ACCU SCOPE 3012 Phase Contrast Microscopy Instruction Manual

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ACCU SCOPE 3012 Phase Contrast Microscopy

Product Information

The product is a microscope that utilizes phase contrast microscopy to enhance the visibility of transparent specimens. It allows users to observe detailed structures of completely transparent specimens by utilizing darkfield illumination, edge scattering, diffraction of light, and polarized light.

Product Usage Instructions

1. Install the Phase Contrast Components and align the condenser to the optic axis of the microscope.
2. Place the object on the object plane.
3. Partially open the field diaphragm until the image of the diaphragm is close to the edge of the field of view.
4. Adjust the condenser carrier centering screws to align the condenser to the microscope’s optical axis.
5. Raise the condenser to its highest position.
6. Replace the stained slide with a phase contrast specimen (e.g., fresh cheek cell preparation).
7. Remove one eyepiece and install the supplied alignment telescope.
8. Focus the telescope on the phase ring inside the 10x Phase Contrast objective.
9. Rotate the condenser-turret to the 10 position and observe through the telescope to see two different rings (Phase Plate and Light Annulus Alignment).
10. Use the adjusting screws on the condenser turret to align annular light rings.

Troubleshooting Guide

Problem	Cause	Corrective Measure
Poor phase contrast image is obtained	The condenser phase annulus image and the objective phase plate are not aligned The condenser phase annulus and the objective phase code do not match. The phase difference of the specimen is too large. The specimen cover glass is incorrect	Adjust the phase annulus to align it with the objective phase plate. Rotate the phase annulus selector wheel to the position that matches the objective in use. Prepare the specimen using a different refractive index immersion fluid. Replace with 0.17mm thick cover glass.

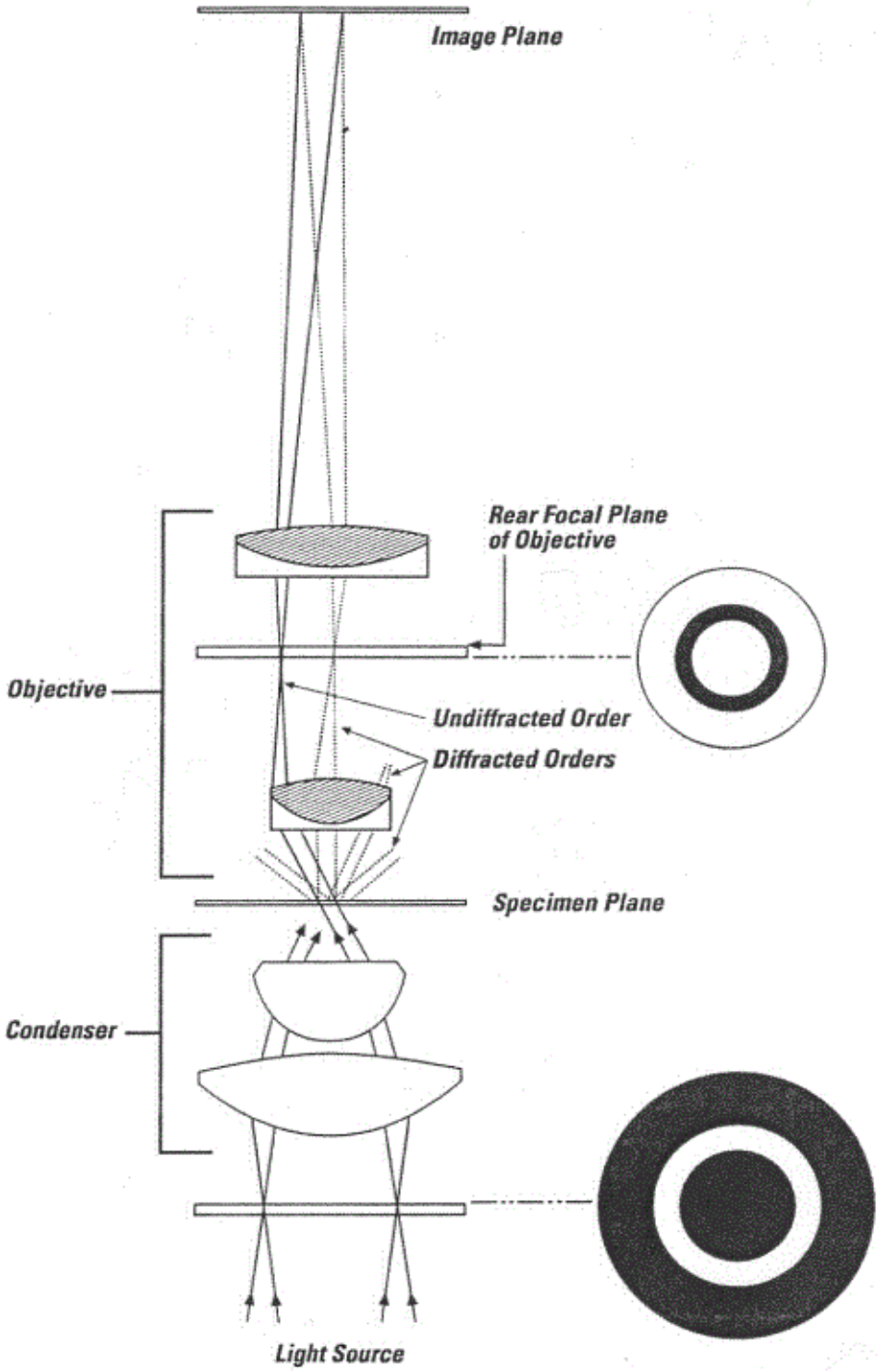
SUPPLEMENTAL INSTRUCTIONS

PHASE CONTRAST MICROSCOPY

- The normal microscopic object is seen because it has regions of varying density. In normal brightfield illumination, a completely transparent specimen is very difficult to observe in detail because all areas of the specimen are equally dense.
- Darkfield illumination displays border effects in completely transparent specimens due to edge scattering and diffraction of light. Polarized light is useful when transparent specimens have directional or crystalline properties.
- Phase contrast microscopy is a type of illumination system to observe transparent media. This form of illumination is utilized extensively in the study of transparent living cells without the need for staining or fixing while being able to obtain good image contrast. The light from phase contrast illumination arrives at the user's eyes at ½ the normal wavelength. This light-altering system produces a visible image of an otherwise invisible, transparent specimen.
- The optical light path necessary for phase contrast is shown in Figure 1. A clear annulus in the focal plane of the condenser is imaged at infinity by the condenser and then re-imaged by the objective in its rear focal plane. The undiffracted light passes through this image. It is reduced in intensity and given a one-quarter wave phase shift by means of an annular phase pattern in the rear focal plane of the objective. These two changes in the undiffracted portion of the beam simulate the phase and intensity distribution which would be present in the objective focal plane if the specimen had density variations rather than refractive index variations. As a result, the image formed by the beam interfering with the diffracted beam simulates that of a specimen having density variations.

IMAGE FORMATION BY PHASE CONTRAST

An annular aperture in the diaphragm placed in the focal plane of the substage condenser controls the illumination of the specimen. The aperture is imaged by the condenser and objective at the rear focal plane or at the exit pupil of the objective. A phase shifting element, or phase plate, is placed in the image plane. Light passing through the phase altering pattern acquires a ¼ wave length advance over that diffracted by the object structure and passes through that region of the phase plate not covered by the altering pattern. The resultant interference effects of the two portions of light form the final image. Altered phase relations in the illumination rays, induced by otherwise invisible elements in the specimen, are translated into brightness differences by the phase-altering plate.

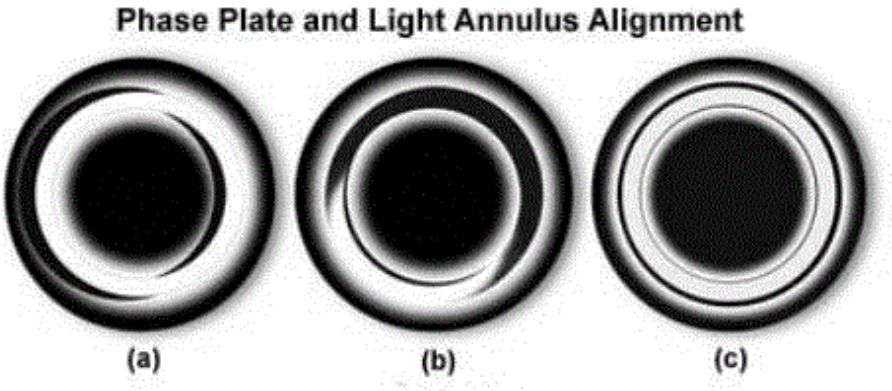


Installation

Installation of the Phase Contrast Components and alignment of the Condenser to the Optic Axis of the Microscope

1. Mount the Phase Contrast objectives onto the nosepiece— clockwise lowest to highest magnification.
2. Install the Phase Contrast Turret-style N.A. 1.25 condenser onto the condenser carrier.
3. Raise the condenser carrier rack to its highest position.
4. Position the condenser turret rotating plate to the “BF” position.
5. Select the 10x Phase Contrast objective.
6. Place a “stained slide” specimen on the stage and focus the microscope.
7. Close the field diaphragm, then lower the condenser until an image of the field diaphragm comes into focus on the object plane.

8. Partially open the field diaphragm until the image of the diaphragm is close to the edge of the field of view. Now adjust the condenser carrier “centering screws” to align the condenser to the microscope’s optical axis.
9. Raise the condenser to its highest position.
10. Replace the “stained slide” with a phase contrast specimen (for example, a fresh “cheek cell” preparation).
11. Remove one eyepiece and install the supplied alignment telescope. Focus the telescope on the phase ring inside the 10x Phase Contrast objective.
12. Rotate the condenser-turret to the “10” position. As you observe through the telescope you will see two different rings, See illustration Phase Plate and Light Annulus Alignment.
13. The condenser turret has two adjusting screws. These are used to align annular light rings in the condenser-turret to the corresponding phase ring in the objectives. Adjust the 10 annulus so that it is aligned – see Figure C below.(The 10 annulus will now function with both the 10x and optional 20x Phase objective on 3012 models).
14. Repeat the above annulus alignment procedure for the remaining Phase objectives on the microscope.



TROUBLESHOOTING GUIDE

PHASE CONTRAST MICROSCOPY

PROBLEM	CAUSE	CORRECTIVE MEASURE
Poor phase contrast i mage is obtained	The condenser phase annulus image and the objective phase plate are not aligned	Adjust the phase annulus so that it is aligned with the objective phase plate.
	The condenser phase annulus and the ob jective phase code do not match.	Rotate the phase annulus selector wheel t o the position that matches the objective i n use
	The phase difference of the specimen is t oo large.	Prepare the specimen using a different ref ractive index immersion fluid
	The specimen cover glass is incorrect	Replace with 0.17mm thick cover glass

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Documents / Resources

	**ACCU SCOPE 3012 Phase Contrast Microscopy** [pdf] Instruction Manual 3012 Phase Contrast Microscopy, 3012, Phase Contrast Microscopy, Contrast Microscopy, Microscopy

References

- Accu-Scope