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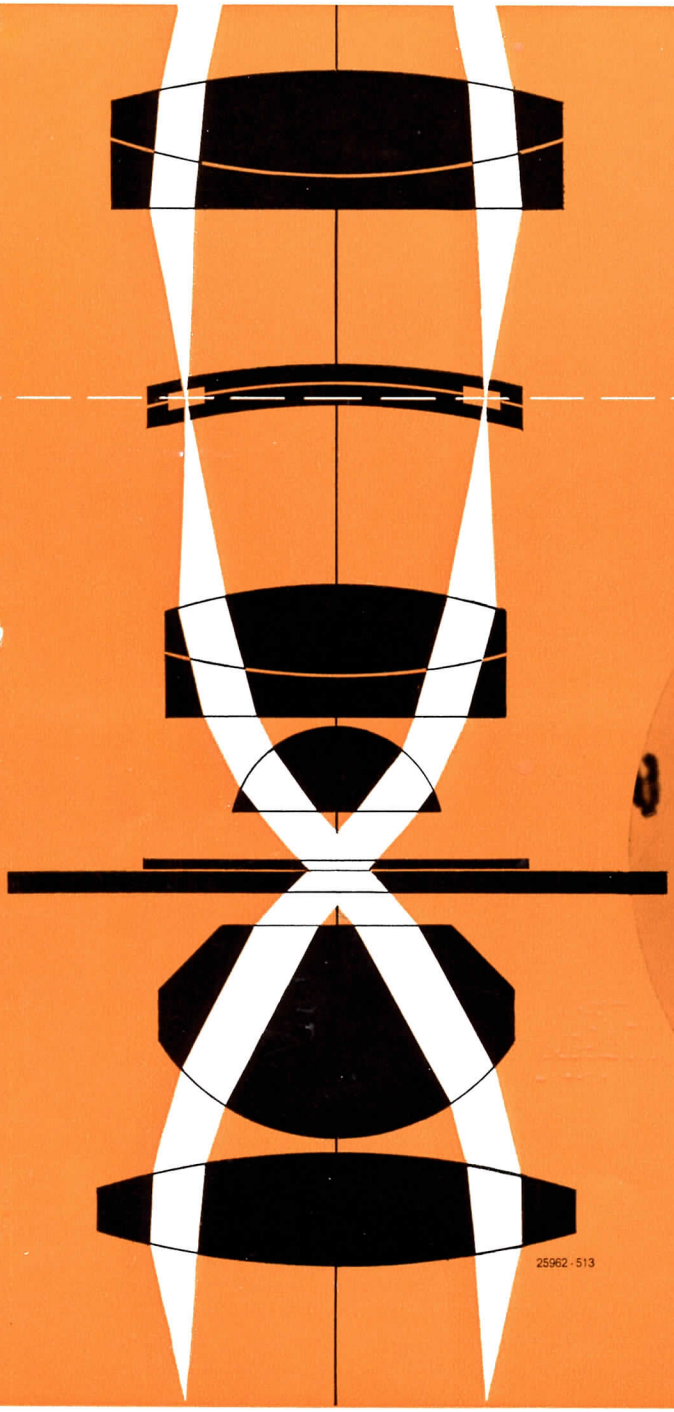

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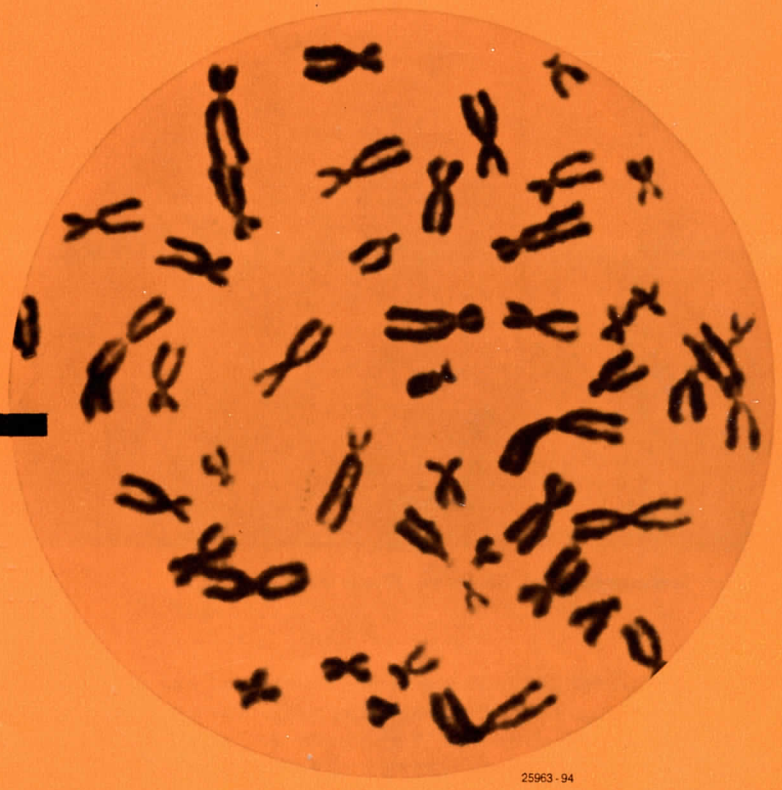

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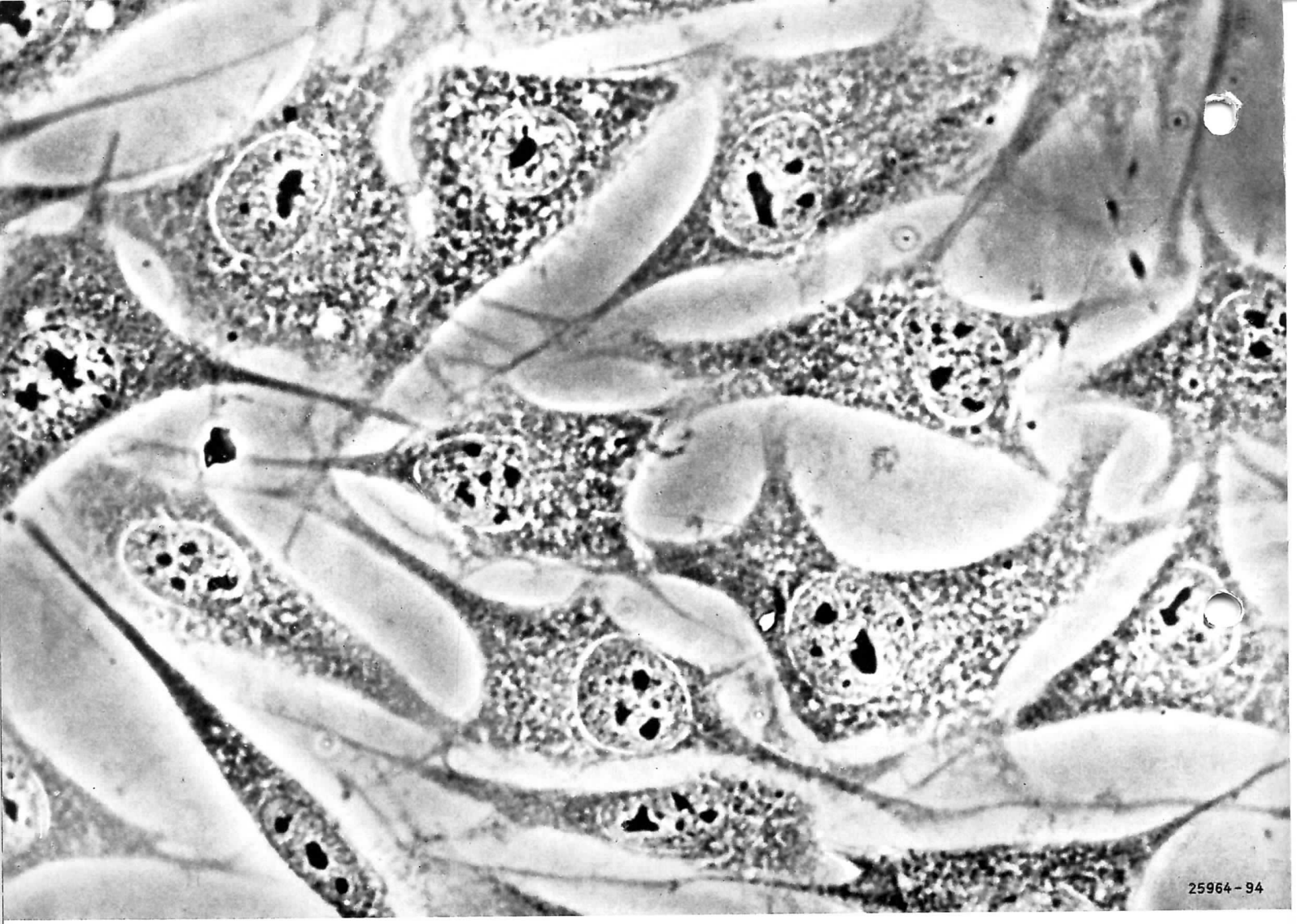
Phase contrast equipment



Fibroblasts in phase contrast. Objective: Phaco 40/0.75

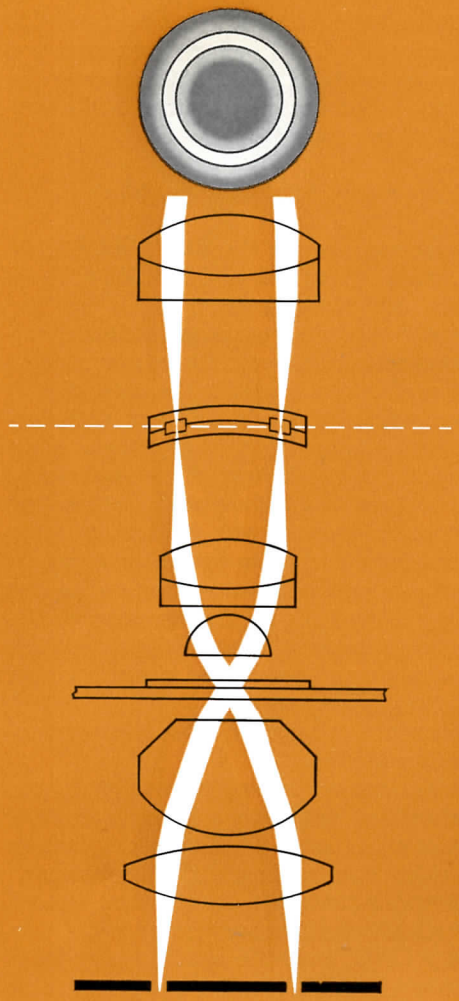
Phase contrast microscopy

If living objects or unstained sections are observed according to the methods of classical microscopy, the images will 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 empty, i. e. practically invisible. Naturally, such objects can be stained in order to obtain a certain differentation, 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 darkfield- 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

after Zernike



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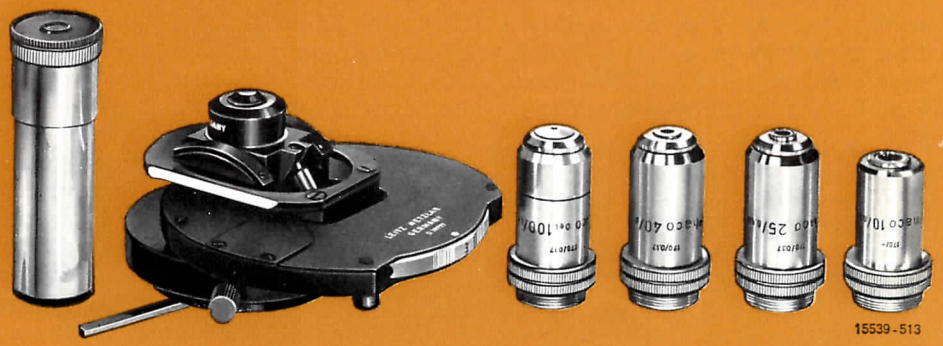
The equipment includes:
Achromatic phase contrast condenser No. 402a, A 0.90
Phaco objectives 10/0.25, 25/0.50, 40/0.65, Ol 100/1.30
or Phaco-NPl phase contrast plano-achromats
Auxiliary (focusing) magnifier
Centring key.



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With this outfit objects can be examined in phase contrast, brightfield, and darkfield. 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 darkfield. 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 it suitable also for routine investigations in the haematological, urological, and pathological laboratory.

Phase contrast condenser

The phase contrast condenser No. 402a (for LABORLUX: No. 402a L) corresponds to ZERNIKE'S basic conception. A turret in the condenser containes 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 darkfield 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.

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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 the

rear focal plane; its absorption has been determined at 75 ± 5% for positive phase contrast. The following objectives are available at present:

Phaco phase contrast objectives

Designation of objectives (Reproduction ratio/aperture)	Focal length mm	Free working distance mm	Coverglass correction	Type of eyepiece
Phaco 10/0.25	16.9	7.7	DO	P
Phaco 25/0.50	7.2	1.1	D	P
Phaco 40/0.65	4.7	0.71	D	P
Phaco Öl 100/1.30	1.9	0.17	D	P

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

Phaco-NPl phase contrast plano-achromats

For very difficult microscopical work and for photomicrography we recommend our phase contrast plano-achromats whose fields of view are flat from corner to corner and free from colour fringes. Their standard of definition, contrast, and resolution is equal to the demands even of very complex research problems. They are used with PERIPLAN® GF widefield eyepieces or ordinary PERIPLAN eyepieces on our stands with standard (23.2mm) tubes; on the ORTHOPLAN*, PERIPLAN widefield eyepieces GW can also be used.

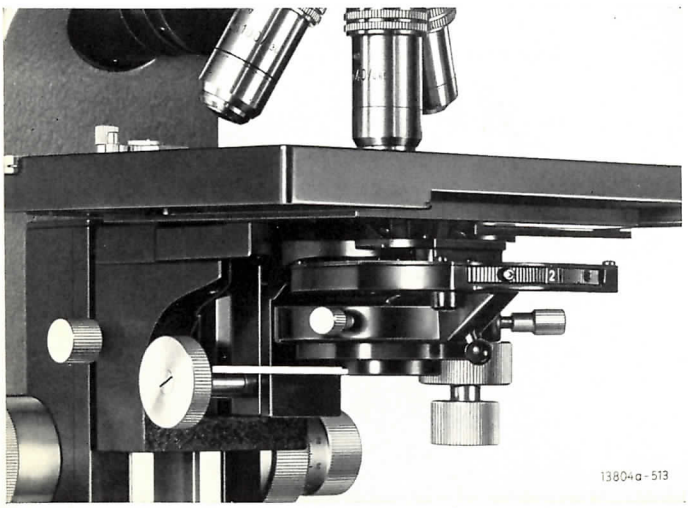
Designation of the objectives (reproduction ratio/aperture)	Focal length	Coverglass correction
Phaco-NPl 10/0.25	15.5	DO
Phaco-NPl 16/0.40	10.8	DO
Phaco-NPl 25/0.50	7.0	D
Phaco-NPl 40/0.65	4.5	D
Phaco-NPl Öl 100/1.30	1.7	D

* Details see List ORTHOPLAN

Combined phase contrast methods

Phase contrast fluorescence

The phase contrast fluorescence method combines the advantages of image reproduction by phase contrast with those of the fluorescence technique. A phase contrast image is superimposed on the fluorescent image so that the two images, which can be well differentiated by filtering, are simultaneously visible in the microscope. This combination calls for a mixture of ultra-violet or blue radiation with visible light in the microscope illuminator. Our Lamp Housing 250 meets this demand. A glass plate built into the mirror housing is used to produce mixed light, consisting of UV or blue radiation and an adjustable proportion of tungsten light necessary for fluorescence excitation and for the reproduction of the non-fluorescing portions in phase contrast.
The outfit consists of:
The phase contrast fluorescence condenser No. 402aa
the Phaco objectives
a modern LEITZ microscope such as the SM, LABORLUX®, ORTHOLUX® with Lamp Housing 250 or ORTHOPLAN® research microscope with Lamp Housing 250.
By and large the phase contrast fluorescence condenser corresponds with the condenser for phase contrast microscopy; however, the opaque annular stops used in the latter are replaced by U.V.- or blue-transmitting annular filters, with ring-shaped apertures corresponding to the objectives used. These apertures transmit the visible light of the low-voltage lamp, while the exciting light passes through the entire filter area (the apertures thus correspond with the annular stops in the phase contrast condenser). This optical arrangement permits the full utilization of the condenser aperture, so that a high radiation energy is available for fluorescence excitation. This is of special importance, since in this case the fluorescent object parts cannot be viewed against a dark background as in darkfield fluorescence.
Special catalogues contain information about our microscopes and the Lamp Housing 250.
Our Opak vertical illuminator in combination with the phase contrast equipment after Zernike* offers a further possibility of microscopic examination, in which the fluorescent portions are investigated in incident light, while phase contrast observation takes place in transmitted light. This method is used mainly with our ORTHOLUX microscope. Further information about it will be found in LEITZ Scientific and Technical Information, 1/5 English Edition, pp. 128–131. The instrument setup is described in our outfit key.



Phase contrast equipment on the ORTHOPLAN microscope

Phase contrast and polarized light**

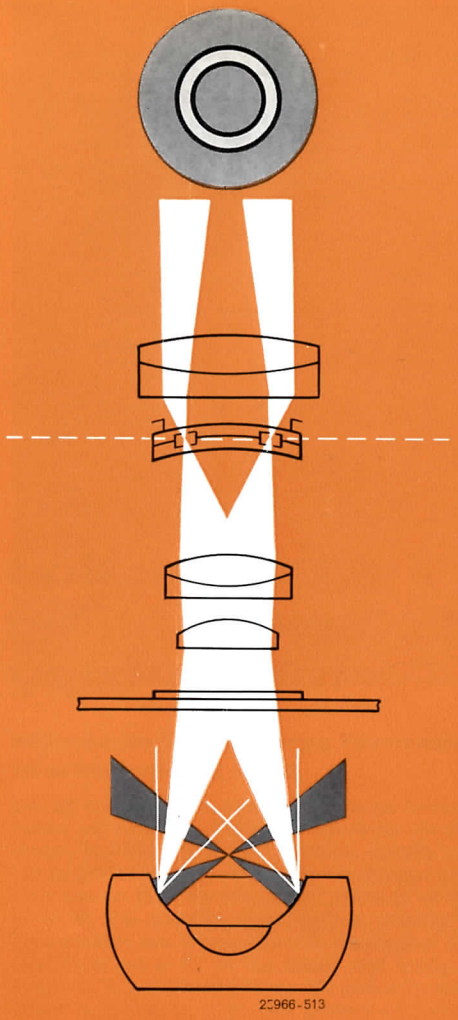
With the polarized-light outfit, consisting of holder, polarizer, analyser, and compensators the observer can demonstrate the anisotropy of birefringent preparations and obtain qualitative information about the position of the optical axes. The phase contrast equipment after Zernike is of particular advantage here. Since the light ring is produced only by the annular stop, the vibration direction of the light, linearly polarized by the polarizer, is largely maintained up to the object plane.
However, if the phenomenon of anisotropy is to be **measured,** the combination of phase contrast and polarized light will no longer be adequate. In this case a special polarizing microscope will be essential.

* Instead of the phase contrast equipment after Zernike the equipment with the Heine condenser can be used.

** Further detail on request. Please state serial No. of the microscope and type of condenser.

Phase contrast equipment

with condenser after Heine



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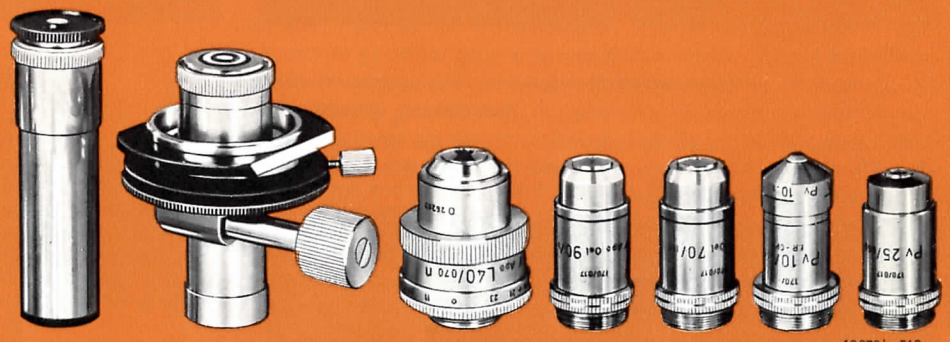
The equipment includes:
Phase contrast condenser after Heine No. 64 or 63
Choice of achromatic or apochromatic Pv phase contrast objectives
Auxiliary (focusing) magnifier



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

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

Condenser after 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 of 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. By raising the mirror component 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 darkfield is obtained when the hollow light cone cut out of the brightfield beam is intercepted by the aperture diaphragm of the objective. Normal darkfield illumination exists when the mirror component is in its highest position.

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Objectives for positive phase contrast

All objectives for positive phase contrast are supplied with a phase ring of normal absorption 75 ± 5% (designation "n"). The achromat Pv 10/0.25 n serves as a scanning objective. With the mirror component in the lowest position it already produces a phase contrast image, which turns into a darkfield 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 Fl Öl 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, brightfield, phase contrast, and darkfield with their transitions can be employed.

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

The apochromatic oil immersion objective Pv Apo Öl 90/1.32 n is provided for the reproduction 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 darkfield, particularly useful in orientating observation.

With the water immersion objectives Pv WE 22/0.65 n and Pv WE 80/1.00 n the distance between front lens and object is so short that particles floating in between cannot practically disturb observation.

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 coincides with 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 the absorption of the phase ring of these objectives is increased.

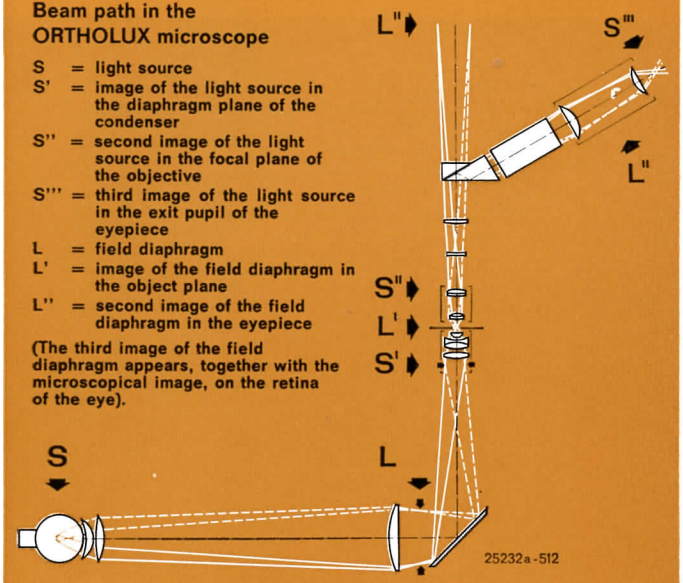
For objects in which the difference in the refractive index of the structure and of its surrounding field is very minute, phase contrast objectives of higher absorption (88 ± 2%) also for positive phase contrast are supplied (designation "h").

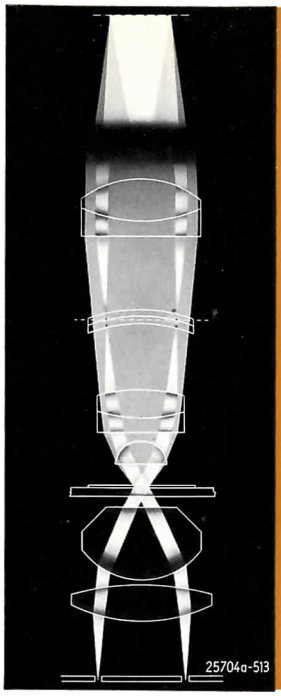
Description of phase contrast microscopy

Beam path in the ORTHOLUX microscope

S = light source
S' = image of the light source in the diaphragm plane of the condenser
S'' = second image of the light source in the focal plane of the objective
S''' = third image of the light source in the exit pupil of the eyepiece
L = field diaphragm
L' = image of the field diaphragm in the object plane
L'' = second image of the field diaphragm in the eyepiece

(The third image of the field diaphragm appears, together with the microscopical image, on the retina of the eye).





Positive phase contrast

As in the amplitude object illuminating ray and diffracted ray interfere in the image plane to form the microscopical image. $\Delta\varphi$ is now 180°, i.e. it has the same value as with the amplitude object.

The phase ring situated in the focal plane of the objective advances the annular illuminating beam by $\lambda/4$, at the same time attenuating it. The diffraction beam is distributed over the entire focal plane; it is practically unaffected by the phase ring.

Light is diffracted on the object. In a phase object the illuminating wave is displaced through $\Delta\varphi = 90°$ compared with the diffraction wave in the object plane. (With the amplitude object $\Delta\varphi = 180°$).

The illuminating beam emerges from the annular stop of the condenser as a hollow cone.

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 sources, 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, in which the aperture diaphragm is 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 images 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, which 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 first-order secondary images.

Microscopical image formation in phase contrast

Conditions are analogous in the case of a 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 surroundings 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.

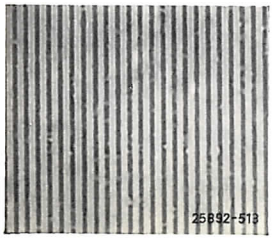
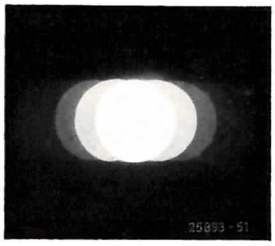
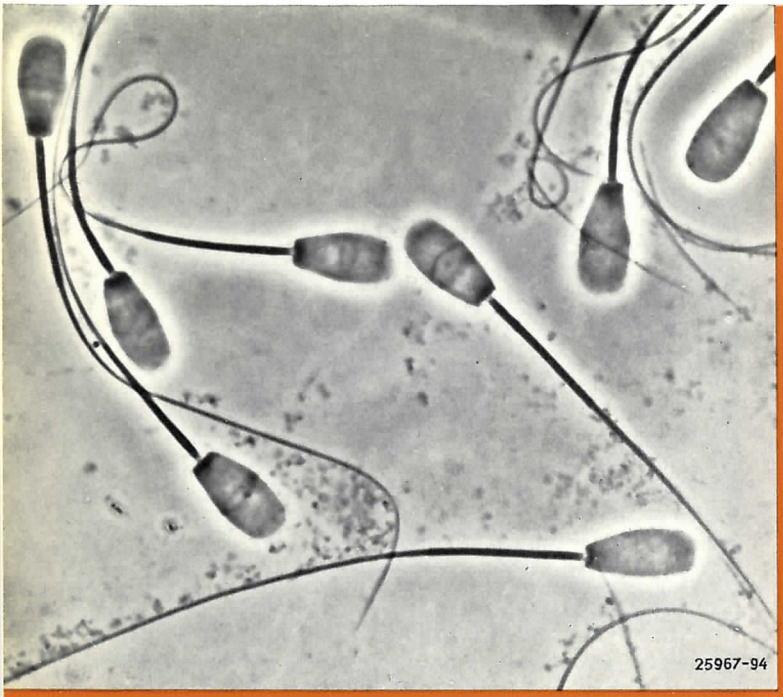
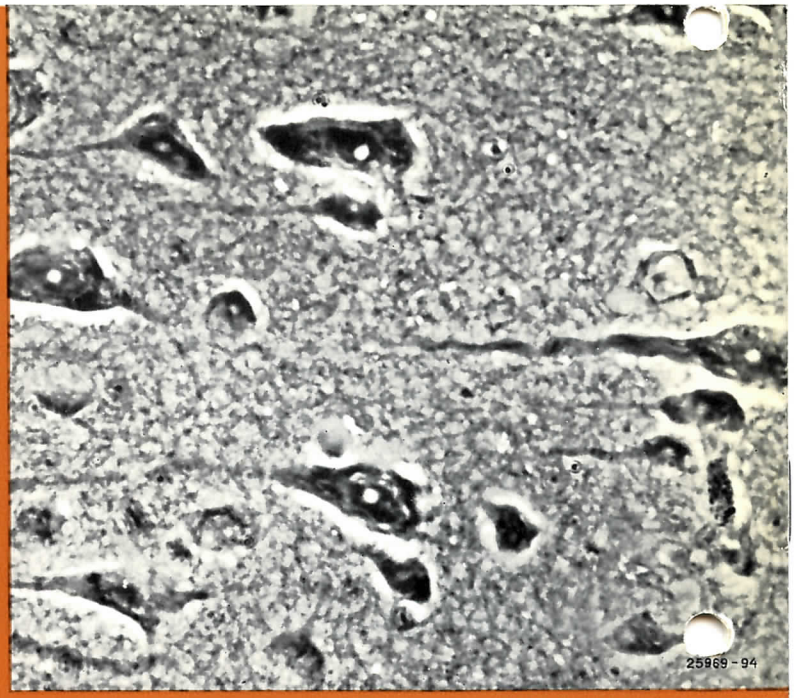


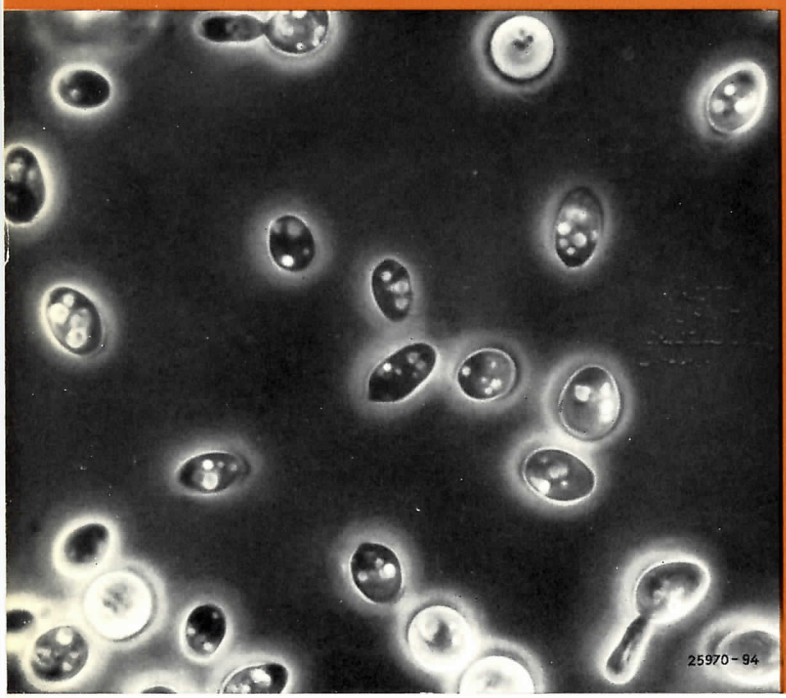
Image of the grid as seen in the eyepiece

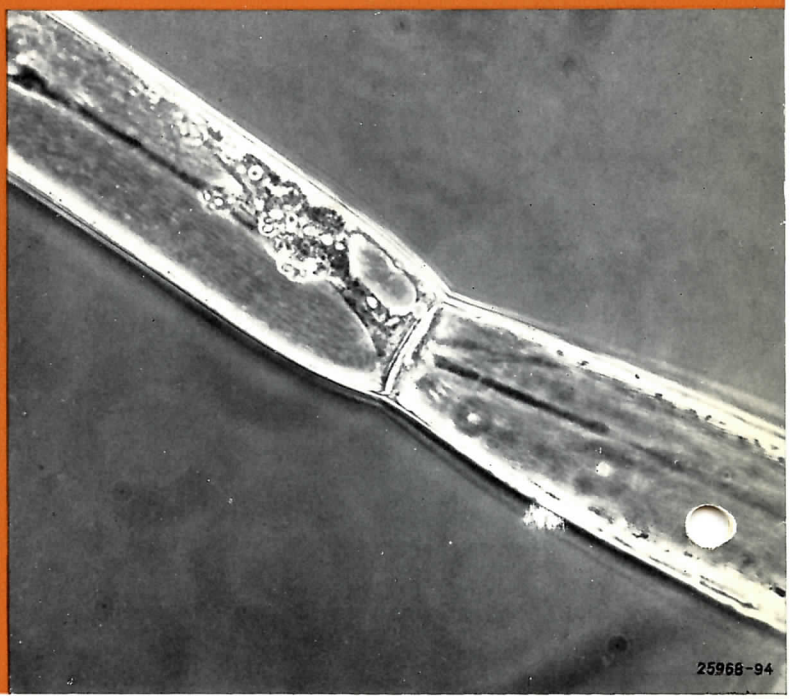


Diffraction pattern of the radically closed condenser diaphragm in the rear focal plane of the objective. The main principal image is seen in the centre, with the secondary images on either side.









Top left: Sperm cells in phase contrast, objective Phaco 100/1.30

Bottom left: Brewer's yeast cells in darkground, Objective Pv Fl Öl 70/1.15 n

Top right: Brain section in phase contrast, objective Phaco 40/0.65

Bottom right: Hair of a commelinacea, objective Phaco 40/0.65

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