

Lab #1 Introduction to Hyphae, Isolation of Fungi and Calibration of Microscopes Fungal Kingdom, Fall 06

Objectives

The objectives of this lab exercise are:

To become comfortable using compound microscope. As part of this, you should be able to identify the following parts of the microscope:

- Adjustable eyepieces (know how to focus individual eyepieces)
- Objectives: These are the lens or system of lenses that forms an image of an object. (know how to select different objectives)
- Stage and iris diaphragm
- Illuminator (know how to adjust it and maximize bulb life)
- Learn how to calculate magnification and calibrate your ocular micrometer
- Become familiar with preparing slides for microscopic observation and observe a variety of fungal hyphae and conidia.
- Be able to confidently identify clamp connections.
- Learn the basic techniques for isolation of fungi

Bring your copy of *How to Identify Mushrooms to Genus III: Microscopic Features* by David Largent, et al to class.

Exercises

Calibration of the ocular micrometer

Refer to pages 7 and 8 in Largent et al.

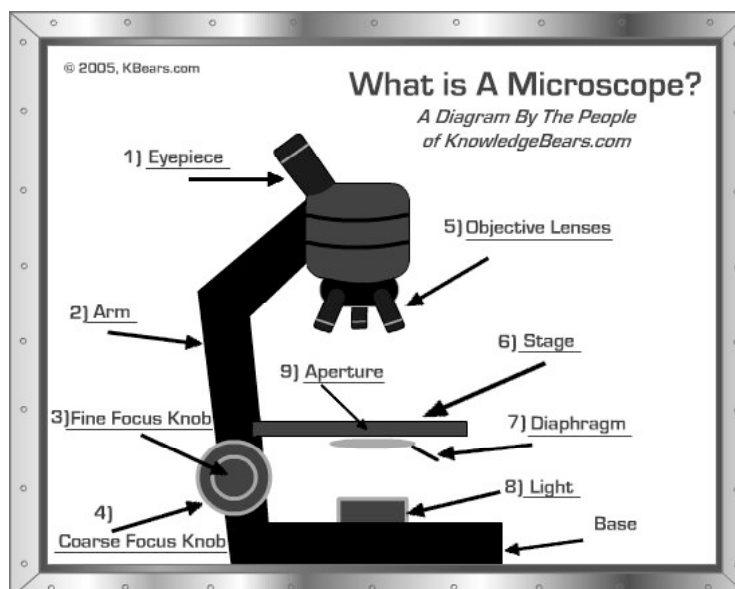
Direct observation of fungal cultures

After you have prepared your first slide, use the following directions to get the most out of your

microscope. **ALWAYS CARRY MICROSCOPES WITH TWO HANDS, ONE ON THE ARM, THE OTHER SUPPORTING THE BASE.**

Setting your microscope for maximum contrast and ease of viewing.

1. Position the microscope so that the arm of the frame is facing away from you.
2. Locate the small set screw that permits the eyepieces to swivel, loosen it, and rotate the eyepieces to face you. **It is mandatory to rotate the eyepieces back over the arm before putting the scope back in the locker.**
3. Locate the focusing eyepiece and move it to a neutral position, usually indicated by a “0”.
4. Move the substage condenser all the way to the top and then move it down about 1 cm (not shown on diagram, but located at #7).



5. Use the coarse focus knob to lower the stage down a couple of cm.
6. Turn the light on so it is moderately bright (1/3 of the way on). Generally, students crank the light up too high, which decreases the contrast and burns out light bulbs quickly.
7. Open the diaphragm (small lever or ring on condenser) all the way.
8. Place your slide in the slide holder on the stage and use the mechanical knobs to position your specimen in the center of the light beam.
9. Rotate the objectives lenses to the 10X objective.
10. Look through the eyepieces and use the coarse focus to bring your specimen into focus. Don't bother adjusting the light unless it is way too bright (turn down the power). *Power user tip: I always have one hand on the knobs that move the mechanical stage and the other on the focus. By slightly moving the slide back and forth, it is easier to quickly focus on the slide instead of some other level in the light path (focusing on the top of the condenser is common).*
11. Adjust the focal length of the eyepieces. Using one eye, focus on some part of the specimen through the non-focusing eyepiece using the coarse focus knob, then using the other eye focus on the same part of the specimen using the focus on the eyepiece.
12. Rotate the 40X objective into position. **AT THIS POINT, YOU SHOULD ONLY USE THE FINE FOCUS KNOB—USE THE COARSE FOCUS ONLY WITH THE 10X OBJECTIVE.**
13. Scan the slide and locate an area of interest, adjusting the focus as needed. You may need to slightly increase the power to the light if it is too dark, but make sure the diaphragm is fully open first!
14. Put one hand on the iris diaphragm lever and look through the eyepieces. Slowly close the diaphragm until you observe an increase in contrast.

Your microscope is now set up for optimal viewing at 400X. If you are using the oil immersion lens, repeat the light adjustment steps 13 and 14. Adjusting the iris diaphragm as outlined above decreases the amount of light passing through the specimen at an angle, which will increase the contrast. This is especially critical when viewing hyaline structures like most fungal mycelium and many spores.

Prepare slide of mycelium for viewing

1. Get several slides, cover slips, a dissecting needle, and dropper bottles of KOH and phloxine.
2. Place a paper towel on the lab bench and prepare all your slides on the paper towel. This will absorb any stain that leaks out and will keep the microscope stage clean.
3. Put a ½ drop of KOH and ½ drop of phloxine on a microscope slide next to one another, **but not touching**. Do this by lightly touching the dropper tip to the slide. Full drops that will fall from the dropper tip are too big.
4. Using a dissecting needle, collect a small amount of mycelium (often with a little agar) and/or conidia from a Petri plate and place it in the KOH on the slide. Lightly mix the specimen with the KOH and then mix in the phloxine.
5. Place a cover slip over the specimen and lightly smooch the cover slip down by tapping with the handle end of the probe or with a pencil eraser.
6. Observe with the compound scope, scan the edges to locate an area where the hyphae are spread out.

7. Measure the average diameter of the hyphae. Sketch what you see in your lab book. Be sure to label each sketch with the identity of the fungus and the magnification. Identify any hyphal structures in your sketches.
8. Repeat for the various fungi available. Make sure you observe a culture with clamp connections

Did you find any consistent differences between the Ascomycetes and Basidiomycetes that you observed? If so, outline them in your lab book.

Isolation of pure fungal cultures

There are many different methods of isolating fungi. The particular method chosen depends on the fungus, its nutritional needs, and its habitat. One of the most common strategies for most saprophytic fungi is direct transfer, which involves getting some fungal tissue or spores that are relatively free of contaminants and putting them on a suitable sterile medium. Once the mycelium begins growing, you can transfer it into a new plate of medium, repeating as needed. Special selective media containing various antimicrobial compounds can be used to reduce competition from other fungi.

Direct transfer can be used to obtain pure cultures of mushrooms and other large fungi. The fruiting body is broken open, without touching the newly exposed flesh, and some of the tissue is aseptically transferred to a sterile culture medium. If this has been done carefully, the tissue may give rise to a colony after a few days. Depending on the toughness of the fruiting body, sharp forceps may be suitable to remove the tissue, but a small chisel may be needed for tough shelf fungi.

Many wood-inhabiting fungi can be isolated by breaking open a slice of the wooden substrate and aseptically removed small pieces of wood and embedding them in a suitable medium. Increment cores are also an effective method for collecting wood for culturing. Cores can be placed in labeled straws in the field, refrigerated and plated upon return to the lab. Each core can be divided into smaller pieces which are then lightly flamed with an alcohol flame to kill any contaminants that may have arrived during sampling.

Isolation from spores works well for most Ascomycetes, Basidiomycetes, and imperfect fungi. However, the resulting cultures are not genetically identical to the parent colony. If one is collecting material to analyze population genetics or interactions, spore isolations are unsuitable. For mushrooms and bracket fungi a piece of the gill or tube tissue can be attached, perpendicular to the agar, on the lid of a Petri dish. The spores will then float down on to the agar surface and later germinate (if one is lucky). With large mushrooms it is sufficient to attach a single gill, flat side down, to the lid; with small ones the whole cap can be stuck on. Petroleum jelly (Vaseline) or masking tape works well to attach the fruiting body to the lid of a Petri plate.

Ascomycetes, such as cup fungi, can also be suspended above an agar surface for spore printing. Some of them, however, are so small that they have to be left attached to the material they are growing on and that stuck down as well. The problem of contamination from loose particles dropping down then arises, however, which can be avoided by turning the plate over and letting the spores shoot up. Most Ascomycetes can shoot their spores far enough to reach the lid of a Petri dish, but Basidiomycetes cannot.

Transferring spores from a spore print using an inoculating loop can also be a successful strategy. Taking spore prints onto sterile glass microscope slides (flame them lightly and then cool in a sterile glass Petri plate) which are stored in a sterile dish until plating will maximize success, although I've had good results from spore prints taken onto paper.