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PHASE - EN

ANOPTRAAL -
CONTRAST

E404



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ZETOPAN

Phase-Contrast
Anoptical-Contrast

INSTRUCTION MANUAL

We are constantly endeavouring to still further improve our instruments and to adapt them to the requirements of modern test and research methods. This involves, in certain cases, modifications in the mechanical and optical structure of our instruments. All descriptions and illustrations in catalogues and instruction manuals as well as specifications relating to the mechanical features and optical data **must not be regarded as binding.**

Phase-Contrast and Anoptral-Contrast Equipment

with the Contrast Condenser No. 00.62.42
on the "ZETOPAN" Large Research Microscope

Instruction Manual

Phase-Contrast and Anoptral-Contrast Objectives Contrast Condenser

1 Objectives

Each of the following phase-contrast and anoptral-contrast objectives is suitable for use with the contrast condenser No. 00.62.42:

Transmitted-light phase-contrast objectives

		Magnification/ Numerical aperture	Ring No.
Dry achromats		Ph 10/0,25	1
		Ph 16/0,32	1
		Ph 25/0,45	2
	iris	Ph 40/0,65	3
	iris	Ph 63/0,75	4
Oil immersion achromat	oil iris	Ph 63/1,00	4
Oil immersion fluorite	oil iris	Ph 100/1,30	5

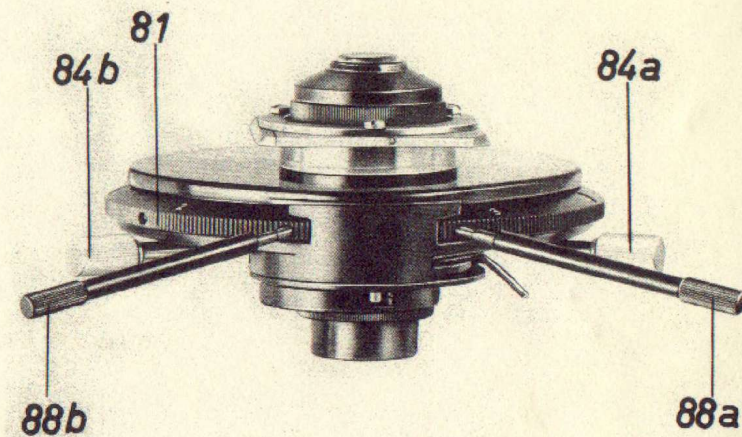
Transmitted-light anoptral-contrast objectives

Dry achromats		PhA 10/0,25	1
		PhA 16/0,32	2
		PhA 25/0,45	3
	iris	PhA 40/0,65	4
	iris	PhA 63/0,75	5
Oil immersion achromats	oil iris	PhA 63/1,00	5
Oil immersion achromats	oil iris	PhA 100/1,25	5
	oil	PhA 100/1,25	6

The phase contrast objectives are marked with a red ring and the letters Ph, the anoptral-contrast objectives with a yellow ring and the letters PhA.

The ring number shown against each objective is also engraved on the objective mount (see the numbers 2, 3 and 5 on the objective mounts in Fig. 1). They refer to the annular diaphragms of the contrast condenser (marked with the corresponding numbers 1 - 6) with which the phase-contrast or anoptral-contrast objectives have to be used.

Fig. 8



8 Individual centering of the annular diaphragms in the contrast condenser

Once the annular diaphragms in the apertures of the contrast condenser have been correctly centered (where possible this is done at the factory) each phase or anoptral ring should be concentric with the image of its corresponding annular diaphragm when changing objectives, without requiring readjustment of the two centering screws (84a) and (84b). If this is not the case, the annular diaphragms mounted in the rotating disc (81) of the condenser have to be readjusted.

This is done as follows: If a 100X anoptral-contrast oil-immersion achromat without iris diaphragm, ring 6, is available, anoptral-contrast illumination is set up with this objective in accordance with the directions given in Section 7. The annular diaphragm No. 6 used with this objective cannot be centered but is rigidly cemented into the rotating disc of the condenser. The objective with the next lower magnification and ring 5 is now brought into position. If the annular diaphragm corresponding to this objective is not accurately concentric with the phase or anoptral ring, the two centering keys (88a) and (88b) are inserted into the openings corresponding to the diaphragm in use; these are located at the edge of the rotating disc (81). By rotating these two keys the annular diaphragm is now centered accurately to the phase or anoptral ring of the objective as seen through the auxiliary microscope. The same procedure is followed with the subsequent lower magnification objectives and their corresponding annular diaphragms.

If the 100X anoptral oil-immersion achromat without iris diaphragm, ring 6, is not available the centering procedure starts by adjusting the phase or anoptral-contrast illumination with the most powerful objective, e.g. that with ring 5. The annular diaphragms of the lower magnification objectives are then adjusted individually to this reference position of the rotating disc (81) in accordance with the instructions given above.

The number "160" which appears on the objective mount after the magnification and the numerical aperture, indicates that the objectives are corrected for a mechanical tube length of 160 mm. The next marking which follows the oblique stroke indicates the cover glass correction of the objective, as follows:

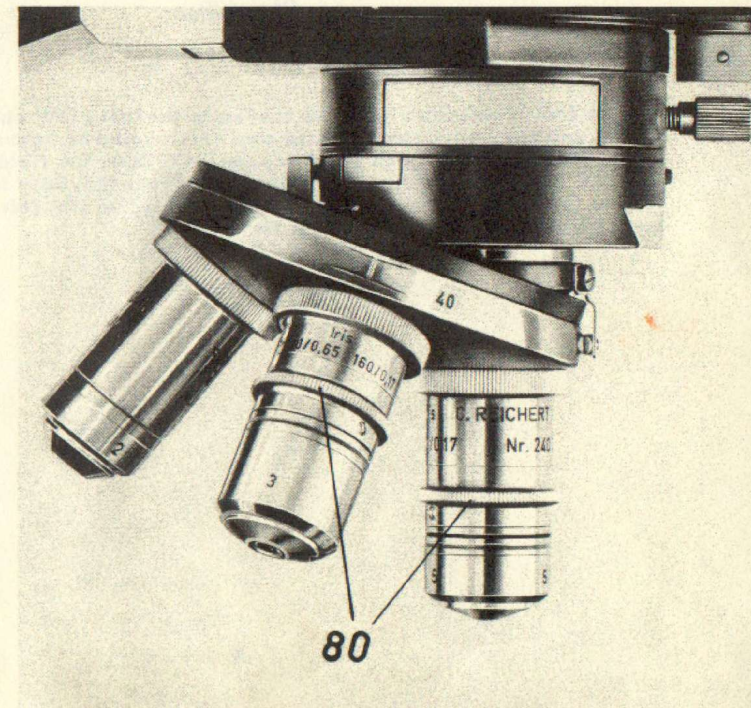
- for specimen with or without cover glass
- 0.17 for specimen with 0.17 mm thick cover glass

The aperture iris diaphragms of the phase-contrast and anoptral-contrast objectives with 40X, 63X and 100X magnifications are used for dark ground work especially with the "POLYPHOS" universal condenser; in all other applications the aperture iris diaphragms of these objectives have to be opened fully by rotating the ring (80) to the right.

The phase-contrast or anoptral-contrast objectives are screwed into the appropriate quadruple or sextuple nosepiece. The positions of the individual objectives are indicated on the nosepiece by the magnifications engraved on it; red numbers refer to phase-contrast objectives and yellow numbers to anoptral-contrast objectives.

The nosepiece on its slide is fitted into the guide on the "ZETOPAN" microscope body as described in Section 7 of the "ZETOPAN" operating instructions.

Fig. 1



2

Contrast Condenser, auxiliary microscope

The condenser on its slide is fitted into the guide on the condenser carrier as explained in Section 5 of the "ZETOPAN" operating instructions.

The contrast condenser carries a rotating disc (81) with six annular diaphragms marked with the numbers 1 to 6. The diaphragm in use is the one whose number appears opposite the mark (82) on the condenser. When changing diaphragms it is essential to ensure that the rotating disc engages accurately in its click stop. The disc has an empty position after every two annular diaphragms; this is intended for comparative bright ground observation. The position "0" has a specially large clear opening for bright-ground work using objectives of large aperture, i. e. immersion objectives. For this type of work the condenser carries an aperture iris diaphragm which is operated by the lever (83). Please note that this iris diaphragm has to be opened fully before starting phase-contrast or anoptral-contrast observations. The rotating disc with its annular diaphragms is adjusted by means of the screws (84a) and (84b).

When used on the "ZETOPAN" microscope the contrast condenser carries on its underside a swing-out wide-field lens (85) which is required with the phase-contrast objectives Ph 10/0,25, Ph 16/0,32 and the anoptral-contrast objectives PhA 10/0,25 and PhA 16/0,32, to ensure complete illumination of the field. When using objectives with a magnification greater than 16X this wide-field lens is swung out.

An auxiliary microscope (86) is supplied with the contrast condenser; it is fitted into the eyepiece tube in place of the eyepiece to permit the adjustment of phase-contrast or anoptral-contrast illumination.

Observation

3

Adjustment of illumination

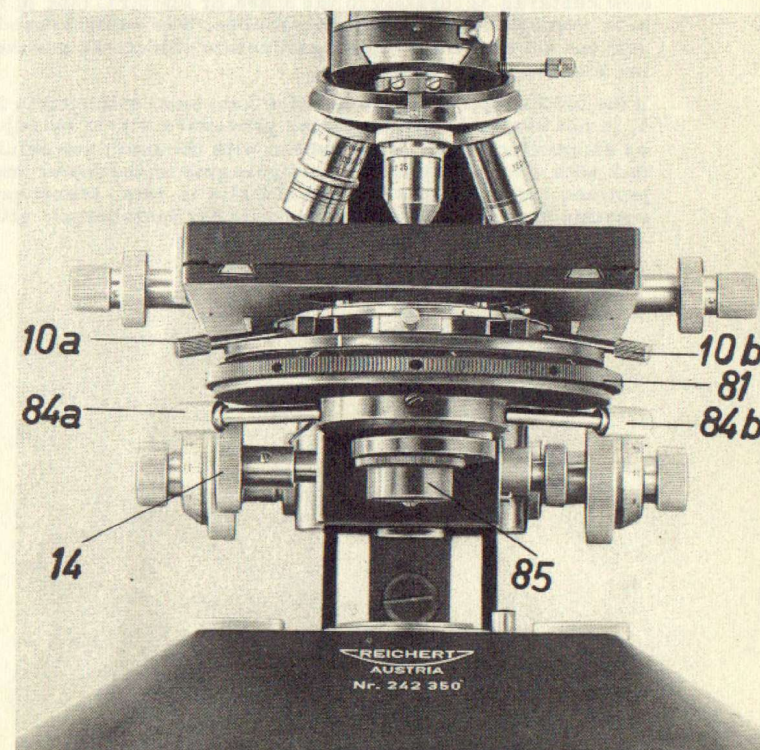
Set the lamp condenser close to the lamp; the lever (28) whose movement can be locked by rotation about its own axis should be approximately vertical. Swing in the ground glass screen (29). Open the field diaphragm on the "ZETOPAN" lamp housing by moving lever (35) fully to the left. The levers (40) and (41) are turned outwards, i. e. to the left and right respectively. The lever (15) is set to "EX".

The rotating disc (81) is then set to "6" and the inner edge of the bright ring centered with respect to the anoptral ring by means of the adjusting screws (84a) and (84b). The specimen is now focused in the eyepiece if this should have proved impossible before. Slightly raising and lowering the condenser with the knob (14), and adjusting the lamp collector with the lever (28), Fig. 4, the bright ring in the auxiliary microscope is adjusted accurately to the size of the anoptral ring and set to optimum brightness. The auxiliary microscope is then removed, the eyepiece replaced in position, and observation can now begin.

When working with the "BIOTHERM" please note:

The anoptral oil immersion achromat PhA 100/1,25 without iris diaphragm, ring 6, is the only one of the phase-contrast and anoptral-contrast objectives which cannot be used with the "BIOTHERM" biological hot stage. To obtain the highest magnifications in anoptral contrast we recommend in this case the anoptral oil-immersion achromat 100/1,25 with iris diaphragm, ring 5.

Fig. 7



6 Change to phase-contrast or anoptral-contrast objectives of higher magnification

When changing to the 16X objective the wide-field lens (85) remains in position; when changing to objectives with a magnification of 25X or more the wide-field lens underneath the contrast condenser is swung out. In the case of objectives fitted with an aperture-iris diaphragm it is advisable to check that this is opened fully (see also Section 1). The ring number marked on the mount of each objective is read off and the corresponding annular diaphragm on the rotating disc (81) is brought into position (the only exception is the 100X anoptral-contrast oil immersion achromat without iris diaphragm, ring 6; see Section 7). The auxiliary microscope is used to check that the diaphragm is concentric with the phase or anoptral ring. If necessary, the position of the bright diaphragm ring can be corrected by means of the two centering screws (84a) and (84b). (If large centering corrections are required, it is preferable to center the annular diaphragms individually in the rotating disc in accordance with Section 8). As in the case of Ph 10X or PhA 10X, the specimen is focused and the field diaphragm (35), Fig. 4, the condenser drive (14), and the collector (28), Fig. 4, are readjusted; in other words, with phase-contrast or anoptral-contrast illumination it is necessary to readjust for correct Köhler illumination after every change of objectives.

After swinging out the wide-field lens (85) it may also be necessary to readjust slightly the image of the field diaphragm by means of the screws (10a) and (10b).

When using phase-contrast or anoptral-contrast objectives of higher magnification, the ground glass screen (29), Fig. 4, on the lamp may be swung out if required.

7 Adjustment of illumination with the 100X anoptral-contrast oil-immersion achromat without iris diaphragm, ring 6

Set the rotating disc (81) to position "0". Swing in the 100X anoptral-contrast objective without iris diaphragm, immerse onto the specimen and focus. Open the field diaphragm by means of lever (35), Fig. 4, lower the contrast condenser slightly by means of the knob (14) and then raise it again slowly a little, at the same time observing through the auxiliary microscope (86), Fig. 3, which is fitted in place of the eyepiece and focused on the anoptral ring of the objective. When the condenser is raised, the circular illuminated field grows at first and then divides into a circular central field which decreases again, and an illuminated ring which continues to grow and which has to be set to the size of the anoptral ring by means of the knob (14). The bright ring is centered to the anoptral ring of the objective using the two adjusting screws (10a) and (10b). This adjustment replaces the focusing and centering of the field diaphragm which is normally carried out when changing over to objectives of higher magnification.

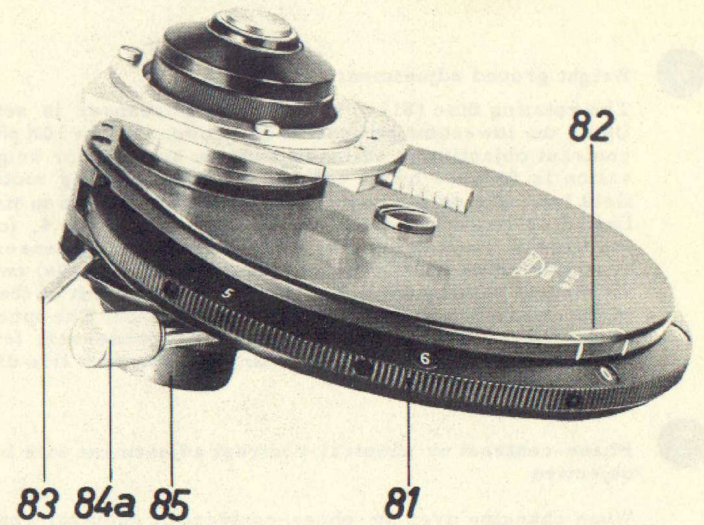
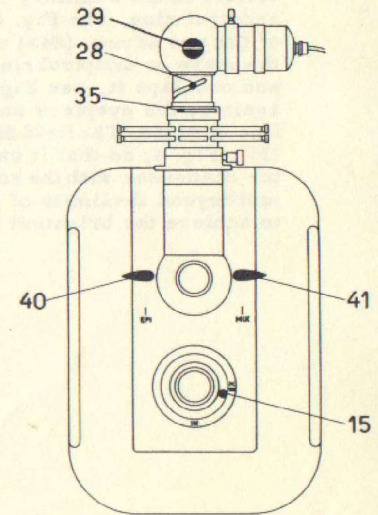
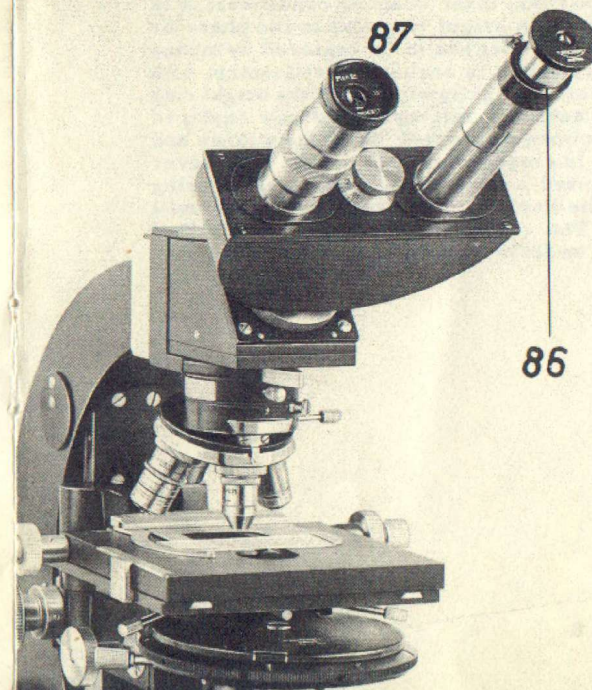


Fig. 2

Fig. 3

Fig. 4



4 Bright ground adjustment

The rotating disc (81) of the contrast condenser is set to position "0". Using the lowest magnification objective, i.e. the 10X phase or anoptral-contrast objective, a stained specimen suitable for bright ground observation is focused by the coarse and fine focusing motions, the wide-field lens (85) on the condenser being swung in up to its stop. Close the field diaphragm by means of the lever (35), Fig. 4, focus its image in the field of view by raising or lowering the condenser drive (14), and centralise it by means of the adjusting screws (10a) and (10b). The field diaphragm is then opened to just beyond the limit of the field of view, as in the usual adjustment for Köhler illumination. The optimum illumination of the field is set by means of the lamp condenser, lever (28), Fig. 4, and the clearest image by adjusting the aperture iris diaphragm - lever (83) - .

5 Phase-contrast or anoptral-contrast adjustment with low-magnification objective

When changing over to phase-contrast or anoptral-contrast adjustment with the objective Ph 10X or PhA 10X respectively, the aperture-iris diaphragm of the contrast condenser is opened completely using lever (83). Without changing the setting of the coarse and fine motions, replace the stained specimen on the stage which was used for bright ground adjustment, by the phase specimen to be examined. The auxiliary microscope (86), Fig. 3, is fitted to the eyepiece tube in place of one of the eyepieces; using the eyepiece extension (which can be clamped by the screw (87), Fig. 3, it is focused on to the grey phase ring or the golden brown anoptral ring of the 10X objective. The rotating disc (81) to position "1" brings the annular diaphragm No. 1 into position in the contrast condenser; it is visible in the auxiliary microscope as a bright ring next to the phase or anoptral ring, see Fig. 6a. This bright ring has to be centered by means of the two screws (84a) and (84b) so that it is accurately concentric with the phase or anoptral ring; the latter is slightly wider than the bright ring and overlaps it, see Fig. 6b. The auxiliary microscope is now replaced again by the eyepiece and the specimen is focused using the coarse and fine motions. The field diaphragm is closed slightly by means of the lever (35), Fig. 4, so that it can be focused accurately by raising or lowering the condenser with the knob (14); the diaphragm is then opened again until just beyond the limits of the field. The collector (28), Fig. 4, is adjusted to achieve the brightest and most uniform illumination of the field.

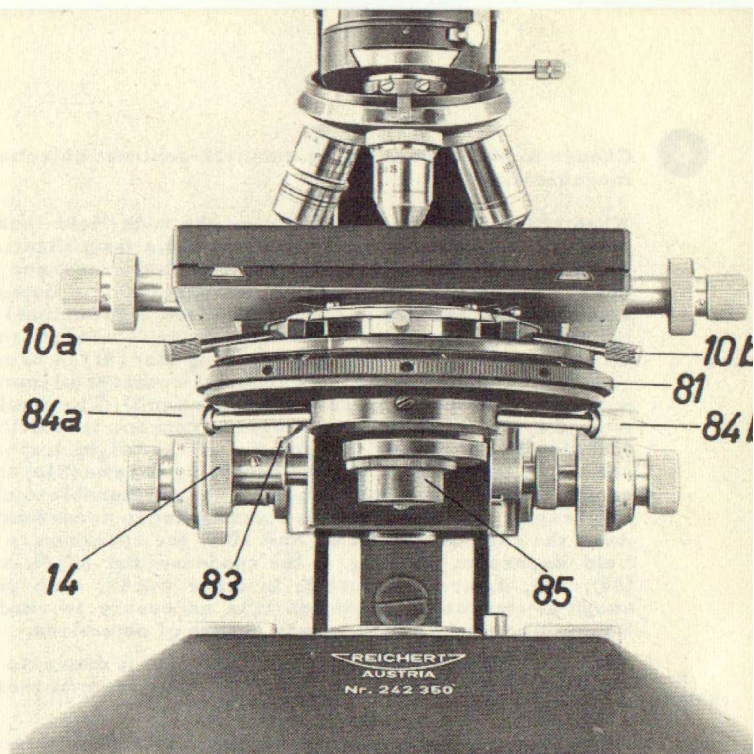


Fig. 5

Fig. 6

