E. LEITZ
WETZLAR

Instructions for Use

Leitz

Phase Contrast Equipment

with the Heine Condenser

ERNST LEITZ GMBH WETZLAR

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The LEITZ Phase Contrast Equipment permits the setting of the following types of microscope illumination:

bright field illumination
phase contrast after Zernike
dark field illumination

The Heine condenser used in the equipment renders it possible to set these modes of illumination while observing the specimen, by operating a control knob on the condenser, and thus to use every intermediate adjustment in continuous transition from bright field to phase contrast or to dark field.
The possibility thus offered of continually changing the illumination is of particular advantage in cases where the structures visible in phase contrast are to be compared with the structures visible in an ordinary bright field or a dark field. According to the particular nature of the specimen, the intermediate settings can also be important for differentiating, and can assist in the judgement of the specimen.

The illustration shows the phase contrast equipment with a selection of Pv objectives.

The LEITZ phase contrast equipment is supplied in two models to fit any LEITZ microscope: one with dovetail slide fitting the horizontal condenser holder of the ORTHOLUX, DIALUX and PANPHOT microscopes and another with cylindrical sliding mount fitting the condenser sleeve (of 39.5 mm. internal diameter) on all other stands. The latter model can also be used on the simple student's microscope stand H only an extension piece for the mirror holder being required.

As a suitable source of light for microscopes without built-in illuminating system the MONLA low-voltage lamp (6 volts 5 amps) is particularly recommended, preferably the MONLAFIX attachable model which fits all ordinary stands; a special design is available for the LABORLUX III. Full particulars will be found in special leaflets.

THE LEITZ PHASE CONTRAST EQUIPMENT

consists of:

	Condenser with dovetail slide	cylindrical mount
Phase contrast condenser after Heine (available in two models for large microscopes with horizontal condenser holder and ordinary microscopes with cylindrical substage sleeve of 39.5 mm. internal diameter)		
with mirror component, vertically adjustable by rack and pinion, and screw-on immersion cap	PHAKY	PHARF
Filter holder with daylight and photo filters	PHAFT	PHAFT
Auxiliary magnifier	PHADS	PHADS
Case for condenser and 4 objectives	PHAMB	PHAMB
Phase contrast condenser complete		
(a) No. 74 for horizontal condenser holder	PHAHT	
(b) No. 75 for cylindrical substage sleeve		PFAGS

For suitable Pv objectives see table on page 3.

PHASE CONTRAST OBJECTIVES

Designation [1]		Free working distance mm.	Micrometer value with H 6 x	Cover glass [2] correction	Type of eyepiece [3]	Code word
Dry system	Pv 10/0.25	5.8	15	DO	P	PHALZ
	Immersion attachment for Pv 10/0.25	0.3				PHAWK
Dry system	Pv 20/0.45	2.8	7.6	D	H (P)	PHANC
Dry system with extra long working distance	Pv Apo L 40/0.70 with correction mount, automatic focusing compensation	0.38	3.8	D !	P	PHASG
Dry system with extra long working distance	Pv Apo L 63/0.70 with correction mount, automatic focusing compensation	0 35	2.4	D !	P	PHERG
Water dipping objective	Pv WE 22/0.60	0.05	6.5	O	P	PHESH
Water dipping objective	Pv WE 50/0.70	0.05	2.8	O	P	PHEWL
Water dipping objective	Pv WE 80/1.00	0.06	1.9	O	P	PHEYN
Oil immersion	Pv Fl Oil 70/1.15	0 20	2.0	DO	P	PHELB
Oil immersion	Pv Apo Oil 90/1.15	0.12	1.6	DO	P	PHATH

1) The figure in front of the oblique stroke indicates the initial magnification and the one after it the numerical aperture of the objective.

2) D: for cover glasses 0.17 mm. thick (to be adhered to within ± 0.05 mm.), O: for use without cover glass, DO: for use with or without cover glass. D!: Sensitive to cover glass variations over ± 0.01 mm. which are compensated for by operating the correction mount.

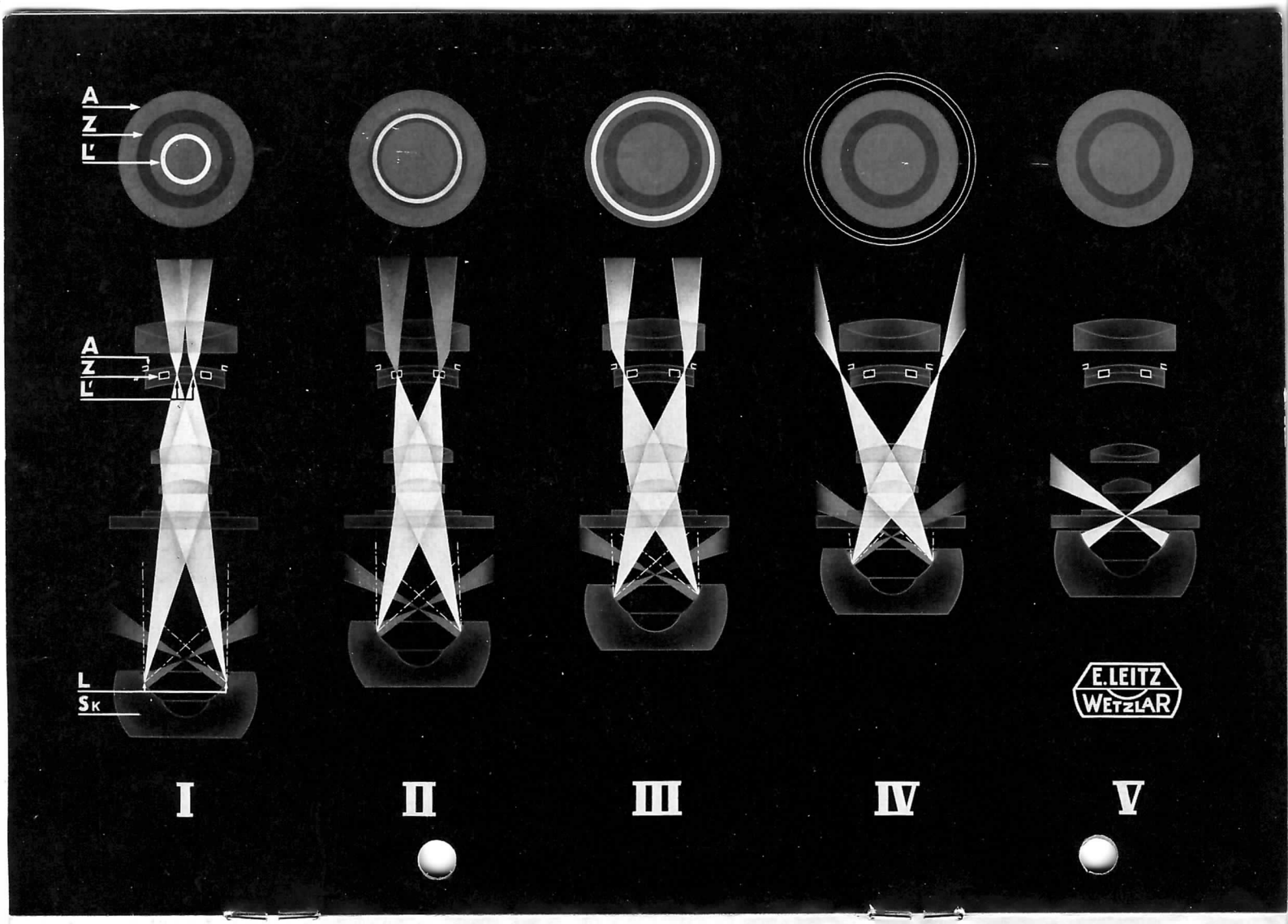
3) H = Huygens eyepieces, P = Periplanatic eyepieces.

All objectives are supplied for **positive phase contrast** with normal absorption (75 % ± 5 %) which is indicated by the engraving "n". On special request the objectives can also be made with a higher absorption (88 % ± 2 % with designation "h") to facilitate the observation of phase structures which cannot easily be differentiated from the surrounding media.

For **negative phase contrast** the following objectives can be supplied with high absorption (88 % ± 2 %) their special purpose being indicated by the engraving "–h"

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

For details on the use of the various objectives see page 6.



THE VARIOUS TYPES OF ILLUMINATION

in relation to the position of the mirror component. Objective Pv 20/0.45.

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 nowbeen reached with phase contrast after Zernike.

Further raising of the mirror component Sk permits the image of the illumination 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.

Further raising of the mirror component Sk causes the image of the light ring to vanish 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 in place of the eyepiece. The light ring is focused by turning the eyelens and, if neccessary, by raising the auxiliary magnifier and adequately setting its stop collar.

FEATURES OF THE Pv OBJECTIVES

Pv 10/0.25. This achromatic objective is intended for examining general features of specimens. It yields a phase contrast image already with the mirror component in its lowest position and subsequently a dark field image. The objective is supplemented by an immersion attachment when the work carried out also calls for the use of a high-power oil immersion system. The objective Pv 10/0.25 is intended for finding the areas or structures of the specimen to be examined more closely under high magnifications. The immersion attachment reduces the working distance of this low-power objective to that for which all immersion objectives are uniformly matched so that a convenient change-over is ensured.

Pv 20/0.45. The adjustment of the various modes of illumination with this objective and the following systems is fully dealt with on the preceding page.

Pv WE 22/0.60, Pv WE 50/0.70, Pv WE 80/1.00. The working distance with these special water dipping objectives has purposely been reduced to a minimum so that observation will not be impaired by particles possibly floating between the objective front lens and the specimen.

Pv Apo L 40/0.70, Pv Apo L 63/0.70. Efficient high-power dry objectives can only reveal their optimum performance if they incorporate means for cover glass correction. The conventional cover glass correction mounts have the marked disadvantage that the focusing adjustment must be repeated whenever the attempt is made to set the correction mount in accordance with the deviation of the cover glass from the standard thickness (0.17 mm.). This procedure may cause the image to disappear completely and refocusing may turn out to be in the wrong direction. To do away with such inconveniences the Pv Apo dry objectives have been designed with automatic focusing compensation. To start observations, the correction mount is set to the standard cover glass thickness 0.17 mm. After focusing the microscopic image optimum image quality (in the bright field) is reached by adjusting the milled mount of the objective and a subsequent check on the setting of the micrometer screw.

Pv Fl Oil 70/1.15. This objective allows observations on ordinary microscope sections and thicker specimens. All modes of illumination can be set if the phase contrast condenser is also used with its immersion cap. Without this dark field illumination is not possible but object slides up to 7 mm. thick may be employed in this instance, also 7 mm. Carell flasks and oil chambers such as they are customary with micro-manipulators.

Pv Fl Oil 70/1.15, Pv Apo Oil 90/1.15. The mounts of these objectives are somewhat longer than ordinary oil immersion types to prevent the high-power dry systems from making contact with the oil layer on the cover glass. On the other hand special precaution is required when turning the revolving nosepiece to avoid their collision with the object slide.

Pv Apo Oil 90/1.15. This objective takes full advantage of the maximum aperture of the Heine condenser when using it in the dark field setting IV. It can also be successfully used for critical dark field work with the standard dark field condenser D 1.20 A.

Pv objectives should preferably be obtained specially adjusted on a separate nosepiece when required for use on microscopes with detachable nosepiece bracket (stands B, LABORLUX II, DIALUX, ORTHOLUX, PANPHOT) to ensure best results.

DIRECTIONS FOR USE

1. Adjust the light in the microscope, most expediently without condenser, without objective and without eyepiece, on looking into the body tube (with the ORTHOLUX and PANPHOT the pivoting illuminating lens should be swung out of action). With microscopes with built-in source of light, or when using the MONLAFIX microscope lamp, the centration of the source of light can be easily checked with the standard centred bright-field condenser.
2. Insert the Heine condenser and attach the set of objectives to the microscope. Operate the rack and pinion for the vertical adjustment of the substage upwards against the stop. This position remains unaltered during the examination. The mirror component Sk should be set in the lowest position against the stop in its cylindrical guide by means of the control knob Tr. Objective Pv 10/0.25 on the revolving nosepiece should then be swung into position. The eyepiece must be of the periplanatic type.
3. With the mirror component in its lowest position, focus the image sharply with the coarse and fine adjustment of the microscope.
4. Regulate the brilliance of the lamp; illuminate the field of view uniformly by focusing the illuminating lens on the microscope lamp.
5. Remove the eyepiece and insert the auxiliary magnifier in the body tube. Focus the eyelens sharply on the light ring L and centre the latter to the edge of the aperture diaphragm A by operating the two lateral screws S of the condenser. The centring is simplified if the mirror component is raised so high that the light ring just touches the diaphragm edge; as the mirror component is further raised, the light ring should vanish concentrically from the field of view. The mirror component is then returned to the lowest position by means of the control knob Tr. When centring, reduce the brilliance of the lamp by regulating the transformer if necessary. Replace the eyepiece.
6. If the objectives are not supplied parfocally adjusted on a special revolving nosepiece, a slight subsequent focusing may prove necessary when changing over from one objective to another. The best centration is achieved if the shadow spot which becomes faintly visible appears in the centre of the field of view after the mirror component has been vertically adjusted (by means of control Tr). Slight subsequent centring to this shadow spot may be necessary.
7. Phase contrast is obtained with the objective Pv 10/0.25 with the mirror component in its lowest position; with the other objectives the mirror component must be set correspondingly higher. (If bright field observations are to be carried out with the objective Pv 10/0.25 the complete condenser should be lowered, the mirror component remaining in its lowest position.)
8. When using the oil immersion objectives the immersion cap is screwed onto the Heine condenser, given a drop of oil, and brought upwards to the object slide from beneath by means of the rack and pinion of the substage, until the oil drop comes into contact with the object slide. Take care that the object slide is not pushed up by the immersion cap. The condenser then remains in this position during the observation with

immersion. The adjustment of the bright field, phase contrast or dark field image is effected by means of the mirror component control knob Tr (with the objectives Pv Fl Oil 70/1.15 and Pv Apo Oil 90/1.15 without the immersion cap on the condenser, a phase contrast image can still be achieved, but neither a dark field nor a bright field image is possible). Before bringing the immersion objective into use, the body tube must be raised, or the object stage must be lowered according to the type of microscope. The drop of oil applied to the specimen should not be too small, and the oil immersion should then be lowered until it dips into the oil drop (distance from cover glass 0.20 and 0.12 mm respectively). Focus the image sharply by means of the micrometer screw. If objective Pv 10/0.25 is used with the immersion attachment for locating sites of the preparation to be studied, no adjustment to the coarse focusing is necessary on changing over to oil immersion objectives (see also page 6).

9. Although as a rule work is carried out without the pivoting supplementary illuminating lens on the ORTHOLUX, DIALUX and PANPHOT and without the front lens of the MONLAFIX lamp, when setting normal dark field (Setting V) with the two low power objectives, it is advisable to bring these lenses into action in order to achieve uniform illumination of the image field.

10. The cover glass and the front lens of the objective must always be spotlessly clean. The eyelens should similarly be cleaned frequently, since with the small circular exit pupil the image may already be affected through slight soiling by the eye-lashes.

11. For work in polarized light a filter analyser is fitted over the observation eyepiece or placed on the tube lens accommodated on the nosepiece bracket of microscopes with interchangeable body tubes. The filter polarizer is clamped to the lower condenser mount and so oriented that the directions of vibration in the polarizer and analyser are at right angles to each other.

12. When working with tissue cultures it will often be found necessary to make use of chambers with ample accommodation for the cell growth. The Heine condenser allows the use of and observation in culture chambers up to 7 mm. high in conjunction with the oil immersion objective Pv Fl 70/1.15. This system also enables the light ring to be centred to the phase ring of the objective and a phase contrast after Zernike to be obtained when examining cover glass cultures, i. e. cell cultures growing along a cover glass of a maximum thickness 0.17 mm.

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