# EXERCISE 2
# USE OF PHASE CONTRAST OPTICS

## INTRODUCTION

In the last exercise, you used the brightfield optics of the compound microscope to view stained, fixed specimens of various microbes. With brightfield optics, it is necessary to stain most microbes because they lack enough color to be visible with transmitted light. Unfortunately, this prevents us from studying live organisms, and often obscures interesting details that are not visible in stained specimens.

Phase contrast optics are a way that we can enhance the contrast of living organisms enough that we can see them with the microscope. A full discussion of the physics of phase contrast optics isn't possible in this brief space, but briefly, we take advantage of the wave nature of light and the fact that the speed of that light will vary as it passes through different substances.

We use an annular ring in the condenser to set up light waves that are in phase with each other (that is, the crests and troughs of the waves are occur parallel to each other). These phase-coherent waves then pass through the specimen, and as they do, some pass through cytoplasm, others through lipid, or glycogen, or other cellular components. The speed of light through each component is a little different, and the light waves drift out of phase. A second annular ring in the objective lens helps resolve the light waves and makes constructive and destructive interference of the waves. Constructive interference enhances the brightness of the sample, and destructive interference enhances the darkness. Thus, a sample that has a refractive index near that of water, and is nearly invisible in brightfield optics, can be made more visible and can be studied without fixing and staining it.

In this exercise, you will learn how to adjust the phase contrast microscope and use it to observe living, unstained specimens.

### Centering the Phase Rings

If you have changed the centering on ANY objective, you will then need to reset the phase rings. Luckily, if the phase rings are set for the highest power objective, they are set for all the other objectives, too. Raise the condenser to its highest position. Swing the 40x objective into place and rotate the phase contrast turret so that the "40" on the turret clicks into place on the detent. Remove the right eyepiece and replace it with a phase telescope. Focus the phase telescope on the bright ring of light by loosening the set screw and raising or lowering the eyepiece until the ring is in sharp focus. Now, adjust the condenser height until the two phase rings are in focus. The darker of the two rings is the phase retardation annulus located in the objective. The brighter one is the illumination annulus located in the condenser. You must use the two knurled centering screws located toward the rear of the phase condenser to bring the two annuli into alignment.

### Adjusting the Phase Contrast Microscope

Make sure your microscope is set up with the proper phase condenser matched up with the objective (phase

condenser matches the magnification of the objectives).

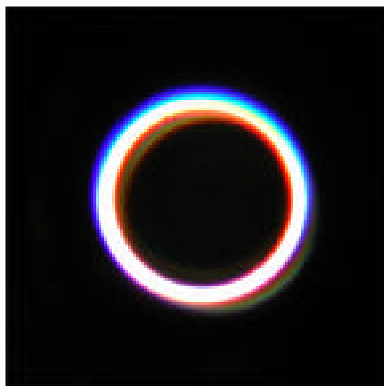
First, make sure your condenser is centered (like we did when we set up the microscopes).

Remove one eyepiece and put it safely away.

Insert the phase telescope into the eyepiece tube, and bring the phase rings into focus. If the rings are out of alignment, but in focus, they will look like this:

Pull out the phase adjustment knobs (the ones that come straight out from the condenser, not the ones that come out at an angle) and turn them until the rings match up.

These rings are close, but not quite matched:



When the rings are aligned, you will see only a single ring:

You should only have to adjust one set of rings; the others are adjusted the same way.

If you readjust, realign, or bump the condenser, you will have to readjust your phase rings.

## Viewing a specimen with phase contrast optics

The stage on the ATC 2000 doesn't move up and down; instead, the objective lenses move. Note that each objective has its magnifying power, listed as Ph 10, Ph 40, and Ph 100 stamped on the side. The condenser has a rotating disk that is marked 0, 10, 20, 40, and 100. To use phase contrast optics, you must match the setting on the condenser with the objective you are using.

The first time you use the phase contrast optics, try using one of the stained specimen slides that you used in Exercise 1. This will make it easier for you to get the feel of how to use phase contrast optics. We will make a live preparation after you feel confident about the settings.

1. Place the specimen on the microscope stage.

2. Open the iris diaphragm (on the condenser) all the way.

3. Put the 10x objective in place, and observe the specimen with the

condenser in the brightfield (0) position.

4. Next, pull out the centering keys (the silver knobs that extend directly out from the condenser), and rotate the condenser to the 10 position.

5. Observe the specimen using the phase contrast optics.

*Describe in a couple of sentences how the phase contrast image differs from the brightfield image.*

6. Next, change to the 40x objective and change the condenser to the 0 position. Observe the specimen under these conditions, then rotate the condenser to the 40 position. *Compare the images as seen through the two different types of optics.*

Once you feel confident using the phase contrast optics, move on to the next section.

## Preparing a live sample for phase contrast optics.

1. Obtain a broth culture of the yeast *Saccharomyces cerevisiae* or *Ustilago maydis.* Using your inoculating loop, transfer a small amount to a clean microscope slide.

2. Place a cover slip over the sample to make a wet mount.

3. Blot using your bibulous paper to make sure that the sample is very thin. Phase contrast works best on very thin preparations.

## Viewing a live sample with phase contrast optics

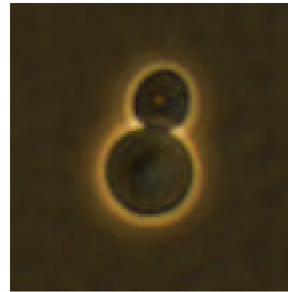
1. Place the specimen on the stage and adjust your microscope for phase contrast with the 10x objective.

2. Focus on the specimen. (Note: it may be easiest to focus on an air bubble or other large object to find the correct plane of focus)

3. Draw what you see. (Compare to the slide shown on the video screen so that you can identify the cells)

4. Compare what you can see if you reset the condenser to the 0 position.

5. Repeat the process with the 40x and 100x objectives. Draw what you see. Can you see internal organelles, such as the nucleus and vacuoles?



## Extend your observations

Now that you are an expert at phase contrast microscopy, obtain samples of pond or aquarium water and make wet mounts of them. (You may need to put a drop of Proto-Slo on the slide to slow the swimming protists down.) Identify as many protists as possible and sketch what you see. Pay special attention to any internal structures you can see.