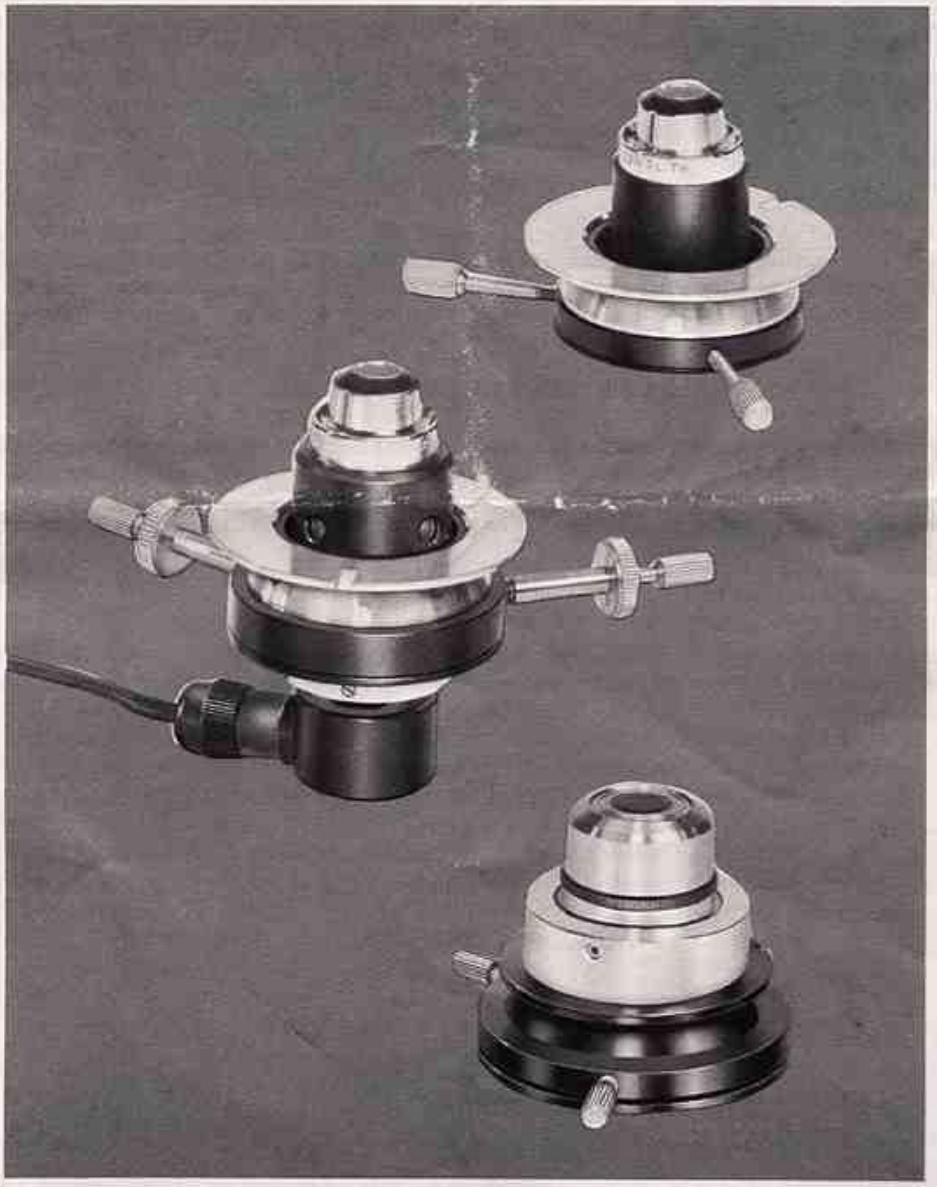
AO® DARKFIELD CONDENSERS
Models 214F, 218F, and K2172

REFERENCE MANUAL



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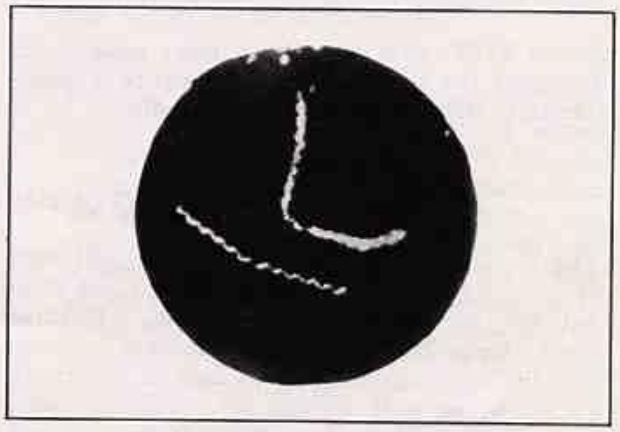
INTRODUCTION

The darkfield condenser is an important accessory to the microscope for a wide variety of applications. In medical laboratories it is routinely used to examine body fluids and colloidal material taken from body lesions in the search for specific disease causing microorganisms, such as in diagnosing syphilis. For fluorescence applications the darkfield condenser is used in combination with a controlled source of ultraviolet for antibody-antigen work (FA) and in the fluorescent treponemal antibody absorption test (FTA-ABS).

In biology, a microscope with darkfield condenser may be used to demonstrate ciliated and flagellated protozoans. In food and beverage laboratories it is useful for checking products for clarity or turbidity and for the presence of microscopic particles such as yeasts or molds. Industrially the darkfield condenser can be used to check fluids such as fuels, lubricants, and solvents for submicroscopic particles, e.g., dust, grit, or other undissolved material.



Darkfield Photomicrograph

DARKFIELD CONDENSERS

AO darkfield condensers are of bispheric design and require the use of immersion oil between the top of the condenser and the specimen slide. These condensers are for use with AO MICROSTAR® Microscopes Series 2, 4, 10, and 20.

Three different models are supplied:

1. Model 214F - Darkfield condenser has a centerable mount with two centering screws, and is primarily for use with objectives equal to or greater than 40X.

2. Model 218F - Darkfield condenser has a centerable mount, a separately centerable built-in light source, 6.5 volt, 1.7 ampere lamp, and two double sets of coaxial centering screws. Model 218F is primarily for use with objectives equal to or greater than 40X. A step-down transformer, not included with the outfit, is needed to operate the lamp. AO transformer nos. 365, 651, or 1051 may be used.

3. Model K2172 - Large area darkfield condenser with toric lens is a special model for viewing large areas and can be used with objectives equal to or greater than 10X. It is supplied in a centerable mount with two centering screws. This condenser is primarily intended for use on AO Series 10 and Series 20 microscopes.

Figure 1. Model 214F Darkfield Condenser

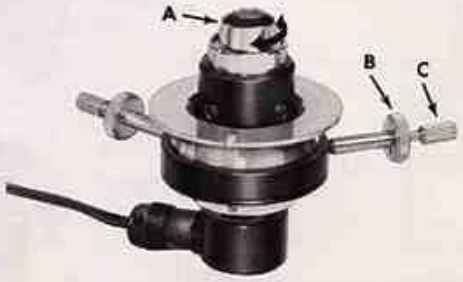


Figure 2. Model 218F Darkfield Condenser with Centerable Built-In Light Source

Figure 3. Model K2172 Large Area Darkfield Condenser

All of the above darkfield condensers can be used for routine darkfield work. Models 214F and K2172 are for use either with a high intensity illuminator in the microscope base, or with and adjustable plano mirror in the microscope base in combination with a separate, focusable, high-intensity illuminator.

Model 218F with a built-in light source, is designed for use in situations where a high-intensity illuminator is not available.

For fluorescence darkfield work, Model 214F or Model K2172 are used on AO MicroStar microscopes in combination with an ultraviolet source such as the AO Model 645 Fluorolume Illuminator or the AO Series V20 FLUORE-STAR[T.M.] Microscope, which has a UV source in its base.

PRINCIPLES OF DARKFIELD ILLUMINATION

The darkfield condenser has a central dark stop that prevents direct rays of light from entering the microscope objective. Parallel light from below the microscope passes into the darkfield condenser and is internally reflected to exit above the condenser as a hollow cone of light having a dark background. When the cone of light is precisely focused on the specimen, the object particles within the specimen are seen by the light they scatter and reflect into the objective. Such particles are seen, like stars in the night sky, against a nearly black background. The more intense the illumination, the smaller the particles that may be discovered.

Small bright objects are more readily seen against a dark background than similarly small dark objects on a bright background. The darkfield method reveals objects smaller than may be seen with bright field, including particles too small to be resolved. Because these particles are seen only as reflected points of light it is not possible to accurately measure them or to see their structure.

The diagrams that follow (Figures 4 and 5) illustrate the paths of light passing through the standard bispheric condensers, i.e., AO Model 214F and Model 218F, and through the special AO Model K2172 Large Area Darkfield Condenser with toric lens.

A good darkfield image can be obtained only if none of the direct rays of light pass through the objective. To accomplish this result the useful aperture of the objective must be smaller than the inner aperture of the darkfield condenser. This is accomplished by inserting a funnel stop, a small tube-shaped device, into the N.A. 1.25 oil immersion objective in place of the regular diaphragm. The stop reduces the objective aperture to about N.A. 0.85, and also prevents passage of peripheral direct rays through the objective.

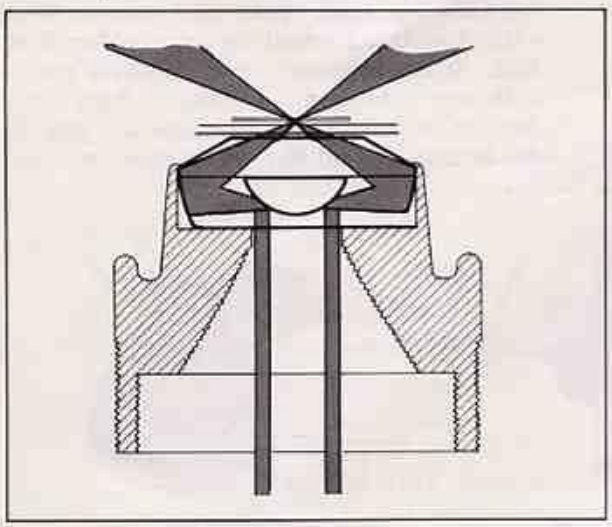


Figure 4. Light Path Through Models 214F and 218F Darkfield Condensers

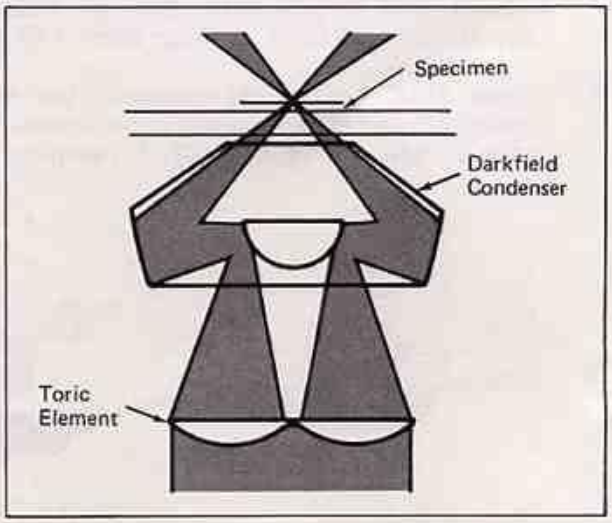


Figure 5. Light Path Through K2172 Large Area Darkfield Condenser

SPECIMEN PREPARATION

1. It is extremely important in darkfield work that all material be kept very clean. Slides and cover glasses should be free of all finger marks, dirt, or grease of any kind. Foreign substances 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.

2. Use slides having a thickness range of 1.10mm and 1.25mm with Models 214F and 218F darkfield condensers. With Model K2172 condenser use slides not exceeding 1mm thick, and not appreciably thinner.

   Use No. 1 or No. 1-1/2 thickness cover glasses.

3. An effective test specimen for darkfield can be prepared using a toothpick to gently scrape off a few epithelial cells from inside the check or a few leukocyte cells from gum tissue between the teeth. Eliminate food particles, if possible, as they introduce debris which scatters light and reduces image contrast.

4. Place a small amount of specimen with saliva or equivalent on a clean slide. Touch a cover glass to the slide by one edge, and then drop it into place over the specimen. (This method helps reduce air spaces between the slide and cover slip.) Press down on the cover glass with a toothpick or tweezers to force out excess liquid which then can be removed with filter paper. The specimen preparation must be very thin to avoid scattering light by an over-abundance of particles just above or below the focus.

INSTALLATION

1. Lower the condenser by turning the condenser focusing knob. (See Figure 6.)

2. Remove the standard condenser assembly from the fork mount by backing off the thumb screw and pulling the entire condenser assembly forward.

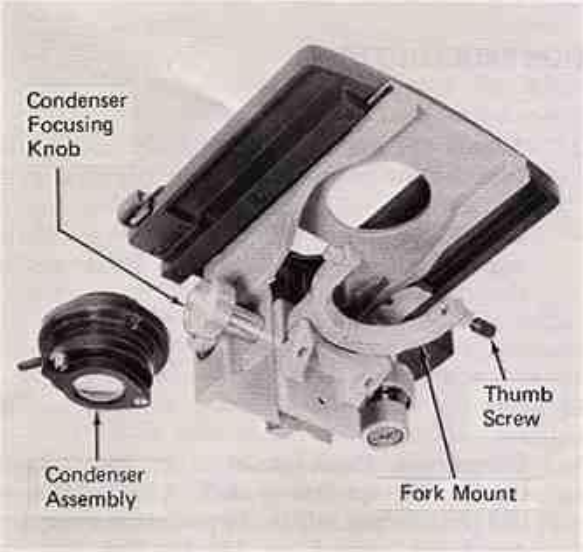


Figure 6. Removal of Condenser Assembly

3. Insert the darkfield condenser into the fork mount. Rotate the condenser until the slot in its flange (see Figure 7) engages the pin in the back of the fork mount. Tighten the thumbscrew to hold the darkfield condenser firmly in the fork mount.

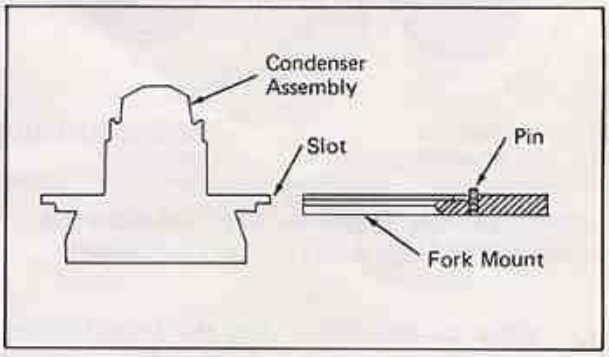


Figure 7. Fork Mount

4. (a) Re Models 214F and 218F

   Turn the condenser focusing knob (Figure 6) to raise the darkfield condenser until it stops. The top lens of the condenser should stop slightly (approximately the thickness of ordinary writing paper) below

stage level. The height of the lens (A) (see Figure 2) can be adjusted, if necessary, by screwing it up or down within its threaded cell.

(b) Re Model K2172

Turn the condenser focusing knob (Figure 6) to raise the darkfield condenser until it stops. Then lower the condenser until the top lens is just slightly below stage level.

5. Remove the oil immersion objective from the microscope nosepiece. Unscrew the knurled diaphragm (Figure 8) from the back of the objective. Replace this diaphragm with a funnel stop (Figure 9) supplied with the darkfield condenser. Place the objective back on the nosepiece.

NOTE

No. 214-79 Funnel Stop fits No. 1079 100X objective; No. 214-127 Funnel Stop fits No. 127 97X objective.

For brightfield work the knurled diaphragm should be placed back in the objective in place of the funnel stop.

NOTE

Cat. No. 1024 100X plan achromat objective is not intended for darkfield work. Check the number on your oil immersion objective.

Cat. No. 1014 100X and Cat. No. 1016 50X plan achromat objectives have built-in iris diaphragms and may be used with darkfield condensers. They require no funnel stop.

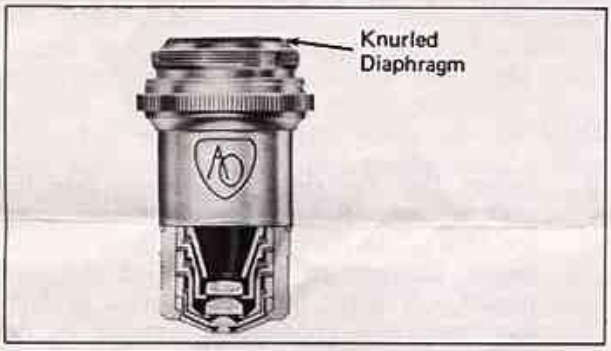


Figure 8. Cross Section of Oil Immersion Objective, 100X

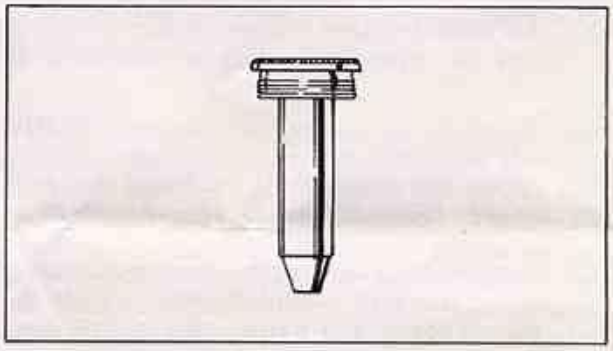


Figure 9. Funnel Stop

CENTERING AND OPERATION PROCEDURES

Models 214F and 218F Darkfield Condensers and Model K2172 Large Area Darkfield Condenser

1. Turn on the light. Set the transformer voltage as desired; it can be adjusted later for best results. Remove all filters from the light path, and make sure the field diaphragm in the microscope base illuminator is open.

   If using the Series 20 microscope, adjust the base reflector knobs and base condenser knob to make sure the light is directed straight upward from the base in a parallel beam.

2. Lower the darkfield condenser about 1/8-inch below stage level, and place a generous drop of immersion oil on top of it. Allow time for tiny bubbles, which appear as bright dots, to float out of the oil.

3. Prepare a clean glass slide about 1mm thick with specimen and cover glass on it. Place the slide in position over the darkfield condenser. Raise the condenser to make oil contact with the slide.

4. For Models 214F and 218F:

   a. With the 10X objective, focus on the specimen. A disc of light will appear against a dark background. Gently adjust the height of the condenser to establish a well defined border between the disc of light and the dark area (Figure 10).

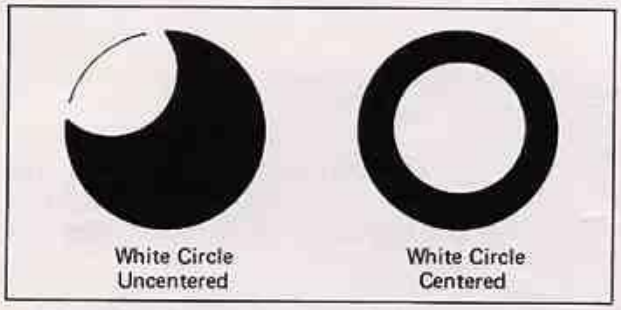


Figure 10. No.'s 214F and 218F Darkfield Condensers

   If you cannot see any light, check the stage to make sure it is up high enough.

   Stage height can be adjusted, if necessary, by resetting the autofocus stop screw, and on the Series 10 and Series 20 microscopes by loosening the stage locking lever.

   b. With the white disc focused, move it to the center of the field by means of the two centering screws on the condenser.

   In the Model 218F, center the white disc using the two larger diameter knurled knobs (B) (see Figure 2). Then move the built-in light, using the smaller knurled knobs (C) (see Figure 2), until the white area is evenly illuminated.

   c. Turn the coarse adjustment to separate the 10X objective and specimen. Place a drop of immersion oil on the cover glass. Turn the oil immersion objective into position, making sure it has a funnel stop in it, and focus to the specimen with the coarse adjustment. Then focus with the fine adjustment until a clear image is attained.

   The material being examined should be crisp and bright against a dark background.

   A 40X or 45X objective may also be used, and these do not need a funnel stop, nor do they need immersion oil on the cover glass.

   d. If your oil immersion objective has a built-in iris diaphragm, close the diaphragm to eliminate light around the periphery of the field of view. Then adjust it for best results.

5. For Model K2172 Large Area Darkfield Condenser:

   a. With the 10X objective, focus on the specimen. A few bright areas will be visible in the field. (It may be helpful to turn the transformer to a high voltage.) Lowering the condenser slightly produces a darkened area covering about two-thirds of field. Move this darkened area to the center of the field (Figure 11) by means of the two centering screws on the condenser.

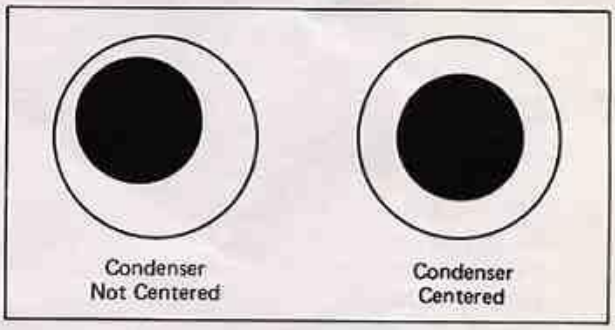


Figure 11. No. K2172 Large Area Darkfield Condenser

6. Raise the condenser until the darkened area disappears and the specimen is brightly illuminated. (On Series 20 microscope, adjust the condenser knob at rear of base and the centering knobs near front of base to obtain the brightest and most uniform illumination.) The condenser is now centered and ready for operation.

Operation of Darkfield Condenser Using External Light Source

Repeat the above steps with the exception of adjusting the light source itself. The lamp should be very intense, preferably having a 100-watt projection type bulb and adjustable to obtain parallel rays. In the case of the AO Models 370 or 735 lamps, 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 inches 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.

TROUBLESHOOTING CHECKLIST

1. Cleanliness - It is extremely important in darkfield work that all material be kept clean. Slides and cover glasses should be free of all finger marks, dirt or grease. 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.

2. Slides and Cover Glasses - It is important that slides having a thickness of between 1.15 and 1.25mm be used with your AO darkfield condenser. Use No. 1-1/2 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.

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

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

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

   Avoid air bubbles when preparing your specimen as they cause an undersirable 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.

   If field does not appear dark and give good contrast, return to the 10X objective. Move 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 circular light, dark in the center. 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.

   If the bright outlines of specimen detail show a pronounced broadening in one direction, the illumination is oblique from one side. This is corrected by centering the illumination or by adjusting the substage mirror.

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214F-101 7/76

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