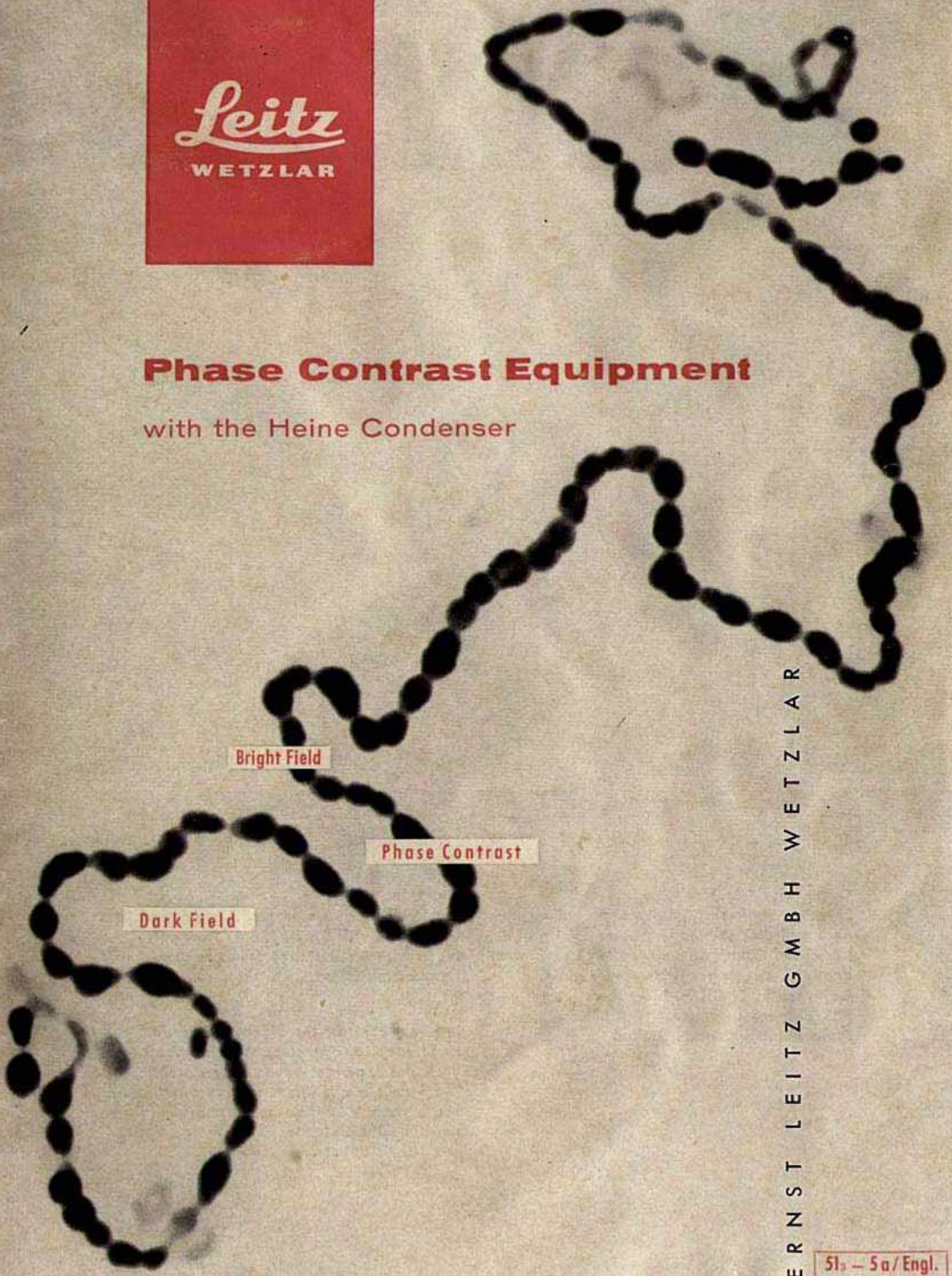
Leitz
WETZLAR

Phase Contrast Equipment

with the Heine Condenser



ERNST LEITZ GMBH WETZLAR

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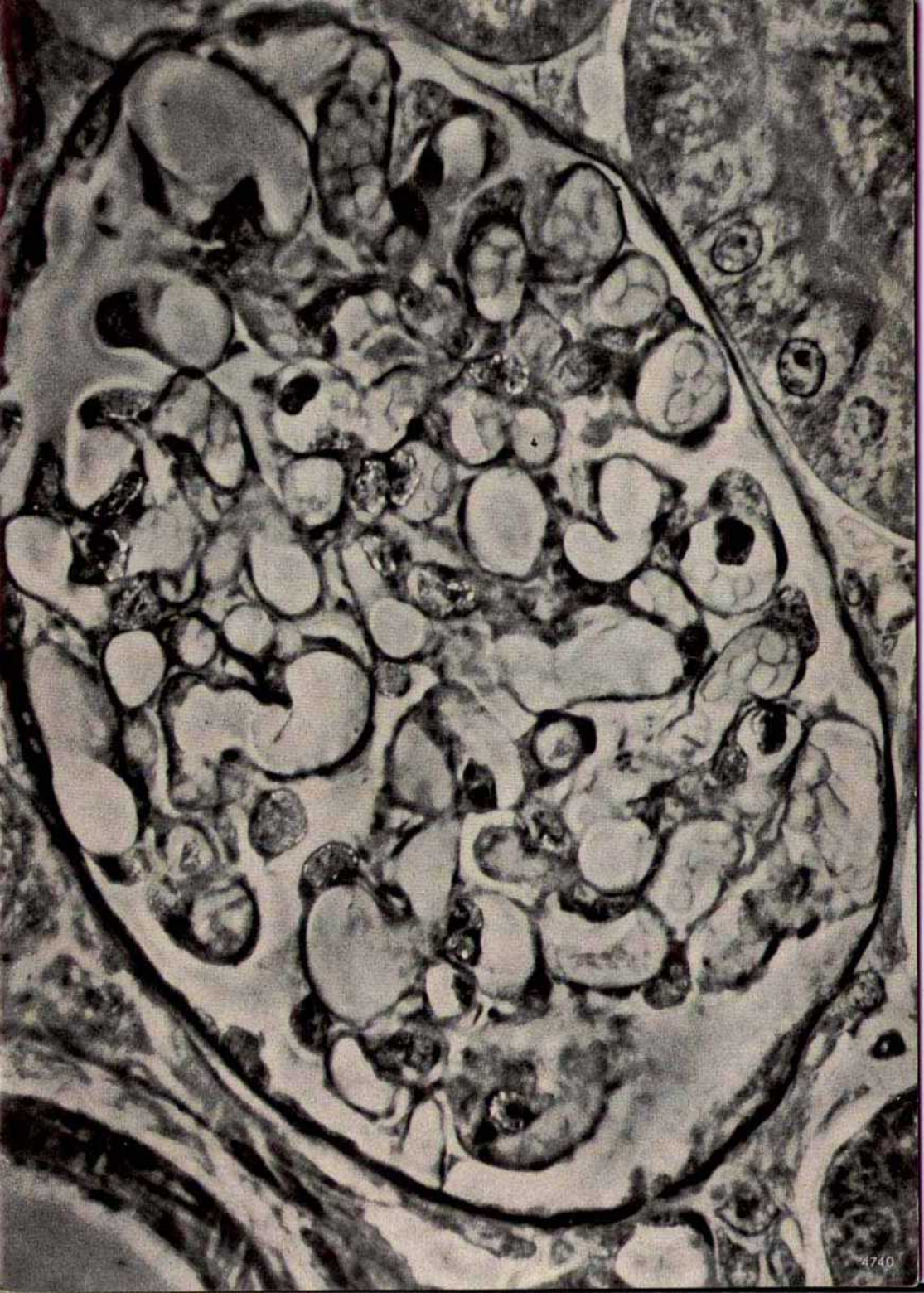
Cover page (Fig. 1):
Spherical shapes of Streptococcus salivarius; oval and disk shapes can be recognized in the chain. Culture in culture trough of 1/10 mm. depth on clear agar under cover glass. Objective Pv Fl Oil 70/1.15 n. MIKAS micro attachment with LEICA. Scale of magnification 3900:1

Fig. 2 (on right):
Rat kidney, phase contrast. Unstained section. Objective Pv Fl Oil 70/1.15 n; Panphot; taken with 9x12 cm. plate camera; scale of magnification 1100:1.

In phase contrast the kidney glomeruli, tubules, cell nuclei, nucleus membrane and nucleoles come out particularly clearly. The arteriola afferens is seen at top right. The Bowman's capsule, membrana propria and the glomerulus loops can be recognized in all their details.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The Phase Contrast Process

The phase contrast process represents a new way in microscopy to make features visible which until now remained hidden to the human eye. Its principle is based on considerations for which F. Zernike was awarded the Nobel Prize in 1953.

Before staining, most biological preparations are full of structural details which are distinguished from their surroundings only in respect of refractive index and thickness, but not as regards light transmission. Light waves passing through these sites travel more slowly or more quickly as compared with the light waves which pass through the surrounding field. This phase variation is recorded in the microscopic image of the specimen. But the structural details of a "phase specimen" cannot be seen or photographed; the human eye and photographic film only register differences in colour and brillance, but not differences in phase.

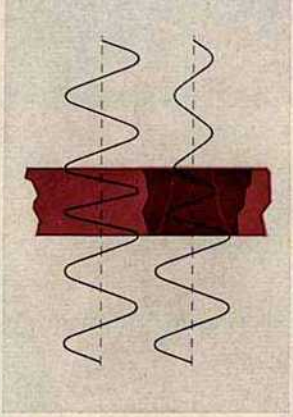
What we see with normal bright-field observation in the microscope are "amplitude specimens", i. e. structural elements which have weakened the light waves passing through them to a greater or lesser extent, and which now appear correspondingly darker or lighter in the image field. Only with dark field observation or oblique illumination can the outlines be dimly perceived – at least of thick phase specimens. But if it is required to study their structure by means of images with really adequate contrast, it is necessary to convert their variations in thickness and refractive index into differences of amplitude, i. e. brightness, by carrying out a differentiating staining process.

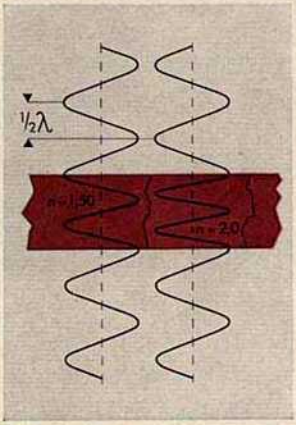
The scientific results obtained with bright field microscopy of stained preparations is excellent, but the preparation technique of fixing and staining, whereby the phase specimens are converted into amplitude specimens, represents a dangerous operation. For this process can change the morphological details in an uncontrollable manner, and a preparation can hardly survive after such treatment.

Zernike's phase contrast process has overcome this problem, and differences in refractive index and thickness appear directly as light-dark contrasts in the unchanged and living specimen. With the Zernike process, we also convert phase objects into amplitude objects. But this is no longer done by a chemical treatment of the preparation, but by means of optical manipulation of the beam path – a process which transforms the phase differences in the specimen into

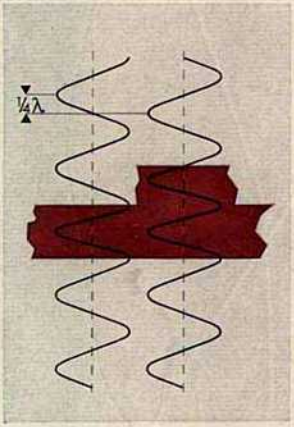
Fig. 3

Example of a pure amplitude specimen: the wave passing through is weakened by a structural element, but is not displaced in its phase.





Example of a phase specimen: The wave passing through a specimen of a higher refractive index is retarded (here ½ wave-length = ½ λ).



Example of a phase specimen: The wave which has passed through a thicker section of the specimen is retarded (here ¼ wave-lenght = ¼ λ).

amplitude differences, i.e. differences in brillance in the image. Zernike realized that in order to achieve this, in the microscope the direct light which is not deflected at the specimen must be allowed to travel ¼ wave length faster or slower than the deflected light, while further weakening of the direct light gives still more contrast. This operation is possible. For the direct light comes together again after it has passed the object in the rear focal plane of the specimen. On the other hand, the

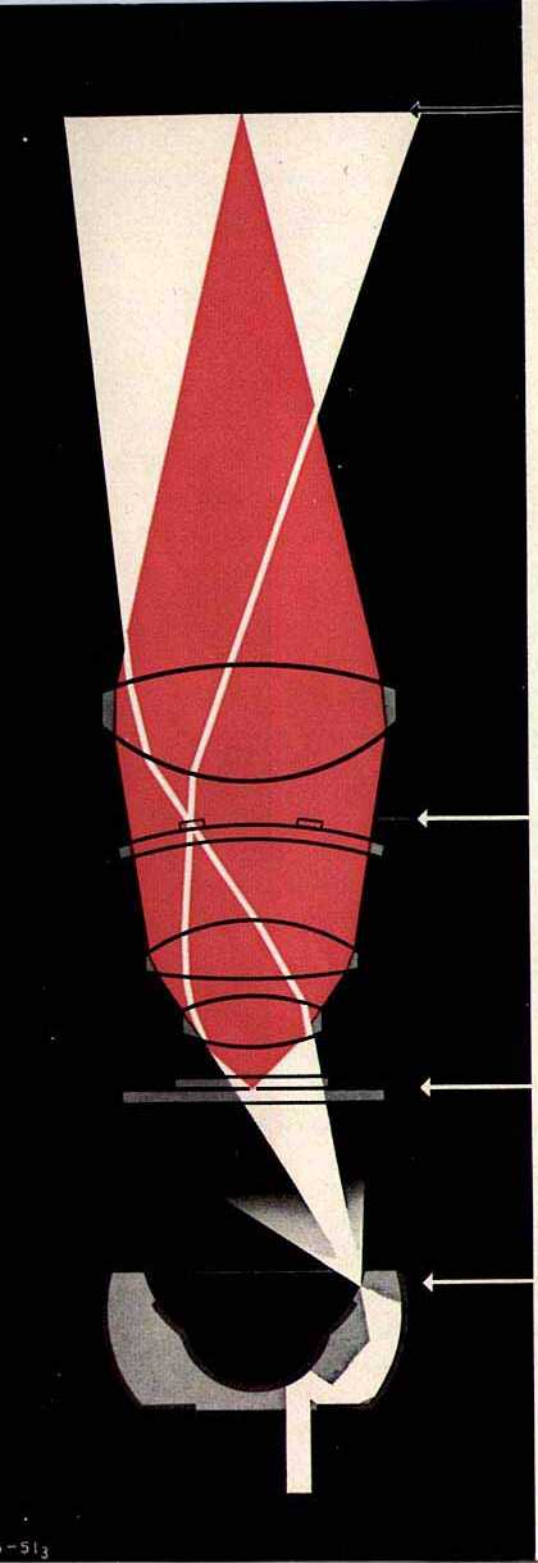


Fig. 4

Coincidence of the direct light (accelerated by 1/4 wave-length at the phase ring and at the same time weakened) with the deflected light at the specimen gives positive phase contrast in the image field.

The beam of light focused in the aperture plane in ring form is accelerated by 1/4 wave-length and at the same time weakened by the phase ring. The light deflected at the specimen is distributed over the rear focal plane. It is virtually uninfluenced by the phase ring.

Illuminating light is deflected at the specimen (shown as green here), and with a phase specimen its phase is also displaced as compared with direct light. (shown as red here)

The illuminating beam emerges from the mirror component of the Heine condenser as a wide hollow cone. For the sake of clarity in this illustration, only half of the illuminating light is shown.

deflected light is generally distributed over the whole rear focal plane. If there is a ring-shaped aperture diaphragm in the condenser, then the direct light appears in the rear objective focal plane as a light ring. The phase ring, a ring-shaped disk of suitable thickness and absorption, is placed at this point. If this phase ring accelerates the light flowing through it by $^1/_4$ wave length, and at the same time weakens the light, then "positive phase contrast" is achieved with the coincidence of direct and deflected light. Specimens which have higher refractive indices and are thicker than their surroundings generally appear darker in the image, while specimens with lower refractive indices and thickness appear brighter than the surrounding field. If the phase ring retards the direct light by $^1/_4$ wave length, then "negative phase contrast" is obtained, and the contrast relationships are reversed.

Every microscopist who uses phase contrast microscopy and sees how scarcely suspected details of a living specimen appear before him full of contrast and apparently plastic will readily appreciate the great value of this process. He will not want to be without phase contrast once he has experienced it. But he will still require one further facility: to compare the image in phase contrast with the familiar image of the classical types of illumination, i. e. particularly with normal bright field and dark field, without having to interrupt his observation. For there are few specimens which contain phase objects only. Structural details, which displace the phase, are usually intermingled with other details which absorb. And these would be seen more effectively and with greater contrast as amplitude features in bright field. Other details would perhaps appear more clearly in dark field.

This requirement, to be able to study a specimen according to its particular structure and the aspects of most interest to the microscopist under the most favourable observation process, is fully met by the LEITZ phase contrast equipment with the Heine condenser.

With the Heine condenser, the bright field beam flowing from the mirror component illuminates the specimen with its wide open cone of light (see illustrations on pages 6 and 14). From this beam, the objective cuts out a narrow cone and gathers it together in a light ring in the vicinity of the rear focal plane of the objective. When the mirror component is in its lowest position, the diameter of this light ring is smaller than the diameter of the phase ring. The direct light flows undisturbed past the phase ring, giving bright field observation (I). If the mirror component is raised by using the control on the Heine condenser, then the small cone of light covered by the objective apparently opens up like a pair of scissors. At the same time, the diameter of the light ring also increases. If the light ring and phase ring coincide, the illuminating beam is displaced in its phase and is weakened, thus giving phase contrast observation (II). If the light ring becomes wider than the phase ring, then bright field observation is obtained with concentric oblique illumination but larger aperture (III), and finally the first dark field (IV) is obtained when the hollow cone of light cut out of the bright field beam is stopped by the aperture diaphragm of the objective. With the mirror component in the highest position, the rays of a second illuminating beam – the real dark field beam – cross in the specimen. The rays of this beam are no longer taken up by the objective. This gives normal dark field observation (V).

Thus by raising and lowering the mirror component while observing the specimen, the microscopist can alternate at will between the various types of observation.

Every phase contrast equipment includes a focusing magnifier which is interchangeable with the eyepiece, by means of which the rear focal plane of the objective with the phase ring and the light ring (narrow or wide according to the condenser setting) is observed. By this means, the microscopist can select and control the illuminating methods described above. But he will soon be able to dispense with this aid, and will be able to apply the most expedient method of observation for an interesting detail quickly, surely and repeatedly, using the image itself and its contrasts.

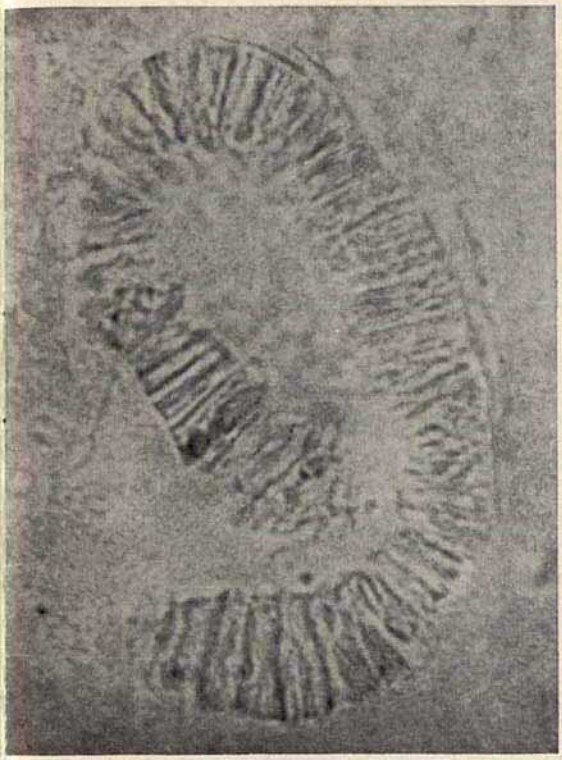


Fig. 5 Bright field: Silvary gland chromosome from the Chironomus

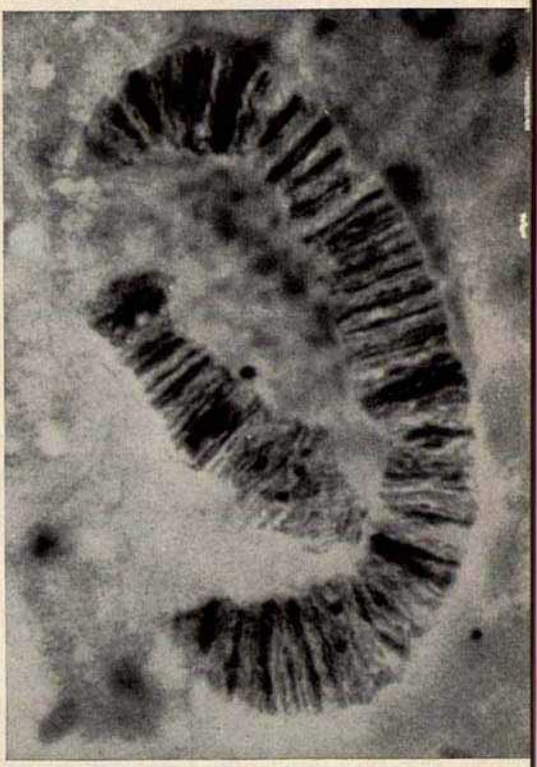


Fig. 5a Phase contrast: Salivary gland chromosome from the Chironomus

Phase preparation: unstained salivary gland, embedded in n-methacrylic acid butyl ester, which is changed into a polymer with the aid of a catalyst.
Objective Pv Apo Oil 90/1.15n, scale of magnification 1830:1

In the bright field image, the cross-section disk pattern of the salivary gland chromosome can only be roughly recognized. The chromomere disks are more strongly refractive than the intervening sections of the giant chromosomes. In phase contrast the disk structures are depicted with the same clarity as with a stained preparation. But in contrast to the stained preparation, in the unstained preparation the composition of the fine disks from individual chromomers is far clearer. Similarly, with observation in phase contrast, the heterochromatin in the living cell shows its vacuolic structure more clearly. The intermediate settings of the condenser provide cytologists with new possibilities for observing and depicting cell structures.

Fields of Application of Phase Contrast Microscopy

The main field of application of phase contrast microscopy is still that of its original purpose, i. e. to make visible cells, tissues and micro-organisms which are too delicate and too transparent when unstained to be shown by other microscopic methods. For the observation of fungi and bacteria, algae and protozoa in embryology, cytology, histology and haematology, and for clinical diagnosis, the phase contrast microscope has become an indispensible aid in both research and routine examination.

But the possibility of depicting minute differences in thickness and refractive index as light contrast soon opened up wide fields of research in the natural sciences for the phase contrast process. The following represent just a few possible uses: dust research, study of crystal growth and presentation of emulsions, sols and gels; determining refractive indices when embedding in immersion liquids; observations on fibres, textiles and films, plastics and glasses; examination of surface structure – also of opaque specimens – with lacquer imprints; comparative and general survey of ultra-microscopic specimens.

Perception Limits

The thickness and refractive index at which a phase object can be just recognized depends on the size and structure of the object, the structure of the surrounding field, and the absorption of the phase ring. With our normal positive phase contrast, the perception limit lies at an optical thickness of the object of $\lambda/200$, i.e. at about 25 ÅE (10 ÅE = 1 mμ). Here the optical thickness of the object is given by the following formula:

$$(n_{object} - n_{surrounding\ field}) \cdot d_{object}$$

For example: a crystal block with a thickness of 2.5μ (d) appears just perceptibly darker than its surrounding field, if its refractive index (n) is 0.001 higher than the refractive index of the homogeneous surrounding. The optical thickness formula is then evaluated as follows:

$$(n_{object} - n_{surrounding}) \cdot d_{object} = 0.001 \cdot 2.5 \cdot 10^4 \text{ ÅE} = 25 \text{ ÅE}.$$

Combination of the Phase Contrast Process with Traditional Observation Methods

With the polarization attachment on the phase contrast microscope, the observer can demonstrate the anisotropy of double refractive preparations and obtain data – at least qualitative – as to the position of the optical axis and the extent of the double refraction. If it is necessary to measure the anisotropy phenomenon, then the combination of phase contrast and polarizing attachment is no longer sufficient, and recourse must be taken to a special polarizing microscope.

The microscopist knows that every variation in the illuminating process can supply new data on the fine structure of a preparation. Under certain circumstances it is therefore useful to supplement the observation in the bright and dark field of the Heine condenser by comparative observations in normal bright and dark field with the aid of a special condenser. This illuminating process will be used additionally when examining specimens in which purely phase objects and purely amplitude objects are situated next to each other, or when the structure detail to be analysed acts as both a phase object and an amplitude object. Thus the Pv objectives, for example when using a bright field condenser with aperture diaphragm, can be fully used in all aperture ranges. In dark field observation with the Pv oil immersions, under certain circumstances the dark field condenser D 1.20 A may be preferable to the Heine condenser.

The various condensers can be easily interchanged on all LEITZ microscope stands.

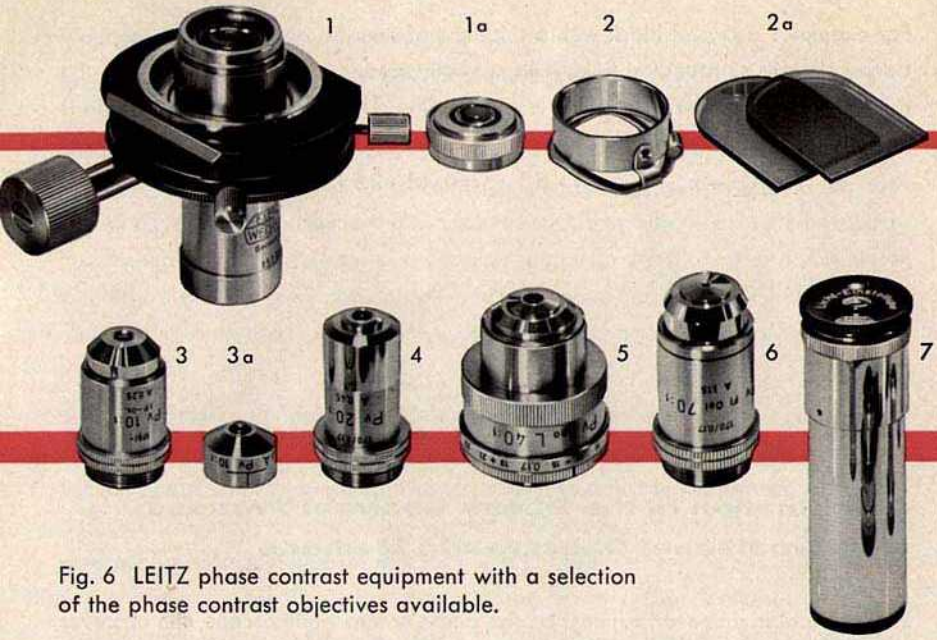


Fig. 6 LEITZ phase contrast equipment with a selection of the phase contrast objectives available.

The *Leitz* Phase Contrast Equipment

consists of the following items

The Heine ***phase contrast condenser*** (1) with control knob for the vertical adjustment of the mirror component (num. aperture 0.25 to 0.70). By means of this vertical adjustment, the various types of illumination, i. e. bright field, phase contrast and dark field, are obtained with the corresponding intermediate settings. There is a screw-on immersion cap (1a) (num. aperture 0.50 to 1.40) for examinations in an oil immersion.

Phase contrast objectives with suitable graduated apertures and magnifications. The illustration shows the achromatic dry system Pv 10/0.25 (3); immersion attachment for this objective (3a); in combination with this immersion attachment, the objective can be used, preliminary survey of the preparation for the with immediate change-over to oil immersion. Conversly, it is also possible to change over from oil immersion to the general survey objective. Achromatic dry system Pv 20/0.45 (4). Apochromatic dry system Pv Apo L 40/0.70 in correction mount with automatic focusing compensation (5). Fluorite oil immersion Pv Fl Oil 70/1.15 (6). Filter holder (2) with daylight filter and photographic filter (2a). ***Auxiliary focusing magnifier*** (7).

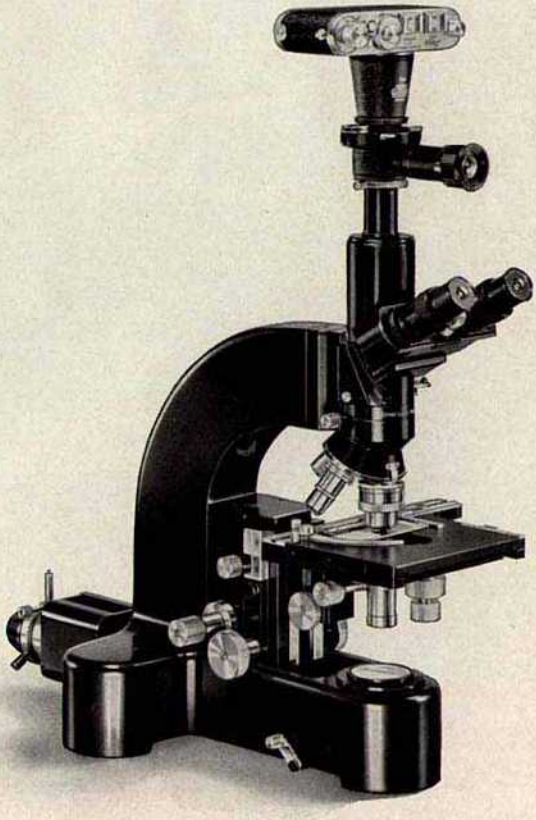
The range of objectives available is specified on pages 16, 17 and 20, while on pages 18 and 19 suitable outfits are recommended.

The LEITZ phase contrast equipment can be supplied to fit any LEITZ microscope:

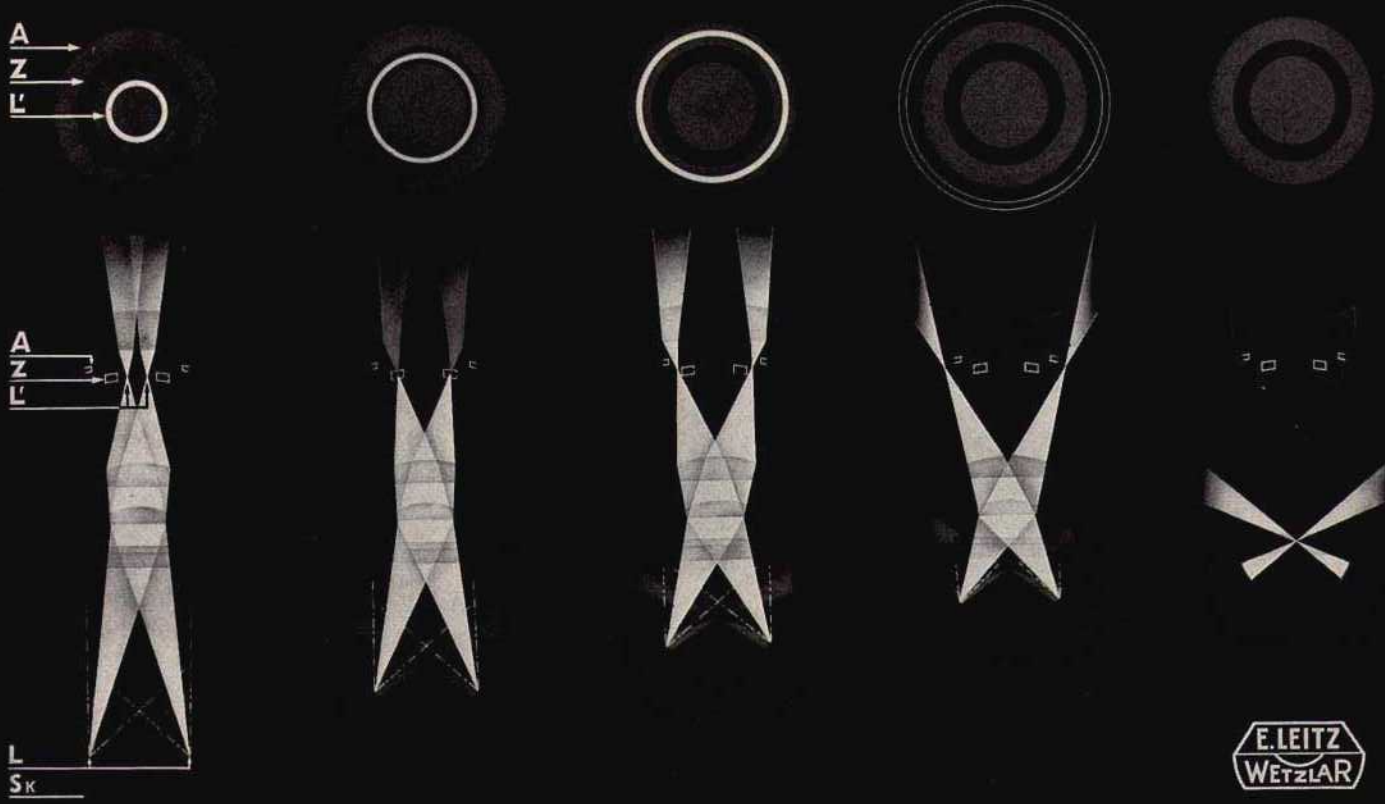
The LEITZ phase contrast equipment can be supplied with a dovetail slide or cylindrical mount for the condenser of any LEITZ microscopes.

The optimum conditions for phase contrast observation are offered by stands with interchangeable revolving objective nosepieces. For these microscopes the phase contrast objectives are supplied fitted parfocally on an objective nosepiece, so that on interchanging this nosepiece with a nosepiece arrying normal objectives, the constant centration of the individual objective systems is ensured.

The built-in source of light of our microscopes is particularly suitable for phase contrast observation. For the SM microscopes we recommend the use of the microscope lamp 6 volts 2,5 amps NANAW-REROW



The ORTHOLUX microscope with built-in illumination is the ideal instrument for research work, in view of its outstanding advantages as regards optical performance and manipulation. The built-in source of light ensures permanently centred illumination, while the high intensity low-voltage lamp (6 volts, 5 amps) provides an exceptionelly great reserve of brilliance which is sufficient even for photomicrography.
Fig. 7 on the right shows the ORTHOLUX fitted with the LEITZ phase contrast equipment, the photo tube with lateral binocular observation, and the LEICA camera for photomicrography.


A
Z
L'
A
Z
L'
L
SK
E.LEITZ
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I
II
III
IV
V

Bright field	Phase contrast	Bright field	Dark field	Dark field

The Various Types of Illumination

in relation to the position of the mirror component. Objective Pv 20/0.45. (Fig. 8)

With the mirror component *SK* in the lowest position (*I*) the narrow ring of light *L* produced by the condenser is reduced to *L'* within the Zernike phase ring Z. Bright field observation is given.	As the mirror component *SK* is raised, the image *L'* of the ring of light widens until it is completely covered by the dark-looking phase ring *Z*. Position *II* has now been reached with phase contrast according to Zernike.	Further raising of the mirror component *Sk* permits the image of the illuminating ring to increase still further, until it is no longer influenced by the phase ring. This position (*III*) gives bright field images with very rich contrast qualities.	Continued raising of the mirror component *Sk* causes the image of the light ring to vanis beyond the edge *A* of the aperture diaphragm. In this position (*IV*) with the ring *L* as source of light, a particular dark field is achieved which in many cases reveals special structures more clearly than ordinary dark field.	Finally, in position *V* the cone of illumination convergent in the object field becomes effective; this gives normal dark field illumination.

The transition from one of the characteristic settings to the other is continuous.

The phenomena in the aperture diaphragm of the objective can be observed with the auxiliary magnifier, which is inserted into the body tube instead of the eyepiece. Fine focusing on the light ring is effected by adjusting the eyepiece, and if necessary by partially withdrawing the auxiliary magnifier (displacing the stop ring). For this purpose it is expedient to slightly reduce the intensity of the light.

Phase Contrast Objectives Type n for Positive Phase Contrast

All objectives for positive phase contrast are supplied with a phase ring of *normal* absorption of 75% ± 5% (distinguished by the letter "n").

The Objective Pv 10/0.25 n serves as a general survey objective. In the lowest position of the mirror component, it already shows a phase contrast image, and subsequently a dark field image. The immersion attachment for the lens is used, if simultaneous oil immersion work is required. The objective 10/0.25 is then used as an oil immersion for selecting suitable sites in the preparation, and the immersion attachment gives the same working distance as that for which the oil immersion objectives are adjusted. It is thus possible to switch over directly between these objectives. When using the objective in bright field, it is necessary to slightly lower the condenser carrier.

The Objective Pv 20/0.45 n is particularly suitable for surveying preparations in the various types of illumination.

Objectives Pv Apo L 40/0.70 and Pv Apo L 63/0.70 n are apochromatics which permit correction of the cover glass thickness by means of the correction mount. The large free working distance permits good observation also in instances where thick cover glasses are used, e. g. for tissue cultures. These objectives are therefore particularly popular for use in cytology and histology.

The Objective Pv Fl Oil 70/1.15 n (immersion) is used primarily in cases where a long working distance is vital. In combination with the immersion cap for the phase contrast condenser, all types of illumination can be used (bright field, phase contrast, dark field). If the immersion cap is not used, it will not be possible to carry out observations in peripheral bright field or in dark field. At a distance of 7 mm above the stage plane, the phase contrast condenser will then still fill out the aperture of the phase ring. It is thus possible to carry out phase contrast observations in cells with an upper cover glass 7 mm above the level of the object stage, and in which the cultures grow on the lower surface of the upper cover glass. In the tropics, Pv Fl Oil 70/1.15 only should always be used as the oil immersion objective.

The Objective Pv Apo Oil 90/1.15 n (oil immersion) is designed for the observation of finest structures, such as lattic fibre, chromosome structure, flagella, membranes, etc. With the immersion cap on the condenser at the upper stop, this objective also gives a dark field illumination which is of particular value for general survey work.

With the **water immersion objectives Pv WE 22/0.60 n, Pv WE 50/0.70 n and Pv WE 80/1.00 n** the distance between the front lens and the specimen is kept so small that any intervening floating particles are virtually incapable of disturbing the observation.

Phase Contrast Objectives Type h for Intensified Positive Phase Contrast

All phase contrast objectives can be supplied with a phase ring of ***high*** obsorption (88 % ± 2%) on request, and are distinguished by the letter "h". These objectives should be used in instances where the difference in refractire index between the structure to be observed and the surrounding field is very small. For example, with living cultures it is not always possible to prepare a culture solution with the suitable difference in refractive index between the structure and the surrounding field. In such çases the objectives with a high absorption phase ring are particularly suitable. For example, when using objectives with normal absorption, the mitochondria in tissue cultures appear pale, although the resolution is good, whereas with high absorption objectives they stand out much more clearly.

If the contrast obtained with "n" or "h" objectives still does not meet the requirements, then the embedding material must be changed.

Phase Contrast Objectives Type -h for Negative Phase Contrast

For examinations where negative phase contrast is desired, the following phase contrast objectives are supplied with a negative phase ring (distinguished by " – h"):

Pv 20/0.45 – h
Pv Apo L 40/0.70 – h
Pv Apo L 63/0.70 – h
Pv Fl Oil 70/1.15 – h

These objectives also have a phase ring of high absorption, viz. 88% ± 2%. Through their higher contrast effect, the observer is given the subjective impression of a "more plastic" image.

Leitz Phase Contrast Equipment

	Condenser designed for dovetail holder	Condenser designed for cylindrical mount
Heine Phase contrast condenser, with rack and pinion for vertical adjustment of the mirror component; with immersion cap to screw on the condenser	PHAKY	PHARF*
Filter holder with daylight and photographic filters, and groudglass (Outfit E)	PHAFT	PHAFT
Auxiliary magnifier for centring the light ring	PHADS	PHADS
Case to hold the condenser and four objectives	PHAMB	PHAMB
Phase contrast condenser No. 74 (for dovetail holder)	PFAHT	
Phase contrast condenser No. 75 (for cylindrical mount)*		PFAGS

The following Optical Outfits "C" are recommended for phase contrast**

	Code words and range of magnifications monocular	Code words and range of magnifications binocular
Optical outfit C 1 (for thinnest section histology, bacteriology and cytology) Achromatic Pv 20/0.45 Apochromatic objective Pv Apo L 40/0.70 Fluorite immersion Pv Fl Oil 70/1.15 Apochromatic Immersion Pv Apo Oil 90/1.15 Periplanatic eyepieces 8x and 12x	160 – 1080x FOACS-FE	200 – 1350x FOADT-FE
Optical outfit C 2 (for general histology, only with dry objectives) Achromatic objectives Pv 10/0.25, Pv 20/0.45 Apochromatic objectives Pv Apo L 40/0.70, Pv Apo L 63/0.70 Periplanatic eyepiece 6x, 8x and 12x	60 – 760x FOAHX-FE	75 – 950x FOAJY-FE
		PHEDT

* Internal diameter of the sleeve 39.5 mm. For the stand "H" class microscope an extension piece for the mirror holder is necessary when using the phase contrast equipment.

** Unless otherwise ordered, "n" phase contrast objectives will be supplied.

	Code words and range of magnification	
	monocular	binocular
Optical outfit C 3 (for general histology, with immersion) Achromatic objectives Pv 10/0.25, Pv 20/0.45 Immersion attachment to fit on objective Pv 10/0.25 Apochromatic objective Pv Apo L 40/0.70 Fluorite immersion Pv Fl Oil 70/1.15 Periplanatic eyepieces 6x, 8x and 12x . . .	60 – 840x FOALB-FE	75 – 1050x FOAMC-FE
Optical outfit C 4 (for bacteriology, general morphology and physiology of bacteria and tissue cultures) Achromatic objective Pv 20/0.45 Apochromatic objectives Pv Apo L 40/0.70, Pv Apo L 63/0.70 Fluorite immersion Pv Fl Oil 70/1.15 Periplanatic eyepieces 8x and 12x	160 – 840x FOAND-FE	200 – 1050x FOAPF-FE
Optical outfit C 5 (for examination of water cultures) Apochromatic objective Pv 10/0.25 Immersion attachment to fit on objectives Pv 10/0.25, Pv WE 22/0.60, Pv WE 50/0.70, Pv WE 80/1.00 Periplanatic eyepieces 8x and 12x	80 – 960x FOECT	100 – 1200x FOEHY
Optical outfit C 6 (for examinations of a general nature, irrespective of the nature of the specimens) Achromatic objective Pv 10/0.25 Immersion attachment to fit on objective Pv 10/0.25 Achromatic objective Pv 20/0.45 Apochromatic objective Pv Apo L 40/0.70 Apochromatic immersion Pv Apo Oil 90/1.15 Periplanatic eyepieces 8x and 12x	80 – 1080x FOARG-FE	100 – 1350x FOEBS-FE
Optical Outfit for examination of tissue cultures and for cinematography Outfits C 1 to C 6, but with phase contrast objectives "h".		

The objectives can also be supplied in any required assortment in accordance with the table on page 20.

Phase Contrast Objectives

Type	Designation [1]	Free working distance	Micrometer value measured with eyepiece H 6x	Cover glass [2] Correction	Type of eyepiece [3]	Code word	Designed with absorption of		
							75±5%	89±2%	88±2%
Dry system	Pv 10/0.25	5.8	15	DO	P	PHALZ	n	h	–
	Immersion attachment for Pv 10/0.25	0.3				PHAWK	–	–	–
Dry system	Pv 20/0.45	2.0	7.6	D	H (P)	PHANC	n	h	– h
Dry system with very long working distance	Pv Apo L 63/0.70 in correction mount with automatic focusing compensation	0.38	3.8	D!	P	PHASG-FE	n	h	– h
Dry system with very long working distance	Pv Apo L 63/0.70 in correction mount with automatic focusing compensation	0.35	2.4	D!	P	PHERG-FE	n	h	– h
Water dipping objective	Pv WE 22/0.60	0.05	6.5	O	P	PHESH	n	h	–
Water dipping objective	Pv WE 50/0.70	0.05	2.8	O	P	PHWL	n	h	–
Water dipping objective	Pv WE 80/1.00	0.06	1.9	O	P	PHEYN	n	h	–
Oil immersion	Pv Fl Oil 70/1.15	0.20	2.0	DO	P	PHELB-FE	n	h	– h
Oil immersion	Pv Apo Oil 90/1.15	0.12	1.6	DO	P	PHATH-FE	n	h	–

[1] The number before the oblique stroke gives the initial magnification, while the figure after the stroke gives the numerical aperture.

[2] D: with cover glatt D = 0.17 (cover glass thickness should be observed accurately to within ±0.05 mm)
O: without cover glass. DO: can be used with or without cover glass.
D!: Cover glass thickness should be observed accurately to within ± 0.01 mm., or should be accurately set with the correction mount.

[3] H = use Huygens eyepieces; P = use Periplanatic eyepieces.

The phase contrast objectives are described in detail on pages 16/17. Unless otherwise ordered, they are supplied in the design "n". The prices for designs "n", "h" and "–h" are the same.

Accessories

	Code word
For microscopes with interchangeable objective revolving nosepieces, the following are recommended:	
Revolving nosepiece for 4 objectives, on which the Pv objectives are mounted parfocally, which bracket*	
for the LABORLUX II microscope (5·4·–·–)	IRBIX
for the LABORLUX III microscope (7·4·–)	NADUR
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We supply also the following items:	
Micro culture troughs for observing tissue explantates and bacteria cultures in phase contrast:	
Culture trough approx. 0.1 mm. in depth	PHOLD
Culture trough approx. 0.01 mm in depth	PHONG
In order to obtain good results, it is of great importance to maintain the correct cover glass thickness (0.17 mm.).	
In order to check or select cover glasses, we recommend:	
Cover glass thickness gauge with graduation 1 interval = $^1/_{100}$ mm.	DIMES
To supplement the phase contrast epuipment we also supply:	
Polarizing equipment, consisting of analyser and polarizer filters, in case	
a) with simple clamp-on polarizer	KAPOP*
b) with clamp-on polarizer, to pivot in and out	KASAN*
for both accessories:	
Gypsum plate, 1 st order red, for use in polarizer mount . . .	KARAM
Mica plate, $^1/_4$ W. L., for use in polarizer mount	KAREN

* When ordering, please state number and if possible designation of the microscope stand to be used.

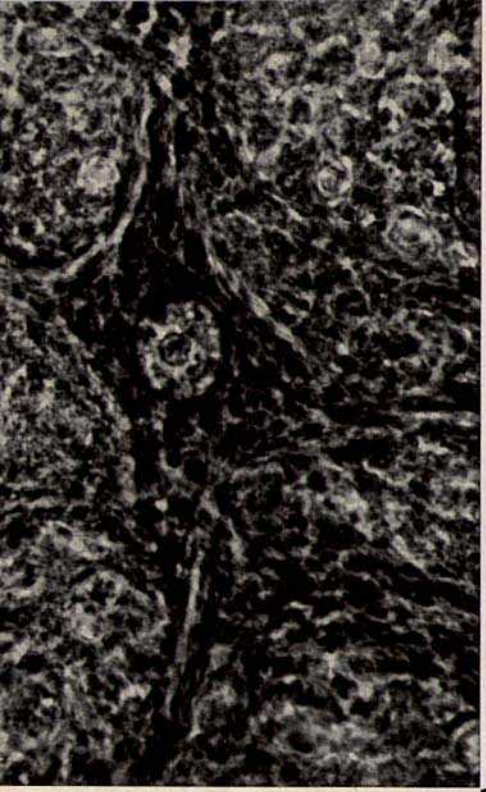

Fig. 9: Frozen section through a rat's brain, nucleus reticularis, phase contrast. Objective Pv Fl Oil 70/1.15 n; MIKAS micro attachment, LEICA; scale of magnification 1210:1.

Nissl corpuscles and endofibrillae can be clearly recognized. As compared with the nuclei, which were depicted by means of special staining processes, the greater extent of the nuclei as shown in the phase optical examination of frozen sections is notable. Widespread vacuole formation in the nucleoles and differences in the refractive index of the nucleoles in different regions can be observed. The phase contrast process provides an important extension in method here.

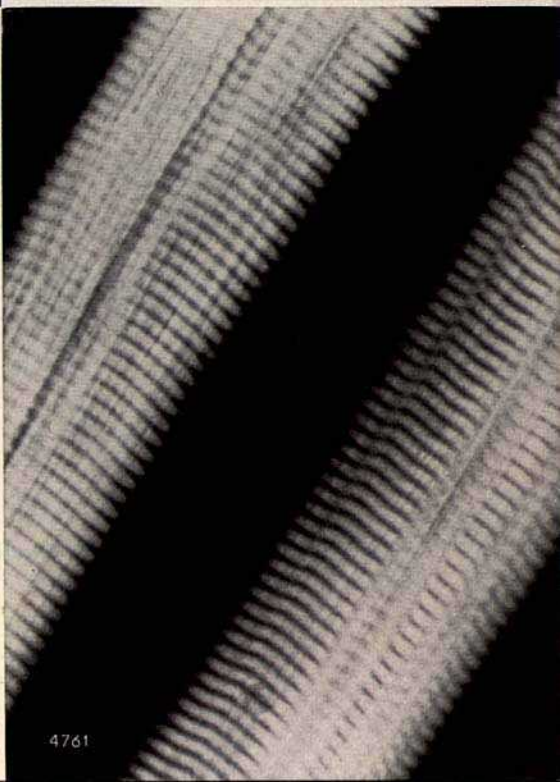

Fig. 10: Stork muscle, polarized light. With polarizing filter.

With crossed polarizing filters, the sarcoplasm coating of the muscle fibres, nuclei and vessels cannot be depicted. But the quality of the image is comparable to that obtained when using a standard polarizing microscope. The differences in the refractive indices of the individual bands (Q-I-Z) are clearly shown.

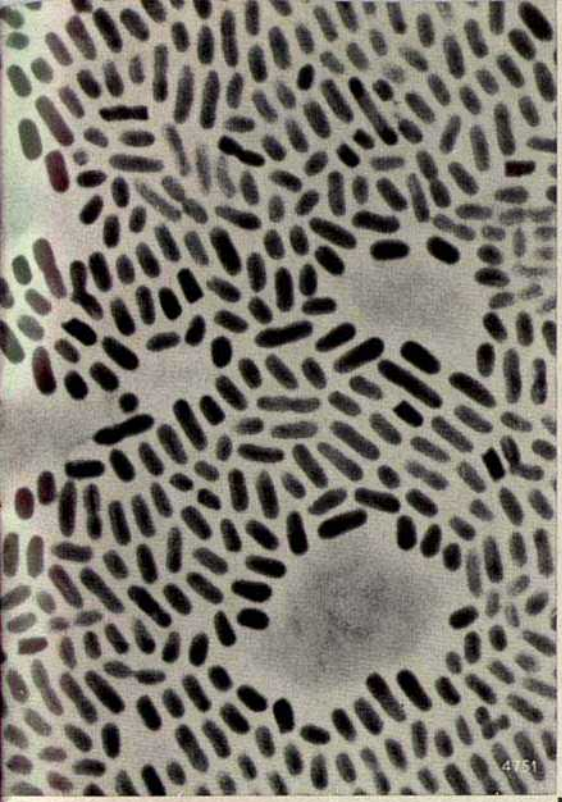

Fig. 11: Culture of Coli bacteria.
Phase constrast.
Objective Pv Fl Oil 70/1.15 h;
MIKAS micro attachment, LEICA;
scale of magnification 3000:1.

The Coli bacteria appear as sporeless bodies of medium size and low motility. The ends are rounded, the one being somewhat narrower than the other, giving commashaped bacteria. Here the bacteria were cultured in a culture trough 1/10 mm. in depth on clear agar under a cover glass.

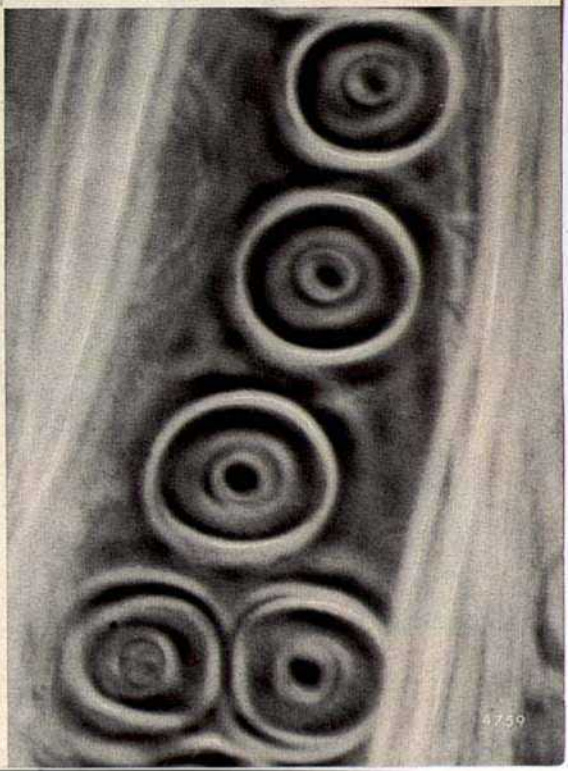

Fig. 12: Bordered pits in pine wood;
radial hand-cut section; negative
phase contrast.
Objective Pp Apo Oil 90/1.15 – h;
MIKAS micro attachment, LEICA;
scale of magnification 1050:1.

In negative phase contrast the entry apertures of the bordered pits are clearly seen. On altering the focusing, the membrane thickening closing the bordered pits can be discerned. The periphery of the closing membrane inside the pits appears in negative phase contrast as a bright ring.

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