LEITZ LABORLUX PHASE CONTRAST MICROSCOPE

I. Reading

BOM - 11: 4.1 Light Microscopy p. 56
4.4 Cell Morphology p. 63
4.13 Endospores p. 87

The following references on are available from the Science library:

Light Microscopic Techniques in Biology and Medicine	James, J.	1976	QH 207 J34
Phase Contrast and Interference Microscopy for Biologists	Ross, K. F. A.	1967	QH 212 I5 R6
Advanced Light Microscopy, vol. 2	Pluta, M.	1989	QH 207 P54 1988 v. 2

Also see the discussion of the optical principles of phase contrast microscopy provided in section VIII of the microscopy notes, available via the course web site.

II. Introduction

You are provided with excellent quality microscopes (compared to those you would ordinarily find in an undergraduate teaching laboratory). They were purchased in 1990 for $ 4,200 each. Replacement cost currently would be on the order of $ 8,000 each. Read and adhere to the few simple instructions for proper care of the microscopes that are given at the end of this handout. Abuse of the microscopes through carelessness or ignorance of these instructions is not acceptable.

This exercise puts the cart before the horse by having you use your microscopes before you fully understand the principles of their operation. Frequently, you will be manipulating them following a rote procedure, an unsatisfying state of affairs that will only be remedied as the course progresses. Most instruction in the principles of optics and microscopy will be presented during lectures (Wednesday morning) rather than in the lab.

The following instructions first describe the steps needed to set up the scopes for brightfield observation, followed by instructions for phase contrast and darkfield observation. Please work through them in this sequence - do not succumb to the temptation to skip steps and jump ahead to making observations, just for the sake of getting "finished". We do not intend that you complete the entire exercise, as written, within one lab period. You have access to your microscope *ad libidum* throughout the quarter for instruction, for practice, and for fun.

At the end of the course there will be both a written and a practical exam that will test your expertise in microscopy. The exam will contribute to your final grade and to your narrative evaluation.

III. Instructions Brightfield Observation with the Leitz Laborlux S Phase Contrast Microscope

The microscopes are capable of brightfield observation of naturally or artificially stained specimens, as well as phase contrast and darkfield observation of transparent specimens.

TABLE A.

OBJECTIVE MAGNIFICATION	BRIGHTFIELD	PHASE CONTRAST	DARKFIELD
4X	YES	NO	YES
10X	YES	YES	YES

40X	YES	YES	YES

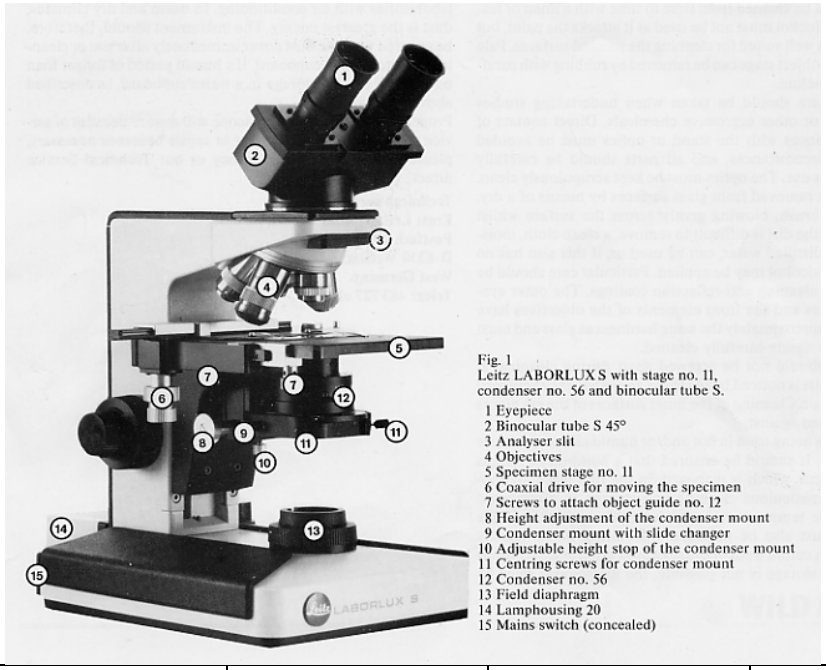


Fig. 1
Leitz LABORLUX S with stage no. 11, condenser no. 56 and binocular tube S.

1 Eyepiece
2 Binocular tube S 45°
3 Analyser slit
4 Objectives
5 Specimen stage no. 11
6 Coaxial drive for moving the specimen
7 Screws to attach object guide no. 12
8 Height adjustment of the condenser mount
9 Condenser mount with slide changer
10 Adjustable height stop of the condenser mount
11 Centring screws for condenser mount
12 Condenser no. 56
13 Field diaphragm
14 Lamphousing 20
15 Mains switch (concealed)

100X	YES	YES	NO

1. Preliminary Focusing of the Image

All other adjustments to the microscope should be made AFTER the microscope has been focused on the specimen. This is at times problematical because, if the microscope is out of adjustment, you will not be able to see the specimen. If you can't see the specimen, you can't focus on it and therefore you can't make the adjustments. An infinitely regressing problem, no? The trick is to begin with a specimen that is very easy to see, even when the scope is far out of adjustment (i.e. something other than a bacterial cell). In wet mounts, air bubbles serve the purpose admirably. Otherwise, the edge of the coverslip works well.

- Place a specimen slide on the stage and mount it securely in the spring-loaded arm of the slide holder. (Notice the vernier X,Y scales that facilitate accurate re-positioning of the slide.) Be sure the coverslip is facing up.

- Select the 10X objective lens by rotating the nosepiece.

  Although It is often suggested to begin with the lowest power objective (4X), this low magnification is essentially useless for bacteriology, so we often ignore it.

- Switch on the illumination and regulate the light intensity with the control knob .

- Bring the condenser to its uppermost position using the height adjustment knob.

- Rotate the condenser turret to the "H" position (for brightfield observation).

- Fully open the condenser aperture diaphragm (to the "PH" position).

- Open the field diaphragm.

- Focus the specimen using the coaxial coarse and fine focus knobs.

  Do this by first lowering the objective toward the specimen until it nearly contacts the coverslip. Then, while looking through the scope, raise the objective to bring the specimen into focus. Wiggling the slide back and forth a bit while raising the objective can help you sense when you are approaching the proper focus.

2. Ergonomic Adjustment of the Binocular Tubes

These adjustments are necessary to minimize "eyestrain".

- Adjust the interpupillary distance by moving the eyepiece tubes together or apart until the images of both eyes perfectly converge.

- To compensate for any bilateral difference in visual acuity:

  First: Look through the right-hand eyepiece with your right eye and focus the specimen with the fine focus knob.

  Then: Observe the specimen with your left eye. Rotate the left hand eyepiece tube until the specimen is in sharp focus. Do not use the fine focus knob to focus your left eye.

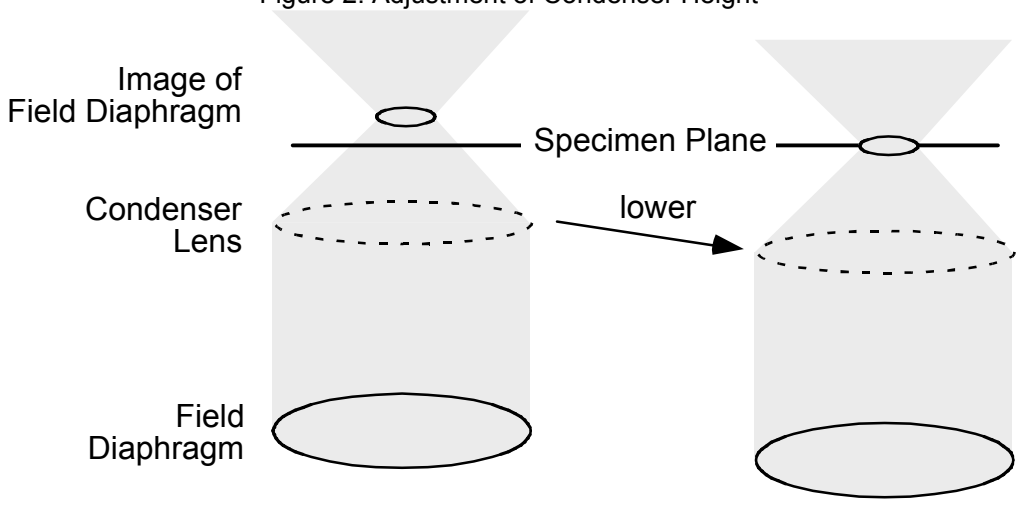
  Now: Both eyes should be in sharp focus.

Note to those who wear corrective lenses: With these scopes it is largely a matter of individual preference whether you leave your glasses on or take them off.

3. Condenser Height Adjustment

When the condenser is set at the correct vertical position, an image of the field diaphragm is superimposed on the specimen plane. Another way of saying the same thing is "the condenser is at the correct vertical position when the narrowest section of the illumination cone is at the same level as the specimen". (See Fig. 2)

Figure 2. Adjustment of Condenser Height



A. Confirm that the specimen is focused and that the condenser is at its uppermost position.

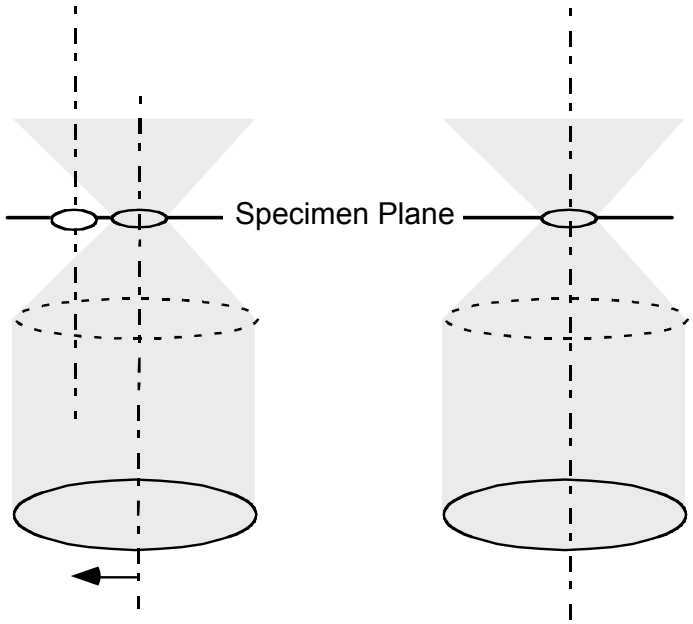
B. Close the field diaphragm.

C. Lower the condenser as necessary to bring the edges of the field diaphragm into sharp focus. (A in Fig. 4)

Opening and closing the field diaphragm as you lower the condenser aids in finding its image.

4. Lateral Centration of the Condenser in the Optical Path.

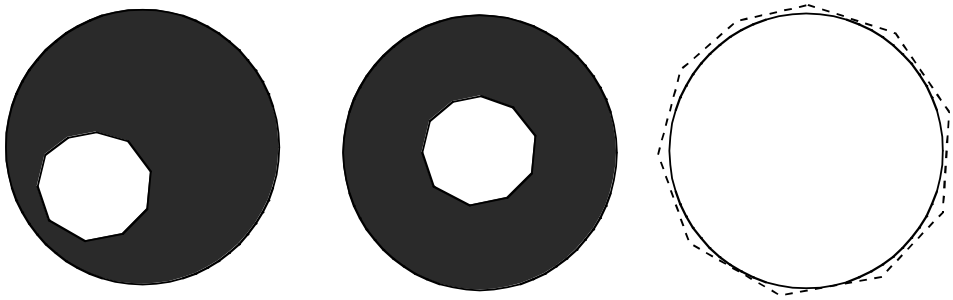
Figure 3. Condenser Centration



Center the image of the field diaphragm (as in B, Fig. 4) using the two centering screws that project at angles from the right and left side of the condenser mount.

Figure 4. Adjustment of Field Diaphragm

This is what you see through the microscope.



5. Set the Field Diaphragm opening.

The field diaphragm controls the diameter of the specimen that is actually illuminated. The field diaphragm allows you to confine illumination to the specific region of the specimen you are actually viewing through a given objective. The knowledgeable microscopist does not illuminate regions of the specimen that are not in view. Heating and photochemical bleaching effects produced by strong illumination are generally detrimental to specimens.

Set the field diaphragm opening so that the area of illumination on the slide is congruent with the view field. Adjust the field diaphragm opening until the edges of its image are just outside the field of view (as in C, Fig. 4).

6. Setting the Aperture Diaphragm

The aperture diaphragm is in the condenser. It is opened and closed by rotating the black serrated disc projecting from the condenser; the one labeled "PH 8 6 4 2 0". The digits represent the diameter of the aperture diaphragn opening in mm. "PH" is the maximum opening, used for phase contrast.

In brightfield microscopy, the aperture diaphragm setting is analogous to The "f-stop" in photography. Therefore, photographers will understand that the aperture diaphragm setting affects spatial resolution and depth of focus in the image. Opening the aperture diaphragm increases resolution and decreases depth of focus. In general, the opening is greater for higher magnification objectives, The aperture diaphragm is opened all the way ("PH" setting) for phase contrast and for darkfield microscopy.

Remember that the function of the aperture diaphragm is totally different than that of the field diaphragm. The aperture diaphragm controls resolution and depth of focus, while the field diaphragm regulates the diameter of the illuminated area on the specimen. Neither diapragm is used to regulate illumination intensity, that is done with the variable electrical transformer of the light source.

A. Remove one eyepiece lens from the binocular tube. Look down the empty eyepiece tube or use the special telescopic eyepiece (described in directions for phase contrast microscopy)

B. Close the aperture diaphragm until you see an image of the diaphragm.

C. Set diaphragm so that the opening appears to fill about 3/4 of the view field.

   Note the setting on the dial for future reference; this will eliminate the need to remove the eyepiece and make this adjustment visually each time you change objective. Each objective requires a different diaphragm setting.

7. Changing Objective Magnification

To change objective magnification, simply rotate the nosepiece.

Don't raise the objective lens away from the slide before changing magnification. The objectives are "parfocal" so only minor readjustment of the fine focus knob should be necessary to refocus the specimen at the new magnification.

Steps 3, 4, 5 and 6 are typically repeated when you change from one objective to another. With a little practice, this becomes a trivial chore.

8. Use of Oil Immersion (100X) Objective

Use the oil immersion objective sparingly. The 40X objective will show you nearly as much detail, without the hassle and mess of the oil.

Do not move from the oil immersion objective back to the 40X objective with the same slide. This transfers oil from the slide onto the 40X lens, where you assuredly don't want it.

1. Assuming the 40X objective is focused on the specimen, simply rotate the 40X out of the optical path, but stop short of rotating the 100X fully into the click stop position.

2. Add a small drop of immersion oil to the coverslip immediately over the specimen.

3. Rotate the 100X objective fully into the click stop position. Check to see that the oil completely fills the space between the coverslip and the lens face.

4. Reset both the field diaphragm and the aperture diaphragm and use the finw focus knob to bring the specimen into view.

Immersion oil has the same refractive index (= 1.515) as the coverslip and the front lens of the microscope objective. This eliminates total internal reflection of light as it leaves the coverslip. The focal length and the working distance of an immersion objective are usually very small. For this reason, working with oil immersion requires care.

At the end of the lab period all surfaces where immersion oil has been applied, intentionally or otherwise, should be carefully cleaned with lens paper and lens cleaner. This may mean that you need to clean the 40X objective.

IV. Instructions for Phase Contrast Observation with the Leitz Laborlux S Phase Contrast Microscope (with Condenser UKL)

The annular stops necessary for phase contrast and darkfield illumination are mounted in a silver disc with a serrated edge that partially projects from the condenser.

Phase contrast is possible with the 10X, 40X and 100X objectives [See Table A].

1. Begin by adjusting the microscope properly for brightfield observation at the desired magnification following the directions given previously.

2. Open the aperture diaphragm fully ("PH" setting).

3. Rotate the condenser turret so that the appropriate annular stop is in the light path [Table B].

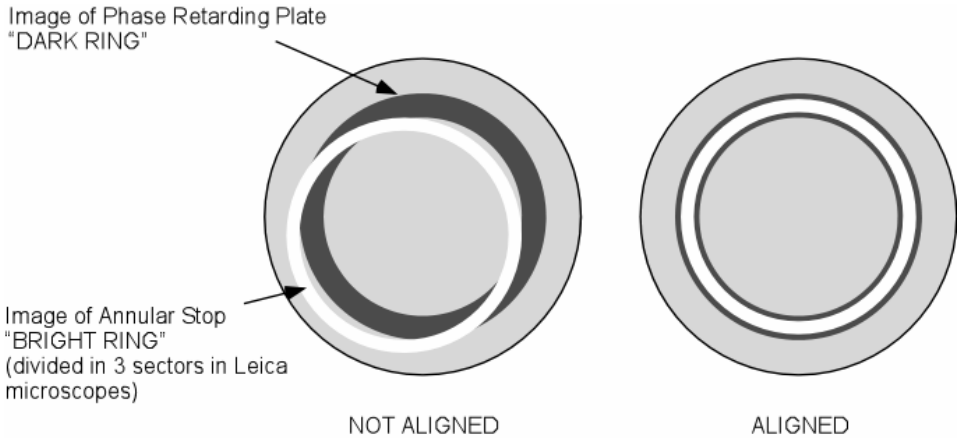
4. To align the annular stop, first remove one of the eyepiece lenses from the binocular tube. In place of the eyepiece lens insert one of the special telescopic eyepieces. Focus the telescopic eyepiece until you see a sharp image of the annular stop (a bright ring, with 3 dark areas at 120° intervals) and a sharp image of the phase retarding plate in the objective (a dark ring).

5. The bright and dark ring may not be concentric but they should be nearly the same diameter. If they are not the same diameter you have the wrong annular stop, so check the condenser turret position.

   Now, the tricky part is aligning the annular stop. Fortunately, this gets easier with practice and is may not be necessary every time you use the microscope.

   Using the alignment knobs, move the annular stop laterally until the bright ring is congruent with the dark ring. The annular stop alignment knobs are hidden on the rear of the condenser and are not depicted in Figure 1. They are silver, not black. You must push inwards gently on the alignment knobs to engage the adjusting screws buried inside the condenser.

Figure 5. Alignment of Phase Annular Stop with Retarding Plate



We have only 2 or three telescopic eyepieces for the class. If you are impatient, then try aligning the annular stop simply by looking at the images of the bright and dark rings directly, down the empty eyepiece tube. This is easier for the lower magnification objectives. By the end of the

quarter you should be so adept that you will be able to align the annular stops without removing an eyepiece, simply by observing the specimen itself.

Table B: Selection of Annular Stops in UKL Condenser

Objective	Application	Condenser Position
All objectives	Brightfield	H
lOX PHACO I	Phase contrast	1
40X PHACO 2	Phase contrast	2
lOOX PHACO 3	Phase contrast	3
4X, 10X, 40X	Darkfield	D

V. Instructions Darkfield Observation with the Leitz Laborlux S Phase Contrast Microscope

Darkfield illumination is possible with the 4X, 10X and 40X objectives only.

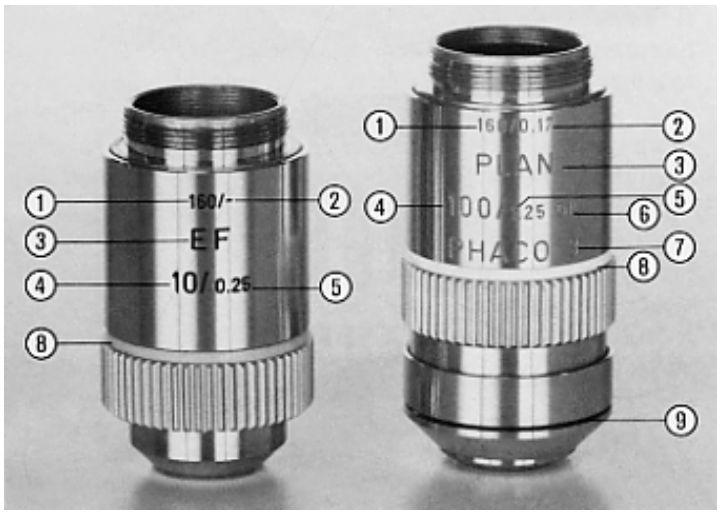
Begin by first setting up the scope properly for brightfield observation according to the directions given previously.

Then, open the aperture diaphragm fully ("PH" setting)

Rotate the condenser turret so that appropriate annular stop is in the light path (position D).

You may need to raise the condenser to the upper limit of its vertical travel.

VI. How to Decode the Identification Markings on the Objectives



1. Mechanical tube length

   The distance in mm from the objective shoulder to the top edge of the tube (where the eyepiece sits). The nearly ubiquitous standard is 160 mm.

2. Coverglass thickness

   The engraving 0.17 is the thickness of the coverglass in mm. A dash (-) instead of a number indicates that the objective can be used for specimens with or without a coverglass.

3. Field flatness of the objective

   This designation is given by the manufacturer to indicate how well the objective lens has been corrected for aberration.

   "EF" Leica objectives are systems with are reasonably well corrected for spherical aberration, and give a more or less flat field of view of up to 18 mm diameter in the intermediate image.

   "PLAN" objectives are more highly corrected, with a flat field of view up to 22.5 mm diameter in the intermediate image. These are more expensive than EF objectives.

4. Magnification (approximate) of the intermediate image.

5. Numerical aperture (N.A.)

6. Immersion medium

   Immersion media can be, for example, immersion oil, water (W), glycerin (GLYC), or air. The objective must <u>always</u> be used with the specified immersion medium.

7. Phase contrast objectives = (PHACO #)

   Objectives with phase rings for phase contrast observation. The Leica objectives also have a number indicating which condenser turret annular stops position # is necessary (e.g. PHACO 3)

8. Colored ring indicating the objective magnification.

9. Black ring indicating immersion objectives.