OPTIKA®
ITALY

Phase contrast

INSTRUCTION MANUAL

Model
B-380
B-500
B-800
B-1000

Version: 2
Issued: 18, 08, 2014

CE

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Introduction to phase contrast

Unstained specimens that do not absorb light are called phase objects because they slightly alter the phase of the light diffracted by the specimen, usually by retarding such light approximately 1/4 wavelength as compared to the undeviated direct light passing through or around the specimen unaffected. Unfortunately, our eyes as well as camera film, are unable to detect these phase differences. To reiterate, the human eye is sensitive only to the colors of the visible spectrum (variations in light frequency) or to differing levels
of light intensity (variations in wave amplitude).

In phase specimens, the direct zeroth order light passes through or around the specimen undeviated. However, the light diffracted by the specimen is not reduced in amplitude as it is in a light-absorbing object, but is slowed by the specimen because of the specimen's refractive index or thickness (or both). This diffracted light, lagging behind by approximately 1/4 wavelength, arrives at the image plane out of step (also termed out of phase) with the undeviated light but, in interference, essentially undiminished in intensity. The result is that the image at the eyepiece level is so lacking in contrast as to make the details almost invisible.

Zernike succeeded in devising a method–now known as Phase Contrast microscopy–for making unstained, phase objects yield contrast images as if they were amplitude objects.
A schematic illustration of the basic phase contrast microscope configuration is illustrated in Figure 1.

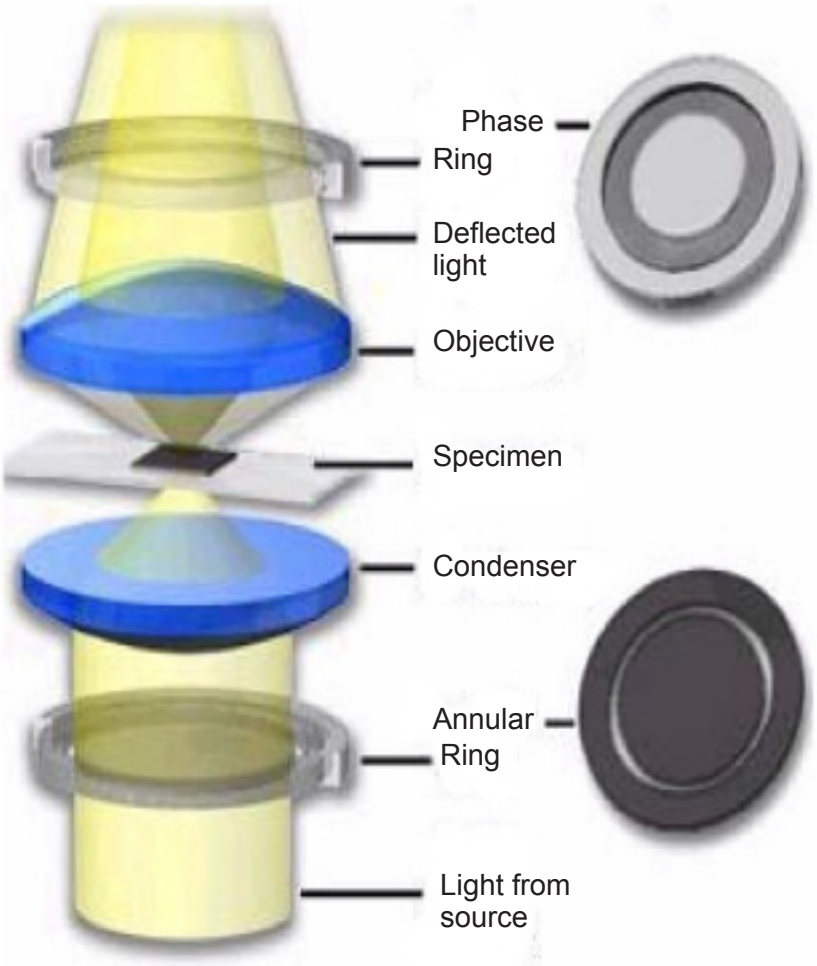


Figure 1

Amplitude objects show excellent contrast when the diffracted and direct light are out of step (display a phase difference) by 1/2 of a wavelength. Zernike's method was to speed up the direct light by 1/4 wavelength so that the difference in wavelength between the direct and deviated light for a phase specimen would now be 1/2 wavelength. As a result, the direct and diffracted light arriving at the image level of the eyepiece would be able to produce destructive interference. Such a procedure results in the details of the image appearing darker against a lighter background. This is called positive phase contrast (see Figure 2).
Another possible course is to arrange to slow down the direct light by 1/4 wavelength so that the diffracted light and the direct light arrive at the eyepiece in step and can interfere constructively. This arrangement results in a bright image of the details of the specimen on a darker background, and is called negative contrast (see Figure 3).

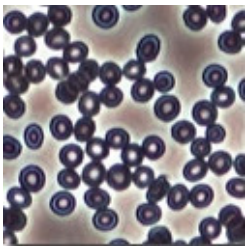


Figure 2

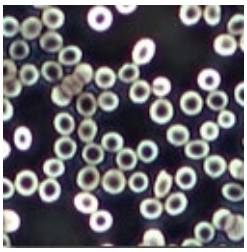


Figure 3

The accessories needed for phase contrast work are a substage phase contrast condenser equipped with annuli and a set of phase contrast objectives, each of which has a phase plate installed. The phase outfit, usually includes a green filter (to increase the resolution) and a phase telescope (to center the annuli).

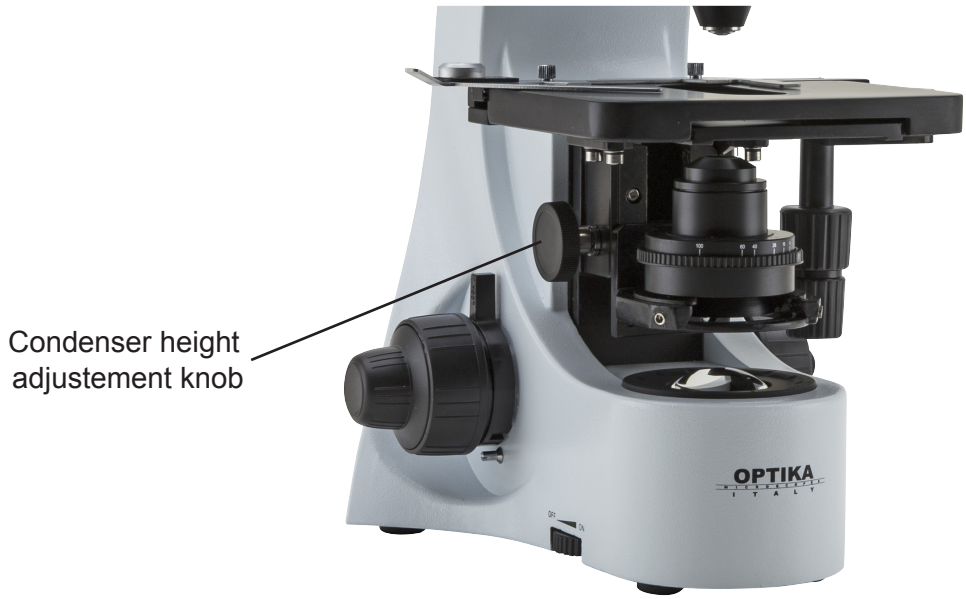
Phase microscopy techniques are particularly useful with specimens that are thin and scattered in the field of view. There are some limitations of phase contrast microscopy:

- Phase images are usually surrounded by halos around the outlines of details. Such halos are optical artifacts, which sometimes obscure the boundaries of details.
- The phase annuli do limit the working numerical aperture of the optical system to a certain degree, thus reducing resolution.
- Phase contrast does not work well with thick specimens because of shifts in phase occur from areas slightly below or slightly above the plane that is in focus.
- Phase images appear gray if white light is used and green if a green filter is used.

Setup on B-380 microscopes

In order to use phase contrast on a B-380 series microscope, follow these steps:

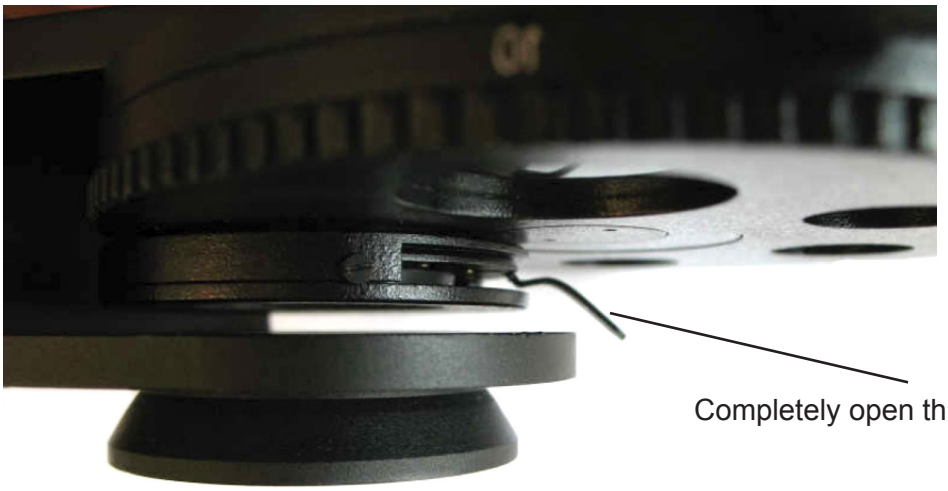
- Make sure that a phase contrast objectives set is mounted on the nosepiece (phase contrast objectives are marked with “PH” writing).
- Lower the condenser using the knob:



- Replace the standard brightfield condenser with the phase contrast condenser:

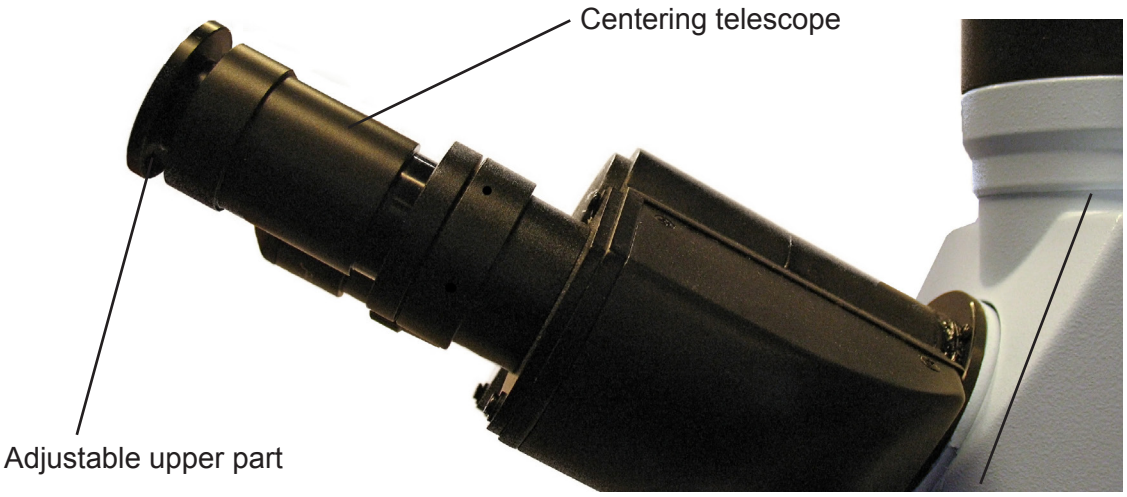
- Fully insert the phase contrast condenser into the holder, pushing it until it is well inserted:

- Rotate the condenser annuli disc to the “0” position (brightfield).
- Completely open the aperture diaphragm:



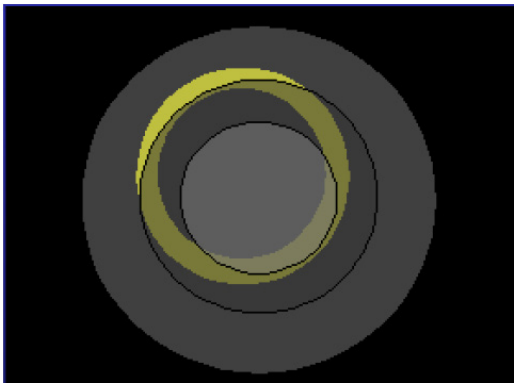
- Turn on the light and put a sample slide on the stage (an opaque specimen will make alignment easier).
- Using the 10x objective, center your sample (moving the stage) and focus using the coarse and fine focus knobs.
- Rotate the condenser height adjustment knob until you obtain a uniform white illumination on the sample. Important: the optimal position corresponds to the top lens of the condenser 1-2mm below the bottom side of the slide.
- Rotate the condenser annuli disc to the “10” position.

- Take one of the eyepieces out of the tube, and insert the centering telescope. Rotate its upper part in order to view a sharp image of the phase ring:

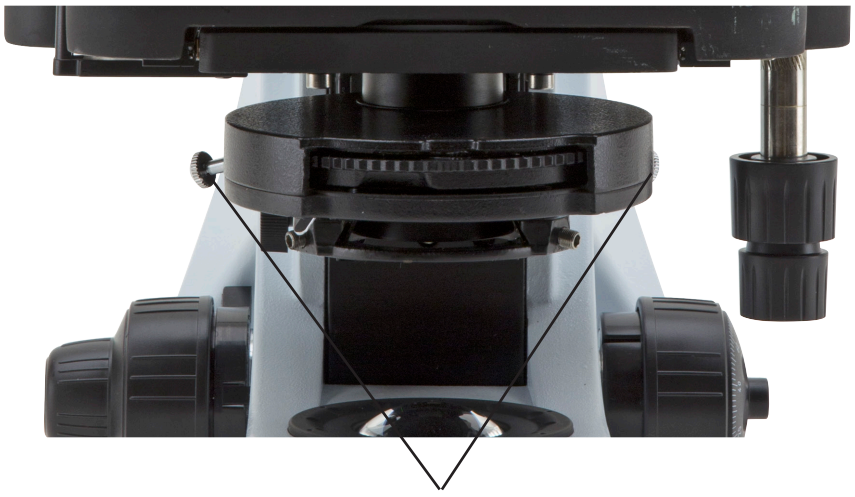


- Typically, you have to see a pair of rings: one brighter and one darker. The bright one is the image of the annulus on the condenser, while the dark one is the phase ring inside the objective:

Image as seen in the telescope

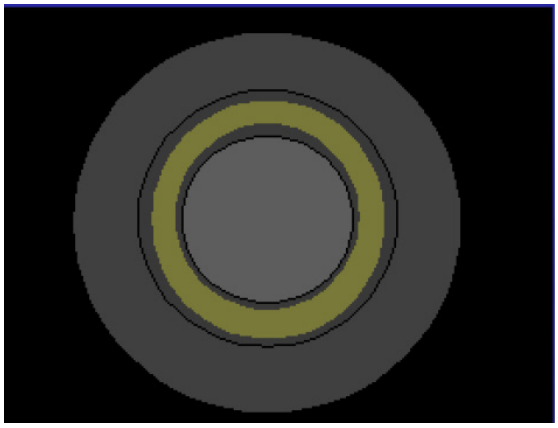


- Rotate the levers on both sides of the condenser. You will notice that the bright ring will change its position:



- The goal is to superimpose this bright ring to the dark one, so that they are in the same position:

Image as seen in the telescope, with centred rings.



- Reinsert the eyepiece.

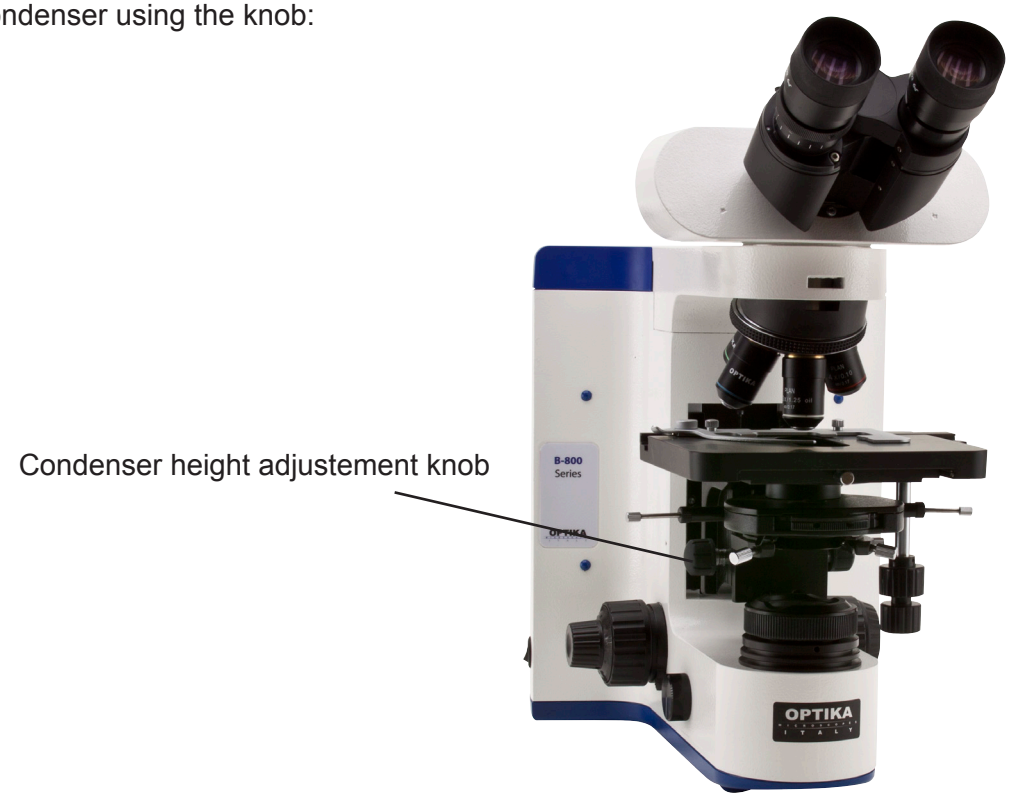
Now you can look at the phase contrast image of your sample.

The same procedure must be followed to align the other objectives (20x, 40x or 100x). Note that in order to obtain a correct image with 100x, you have to use the oil immersion technique (oil between the objective and the specimen coverslip).

Setup on B-500 B-800 B-1000 microscopes

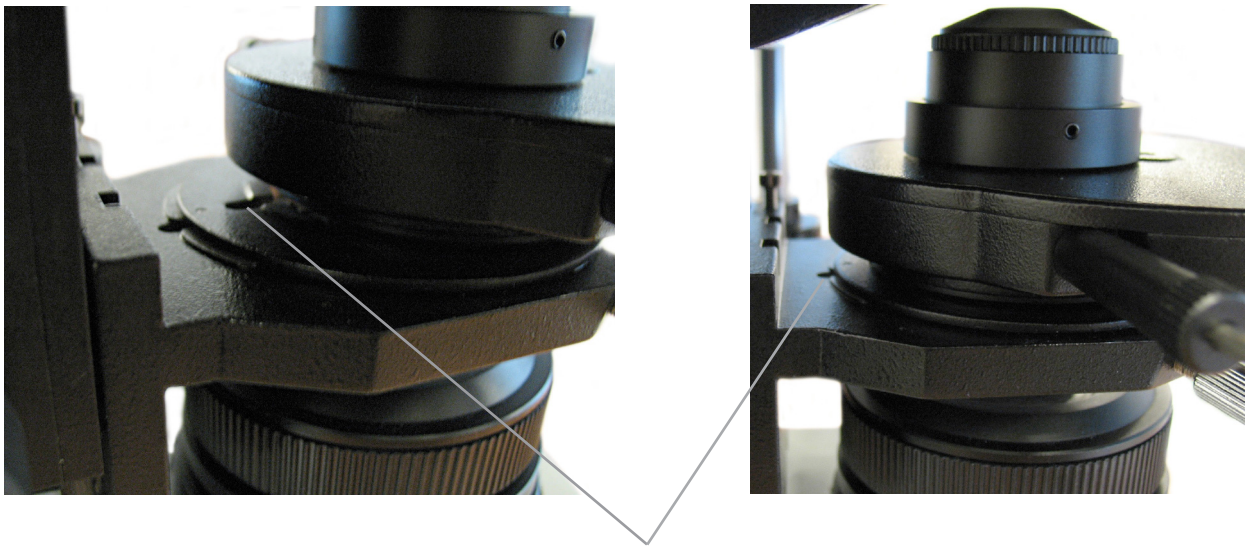
In order to use phase contrast on a B-500, B-800 or B-1000 series microscope, follow these steps:

- Make sure that a phase contrast objectives set is mounted on the nosepiece (phase contrast objectives are marked with “PH” writing).
- Lower the condenser using the knob:



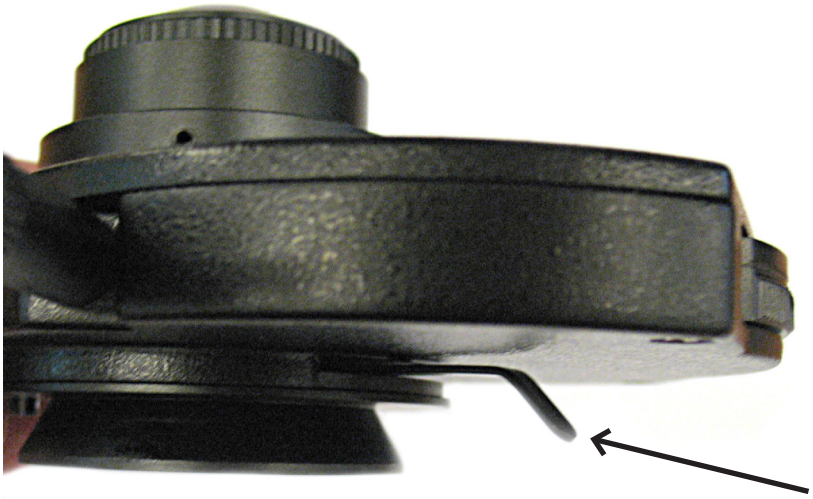
- Replace the standard brightfield condenser with the phase contrast condenser:

- Fully insert the phase contrast condenser into the holder, pushing it until it is well inserted:



Condenser must fully enter into its guide

- Rotate the condenser annuli disc to the “BF” position (brightfield).
- Completely open the aperture diaphragm:

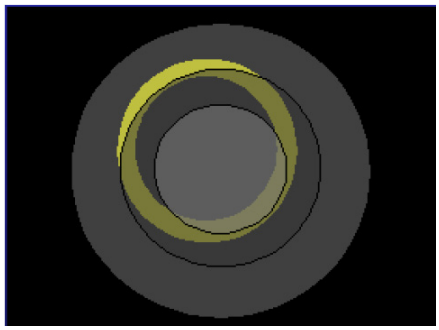


- Turn on the light and put a sample slide on the stage (an opaque specimen will make alignment easier).
- Using the 10x objective, center your sample (moving the stage) and focus using the coarse and fine focus knobs.
- Rotate the condenser height adjustment knobs until you obtain a uniform white illumination on the sample. Important: the optimal position corresponds to the top lens of the condenser 1-2mm below the bottom side of the slide.
- Rotate the condenser annuli disc to the “10” position.

- Take one of the eyepieces out of the tube, and insert the centering telescope. Loosen the screw of the telescope and move its upper part in order to view a sharp image of the phase ring, then lock the screw:

- Typically, you have to see a pair of rings: one brighter and one darker. The bright one is the image of the annulus on the condenser, while the dark one is the phase ring inside the objective:

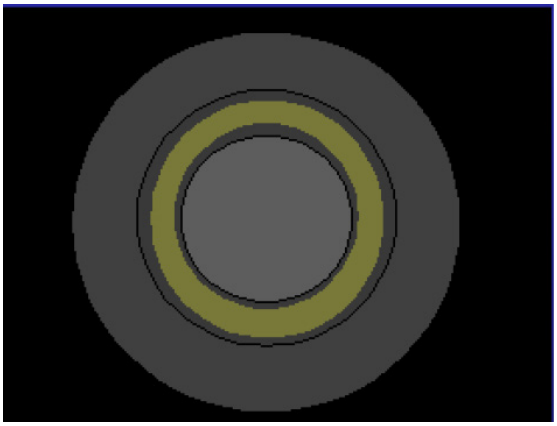
Image as seen in the telescope



- Press both the long screws on the sides of the condenser and then slowly turn them until you notice that the bright ring changes its position:

- The goal is to superimpose this bright ring to the dark one, so that they are in the same position:

Image as seen in the telescope, with centred rings.



- Release the centering screws slowly, guiding them to the rest position.
- Reinsert the eyepiece.

Now you can look at the phase contrast image of your sample.

The same procedure must be followed to align the other objectives (20x, 40x or 100x). Note that in order to obtain a correct image with 100x, you have to use the oil immersion technique (oil between the objective and the specimen coverslip).