BIOLOGY 428/628:
SCANNING ELECTRON MICROSCOPY WORKSHOP
Lab training: 14-16 October 2016

TENTATIVE LAB TRAINING SCHEDULE:

Friday, 14 October, 2016

1 pm: Preparation of material for SEM; identification of samples; introductory lecture. ***(meet in TNR 460, the Parasitology Lab)***
2-5 pm:dehydration to 100% ethanol [EM LAB]
5-7 pm: critical-point or solvent drying of samples for SEM [EM LAB]

Saturday, *15 October 2016*

8:30-9:30 am: Lecture on principles of scanning electron microscopy **(meet in Parasitology Lab, TNR 460)**
9:30 am - 11:30 am: GROUP I: Basic alignment of the SEM; operation & photography (SEM lab, TNR 380A)
11:30 am - 1:30 pm: GROUP II: Basic alignment of the SEM; operation & photography (TNR 380A)
1:30-3:30 pm: Demonstrations: mounting samples on SEM stubs with carbon tape and silver paint; Au/Pd sputter-coating of samples. Lottery for 3.5-hour hands-on sessions. (TNR 460)
3:30 pm - ??? First 3.5-hour hands-on tutorial sessions.

Sunday, 16 October, 2016

9 am-noon: Lecture/Demonstration: freeze-fracturing & tape-ripping for examining internal structure. **(meet in Parasitology Lab, TNR 460)**
noon-??? Scheduled 3.5-hour hands-on tutorial sessions **(SEM lab, TNR 380A)**

Monday, 17 October 2016

TBA: Scheduled 3.5 hour hands-on tutorial session (**SEM lab, TNR 380A**)

★★TBA: There will be a Specimen Collection Day for collecting and preserving samples for SEM. TBA: After the workshop there will be a "Subs and Stereos" party to display Workshop images.

The Workshop.

This workshop is meant to teach the student the following:

(a) the basic methods for preparing material (plants, insects, animal tissues) for scanning electron microscopy, using conventional methods of alcohol or aldehyde fixation, osmium *post-fixation*, ethanol *dehydration*, *critical-point or solvent drying* and *sputter-coating*.
(b) basic principles of photomicrography: identifying and taking pictures of your insects with a stereo light microscope.
(c) the principles of *alignment* and *operation* of the scanning electron microscope, using the UWSP's Hitachi S-3400N-II SEM.
(d) how to create *digital SEM micrographs*, *3-D stereo pairs* and digital *color anaglyphs* from the micrographs for viewing in 3-D.
(e) how to make PowerPoint slides from your micrographs.

After the basic training in the workshop, students will be eligible to use the SEM for *research projects* sponsored by a faculty member.

OBJECTIVES FOR THE COURSE:

Each student will demonstrate their mastery of the basic techniques by presenting:

(1) at least **two dehydrated, well-mounted, sputter-coated specimens**

(2) **12 or more digital micrographs** -- at least 3 low-power (below 200X), and at least 3 high-power (above 500X), and at least **4 stereo anaglyphs**.

(3) the digital images, with specimens properly identified to the level of family of organism, in a **PowerPoint presentation**

THINGS YOU CAN DO TO PREPARE FOR THE WORKSHOP:

1. Pick up a workshop handbook in the UWSP Bookstore. It is about $22. They will be available the third week of the semester.

2. Pick up a large tube of 70% ethanol for preserving samples from the Biology office. I will announce their arrival.

3. Find at least two different types of insect (or plant), several of each kind if possible. (You won't have time look at more than two of them on the SEM during the workshop, but you might lose a sample in the preparation.)

BEST SAMPLE SIZE: Think tiny!! Less than 5 mm x 5 mm and 1-to-2 mm thick – smaller than this ➔ 

We prefer **insects** for this workshop because they are easy to prepare and mount and there is always something interesting to look at. [Suggestions for **plant material** below.] **We will have an insect collection day and I will provide nets.**

Insects. Suggestions: ***in winter***, take your tube of alcohol outside and look for **collembolans ("springtails")**, tiny black insects that hop around on the snow on a sunny day (often, by the hundreds or thousands); they can also be found buried in leaf litter. Numerous grubs, beetles, etc. can be found under heavy bark of some trees in winter. Look under leaf-litter, old fallen wood in Schmeekle Reserve. Other suggestions: our greenhouses have lots of little bugs (ask the greenhouse manager, Mr. John Hardy, TNR 155A, to show you). White flies on leaves are common. Tap a flower head into a dish of alcohol and you will collect a number of tiny insects (thrips, mostly). Leave a piece of ripe banana around your house and catch fruitflies. **Do not use dead, dried insects! Insect Catcher:** In warmer weather, the easiest thing is to take an old pillowcase and throw it over a bush and shake the bush, or drag the pillow through tall grass. Then, snap the pillowcase down and toward one bottom corner and twist to keep everybody inside. To slow down the insects you've caught, put the pillowcase into the freezer for **30 seconds**(!), no more. Then shake them onto a sheet of newspaper and quickly pour insects into the alcohol collecting tube before they wake up! Submerge completely. If it really is an old pillowcase, just cut off the corner and drop the corner into the alcohol tube.

Plants: cut small squares of fresh leaves, or anthers containing pollen grains plucked from flowers or **tiny** whole flowers. Hairy leaves have interesting trichomes for SEM. Immerse immediately in alcohol.

Avoid soft or large samples (caterpillars, grubs, large worms, large flowers, large grasshoppers, animal tissues, etc.). They will not preserve well in our alcohol fixative.

The samples will keep indefinitely in 70% ethanol at room temperature. Make sure the cap is tight and invert tube and store on its cap.

Sol Sepsenwol, Ph.D.
Department of Biology, TNR 439
University of Wisconsin, Stevens Point
Stevens Point, Wisconsin 54481
Telephone & FAX: (715) 346-4256
e-mail: ssepsenw@uwsp.edu

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