INSTRUCTIONS

for

AO SPENCER DARKFIELD ILLUMINATOR

AMERICAN OPTICAL
CORPORATION
SCIENTIFIC INSTRUMENT DIVISION • BUFFALO, N.Y. 14215

INSTRUCTIONS FOR AO SPENCER DARKFIELD ILLUMINATOR

A. For the experienced microscopist, the following check list will insure that proper steps have been taken in setting up the Darkfield Illuminator.

1. Remove bright field condenser from substage fork (Fig. II).
2. Replace with darkfield illuminator (Fig. III).
3. Connect transformer to outlet and darkfield illuminator to transformer.
4. Adjust upper surface of condenser lens to a height just below stage level.
5. Place a clear glass slide in position over the condenser.
6. Focus 10X objective on top of condenser until white ring appears.
7. Center ring concentric with field edge, using centering screws "B" Fig. I.
8. Center light source for evenly illuminated field, using centering screws "C" Fig. I.
9. Remove the clear glass slide.
10. Place funnel stop in oil immersion objective.
11. Place slide on stage having oil contact between it and the condenser.
12. Place drop of oil on slide cover glass.
13. Focus with oil immersion objective until crisp contrast image appears.

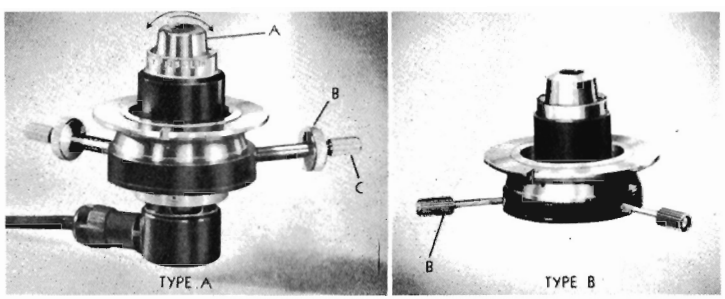


Fig. I.- *No. 218F and No. 214F Darkfield Condensers*

B. The following is a detailed step by step outline of the set-up procedure for darkfield illuminator having a built-in or integral light source (Type A, Fig. I). A full discussion of the use of an outside light source for Type B, Fig. I will follow.

INSTALLING DARKFIELD ILLUMINATOR ON STAND

1. For convenience, reverse and incline the microscope so that the AO Spencer Monogram faces you. Rack the regular condenser down and remove it by pulling it toward you with a slight twisting motion. (Fig. II)

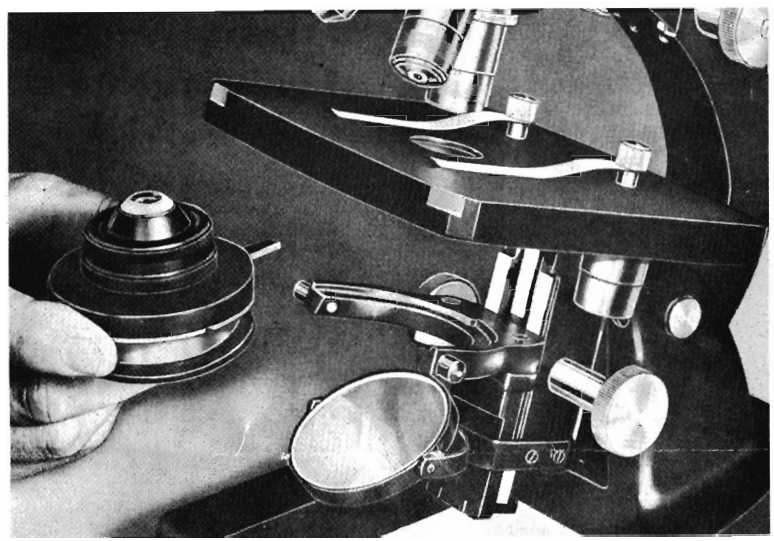


Fig. II—*Removing Standard Condenser*

2. Place the darkfield illuminator in the fork substage mount with the centering screws (B and C Fig. I) toward the microscope arm. (In the case of Spencer Series 15-35 microscopes, be sure the slot in the wide flange ring of the darkfield illuminator is toward the microscope arm and engaged in the lug in the throat of the fork mount).
3. Connect the transformer or resistance to electrical outlet, then connect it to the darkfield illuminator.
4. Rack the substage up to its maximum height. The upper lens surface (A, Fig. I) of the darkfield illuminator should fall slightly below (approximately the thickness of ordinary writing paper) the level of the stage. The height of the lens can be adjusted by rotating it, counterclockwise for raising, or clockwise for lowering (Fig. I A).

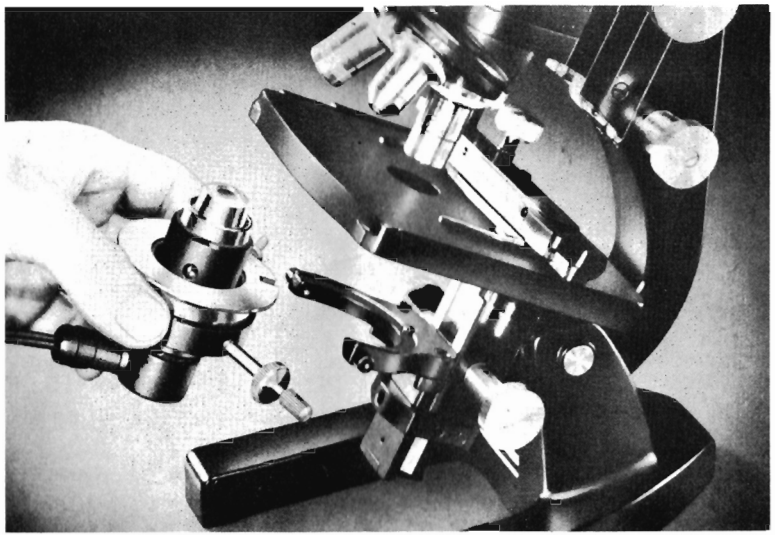


Fig. III—*Inserting the Darkfield Illuminator*

CENTERING PROCESS

5. Turn microscope around in position for normal use, returning it to its vertical position. Place a clean glass slide in position over the condenser. Do not place oil on the condenser at this point since the ground circle will not be visible for centering. Turn on transformer or resistance by means of switch provided. In case of a variable transformer, set the rheostat at approximately 6.5 V., light intensity can be adjusted later for best results.

6. With the 10X objective in place, focus on the top lens of the condenser. A white ring will appear in the field when the proper focus is attained. (In case of the Nos. 35 and 15 series microscope, the coarse adjustment should be at the bottom of its excursion and any residual focusing done with the fine adjustment). By means of the large knurled head centering screws (B, Fig. I) center this ring until it is concentric with the outer periphery of the field. The built-in light source is centerable by means of small centering screws ("C", Fig. I, Type A). Manipulate these screws until field is evenly illuminated throughout. The glass slide (step 5) should be removed at this point since its only purpose was to aid in focusing on condenser top element. The condenser is now in center and prepared for operation.

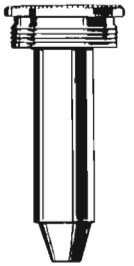


Fig. IV
Funnel Stop

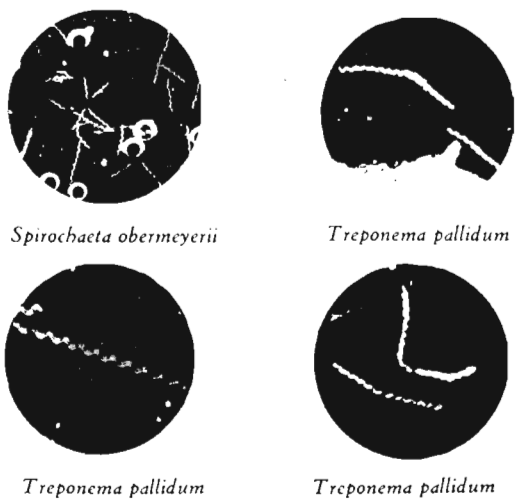
SETTING UP OIL IMMERSION OBJECTIVE

7. Remove oil immersion objective from the microscope by unscrewing it from the nosepiece. Unscrew the knurled black diaphragm from the back of the objective. Replace this diaphragm with the funnel stop which is contained in the darkfield case (Fig. IV). This serves to reduce the numerical aperture of the objective which must be less than that of the condenser. Replace the objective on the nosepiece.

8. Rack condenser down slightly and place a generous drop of oil on the top surface so that it is covered evenly. Take extreme care to avoid air bubbles in the oil. Set the specimen slide in place over the condenser and rack it slowly up to make oil contact with the slide. Again use care to avoid air bubbles.

9. Place immersion oil on top of the cover glass and lower the oil immersion objective to make oil contact with the cover glass.

10. Focus up or down with fine adjustment until a clear image is obtained. The material which is being examined should appear crisp and bright against a very dark background (Fig. V).



Spirochaeta obermeyerii — *Treponema pallidum*

Treponema pallidum — *Treponema pallidum*

Fig. V—*Various Darkfield Photomicrographs*

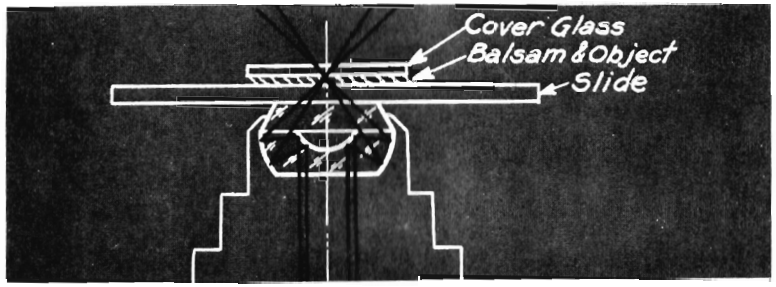


Fig. VI—*Path of light through Darkfield Condensers*

FOR DARKFIELD CONDENSER USING EXTERNAL LIGHT SOURCE

C. The above steps are repeated with the exception of adjusting the light source itself. The lamp should be very intense, preferably having a 100 W or more projection type bulb and adjustable to obtain parallel rays. In the case of the AO Spencer 370 or 735 lamp, close diaphragm down and focus the filaments of the bulb on an object about 15 feet away. The light rays can then be said to be parallel. Place the lamp in position about 12" from the microscope mirror (Plano—not Curved) and center the light beam on the mirror. Set the iris diaphragm at about a 20mm opening for best results. When using a binocular microscope, use the lamp without filters. With a monocular microscope best results are obtained with the ground glass filter. With the lamp set up as above steps 1-10 may be followed substituting the external light source as necessary.

CLEANLINESS

It is extremely important in darkfield work that all material be kept scrupulously clean. Slides and cover glasses should be free of all finger marks, dirt or grease of any kind. Foreign substances will tend to deviate the light path from its normal course, making it impossible to produce an image of good contrast. The objective and condenser should be cleaned thoroughly.

SPECIMEN PREPARATION

It is important that slides having a thickness of between 1.15 and 1.25mm be used with your AO Spencer darkfield illuminator. Use No. 1 cover glasses as the oil immersion objective has a short working distance and cannot be focused through thicker cover glasses. Specimen must be very thin to avoid scattering light by particles just above or below the focus.

CRITICAL FOCUS

If field does not appear dark and give good contrast, return to the 10X objective and if using a darkfield illuminator move outer centering screws (Fig. I, "C") so that field is evenly illuminated, or if using a darkfield condenser adjust mirror position.

Rack condenser very slightly up or down so that a bright spot of light appears in the field. If condenser is too far up or down, this spot will appear as concentric circles alternately light and dark. The condenser is in critical focus when the solid bright spot appears. This spot by no means covers the field and may not even be centered. Use care in focusing condenser and avoid breaking oil contact with slide.

CHECK LIST OF TROUBLE SPOTS

Avoid air bubbles when preparing your specimen as they will cause an undesirable scattering of light. Also use great care to avoid bubbles when making oil contact with both condenser and objective. This is the greatest contributing factor to light scattering with a resultant loss of contrast.

After condenser has been centered with white ring with 10X objective, slight recentering may be necessary when using oil immersion objective to improve definition and evenness of illumination.

If it is difficult to obtain a sharp focus, try a thinner cover glass. The image will tend to be fuzzy if there is not enough oil contact between the objective and the cover glass or the condenser and bottom of the slide.

A halo of light around the outside of the microscope field indicates the aperture of the objective is too great and that a funnel stop with a smaller aperture should be used.

A lack of contrast may indicate the specimen is too thick and the light is being scattered by those particles not in focus.

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