Microscopy - Chapter 1

Lecture 2

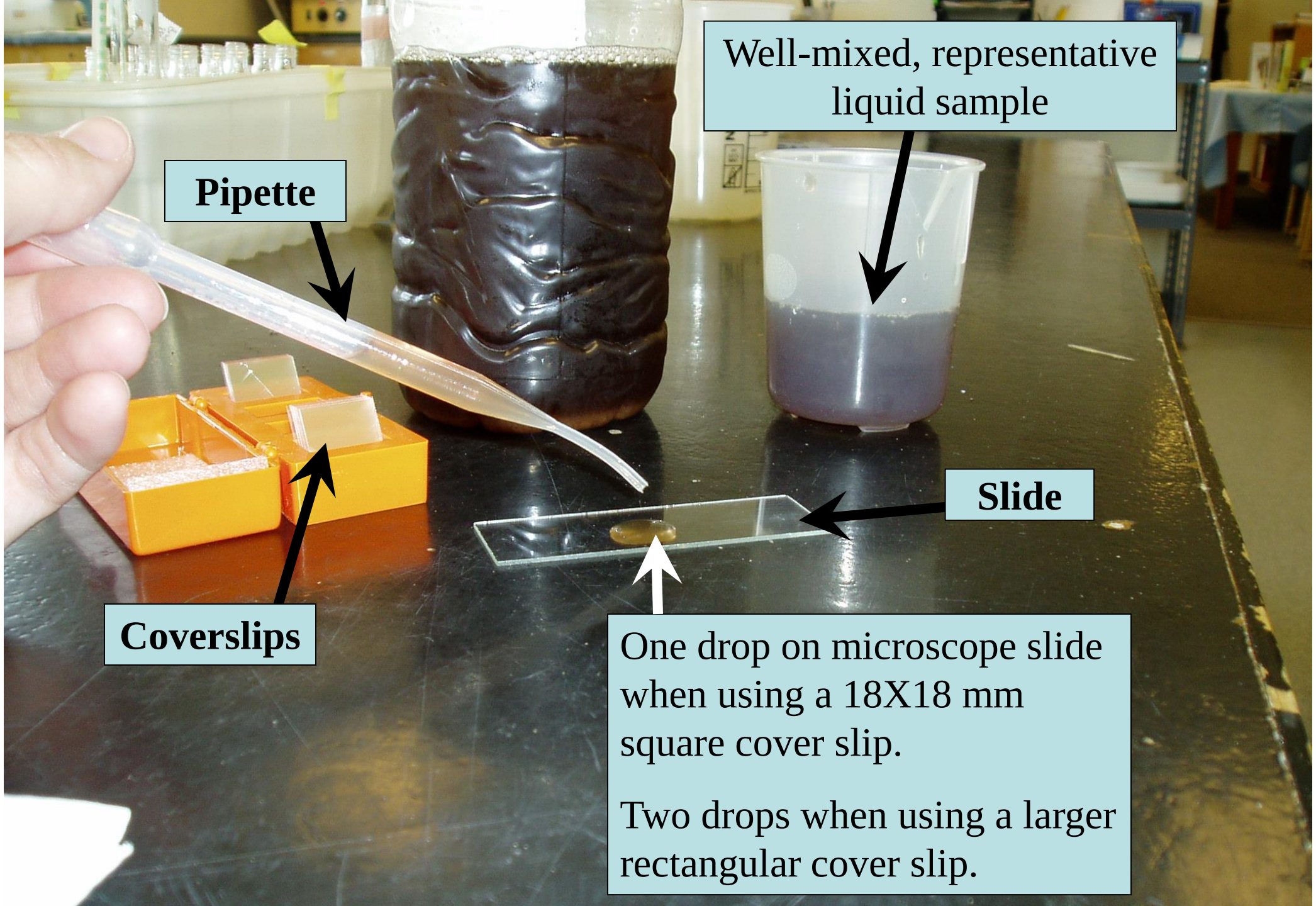
Using the Compound Microscope for Shadowing Microscopy (Part 2)

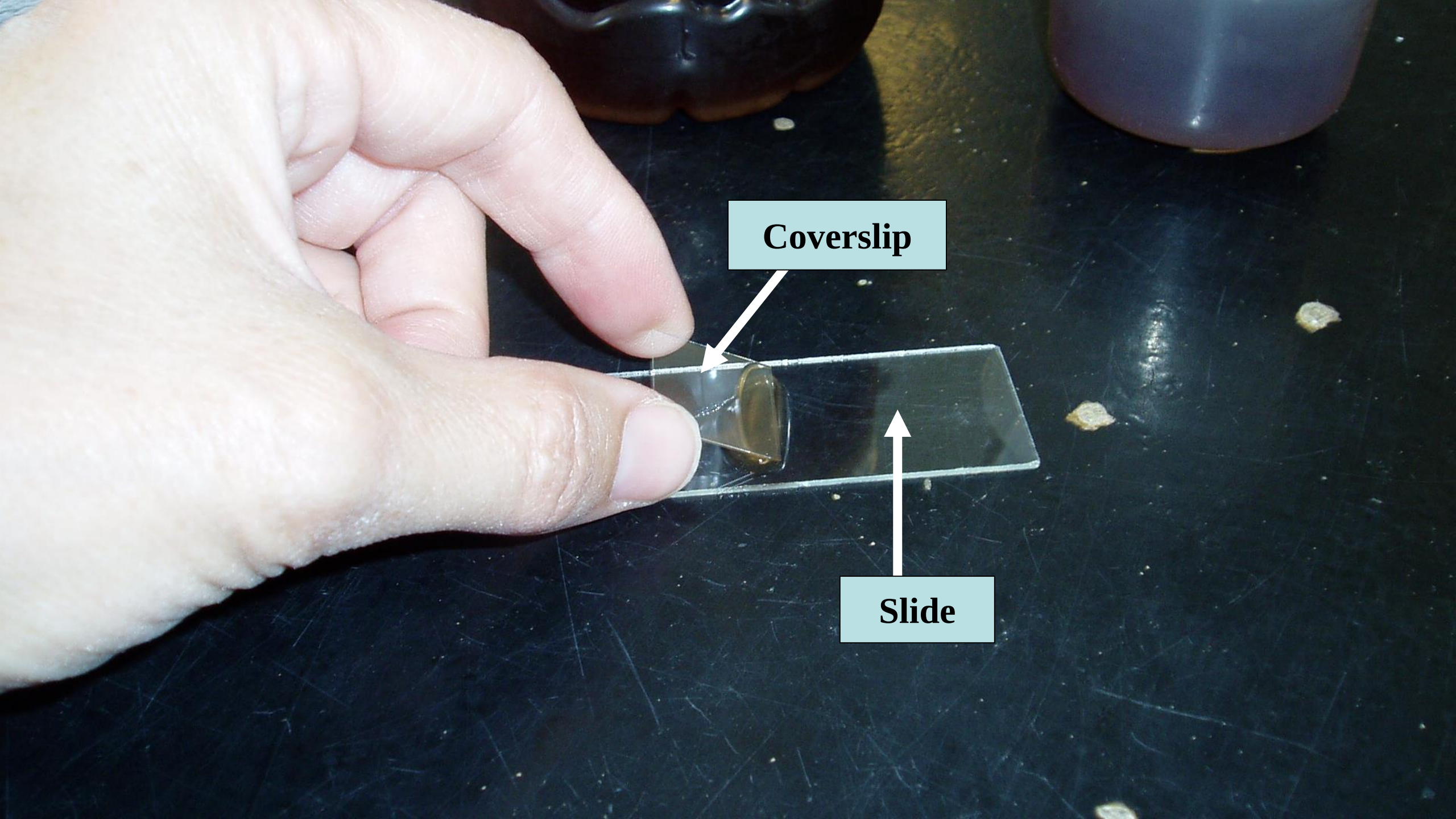
Preparing a Sample for the Microscope

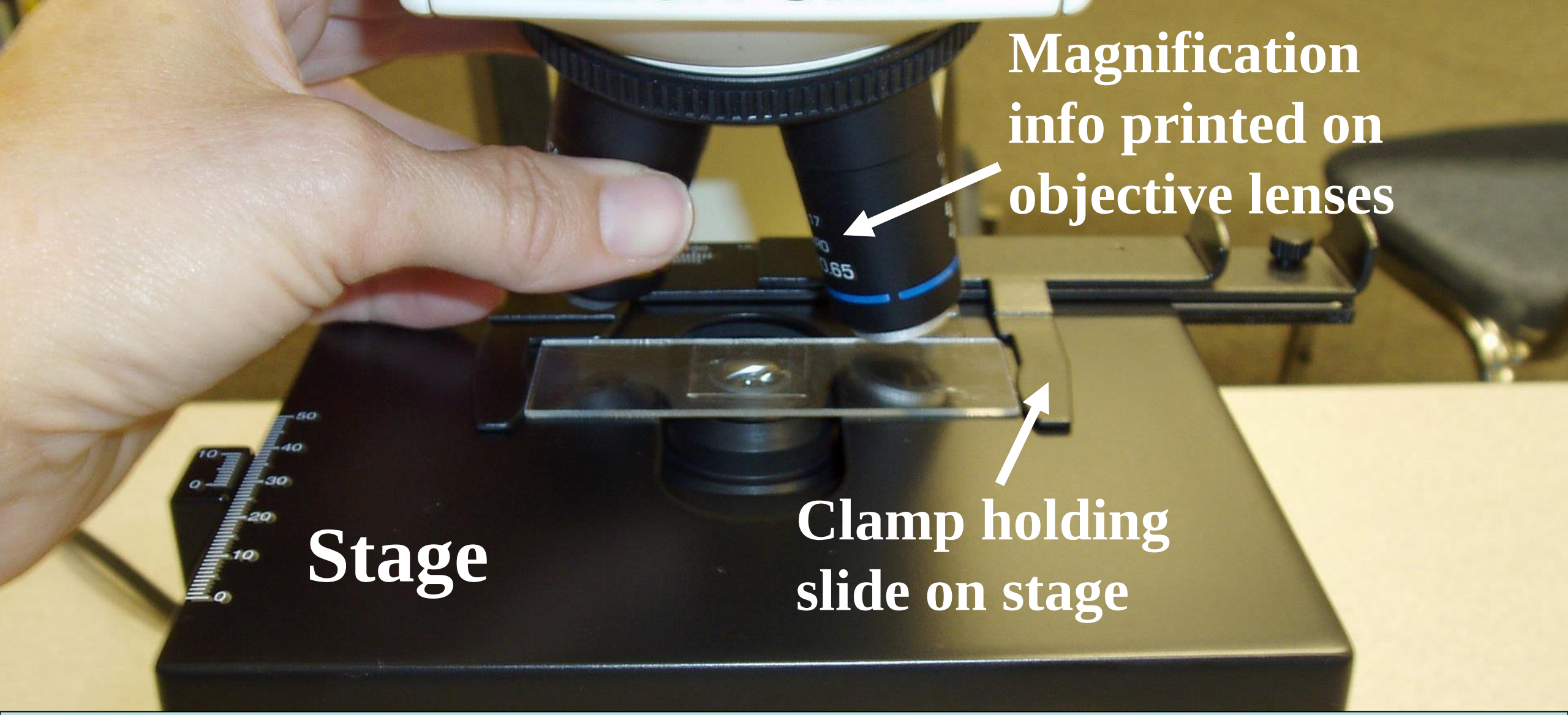
Solid Sample: Gently mix soil, dirt, or compost and break up any compacted parts. Measure 1 ml volume of sample (also equals 1 gram or 1 ml volume) into a test tube. Tap sample in the test tube 10 times on the table top. Add more of solid sample material to make sure sample has 1 ml volume.

Add 4 ml water (treat chlorine/chloramine if needed). Shake sample in a 1 foot arc (forearm parallel to floor, to shoulder and back each second) for 30 seconds. Let settle for 10 seconds.

Place one drop on microscope slide, spread the sample evenly on the surface as was done for the liquid sample. Place the slide on the stage, clamp the slide into place.


Well-mixed, representative liquid sample
Pipette
Slide
Coverslips
One drop on microscope slide when using a 18X18 mm square cover slip.
Two drops when using a larger rectangular cover slip.


Coverslip
Slide



These objectives lenses are “par-focal”, so when one lens is focused, the other lenses are in focus too. Adjust the condenser while using the 10X objective.

A Note About Filters

Often several different colored filters are included when you buy a microscope. These filters are used to alter the background color of the field of view.

Photographers will say they want “daylight” backgrounds, but daylight doesn’t penetrate into the soil more than a few micrometers.

And now, the bad news…. Filters reduce the amount of light getting to your eyes, and thus reduce resolution. Filters are not needed!

Microscope Parts:
Ready to Assess Organisms!

Nematodes – Count and ID nematodes by scanning the entire drop of sample using 4X or 10X objectives. To ID to functional group, use 40X objective.

The number of individuals per functional group per 1 drop of sample should be recorded in the spreadsheet.

Bacteria - Can be seen using the 4X objective, if the sample is not dried, but to distinguish between bacteria and clay particles, 400X total magnification is needed. Count the number of 1µm wide and up to 100µm long, smooth, not-jagged surfaced organism.

Microscope Parts:
Ready to Assess Organisms!

Bacteria (continued) -

Record number of bacteria per field in the spreadsheet. If the bacteria in ¼ field were counted, multiply the number of individuals seen in that ¼ field by 4 to estimate the number per field.

Typically bacteria are so numerous that 1:100 to 1:1000 dilutions are needed to spread bacteria out enough to be able to count them.

Microscope Parts:
Ready to Assess Organisms!

Actinobacteria – Measure the length of the narrow diameter filaments (1 – 1.5 µm), which can branch and look very much like fungi. Often the chain of bacteria inside the protective filament can be seen. Record lengths of actinobacteria observed per field.

Fungi – Measure and record length and width of each strand seen, note color, cross walls (septa), bubbly cytoplasm or lumpy hyphae seen per field. Usually hyphae are not uniformly spread out, so multiple fields need to be assessed to keep standard deviations low.

Microscope Parts:
Ready to Assess Organisms!

Protozoa – Counted using 40X objective, record the number of each functional group seen per field. Correct shadowing must be used to see protozoa. Movement is important to note as well as size. Flagellates bumble, amoebae ooze, ciliates zoom.

Spores – Noting the diversity of fungal spores, protozoan cysts, nematode eggs, and parasite eggs can tell a great deal about past conditions in the sample. We do not count any of the dormant stages as active biomass, however.

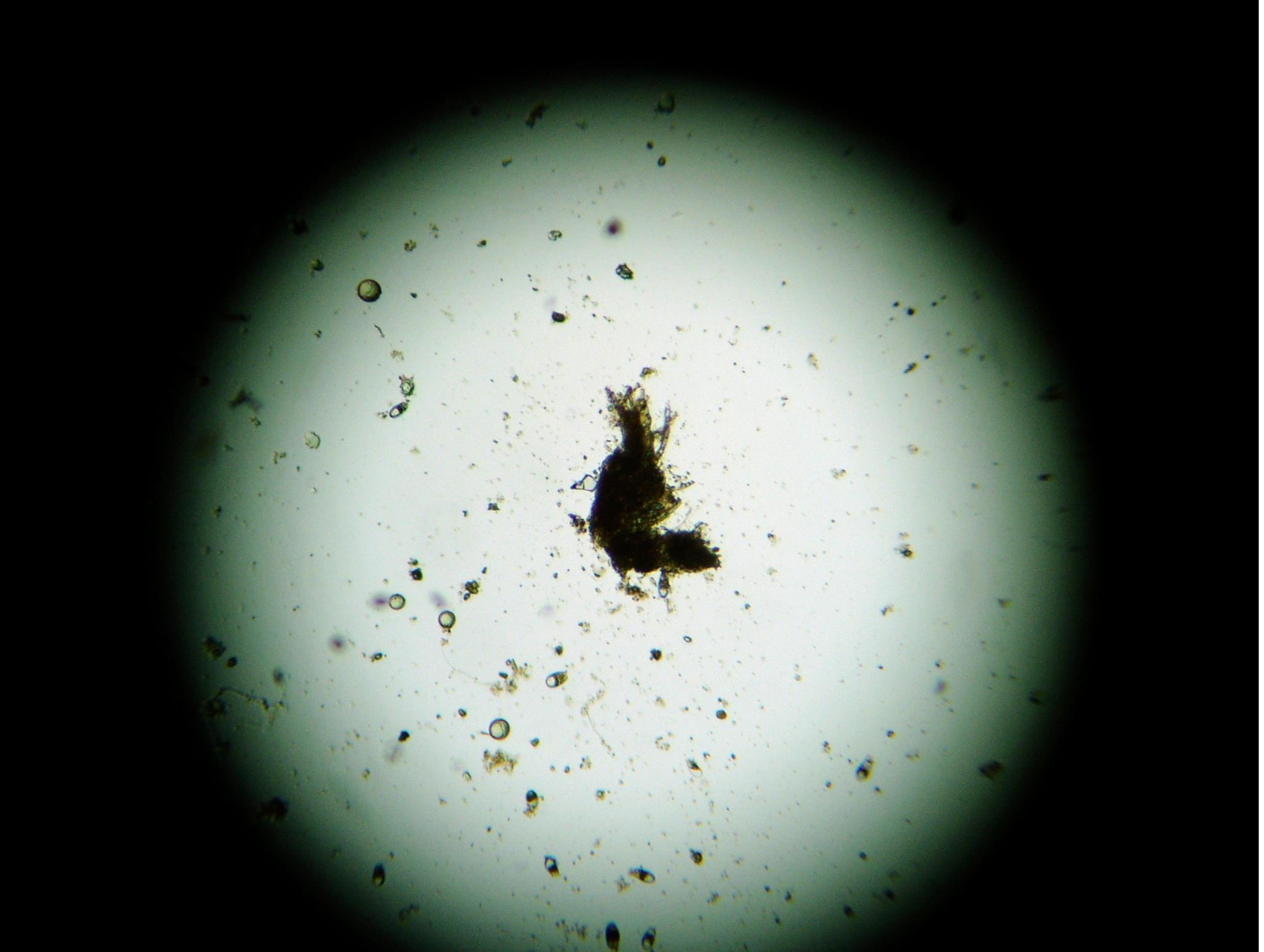
The higher the magnification, less of the sample is seen

The next three slides exemplify how much less of the sample is seen as magnification increases.

Slide 1: 40X Total Magnification - plant debris, cysts or eggs, sand grains, silt, clay, lots of bacteria. Need to increase magnification to resolve.

Slide 2: 100X Total Magnification - quite a bit less of the sample seen.

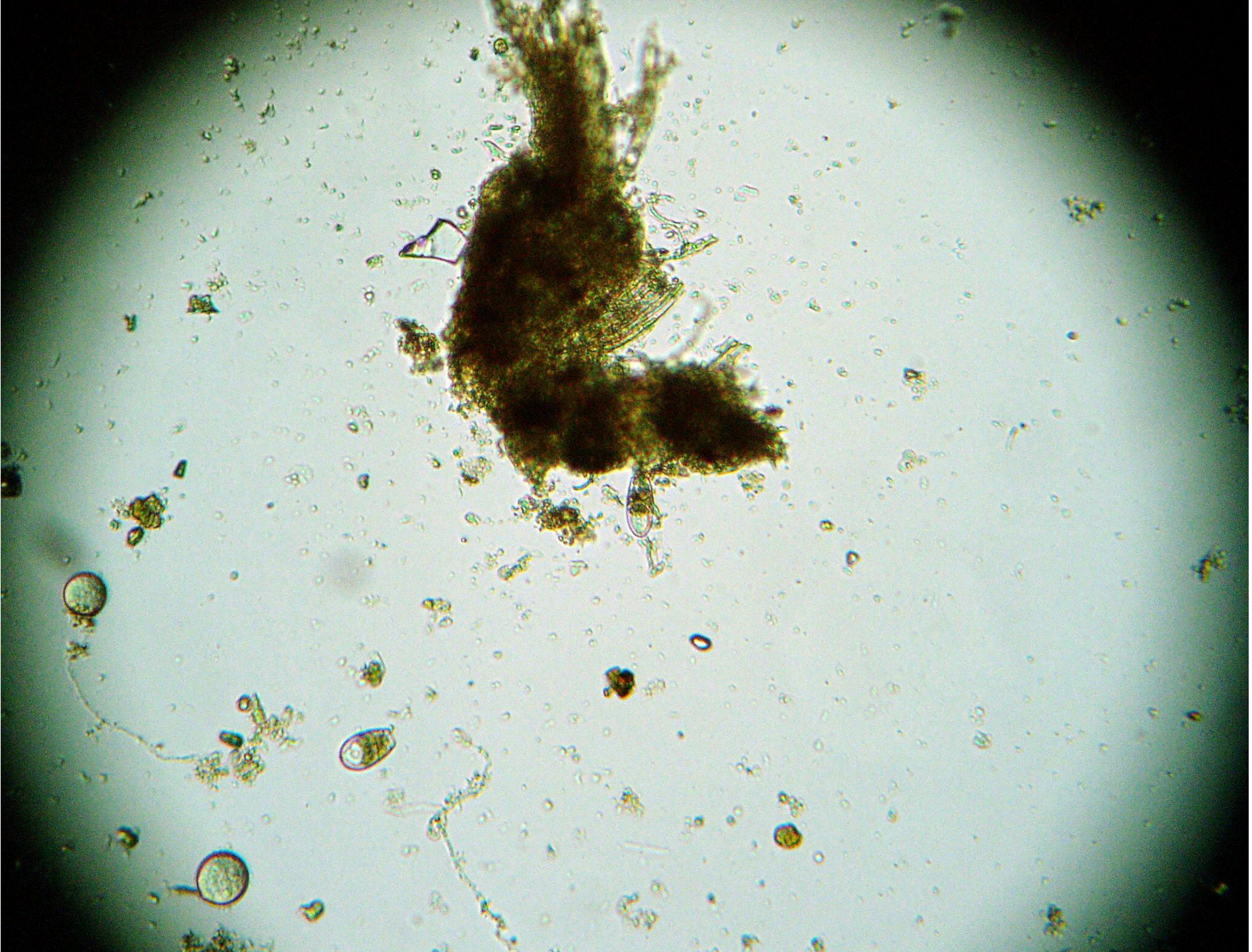
Slide 3: 400X Total Magnification - seriously less to see, but can ID to species to functional groups easily….with training.



Slide 1

40X Total Magnification

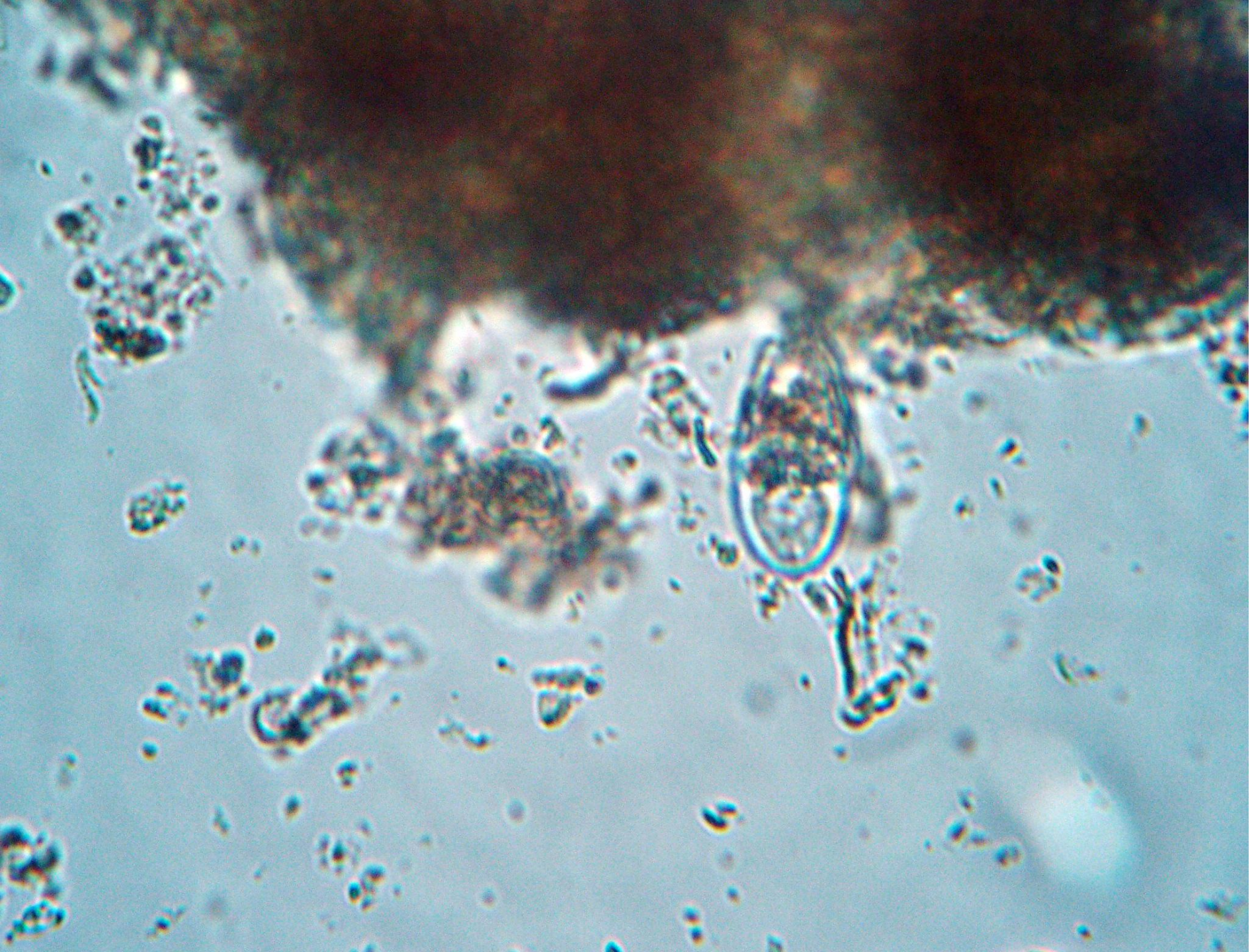
(10X Eyepiece * 4X Objective)



Slide 2

100X Total Magnification

(10X Eyepiece * 10X Objective)

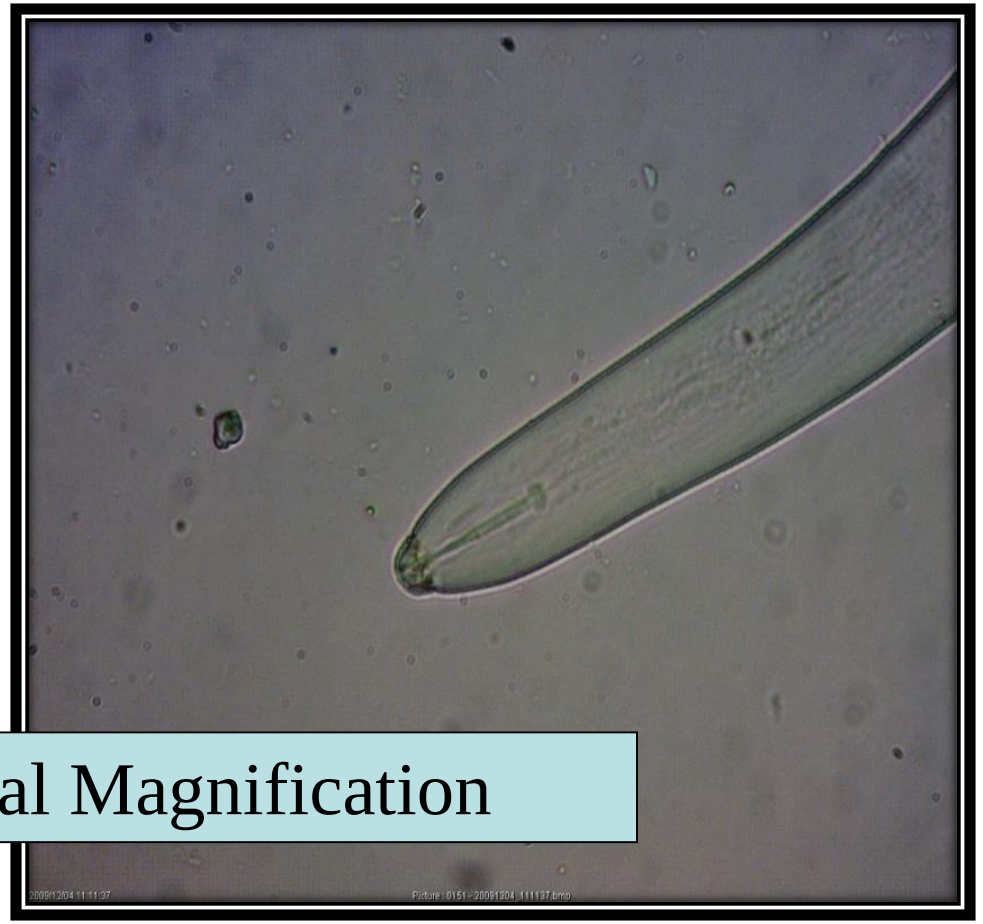


Slide 3

400X Total Magnification

(10X Eyepiece * 40X Objective)

10X Eyepiece * 10X Objective = 100 Total Magnification



10X Eyepiece * 40X Objective = 400X Total Magnification