E. LEITZ
WETZLAR

51a–31 / Engl. III / 56 / LX Printed in Germany Arthur Leipscher, Wetzlar

Leitz

Directions for Dark Field Microscopy

with the Dark Field Condensers D 1.20 A and D 0.80

ERNST LEITZ GMBH WETZLAR
GERMANY

51a–31 / Engl.

The immersion dark field condenser D 1.20 A in centring mount is designed for dark field examination primarily with oil immersion objectives and high magnifications. The achromatic oil immersion "Oil 100/1.30" is highly satisfactory for the purpose, while for most exacting demands in respect of image quality the fluorite oil immersion "Fl Oil 95/1.32" is recommended, or its special design for dark field "Fl Oil 95/1.15"*. Whereas the latter objective can be used without any supplementary means of stopping down its aperture, for the other two objectives the IRTIS adapter with iris diaphragm or a drop-in diaphragm must be used, according to the type of objective mount (with or without screw-on objective head), in order to reduce the objective aperture, which is too large for the dark field condenser D 1.20 A, and to remain below the aperture limit of the condenser (1.20 in this instance). Otherwise part of the illuminating rays would impair the dark field image. Most advantageous as to results and convenience are new type objectives with permanently built-in iris diaphragm.

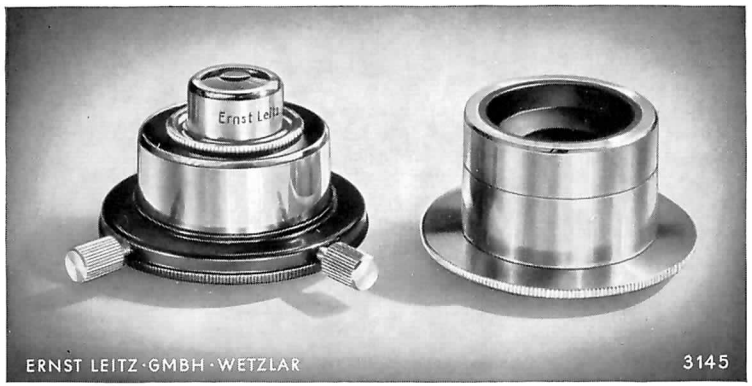

Fig. 1 Immersion dark field condenser D 1.20 A; dry dark field condenser D 0.80.

To obtain a perfect dark field preparation, the following important points must be observed:–

① Condenser, object slide and cover glass must be free from scratches, thoroughly cleaned, and dusted before use.

② The object slide should be between 0.9 and 1.1 mm thick. If it is too thick, the rays converge in the slide, and it is impossible to focus an intensive point of light as a prerequisite for a perfect dark field. If the slide is too thin, the drop of oil between the condenser and the object slide may part.

③ The thickness of the cover glass should be as near 0.17 mm as possible (between 0.12 and 0.22 mm). The microscope objectives specified above have been adjusted to this thickness.

* The three objectives mentioned are identical with those bearing the former designations 1/12. 1/12 Fl and 1/12 Fl N. A. 1.15.

④ The preparation should not be too thick. Otherwise the contrast in the dark field is reduced. Nor should the preparation be too dense or contain too many particles which deflect light; this also leads to a reduction in the contrast effect, which may render the image useless under certain circumstances. It may thus be necessary to dilute the substance to be examined when preparing the specimen for dark field examination. As a protection against evaporation of the liquid, the edges of the cover glass should be sealed with wax or paraffin.

⑤ The cover glass must lie absolutely even, i. e. the layer of preparation should not be wedge-shaped; otherwise it is possible that one-sided deformations of the light deflection phenomena may occur.

⑥ Preparations which are to be examined in a dark field with condenser apertures of over 1.0 must be embedded between the object slide and the cover glass in a substance with a refractive index somewhat higher than the lower limit aperture of the condenser. Thus for condenser D 1.20 A, water is a suitable embedding medium.

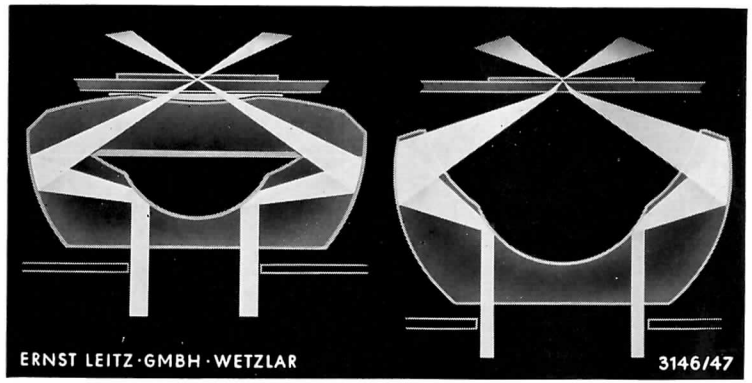

Fig. 2 Path of rays in the dark field condensers D 1.20 A (left) and D 0.80 (right).

The perfect ***adjustment of a dark field*** depends on the accurate centring of the source of light, which should be tested with the aid of the bright field condenser if necessary (on removing the eyepiece and looking into the tube, the rear lens of the objective must be fully illuminated). Before inserting the dark field condenser, the centring mount of the condenser should be set to about the middle position (using both centring screws if required). Only then is the dark field condenser pushed into the condenser holder on the microscope until the stop is reached, but it is not set to the upper position with the rack and pinion.

① A drop of immersion oil (not too small) is placed on the surface of the condenser (with immersion dark field condenser D 1.20 A).

④ The preparation should not be too thick. Otherwise the contrast in the dark field is reduced. Nor should the preparation be too dense or contain too many particles which deflect light; this also leads to a reduction in the contrast effect, which may render the image useless under certain circumstances. It may thus be necessary to dilute the substance to be examined when preparing the specimen for dark field examination. As a protection against evaporation of the liquid, the edges of the cover glass should be sealed with wax or paraffin.

⑤ The cover glass must lie absolutely even, i. e. the layer of preparation should not be wedge-shaped; otherwise it is possible that one-sided deformations of the light deflection phenomena may occur.

⑥ Preparations which are to be examined in a dark field with condenser apertures of over 1.0 must be embedded between the object slide and the cover glass in a substance with a refractive index somewhat higher than the lower limit aperture of the condenser. Thus for condenser D 1.20 A, water is a suitable embedding medium.

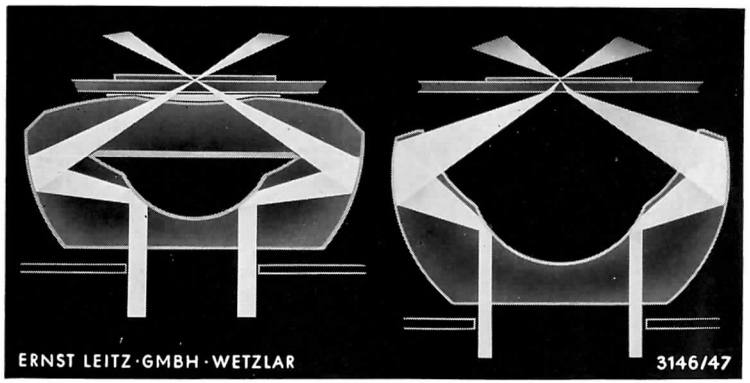

Fig. 2 Path of rays in the dark field condensers D 1.20 A (left) and D 0.80 (right).

The perfect ***adjustment of a dark field*** depends on the accurate centring of the source of light, which should be tested with the aid of the bright field condenser if necessary (on removing the eyepiece and looking into the tube, the rear lens of the objective must be fully illuminated). Before inserting the dark field condenser, the centring mount of the condenser should be set to about the middle position (using both centring screws if required). Only then is the dark field condenser pushed into the condenser holder on the microscope until the stop is reached, but it is not set to the upper position with the rack and pinion.

① A drop of immersion oil (not too small) is placed on the surface of the condenser (with immersion dark field condenser D 1.20 A).

② The dark field preparation is placed in position, and is sharply focused with the objective 10/0.25 (old type No. 3). If the brilliance is inadequate, lower the condenser.

③ The condenser is now raised (with the lateral rack and pinion while observing from the side), until the drop of oil comes into contact with the lower side of the object slide (brief illumination of the slide).

④ Looking into the tube with the preparation sharply focused, the condenser is brought still closer to the slide until the ring of light originally observed becomes as small a point of light as possible. This point of light is now brought into the centre of the field of view by operating the two centring screws of the dark field condenser (see fig. 3). Still more accurate centring can be achieved if the ring of light originally observed is immediately centred in relation to the circumference of the field of view.

⑤ The revolving objective nosepiece is now turned to an objective with high magnification. Objectives with a higher aperture than 1.15 must first be fitted with the adapter with iris diaphragm IRTIS (screw off the lower part of the objective and screw IRTIS on to the objective head in its place, see fig. 4) or a drop-in diaphragm (see fig. 5). The latter diaphragm must be completely inserted into the objective; it is engraved with specifications as to the appropriate objective and condenser.

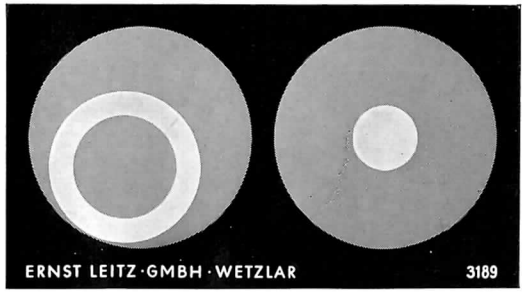

Fig. 3
Left: the ring of light which first appears, not yet centred.

Right: the centred point of light; the dark field condenser is correctly set.

⑥ When using an oil immersion objective, a drop of immersion oil (not too small) is placed on the surface of the cover glass, and with careful observation from the side, the objective is lowered until its front lens just dips into the oil drop. When using the IRTIS adapter with iris diaphragm, maximum brilliance in the field of view is set with the micrometer screw; the structure must become weakly visible at the same time, and the diaphragm is then closed until a perfect dark field is obtained. The structures of the specimens appear framed in a bright periphery (check sharp focusing by means of the micrometer screw). When using a drop-in diaphragm, the image is focused in the usual manner by operating the micrometer screw.

Fig. 4 Objective head and IRTIS adapter with iris diaphragm.

For dark field work with dry objectives of medium power, especially for serial examinations, the ***dry dark field condenser D 0.80*** is recommended; this condenser is simple and easy to use. The individual points for obtaining the dark field image apply here in a corresponding manner; immersion oil is, of course, not used between condenser and slide nor between cover glass and objective. For dry objectives with a higher numerical aperture than about 0.70, the IRTIS adapter with iris diaphragm or a drop-in diaphragm is to be used in the same way as with immersion objectives.

The D 0.80 condenser is supplied with a centre stop which should always be used as it does not affect the lower limit of the numerical aperture 0.80 but effectively suppresses lateral stray light.

On microscopes with built-in source of light and pivot lens in the lower section of the stand, such lenses are brought out of action when using dark field condensers.

A suitable test specimen for adjusting a dark field is a preparation of mouth spirillae, which can be easily prepared at any time.

Fig. 5 Drop-in diaphragm for objective "Oil 100/1.30", for use with dark field condenser D 1.20 A.

ERNST LEITZ GMBH WETZLAR
GERMANY
Branch Works: Ernst Leitz (Canada) Ltd., Midland, Ontario

E.LEITZ
WETZLAR

51₃–31 / Engl. III / 56 / LX Printed in Germany Arthur Leipscher, Wetzlar