BIOLOGY 428/628, 1 cr. , FALL, 2018
SCANNING ELECTRON MICROSCOPY WORKSHOP

Students are expected to achieve the following learning outcomes after completing this Workshop:

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

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

REQUIREMENTS 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) both LM and SEM digital images, with organisms properly identified to the level of Family, in a **PowerPoint presentation**

TENTATIVE LAB TRAINING SCHEDULE:

Note: **Students are required to collect their samples, complete their identification to the Family level and have light micrographs of each before the start of the workshop's lab training on 19 October (Friday)! There will be 4 open sessions before then to do this.**

Monday-Thursday, 8-11, 15-18 October 2018, to be scheduled

Sample collection: plant and insect samples will be collected from Schmeekle Reserve. Two collection trips will be offered before the workshop lab training begins 19 October (to be scheduled). Plant samples may be collected from the Biology Greenhouses under the supervision of Drs. Sun or Sepsenwol or Mr. John Hardy. **Students are required to attend one trip according to the samples they plan to collect.**

Sample identification: Students will identify plant or insect samples with biology faculty in **CBB 326** (3 hr, 2 sessions offered) and take light microphotographs of samples in Purell-ethanol. **Students are required to attend one ID & microscopy session.**

Friday, 19 October 2018

1 pm: Preparation of material for SEM; introductory lecture. **(CBB 376, Human Physiology Lab)**
2-5 pm: Dehydration to 100% ethanol [**CBB 326, EM Lab**]
5-7 pm: Critical-point drying of samples for SEM [**CBB 326, EM Lab**]

Saturday, *20 October 2018*

8:30-9:30 am: Lecture on principles of scanning electron microscopy **(CBB 376, the Human Physiology Lab)**
9:30 am - 11:00 am: GROUP I: Basic alignment of the SEM; operation & photography (**CBB 326, SEM lab**)
11:00 am - 12:30 pm: GROUP II: Basic alignment of the SEM; operation & photography (**CBB 326, SEM lab**)
1:00-4:00 pm: Demonstrations: mounting samples on SEM stubs with carbon tape and silver paint; Au/Pd sputter-coating of samples. Scheduling of 4-hour hands-on sessions. (**CBB 326, EM lab**)
4:00 pm - 8:00 pm One 4-hour hands-on tutorial session (**CBB 326, SEM lab);** two students per session).

Sunday, 21 October 2018

9 am-noon: Lecture/Demonstration: freeze-fracturing & tape-ripping for examining internal structure. Making 3D anaglyphs of SEM images with **3D Stereo Image Factory**; making PowerPoint slides **(CBB 376, Human Physiology Lab).**
12n-4 pm, 4-8 pm Two 4-hour hands-on tutorial sessions **(SEM lab, CBB 326E;** two students per session**). Students attend only one tutorial each.**

Monday - Friday, 22-26 October 2018 [to be scheduled with students]

Three 4-hour hands-on SEM tutorial sessions (**CBB 326; SEM lab;** two students per session)
★★ Open Lab: the SEM Lab will be open for about 1 week after the last tutorial so that students can make slides of their micrographs for the PowerPoint presentation.

★★TBA: After the workshop there will be a "Subs and Stereos" party to display Workshop images.

★ *THINGS YOU CAN DO TO PREPARE FOR THE WORKSHOP:* ★

1. Purchase a workshop handbook (about $23) in the UWSP Bookstore. They will be available by the end of September.
2. Pick up a large tube of 70% ethanol for preserving samples from the Biology office (CBB 302). All samples will be preserved in one tube. We will let you know when they are available.
3. Find at least two different types of samples (insector 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 tutorial, 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 box ➔ 

For SEM materials, we prefer **insects** for this workshop because they are easy to prepare and mount and there is always something interesting to look at. If using plant materials: leaf and floral parts, especially those from drought-adapted species, usually have interesting surface features. All anthers contain pollen grains, always interesting to look at. Ferns have unique reproductive structures. **TBA**: **We will have two insect and plant collection days at Schmeekle; nets will be provided. There will also be two identification and photography days to record each of your samples before preparing them for SEM.**

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, CBB 122, 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 (many varieties). **Do not use dead, dried insects! Insect Catcher:** In warm weather, the easiest thing is to take an old pillowcase and throw it over a bush and shake the bush, or drag the pillowcase 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 creased 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. Bumps on leaves (galls) are often signs of mites or insect larvae inside -- very interesting.

Avoid soft or large samples (do not use 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. We will take pictures of your samples before preparing them for SEM.

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