BIOLOGY 498/698:
SCANNING ELECTRON MICROSCOPY WORKSHOP
12-14 April 2013

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) the principles of *alignment* and *operation* of the scanning electron microscope, using the UWSP's Hitachi S-3400N-II SEM.
(c) how to create *digital SEM micrographs*, *3-D stereo pairs* and digital *color anaglyphs* from the micrographs for viewing with 3-D glasses.

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 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, in a **PowerPoint presentation**

THINGS YOU CAN DO TO PREPARE FOR THE WORKSHOP:

1. Pick up a large tube of 70% ethanol for preserving samples from the Biology office, CNR 167.
Find at least two different types of insect, several of each kind if possible. (You won't have time to do SEM on more than two of them during the workshop, but you might lose a couple in the preparation!)

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

We prefer **insects** for this workshop because they are easy to prepare and to mount and there is always something interesting to look at. Suggestions for **plant material** below.

Insects. Suggestions: in winter, look for collembolans, or springtails, tiny black insects that hop around – very interesting to look at with SEM; on a sunny winter day, you can see them hopping on the snow. Numerous grubs, beetles, etc. can be found under heavy bark of some trees even in winter. Other suggestions: fruit flies from Genetics labs, dermestid beetles from Dr. Yahnke,. Our greenhouses have lots of little bugs (ask John Hardy to show you). Tap a flower head into a dish of alcohol and you will collect a number of tiny insects (thrips). **Do not use dead 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 it through tall grass. Then, snap the pillowcase down and shake toward one bottom corner and twist to keep everybody inside. Put the pillowcase into the freezer for 10-15 min, shake them onto a sheet of newspaper, then fold the paper and pour into the alcohol collecting tube. Submerge completely. PS: If it really is an old pillowcase, just cut off the corner and drop into alcohol tube to make life easier.

Plants: cut squares of fresh leaves, anthers containing pollen grains plucked from flowers, **tiny** whole flowers. Hairy leaves have interesting trichomes for SEM. Put into 70% ethanol before it has a chance to dry.

Avoid soft or large samples (bacteria, caterpillars, 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 top is tight and invert tube and store on cap.

TENTATIVE WORKSHOP SCHEDULE:

Friday, April 12, 2013

1 pm: Preparation of material for SEM; identification of samples; introductory lecture. ***(meet in TNR 380, the EM 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, *April 13, 2013*

8:30-9:30 am: Lecture on principles of scanning electron microscopy **(meet in Physiology Lab, TNR 253)**
9:30 am - 11:30 am: GROUP I: Basic alignment of the SEM; operation; taking digital micrographs
11:30 am - 1:30 pm: GROUP II: Basic alignment of the SEM; operation; taking digital micrographs
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.
3:30 pm - ??? First 3.5-hour hands-on tutorial sessions.

Sunday, April 14, 2013

9 am-noon: Lecture/Demonstration: freeze-fracturing & tape-ripping for examining internal structure. **(meet in Physiology Lab, TNR 253)**
noon-??? Scheduled 3.5-hour hands-on tutorial sessions **(SEM lab, TNR 380A)**

[Monday, April 15, 2013; only if needed for tutorials]

10 am-? 3.5 hour tutorials, continued (if needed)

There will be a "Subs and Stereos" meeting later in the semester to display Workshop images.

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