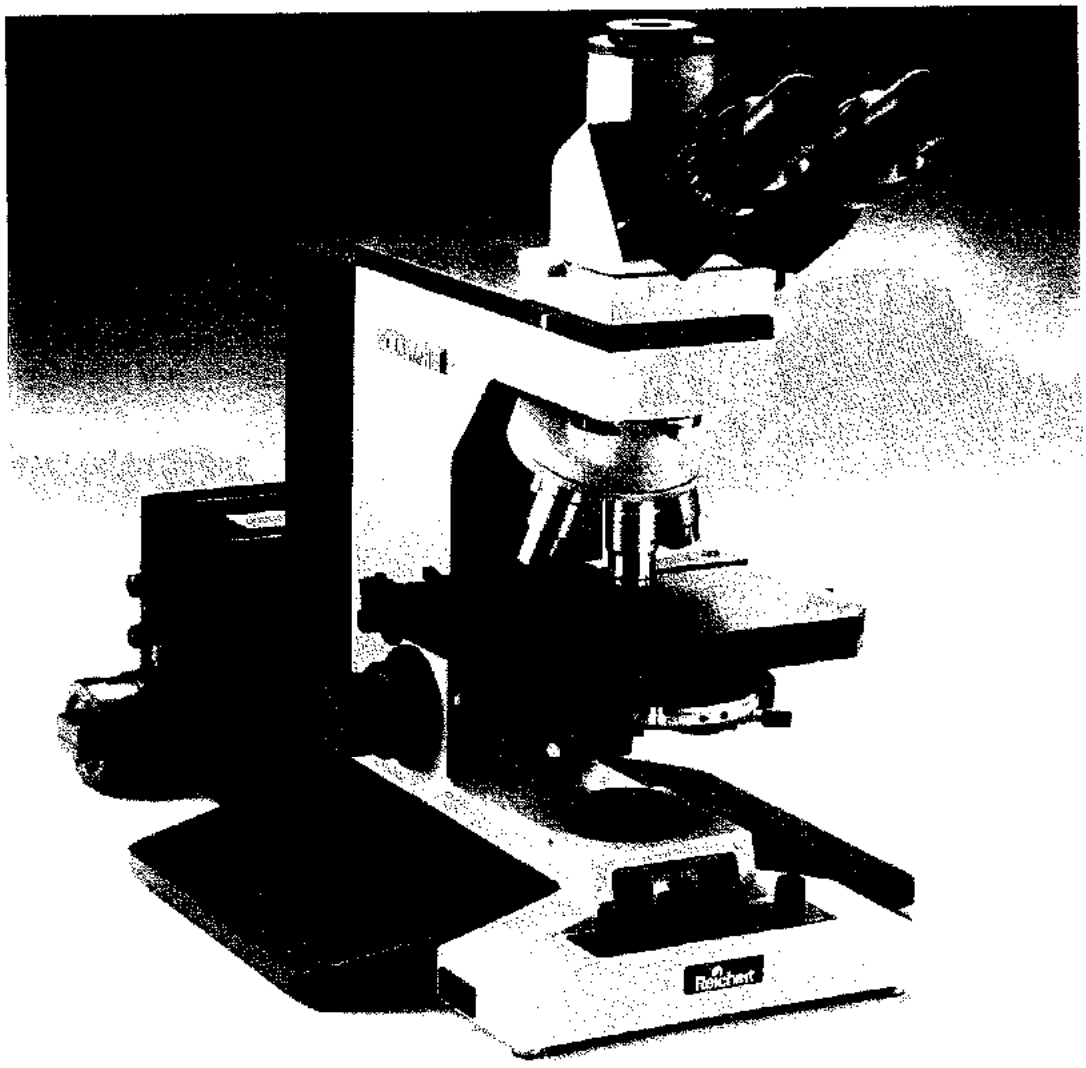
REFERENCE MANUAL

REICHERT-JUNG DIASTAR AND MICROSTAR IV SERIES PHASE CONTRAST MICROSCOPE



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Phase Contrast Microscope	12 months

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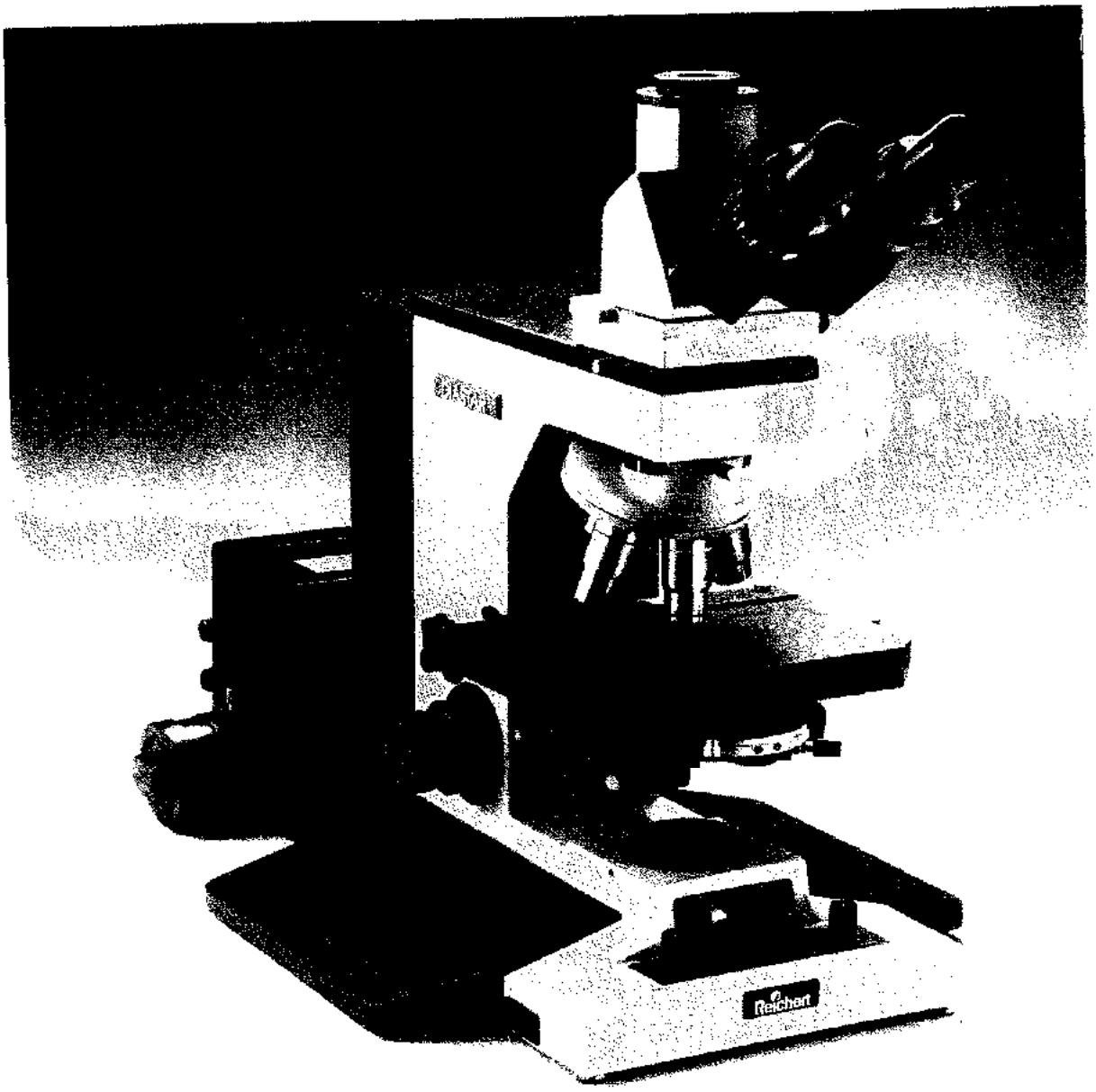


Figure 1. Diastar Phase Contrast Microscope

I. INTRODUCTION

Phase microscopy reveals details in living or unstained specimens too transparent to be seen with ordinary brightfield microscopy. Two distinguishing components of a phase microscope are: (a) an annular diaphragm below the condenser, as shown in Figure 2; and (b) a diffraction or "phase" plate at the rear focal plane of the objective.

Dark Contrast Planachromatic phase objectives 10X, 20X, 40X, and 100X are offered. The Dark Contrast phase objectives show details of greater optical path (refractive index times thickness) darker than surrounding details of lesser optical path, as shown in the photomicrographs.

The preliminary procedure outlined on the following pages, while fundamental, is offered to help assure that you gain full advantage of all the advanced features built into your Reichert Microscope, or the phase parts and accessories which you are adding.

In reviewing this basic information, keep in mind that the essential adjustments of a phase microscope, stated briefly, are: (1) the condenser is centered to the field of view; (2) the image of the annulus exactly matches (superimposes on) the diffraction plate; and (3) the illumination is uniform and sufficient with the field diaphragm properly focused.

II. ARRANGEMENT OF OBJECTIVES

When the phase objective is viewed through its back lens, the diffraction or "phase" plate may be seen as a grayish ring (Figure 3). The area of this ring is illumnated by the corresponding annulus in the phase condenser mount.

Phase objectives, alone or in combination with brightfield objectives, can be arranged as desired on the nosepiece. However, keep in mind that when using the turret condenser mount the phase annuli should be in the same sequence as the phase objectives. On complete microscopes, phase objectives and annuli are installed in corresponding sequence at the factory.

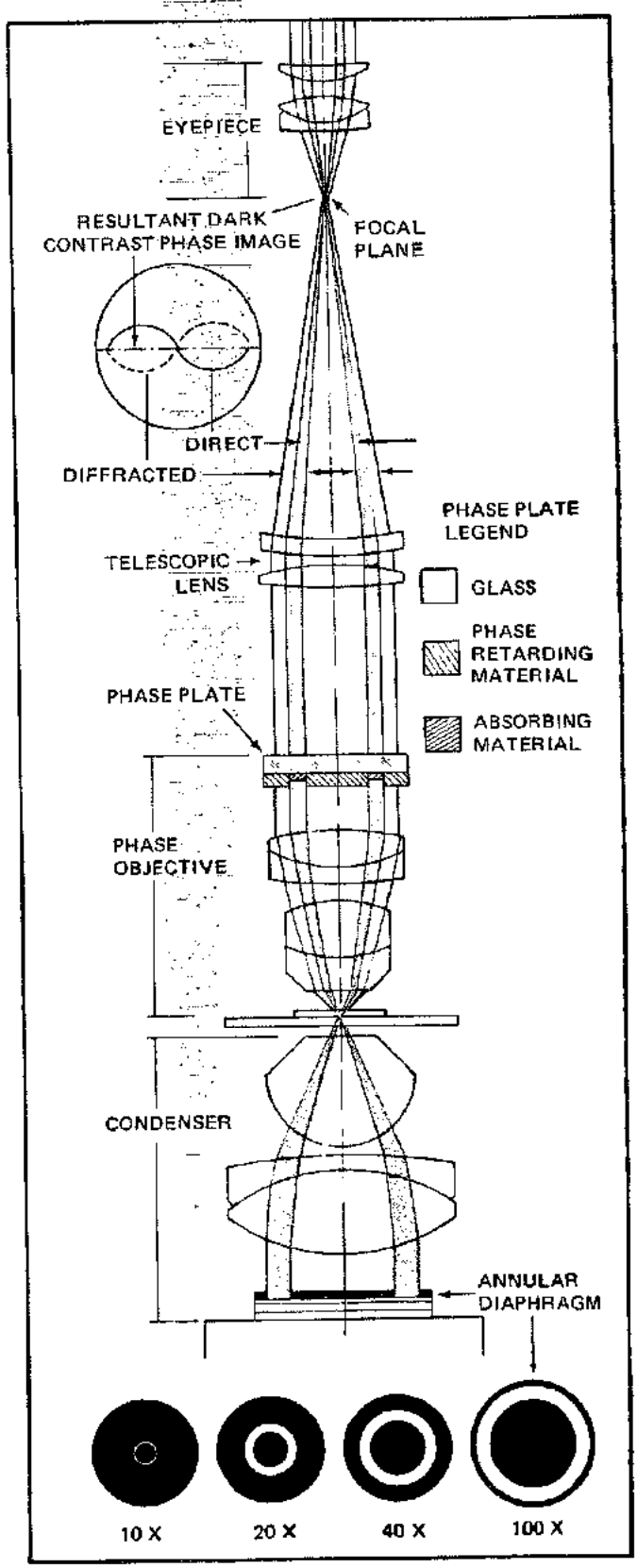


Figure 2.

III. USE OF THE APERTURE VIEWING UNIT

The Phase Aperture Viewing Unit No. 1204 permits binocular viewing and focusing on the diffraction plate of each objective and the image of its corresponding annulus for fast and easy centration. The Unit, which in conjunction with the eyepiece acts as a built-in telescope, is also a convenient and rapid method of checking alignment from time to time.

The Aperture Viewing Unit is easily attached beneath the body of any Microstar IV or Diastar Series Microscope. It is held in place with a knurled thumbscrew as shown in Figure 4.

The microscope body is then placed on top of the viewing unit as shown in Figure 5.

The operating lever can face to either side of the microscope to accommodate your technique and equipment arrangement. Regardless of orientation, the Viewing Unit will be precisely located on the optical axis of the microscope.

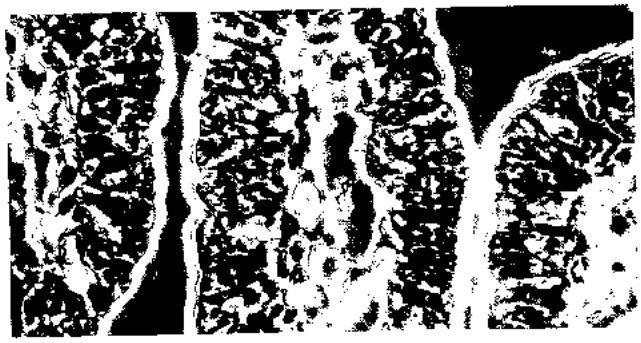
Use the lever to swing lens into optical path to view phase plate (Figure 6). Figure 7A shows the phase plate and annular diaphragm out of focus, as they might appear when the aperture viewing unit is first swung in. Turn the knurled thumbscrew (Figure 6) to bring both in sharp focus as shown in Figure 7B.

IV. OPERATION OF TURRET CONDENSER

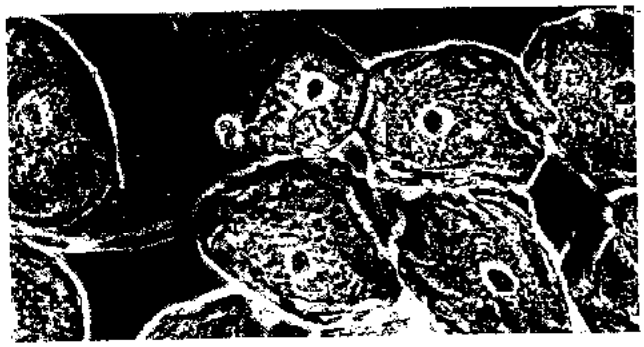
The Phase Turret Condenser Mount No. 1205 (Figure 8) contains provision for four centerable annuli and an open space, any one of which can be positioned by turning the knurled ring of the turret. All annular diaphragm settings are coded to the phase objectives on the face of the knurled ring of the turret condenser. The open aperture has an "0" designation.

Two captive centering wrenches are used to align each annulus to its corresponding objective. Once all objectives and annuli are properly aligned, the

Fork-tailed Arcaria, Phase Contrast 20X Objective



Tissue Section, 2 microns thick, Phase Contrast 40X Objective



Epithelial Cells, Phase Contrast, 100X Objective

Drosophila Chromosomes, Phase Contrast, 100X Objective

turret setting can be changed from one magnification to the next without further adjustment for centration being required. This is a significant feature both in terms of time saved and versatility of objective combinations.

The turret mount accommodates all condensers and annuli. The Standard and Intermediate Working Distance annuli are designed for use with the Standard Working Distance Condenser.

Annuli are easily removed. Turn turret ring to setting for the annulus to be removed. Unscrew condenser. Using wrench provided, unscrew annular diaphragm (Figure 9).

In mounting the turret condenser to the fork, be sure that the screw on the back of the condenser engages in the slot in the fork mount (Figure 10). Insert the condenser all the way into the fork and then rotate until the screw engages the slot (Figure 11).

V. METHODS OF ILLUMINATION

Diastar Series Microscopes are equipped with the 12V Tungsten Halogen Illuminator while Microstar IV Series Microscopes have the 6V Tungsten Halogen In-Base Illuminator and Transformer.

A ground or diffusing glass is helpful for more even illumination. Use of ground or diffusing filters should be confined to the 10X and 20X objectives. For critical microscopy (40X and 100X), control light intensity with neutral density filters. No color filter is required with phase equipment; however, a green filter may be used if desired.

VI. ALIGNMENT OF MICROSTAR IV AND DIASTAR SERIES PHASE EQUIPMENT USING TURRET CONDENSER

In this section it is assumed that the Standard Working Distance mode is being used. Intermediate Working Distance applications are discussed

Figure 3. Diffraction Plate of Phase Objective

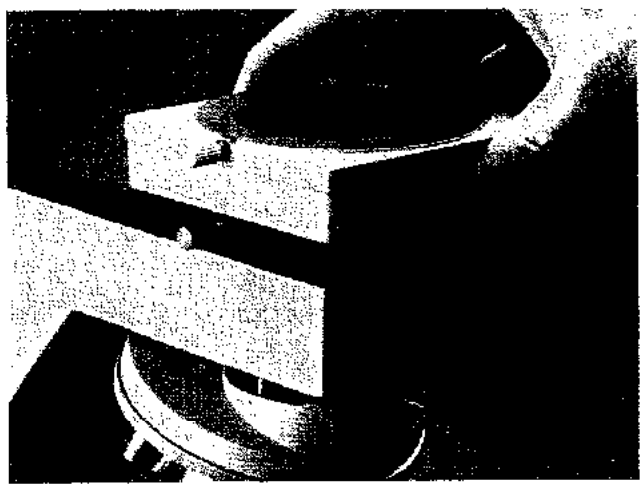


Figure 4. Mounting Aperture Viewing Unit to Stand

Figure 5. Mounting Body to Aperture Viewing Unit

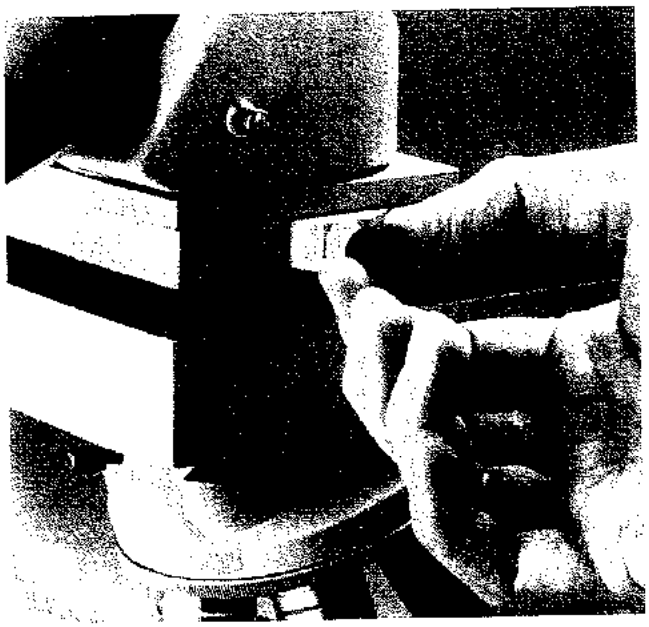


Figure 6. Using the Aperture Viewing Unit

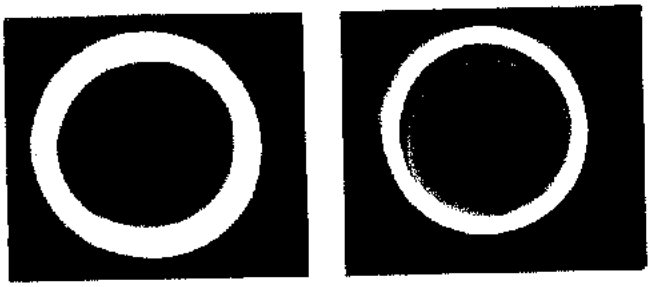


Figure 7. Viewing Diffraction Plate and Phase Annulus

in Section VII. See Figure 15 for applications and diagrams.

Proper optical alignment procedure is of utmost importance in phase contrast microscopy. Once familiarized, it can be rapidly and easily accomplished. The essential steps are outlined below. Review them carefully. It is recognized that set up techniques vary from one individual to the other; however, the basic sequence of procedure is generally the same.

Centration is carefully checked at the factory before each microscope is shipped. If you are using a recently delivered microscope, a quick check should find centration satisfactory with few, if any, adjustments required.

When adding phase equipment to an existing Microstar IV or Diastar Microscope, the set-up procedure should be followed in detail. Also these instructions will be of value when changes are desired in type of condenser, annuli or objectives. Use Figure 12 to identify and locate substage components.

1. Be sure that the instrument is properly set up according to the instructions in the preceding sections.

Figure 8. Cat. No. 1205 Phase Turret Condenser Mount (Shown with Annuli and 1201 Condenser Installed)

Figure 9. Removing Phase Annulus

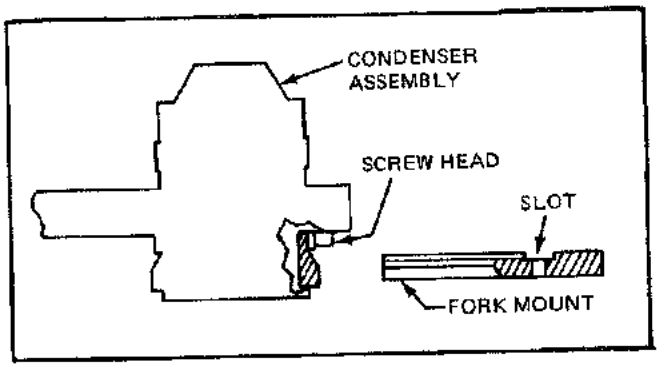


Figure 10. Installing Phase Turret Assembly

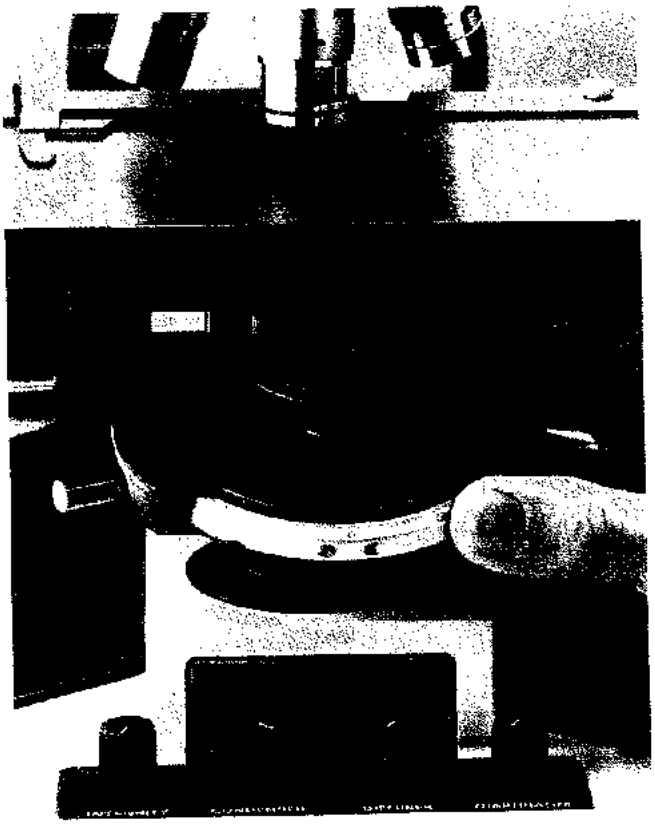


Figure 11. Installing 1205 Turret unto Microscope

2. Cleanliness is essential in phase microscopy. Check carefully to make certain that the equipment and specimen preparation are clean; in particular, the slide and cover glass, top condenser lens, and front lens of the objective.

3. Fully open the iris diaphragm of both the condenser and the illuminator.

4. Withdraw the two captive centering wrenches and rotate the knurled turret ring to the open setting. These wrenches must be withdrawn to permit turret rotation.

5. Place a stained slide (as normally used in ordinary brightfield) on the stage.

NOTE: The top element of the standard working distance condenser should be approximately the thickness of a piece of paper beneath the underside of the slide.

6. Using the 10X objective, focus on the stained specimen.

7. Close the illuminator field diaphragm so that it is visibly superimposed on the specimen image. Focus the image of the field diaphragm at the same plane as the specimen by raising or lowering the condenser. Disregard field diaphragm centration at this point. After focusing, fully open the field diaphragm of the lamp.

8. Leaving the phase turret set at open aperture, rotate the 40X phase objective into operating position. Using the fine adjustment knob, bring the image of the specimen into sharp focus.

9. Fully close the field diaphragm. Without disturbing the fine focus setting, bring the image of the field diaphragm into focus at the same plane as the specimen by raising or lowering the condenser.

   Use the aperture diaphragm of the condenser to enhance contrast of the field diaphragm as desired. Center the field diaphragm to the edge of the field of view by means of the condenser centering screws. Tighten condenser stop retaining screw.

10. Open field diaphragm so that the leaves just disappear from the field of view. The field diaphragm should be so adjusted as each objective is used.

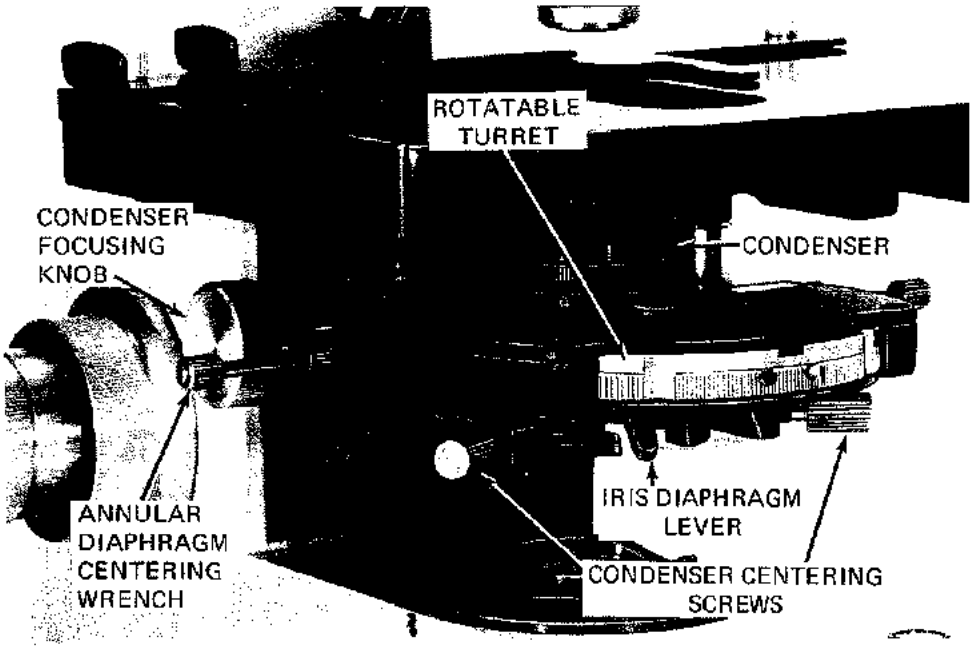


Figure 12. Substage Equipment

Figure 13. Focusing Telescope

11. After centration of the field diaphragm, fully open the aperture diaphragm. All phase microscopy is carried out with the aperture diaphragm of the condenser fully opened.

12. To center the annular diaphragm of the Turret Condenser to the diffraction plate of the objective:

(a) Rotate nosepiece to return the 10X objective to operating position (or lowest power phase objective).

(b) Place an unstained specimen slide on the stage and open the field diaphragm until the iris leaves just disappear from the field of view. (Do not open excessively or stray light will be detrimental to contrast.) Using the 10X objective (or lowest power phase objective), bring the specimen into as good focus as possible.

(c) Turn the knurled disc to the 10X annulus (or lowest power annulus) setting. Push in the two centering wrenches. By turning the wrenches slightly as they are inserted, you will feel them properly engage into the receptacles of the centering mechanism.

(d) Swing in the Aperture Viewing Unit with lever (Figure 6), or remove right eyepiece and insert Phase Telescope.

(e) Bring the image of the annulus of the condenser and the diffraction plate of the objective into simultaneous, sharp focus

by turning the focusing knob of the Aperture Viewing Unit, Figure 6. (If using the Phase Telescope, focus by sliding upper part in and out as shown in Figure 13.)

(f) Adjust the annular diaphragm centering wrenches until the annulus image is superimposed on the diffraction plate as shown in Figure 14.

(g) Swing out Aperture Viewing Unit (or remove Phase Telescope and replace eyepiece).

13. Bring the phase specimen into sharp focus. The microscope is now ready for use with the 10X objective (or lowest power phase objective).

14. Center the other annuli to their respective phase objectives. (Always remember to withdraw the centering wrenches before the turret is rotated.) Once the annuli have been centered to a given set of objectives, they will remain centered for considerable periods of time with careful use of the microscope. Centration should be checked from time to time, particularly for critical observation or photomicrography.

15. In phase microscopy, when changing from one objective to the next, keep in mind the importance of: (a) condenser centration; (b) adjusting field diaphragm so that the field of view is just filled with light; and (c) focusing field diaphragm at the same plane as the specimen. The image of the annulus always should be completely filled with white light. If the annulus image is partially colored, it is an indication of incorrect adjustment of condenser height.

Figure 14. Centering Annulus to Diffraction Plate

16. The 1201 Standard Working Distance Condenser is Achromatic/Aplanatic with a numerical aperture of 0.90. It is designed to be used dry with all objectives.

VII. THE USE OF INTERMEDIATE WORKING DISTANCE PHASE EQUIPMENT

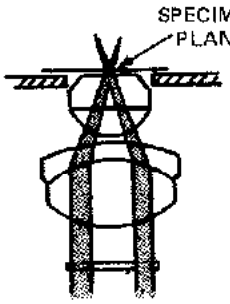
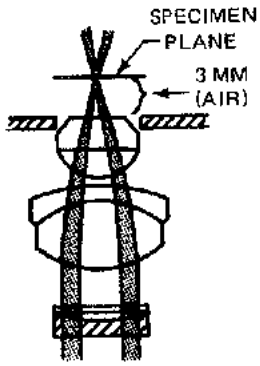
The working distance provided with intermediate phase equipment is 3mm in air (or the optical path equivalent in water or in crown glass). (See Figure 15.) "Working distance" as defined here is the distance from the top of the microscope stage to the focal plane of the specimen. The interchangeability of phase equipment permits fast and easy modification of your microscope from standard working distance usage to intermediate working distance.

Changing from standard to intermediate working distance merely requires the use of intermediate working distance annuli. No change of condenser is necessary. Thus quick change between these two working distances can be accomplished by mounting both types of annuli in the turret condenser.

All objective magnifications (10X, 20X, 40X and 100X) are utilized in intermediate working distance phase microscopy.

The use of plano-plano specimen preparations is of paramount importance in phase microscopy. When utilizing intermediate working distances, the specimen mount should have parallel walls with flat surfaces and be of good optical quality. Irregular, lens shaped (hollow ground) slides or wedge shaped preparations upset the alignment of the phase system. Even with only a small wedge, as when the cover glass is not quite parallel with the slide, it may be necessary to repeat centering procedures each time the specimen is moved to maintain good contrast. Phase microscopy is of little advantage with test tubes because of distortion of the annulus image, making coincidence with the phase plate impossible. Cleanliness

CONDENSER, OBJECTIVE, ANNULAR DIAPHRAGM COMBINATIONS

WORKING DISTANCE DESIRED	CONDENSER	USE WITH OBJECTIVES	USE WITH ANNULAR DIAPHRAGMS
STANDARD For standard thickness microscope slides.	STANDARD  	10X 20X 40X 100X	# 1253A 10X Standard Phase Annulus # 1254A 20X Standard Phase Annulus # 1255A 40X Standard Phase Annulus # 1256A 100X Standard Phase Annulus
INTERMEDIATE For standard thickness microscope slides and chambers (for example, Hemacytometer) not exceeding the equivalent of 3mm of air*	STANDARD  	10X 20X 40X 100X	# 1257A 10X Intermediate Phase Annulus # 1258A 20X Intermediate Phase Annulus # 1259A 40X Intermediate Phase Annulus # 1260A 100X Intermediate Phase Annulus

*One mm of air equals 1.33mm of water or 1.52mm of crown glass. These ratios of refractive indices can be used to obtain equivalent working distance when the specimen includes more than one medium.

Figure 15. Condenser, Objective, Annular Diaphragm Combinations

of specimen preparations is also most important to satisfactory intermediate and long working distance results.

The general set up procedure for intermediate working distance phase microscopy is the same as for standard distance. (See "Optical Alignment Procedure"); however, one additional step is suggested in the set up sequence. This follows Step No. 12, e. (when the image of the annulus in the condenser mount is brought into sharp focus with the diffraction plate of the objective). At this point, adjust the condenser height slightly so as to obtain as good a "match" (same relative size) as possible between the image of the annulus and the diffraction plate as seen through the Aperture Viewing Unit or Telescope. After such adjustment for size, proceed with centration.

VIII. GENERAL COMMENTS ON TECHNIQUE

A few comments on technique are offered here as guidelines in the correct orientation of other annuli to their respective phase objectives.

When using the 40X objective, in some instances the specimen (i.e. a tissue section) may cause light diffusion to a degree which makes focusing the field diaphragm at the plane of the specimen difficult. In such cases, move to a clear area of the slide to accomplish the above; however, do not change the fine focus adjustment of the microscope when using this procedure.

When centering the field diaphragm using the 100X objective, fully close the illuminator iris. Locate the east-west boundaries of the area of illumination by alternately moving them into the field of view by using the centering screw on the condenser mount. When so oriented to the positions of the light boundaries, arbitrarily select the center-most position of the area of illumination.

In adjusting condenser height to bring the field diaphragm into proper focus at the same plane as the specimen, the field of view should be free of color (with perhaps a suggestion of gray only). The presence of color is particularly significant when utilizing the 100X oil immersion objective. A bluish colored area seen in the field of view indicates that the microscope condenser is focused too high. A brownish-orange color indicates that the condenser is adjusted too low. Other color aberration is usually indicative of improper lamp or condenser centration.

The use of plano-plano specimen preparations is essential to clear imagery of the annular diaphragm to the diffraction plate. Figure 16A illustrates an example of distortion caused by a wedge shaped preparation. When such a "cat's eye" or other effect occurs, suspect first that your specimen preparation is not plano-plano. Check by turning the slide or mount 180°. If the distortion of imagery is reversed, this is evidence of irregularity in the preparation (Figure 16B).

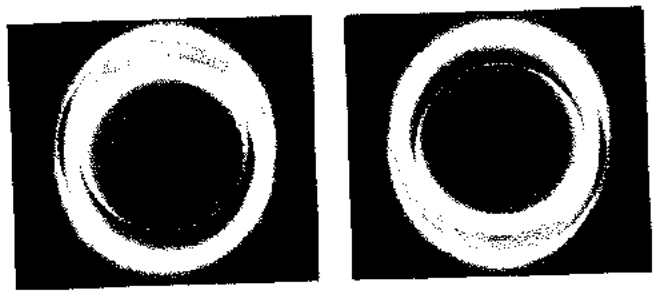


Figure 16. Distortion Due to Wedge Shaped Specimen

IX. PHASE PHOTOMICROGRAPHY

Pictures with excellent detail and contrast can be taken with your Microstar IV or Diastar Microscope with phase accessories. As stated previously, be sure that the annulus and the phase plate of the objective are concentric with no evidence of stray light when viewing through the Aperture Viewing Unit or Telescope.

Exposure times in phase microscopy are generally much longer than those in brightfield work. This is due to the very low light levels that are transmitted to the camera back in a phase contrast system. The Photostar Camera System is especially suited to phase photomicrography since it compensates for film reciprocity failure.

X. PRINCIPLES OF PHASE MICROSCOPY

Phase microscopy puts in the hands of the microscopist a unique technique of immeasurable value in the examination of the structural detail of living, transparent organisms and other specimens of similar optical properties. So significant is its importance that Prof. F. Zernike of Holland was awarded the Nobel Prize in 1953 for his pioneering work in phase microscopy.

Stated in simplest terms, the function of the phase microscope is the conversion, through optical manipulation, of "optical path" differences into visible differences of light intensity. (Optical path being the product of refractive index times thickness.) The American Optical Corporation was among the first to recognize the virtually limitless application of phase microscopy and,

after long and intensive development effort, introduced its initial phase microscopes in 1947.

In conventional microscopy, the details within the specimen are either darker or lighter than one another, in most cases as a result of differential staining. The darker the detail, the greater the amount of light absorbed as it passes through the plane of the specimen. In this manner, each structural element acts as an absorbing medium and weakens, to a greater or lesser degree, the light wave as it passes through. This reduction in intensity results in a corresponding reduction in light wave amplitude (distance from crest to trough of the wave) as shown in Figure A. The selective absorption described here produces a readily visible image because specimen detail appears as differences in brightness or color to which the eye is sensitive.

The phase specimen (e.g. an unstained, small, living organism) is transparent, colorless and, typically, is mounted in a transparent medium of almost identical optical properties. Because of the transparent nature of the fine detail within the phase specimen, the amplitude of the light waves passing through the specimen and its surrounds is not appreciably altered. With very little or no light absorption by the structural elements of the specimen, the intensity of the light waves remain constant. Thus, because varying degrees of light intensity are lacking, an image visible to the eye cannot be formed in the classical manner.

However, as light passes through the phase specimen, another most significant phenomenon occurs, called diffraction. There exists within the fine structural detail of the phase specimen, and the specimen as a whole relative to its surround, what is termed "optical path" differences resulting from differences in index of refraction and thickness. Differences in optical path give rise to diffraction by the specimen detail. The more pronounced the discontinuities in optical path the greater the diffraction effect.

One result of diffraction is that light is scattered as compared to the undeviated light which is transmitted directly through those areas of the specimen where there is insufficient detail to

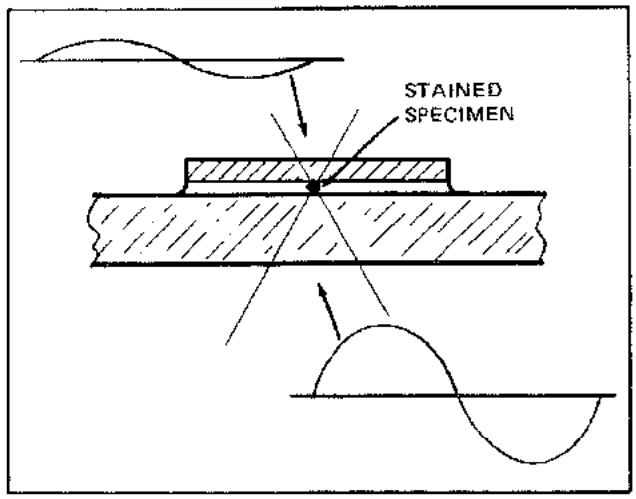


Figure A

cause diffraction. The second important characteristic of diffraction is that there is a difference in phase between the diffracted and undeviated or direct light. The phase microscope makes it possible to convert differences in optical path to which the eye is insensitive into amplitude or intensity differences which the eye can see (without the use of staining).

The phase microscope utilizes two unique components: (1) an annular diaphragm below the condenser which directs a hollow cone of light to the transparent specimen; and (2) a conventional microscope objective which is modified by the addition of a diffraction plate. This plate is constructed and positioned in a manner which separates the diffracted and direct light coming from the specimen and alters their intensity and phase relationships so that they combine in the image plane of the eyepiece to form a visible image.

As the light passes through the phase specimen and its surround, diffraction occurs at each point where internal structural detail (of different optical path) is present. Similarly, the abrupt discontinuity in optical path around the edges of the specimen where it adjoins its surround causes diffraction. This diffracted light is ¼ wave length out of phase with light not diffracted by specimen detail which passes directly through the specimen plane. The objective lens thus receives both

the direct light and that portion of the diffracted orders of light which fall within the limits of the objective aperture (See Figure B).

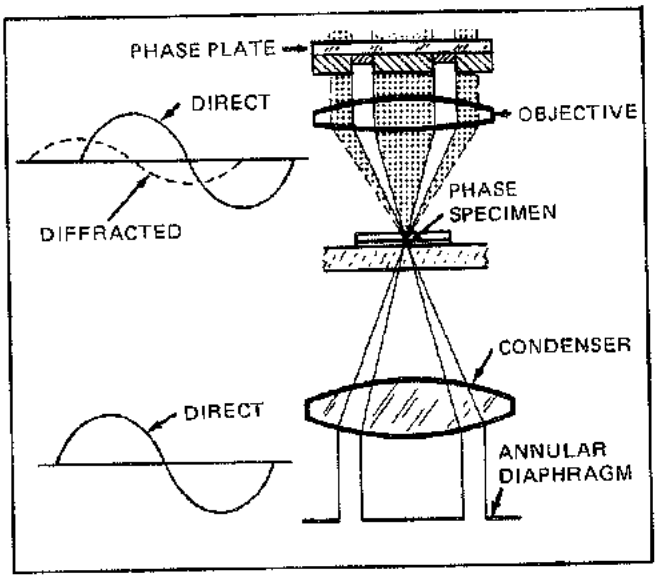


Figure B

We have, then, this situation, as all the light moves from the plane of the specimen toward the diffraction plate. The direct light with considerably greater intensity than the diffracted light moves as a cone of concentrated light toward coincidence with the ring of the diffraction plate. The diffracted light, relatively weak in intensity and retarded in phase by ¼ wave length, moves so as to be distributed over the whole aperture of the diffraction plate.

Since the direct light completely participates in the formation of the image, its intensity would tend to overbalance the weaker, diffracted light and dilute or destroy image contrast. To compensate and bring the direct and diffracted light into balance, a metallic absorbing film in the form of a ring-shaped disc is utilized. This film or coating on the ring of the phase plate reduces the intensity of the direct light. Such absorption tends to equalize intensities or brightness. Thus, when the diffracted and direct light later combine at the eyepiece focal plane, they are balanced to achieve the desired degree of contrast.

Simultaneously, with the absorption procedure described above, the diffraction plate performs a second important function. Utilizing a phase retarding material, the relative phase relationship of the diffracted and direct light is altered by ¼ wave length. If a phase retarding material is placed upon all areas of the diffraction plate other than the ring-shaped disc, a "Dark Contrast" image results. As shown in Figure C, the diffracted light is retarded by ¼ wave length. The effect of this "shift" is to bring the direct and diffracted light together at the image plane of the eyepiece ½ wave length out of phase. The result is subtractive superposition of the light waves whereby the direct and diffracted waves cancel each other to form an image darker than its surround. (This assumes the specimen has greater optical path characteristics than its surrounds, as is generally the case.)

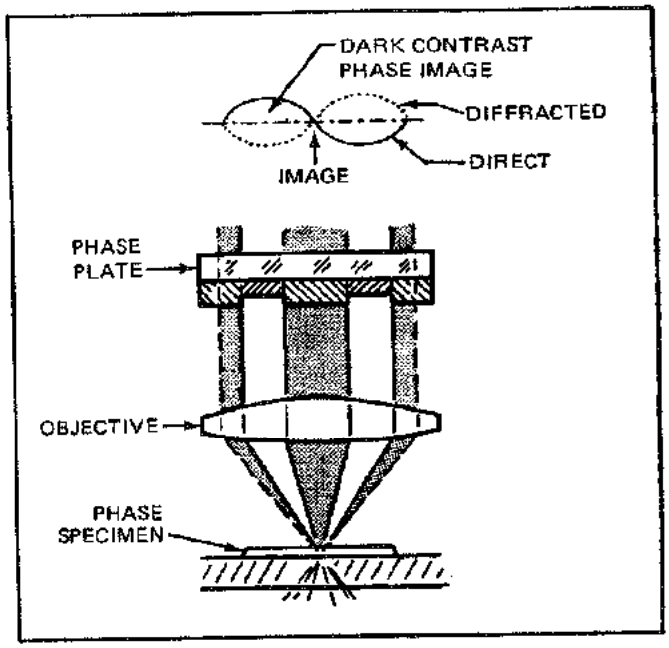


Figure C

This brief and non-mathematical explanation of phase contrast microscopy, with the other information presented in this reference manual, should serve as a sufficient guide for the useful application of the phase microscope. For those who wish to delve further into the theory and mathematics of phase microscopy, refer to "Phase Microscopy", J. Wiley & Sons, 605 3rd Ave., New York, N.Y. 10016.