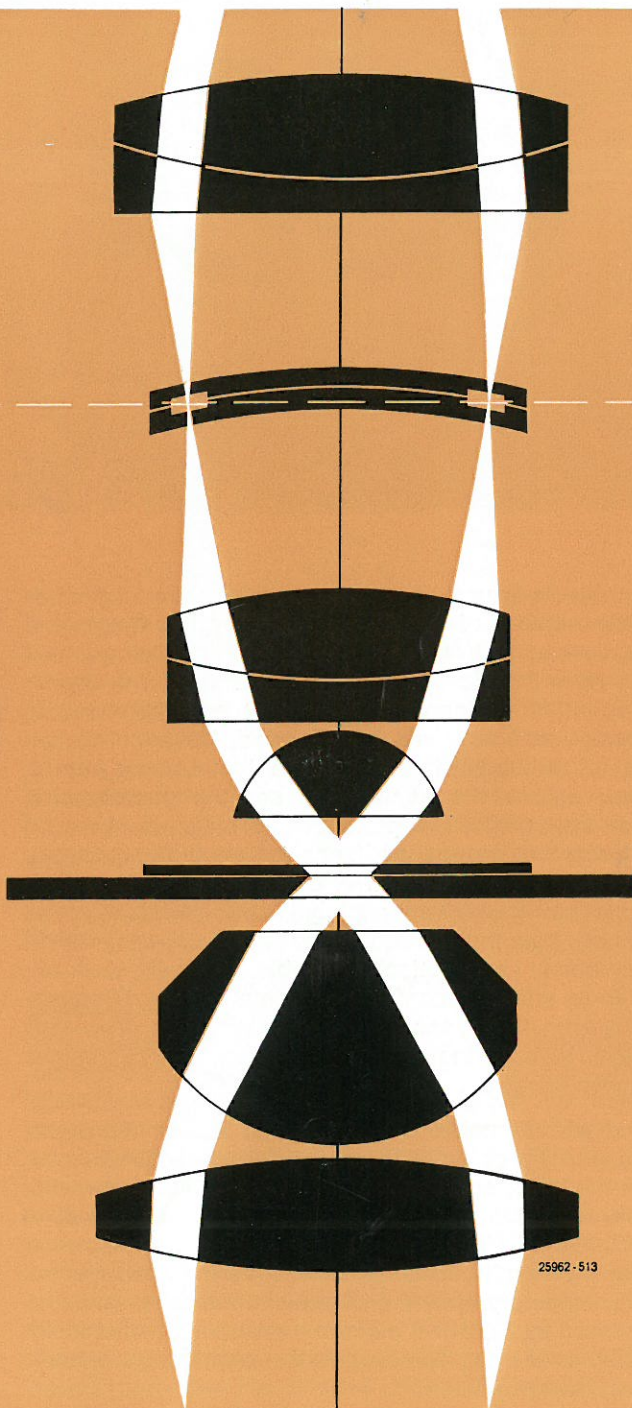


Phase Contrast Equipment



25962 - 513



25963 - 940

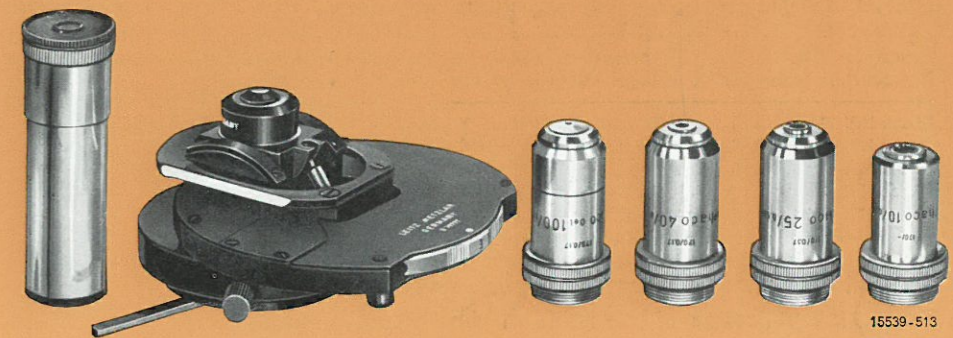
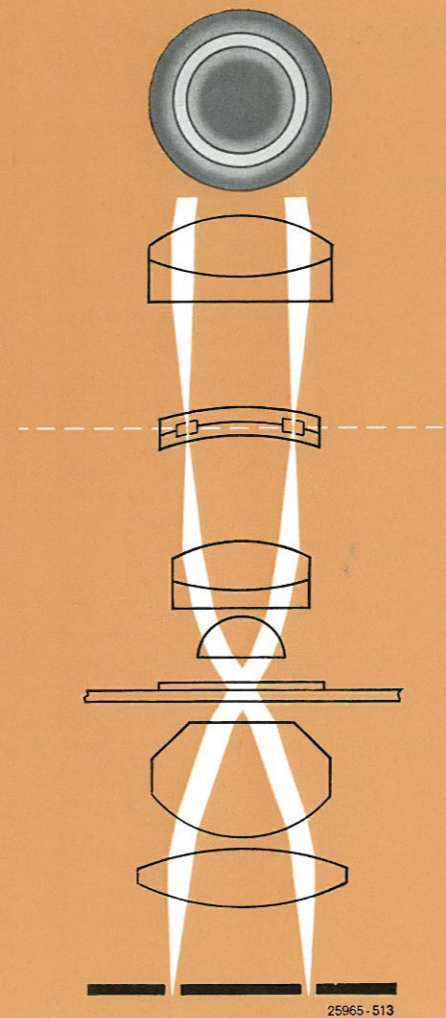


Fibroblasts in phase contrast. Objective: Phaco 40/0.75

Phase contrast microscopy

If living objects or unstained sections are observed according to the methods of classical microscopy, the images will often appear almost empty and structureless. Cell and cell nucleus have practically the same transmission in the visible region of the spectrum, so that no differences in brightness and colour can be observed. Nevertheless, an „image“ of the object is latent in the light coming from it. However, neither the human eye nor the photographic emulsion are suitable receptors for this image. Both record only objects that change the intensity (amplitude) or colour of the image-forming rays, i. e. objects that absorb light. Objects differing only very slightly in their refractive index or thickness from their surroundings (phase objects) remain optically blank, i.e. practically invisible. Naturally, such objects can be stained in order to obtain a certain differentiation, however, fixing, tanning, and staining are processes which even with the most careful treatment involve changes in the morphology of the object.

There is an alternative method, which avoids this interference with the object with its well-known disadvantages. It alters the light beam in the microscope in such a way that the contrast of the image is increased. Every microscopist is sufficiently familiar with darkground and oblique illumination, but the results achieved with these methods are not always satisfactory. Only the introduction of the phase contrast method by ZERNIKE made the observation of unstained as well as of living objects possible producing a hitherto unknown clarity of detail.



Phase contrast equipment

according to Zernike

The equipment includes:
Achromatic phase contrast condenser No. 402a, A 0.90
Phaco objectives or
Phaco NPI phase contrast plan-achromats
Auxiliary (focusing) magnifier
2 centring keys.

With this outfits objects can be examined in phase contrast, brightfield, and darkground. Since each objective calls for a certain position of the annular-stop turret in the condenser, adjustment to the appropriate light ring immediately produces exactly adjusted illumination. The situation is easily controlled and reproducible at any time. The object is observed either in phase contrast, brightfield, or darkground. The widths of the annular stops and phase rings in this outfit have been chosen so that even fairly thick objects are reproduced at good contrast and with a narrow halo. This makes the equipment suitable also for routine investigations in the haematological, urological, and pathological laboratory.

Phase contrast condensers

The phase contrast condenser No. 402a corresponds to ZERNIKE's basic conception. A turret in the condenser contains a number of annular stops which are matched to the phase ring in the appropriate objective. Three annular stops for phase contrast illumination, one for darkground illumination with the 40/0.65 or other objectives up to 63/0.85, and two empty apertures, one for brightfield, are available. All annular stops can be individually centred. The condenser has two screws for centring the field diaphragm, of which it forms an image in the object plane (Köhler's Illumination).

Condensers of longer intercept distances are available for special purposes. For detailed information see table.

No.	Aperture	Designation and description	Focal length mm	Intercept distance above object stage mm	Use
402a	0.90	achromatic phase contrast condenser with bottom 400a and top 002	10	—	phase contrast brightfield with 10x – 100x objectives darkground with 10x – 40/0.65
403c	1.25	achromatic-aplanatic condenser with bottom 400c and top Apl. Oel 003	8.1	—	phase contrast with 40x – 100x Phaco objectives
405e	0.70	achromatized phase contrast condenser with bottom 400e and top 005	13	4	phase contrast with all Phaco objectives
406f	0.60	non-achromatized phase contrast condenser with bottom 400f and top 006	17	11	phase contrast with low- and medium-power Phaco objectives 10x – 40x
407g	0.45	non-achromatized phase contrast condenser with bottom 400g and top 007	20	20	phase contrast with low- and medium-power Phaco objectives 10x – 40x
408h	—	non-achromatized phase contrast condenser with bottom 400h and top 008	27.6	35	Phase contrast with low- and medium-power Phaco objectives 10x – 40x

Phase contrast objectives

The phase contrast objectives (abbreviated Phaco) are achromats of novel design, of 45mm mechanical length. The permanently built-in phase ring is located in their rear focal plane; its absorption has been determined at $75 \pm 5\%$ for positive phase contrast. Phaco phase contrast objectives are designed mainly for use on the LEITZ HM-LUX and SM-LUX microscopes.

Engraved: Reproduction scale/aperture	Free working distance mm	Focal length mm	Coverglass correction	Type of eyepiece	
Phaco 10	0.25	7.6	17	DO	P
Phaco 25	0.50	0.44	7.1	D	P
Phaco 40	0.65	0.08	4.6	D	P
Phaco Oel 100	1.30	0.08	1.9	D	P

D = to be used with coverglass, DO = with or without coverglass
P = to be used with PERIPLAN® eyepieces

Phaco-NPI phase contrast plan-achromats

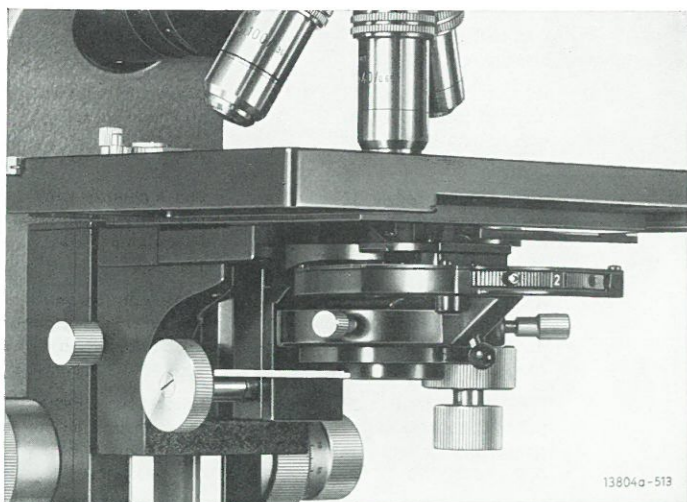
These objectives are corrected for corner-to-corner flattening of the field in eyepieces of field-of-view indices of up to 18. They are therefore particularly suited for phase contrast investigations with the LEITZ SM-LUX, DIALUX® and ORTHOLUX® microscopes and for photomicrography.

Engraved: Reproduction scale/aperture	Free working distance mm	Focal length mm	Coverglass correction	Type of eyepiece	
Phaco NPI 10	0.25	0.53	16	DO	P
Phaco NPI 16	0.40	0.50	11	D	P
Phaco NPI 25	0.50	0.33	7.0	D	P
Phaco NPI 40	0.65	0.15	4.5	D	P
Phaco NPI Oel 100	1.30	0.26	1.7	D	P

PI Apo Phaco phase contrast plan-apochromats

Permit full utilization of the fields of view of up to 28mm dia., which can be achieved only on the ORTHOPLAN® microscope with the GW widefield eyepieces, at extreme resolving power and definition to the very edge of the microscopic image.

Engraved: Reproduction scale/aperture	Free working distance mm	Focal length mm	Coverglass correction	Type of Eyepiece	
PI Apo 16	0.40 Phaco	1.19	10.5	D	P
PI Apo 25	0.65 Phaco	0.65	7.24	D	P
PI Apo 40	0.75 Phaco	0.51	3.98	D	P
PI Apo 100 Oel	1.32 Phaco	0.25	1.7	D	P
PI Apo 40	100 Oel	0.20	4.4	D	P
	Phaco				
PI Apo 63	0.95 Korr.	0.14	2.0	D	P
	Phaco				



Phase contrast objectives for special investigations

For phase contrast observations of living organisms in water at high power several immersion objectives are available. In the table below the following abbreviations are used: W = water immersion, SW = Immersion in salt water (sea water) or in physiological saline solution.

The letter L indicates "long working distance". These objectives are used, for instance, for phase contrast examinations on the LEITZ DIAVERT® inverted microscope.

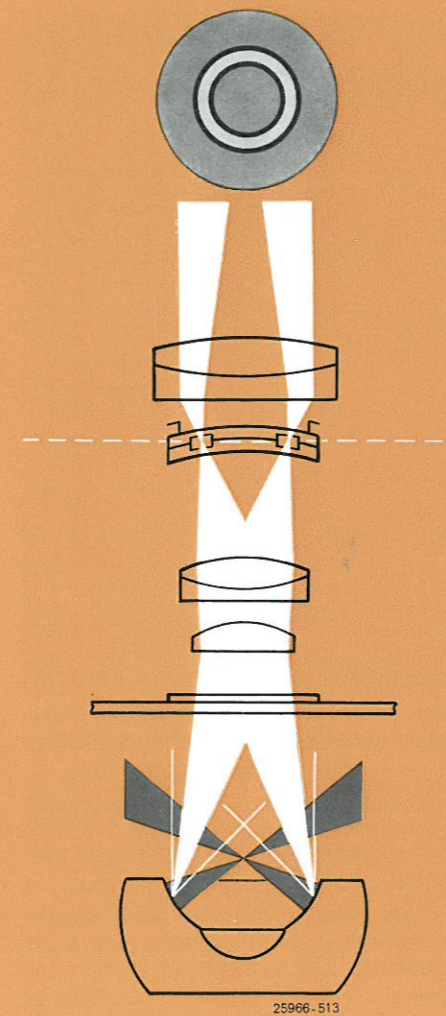
Engraved: Reproduction scale/aperture	Free working distance mm	Focal length mm	Coverglass correction	Type of eyepiece	
Phaco 100	1.20 W	0.18	1.7	D	P
Phaco 25	0.60 SW	1.67	7.1	O	P
Phaco 100	1.20 SW	0.22	1.7	O	P
Phaco L20	0.32	6.73	8.7	DO	P
Phaco L32	0.40	6.45	5.2	DO	P

Phase contrast fluorescence with incident-light excitation

Our PLOEMOPAK fluorescence vertical illuminator offers a possibility of combining phase contrast with fluorescence. Fluorescence is excited and observed in incident light. The phase contrast condenser is used in transmitted light. With this combination very differentiated and brilliant images are obtained. Our ORTHOPLAN and ORTHOLUX microscopes are suitable for this method.

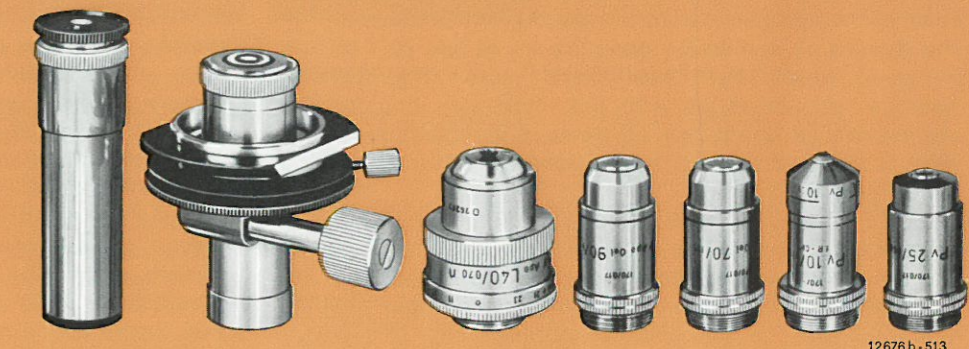
Phase contrast equipment

with condenser according to Heine



The equipment includes:

Phase contrast condenser after Heine No. 64 or 63
Choice of achromatic or apochromatic Pv phase contrast objective
Auxiliary (focusing) magnifier



This equipment offers the possibility of observing objects in phase contrast, brightfield, and darkground, with continuous transition between these types of illumination. It can be used with advantage whenever

- thin sections from the major proportion of objects to be examined,
- a continuous and rapid transition between phase contrast, brightfield, and darkground is demanded, for instance with fine-grain dressing products, dust samples etc.

Condenser according to Heine

With the Heine condenser, the wide-open hollow light cone of the "brightfield beam" emerging from the mirror component illuminates the object. The objective cuts a narrow hollow cone out this beam, concentrating it in a light ring near its rear focal plane. With the mirror component in its lowest position the diameter of this light ring is smaller than that of the phase ring. The direct light bypasses the phase ring undisturbed: observation takes place in brightfield. When the mirror component is raised with the control of the Heine condenser the narrow hollow light cone accepted by the objective opens up like a pair of scissors, increasing the diameter of the light ring. When light ring and phase ring coincide, the object is seen in phase contrast. When the light ring becomes larger than the phase ring brightfield is re-established, with concentric oblique illumination but at a larger aperture. Finally darkground is obtained when the hollow light cone cut out of the brightfield beam is intercepted by the aperture diaphragm of the objective. Normal darkground illumination exists when the mirror component is in its highest position.



Phase contrast equipment on the ORTHOPLAN microscope

Objectives for positive phase contrast

All objectives for positive phase contrast are supplied with a phase ring of normal absorption $75 \pm 5\%$ (designation "n").

The achromat Pv 10/0.25 n serves as a scanning objective. With the mirror component in its lowest position it already produces a phase contrast image, which turns into a dark-ground image when the mirror component is raised. The immersion attachment of this objective is used if it is necessary to work alternately with an oil immersion objective, when the scanning objective with immersion cap serves for the selection of suitable areas in the object. Since both objectives are matched, a change-over to the oil immersion objective is possible immediately after the relevant object area has been selected.

The achromat Pv 25/0.50 n is particularly suitable for scanning in the three different types of illumination and their transitions.

The apochromats Pv Apo L 40/0.70 n and Pv Apo L 63/0.70 n have a correction mount. Both objectives have been computed for a long free working distance, which permits the use of thicker coverglass, customary, for instance, with tissue cultures. These objectives are therefore particularly popular with cytologists, bacteriologists, and histologists.

The fluorite oil immersion objective Pv FI Oel 70/1.15 n is preferred mainly for observations at a long working distance. In connection with the immersion cap for the phase contrast condenser all types of illumination, bright-field, phase contrast, and darkground with their transitions can be employed.

The Pv FI Oel 70/1.15 n is the only oil immersion objective suitable for use in the tropics.

The apochromatic oil immersion objective Pv Apo Oel 90/1.32 n is provided for the demonstration of most delicate structures such as flagellae, membranes etc. This objective, too, with the immersion cap mounted on the condenser in its top position, produces darkground, particularly useful in orientating observation.

Unless stated otherwise, we supply objectives for positive phase contrast (designation "n"). However, on request the objectives 10/0.25 to Pv FI Oel 70/1.15 can be supplied for intensified positive phase contrast (designation "h") or for negative phase contrast (designation "-h"). The table below lists the available Pv objectives.

Description of phase contrast microscopy

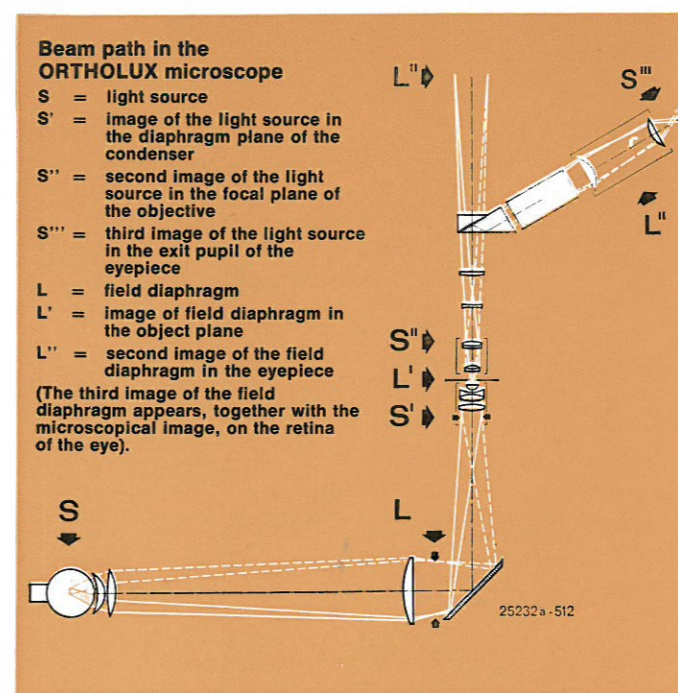
Other phase contrast objectives

Extensive structures when observed in phase contrast show a halo effect. Thus, the edge of a more highly-refracting structure is surrounded by a bright external and a dark internal halo in positive phase contrast; in negative phase contrast the situation is reversed. If the edge of a phase structure is rounded, the boundary between the bright and dark halo no longer outlines that of the object, so that, for instance, the diameter of red blood corpuscles may appear 15% smaller than it is in reality. Minor details may even become submerged in the halo. A displacement of the contrasts inside and outside the object makes it possible to determine its real dimensions more accurately, or to render lost details visible again. For this purpose phase contrast objectives with a negative ring (designation "-h") are available. In addition these objectives have phase rings of increased absorption.

For objects whose refractive index differs very little from that of the surrounding field phase contrast objectives of higher absorption ($88 \pm 2\%$) also for positive phase contrast are supplied (designation "h").

Phase contrast objectives for the Heine condenser

Designation	Reproduction scale/aperture	Adjustment length mm	Coverglass correction	Type of eyepiece	Free working distance mm	Version of absorption		
						$75 \pm 5\%$	$88 \pm 2\%$	$88 \pm 2\%$
Dry system	PV 10/0.25	37	DO	P	5.4	n	h	—
	immersion attachment for PV 10/0.25	38.5			0.3	—	—	—
Dry system	Pv 25/0.50	37	D	H(P)	0.72	n	h	-h
Dry system of particularly long working distance	Pv Apo L 40/0.70	37	D!	P	0.33	n	h	-h
Dry system of particularly long working distance	Pv Apo L 63/0.70	37	D!	P	0.25	n	h	-h
Oil immersion	Pv FI Oel 70/1.15	38.5	DO	P	0.17	n	h	-h
	Pv WE 80/1.00	37	D	P	0.92	n		
Oil immersion	Pv Apo Oel 90/1.32	38.5	DO	P	0.09	n	h	—



Microscopical image formation in transmitted light

Let us consider the process of image formation in the microscope based on Köhler's Illumination. A lamp condenser is situated immediately in front of the light source S, usually combining with it to form the microscope lamp. The lamp condenser produces an image of the light source S in the front focal plane S' of the substage condenser, where the aperture diaphragm is also situated. Further images of the light source are formed by the condenser and the objective in the rear focal plane S'' of the latter, and finally in the exit pupil of the eyepiece S'''. All these planes are optically conjugated.

There is, however, a second system of optically conjugated planes: a reduced image of the aperture of the lamp condenser or of an iris diaphragm situated close to it, the field diaphragm L, is formed in the object L' by the condenser. The objective produces a magnified image of the object in the intermediate image plane L'', which in turn is viewed through the eyepiece at further magnification. We have thus two groups of optically conjugated planes at a regularly alternating sequence. The hitherto independent systems now have to be joined. In the interest of clarity of representation we are using a regular line grating which is transilluminated by parallel coherent light. According to the Laws of Geometrical Optics, an image S'' of the light source would be formed in the rear focal plane of the objective in the absence of an object. However, the grating laterally deflects part of the rays by diffraction, producing in the rear focal plane of the objective diffraction images, secondary to the principal image of the light source (because, according to Huygens, each slit of the grating is the centre of excitation of an elementary wave). The bottom right-hand illustration shows the diffraction pattern of the radically closed condenser diaphragm in the rear focal plane of the objective. The rays forming the principal image have directly passed through the objective according to the Laws of Geometrical Optics, whereas the secondary images are the result of the interference of the light diffracted by the object grating. Elementary waves again originate in the individual points of the diffraction images, and in turn produce, by interference in the intermediate image plane, the magnified image of the object — here the grating. The structure of the object is faithfully reproduced if all the diffracted light interferes. If part of the diffracted light is lost, the image will no longer be an accurate reproduction of the object. The minimum requirement of any image formation at all is that the principal image interferes with the two firstorder secondary images.

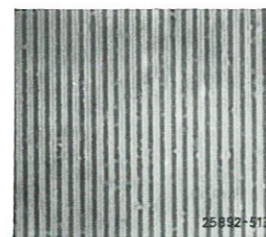
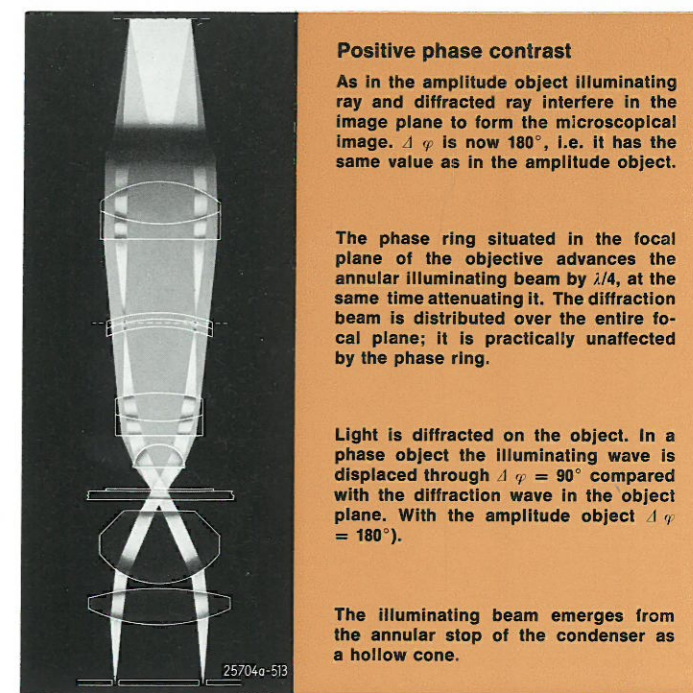


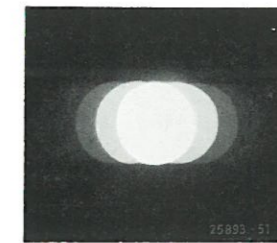
Image of the grid as seen in the eyepiece



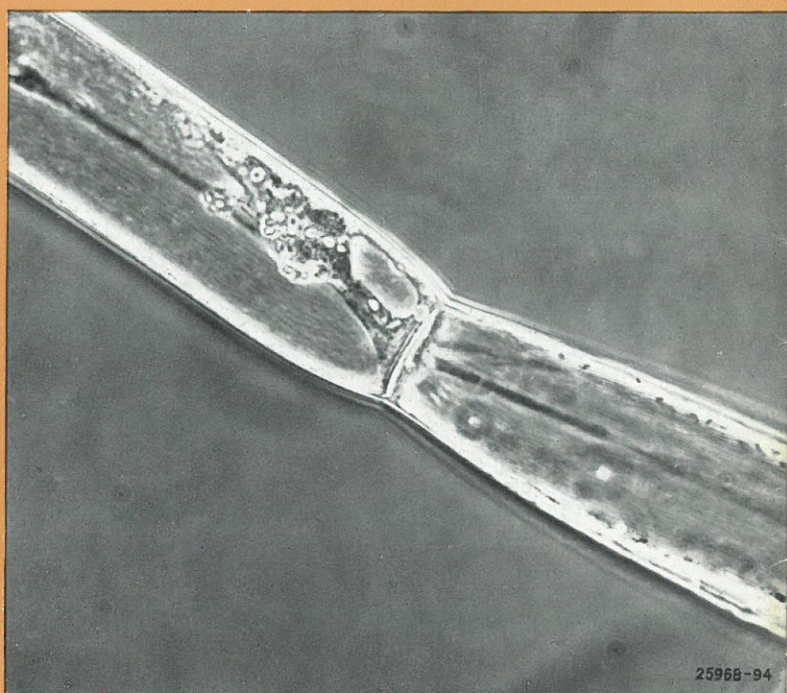
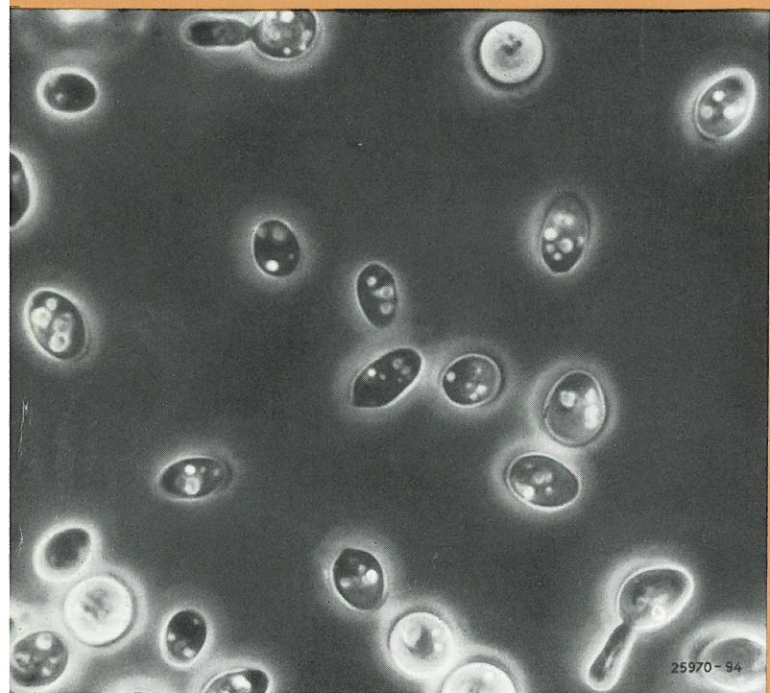
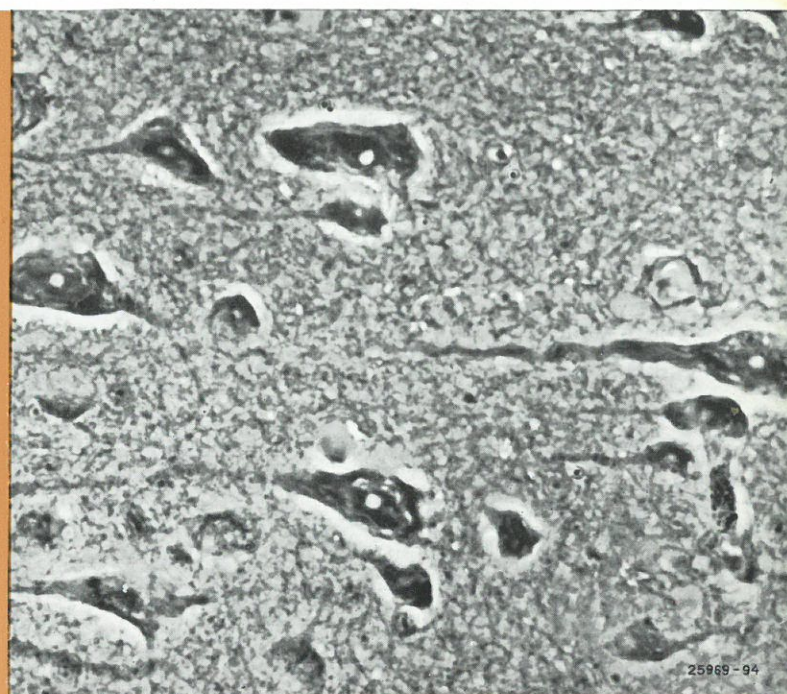
Microscopical image formation in phase contrast

Conditions are analogous with the pure phase object. Here, too, part of the light is diffracted by the object; here, too, illuminating rays and diffracted rays (principal image and secondary images) interfere to form an image which, like the object structure itself, is invisible. However, the diffraction waves of an amplitude object and those of a phase object differ from the illuminating wave only in their phases. This phase difference is $\lambda/2$ with an amplitude object, $\lambda/4$ with a phase object in the object plane and in the image plane. The Dutch physicist ZERNIKE concluded from this that all one had to do to cancel the difference between phase object and amplitude object, and thereby to make phase structures visible exactly like amplitude structures, was to alter the phase of the illuminating rays by $\lambda/4$.

For the practical realization of the phase contrast method ZERNIKE suggested influencing the illuminating beam by means of a phase plate in the rear focal plane of the objective. In practice, one uses phase rings of suitable thickness and absorption for this purpose. These phase rings can be chosen so that more highly refracting or thicker object parts appear darker (positive phase contrast) or lighter (negative phase contrast) than their surrounding in the picture. Such a phase ring, to be effective, requires an annular light source, which is produced by a stop inserted in the condenser. Obviously, the diameter and width of this annular stop influence the aperture of the condenser, which must have a certain relationship with the objective aperture. The phase contrast condenser therefore contains several annular stops conjugated with the objectives and arranged on a turret.



Diffraction pattern of the radically closed condenser diaphragm in the rear focal plane of the objective. The bright principal image is seen in the centre, with the secondary images on either side.



Top: Sperm cells in phase contrast, objective Phaco 100/1.30
Bottom: Brewer's yeast cells in darkground, objective
Pv Fl Oel 70/1.15 n

Top: Brain section in phase contrast, objective Phaco 40/0.65
Bottom: Hair of a commelinacea, objective Phaco 40/0.65

Design subject to alterations without notice.

® = Registered Trademark.

ERNST LEITZ GMBH D-6330 WETZLAR

Subsidiary: Ernst Leitz (Canada) Ltd., Midland, Ontario

List **513-5 e/Engl.**

Printed in W-Germany

XII/74/LX/L