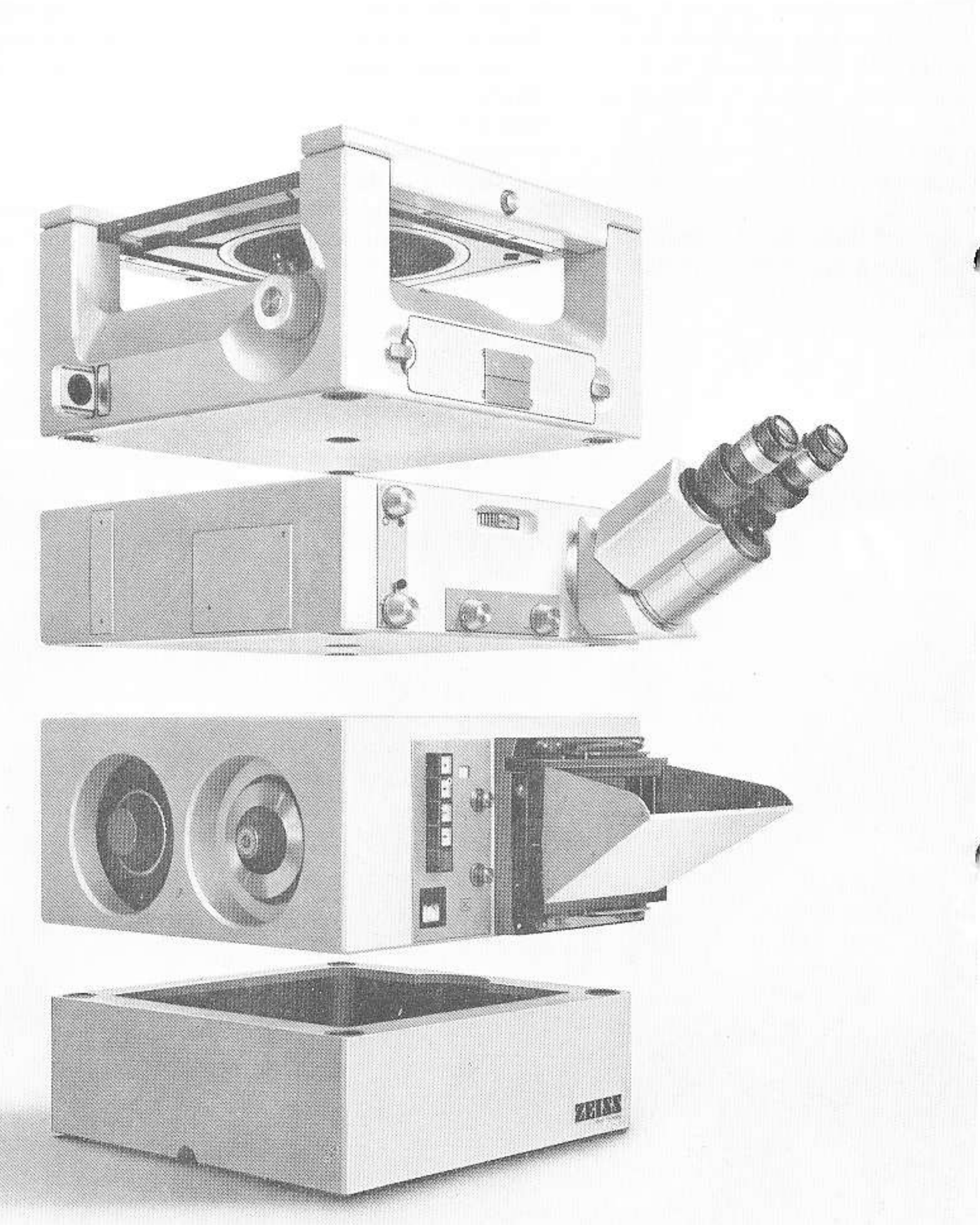


Fig. 51
Zeiss Axiomat IAC and its modules from top to bottom:
stage carrier U
objective module
observation module
camera module
socket U

The newly computed optics is of superior quality. The objectives for medium and high initial magnifications are planapochromatically corrected (Table 9). Furthermore, the maximum field of view number of the eyepieces is 25; which means an optical wide-field system is actually available. Compared with optical systems of conventional incident light microscopes the object fields covered by these eyepieces are larger by about 25%.

Table 9
Incident light objectives
for Zeiss Axiomat NAC and IAC

Objective	Working distance (mm)
Epiplan 5/0.12 HD	8.5
Epiplan 10/0.25 HD	2.5
Epi-Planapochromat 25/0.65 HD	0.5
Epi-Planapochromat 50/0.95 HD	0.18
Epi-Planapochromat 100/1.3 HD oil	0.18

Fig. 52 shows the beam path in the observation module. The wave fronts emitted from the object O are directed by the zoom system partly to a binocular tube, partly to the camera.
Besides for qualitative evaluation and documentation of microscopic objects, Zeiss Axiomat microscopes can be used for quantitative microscopy.

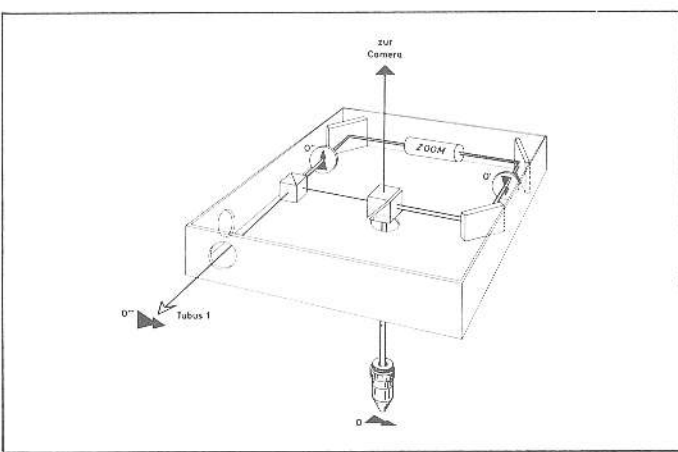


Fig. 52
Beam path in the observation module

Lit. 1 Handbuch der Mikroskopie in der Technik,
Umschau-Verlag, Frankfurt/Main,
Vol. I, part 2, pp. 187 and 443 (1960)
Vol. III, part 1, pp. 37 and 109 (1968)

Lit. 2 Handbuch der Mikroskopie in der Technik,
Umschau-Verlag Frankfurt/Main,
Vol. III, part 1, pp. 90 and 147 (1968)
Vol. III, part 1, p. 17

Lit. 3 Catalogs and operating manuals of
Carl Zeiss, Oberkochen, West Germany

Lit. 4 Optical Systems for the Microscope,
Carl Zeiss, Oberkochen, West Germany

Lit. 5 Anders, H.,
Dünne Schichten für die Optik,
Wissenschaftliche Verlagsgesellschaft Stuttgart (1965)

Lit. 6 Michel, K.,
Die wissenschaftliche und angewandte Photographie,
Vol. X,
Springer-Verlag (1967), pp. 377–384

Lit. 7 Michel K.,
Die Grundlage der Theorie des Mikroskops,
Wissenschaftliche Verlagsgesellschaft Stuttgart (1964),
pp. 248–288

Lit. 8 Michel, K.,
Die Grundzüge der Theorie des Mikroskops,
Wissenschaftliche Verlagsgesellschaft Stuttgart (1964),
pp. 158–164

Lit. 9 Michel, K.,
Die wissenschaftliche und angewandte Photographie,
Vol. X,
Springer-Verlag (1967), p. 98

Lit. 10

Handbuch der Mikroskopie in der Technik,
Umschau-Verlag, Frankfurt/Main (1968),
Vol. III, part 1, p. 187

Peperhoff W. and H.-H. Ettwig,
Interferenzschichten-Mikroskopie,
Steinkopff-Verlag, Darmstadt (1970)

● **Lit. 11**

Kontraststeigerung in der Auflicht-Mikroskopie,
Zeiss Information 66, **15** (1967), pp. 118–123

Lit. 12

Bartz, G.,
Zur Kontrastierung mikroskopischer Objekte in einer
Gasionenreaktionskammer,
Metallographie 10 (1973),
Leitz-Mitteilungen, special issue (1973)

Lit. 13

Michel, K.,
Die wissenschaftliche und angewandte Photographie,
Vol. X,
Springer-Verlag (1967), pp. 406 and 417

Handbuch der Mikroskopie in der Technik,
Umschau-Verlag, Frankfurt/Main, (1969),
Vol. III, part 2, p. 33

● **Lit. 14**

Publications by Carl Zeiss, Oberkochen, West Germany
Zeiss Information 70, **16** (1968), pp. 114–120
Zeiss Information 71, **17** (1969), pp. 12–16
Reprints S 41–210.4 and S 41–210.5 (1970), out of print

Handbuch der Mikroskopie in der Technik,
Umschau-Verlag, Frankfurt/Main (1969),
Vol. III, part 2, pp. 51–53

Lit. 15

Handbuch der Mikroskopie in der Technik,
Umschau-Verlag, Frankfurt/Main,
Vol. I, part 2, pp. 70–128 and 152–159 (1960),
Vol. III, part 2, pp. 39–50 (1969)

Lit. 16

Stach, E.,
Lehrbuch der Kohlenmikroskopie,
Verlag Glückauf, Kettwig (1949), pp. 111–113

Handbuch der Mikroskopie in der Technik,
Umschau-Verlag, Frankfurt/Main,
Vol. II, part 1, pp. 542–545 (1952),
Vol. I, part 2, p. 155 (1960)

Heyse, E.,
Über Anwendung von polarisiertem Licht zur Erzielung von Reflexfreiheit bei Auflichtbeleuchtung,
reprinted from Zeiss-Nachrichten (1934), vol. 7, pp. 3–7

Lit. 17

Heyse, E.,
Über Anwendung von polarisiertem Licht zur Erzielung von Reflexfreiheit bei Auflichtbeleuchtung,
reprinted from Zeiss-Nachrichten (1934), Vol. 7, pp. 3–7

Lit. 18

Michel K.,
Die wissenschaftliche und angewandte Photographie,
Vol. X,
Springer-Verlag (1967), pp. 127–135

Lit. 19

Carl Zeiss, Oberkochen, West Germany,
catalogs and operating manuals for
Photomicrographic cameras
Photomicroscope III
Ultraphot III b
Axiomat
Tessovar

Carl Zeiss
D-7082 Oberkochen
West Germany

K 41-003-e

Printed in West Germany

AW VIII/80 Noo