Course Syllabus: BIOL 4450/6450: Spring 2020

Theory and Practice of Scanning Electron Microscopy

CRN 24699 **and** 24711**;** **Lecture: MW 12:00 – 12:50 p.m. (BC 1202),**
Lab: MW 1:00 – 2:50 p.m. (BC 1075)

Instructor: Dr. Russ Goddard, BC 2090, 249-2642
email: rgoddard@valdosta.edu **Office Hours**: **MW**: M: 3:00 – 4:30 p.m.; W: 10:00 – 11:30 a.m.

Course Catalog Description: BIOL 4450/6450, Theory and practice of scanning electron microscopy, 2-2-4. Prerequisite: BIOL 3200 and 3250 or consent of the instructor (**for 6450**: admission into the graduate program). General principles of scanning electron microscopy operation and theory with comparison to light and TEM optics in a laboratory intensive environment. Topics include fixation and preparation of biological samples for standard, low voltage, variable pressure, and high resolution SEM.

Electronic Key Access: Due to the laboratory intensive nature of this course and the fact that there is only one microscope and each student must use this microscope independently to pass this course, students will need to request OneCard access to the building and to BC 1075. This form is located on the "Key and Electronic Access" page on the VSU website at https://www.valdosta.edu/administration/finance-admin/plant-ops/access-control/. After accessing this page each student should read and adhere to the key shop policies on use of this privilege then click on the "Card access" tab on the right side of the page. Fill in the required fields, note you need access to the Bailey Science Center through the Georgia Avenue side door on nights, weekends, and holidays through the semester, and that you need access to BC 1075. Finally, the department head's email that must be entered is rlgannon@valdosta.edu. Submit the form, find your PIN code on BANNER and then start accessing the lab for your work.

Recommended Texts:

Goldstein et. al. 2018. Scanning electron microscopy and x-ray microanalysis, 4e. Springer Science Business Media LLC. New York. ISBNs: 9781493966745 and 9781493966769

Goldstein et. al. 2003. Scanning electron microscopy and x-ray microanalysis, 3e. Kluwer Academic/ Plenum Publishers. New York.

Several text books will be placed in BC 1075 for use in BC 1075. **These books may not be removed from the SEM lab or Dr. Goddard will remove the privilege of use!**

Many links are also available through the internet for explanation of topics covered. You should be able to pull lots of information when you search for lecture topics.

Grading: There are two parts to this course, the lecture and the laboratory, but students must understand that this course is a laboratory intensive course and that they will need to spend significant independent time in the laboratory. There are slightly more points available through laboratory assignments than lecture exams that count toward your course grade.

Lecture Exams (300 pts): There will be 3 one-hour exams in this course. Each exam mainly will cover approximately 1/3 of the lecture material but each exam is comprehensive and can ask questions from any material covered since day one. Each of the three exams will be worth 100 pts.

Lab Image Portfolio (200 pts.): Throughout the course, students will be assigned comparative parameters that they will use to photograph specimens. Students will be required to make a high resolution electronic portfolio of the comparative images to be turned in to Dr. Goddard. Due dates for parts of the portfolio are listed in the calendar section of this syllabus. The full portfolio requirements and

submission requirements will be provided separately. The final portfolio must be in working form on the computer in BC 1076 by 5:00 p.m. **on Friday, April 24, 2020**. There is a **10 point deduction** per day from the 200 point score for submissions turned in late for portfolios; turned in during the weekend, and for the first three days after the deadline. There is a **25 point deduction per day** for portfolios turned in **on or after April 30, 2020.**

Basic Check-out Exam (50 pts): After their first instruction on the SEM, students are required to use the microscope only during business hours following the rules outlined during lab. It is preferred that students work in pairs or small groups for their first 8 hours of logged use on the microscope. Early independent use (no partner) also may be allowed but students should make sure Dr. Goddard is available to handle problems encountered. Once a student has logged eight hours of use and feels confident in their use of the microscope, you must schedule a basic check-out exam with Dr. Goddard. The requirements for this checkout are posted at the end of this syllabus. Each student is graded on their performance using the SEM using either their own required fixed and processed samples (e.g. *Drosophila virilus*) or other sample that is provided. For the student sample, the quality of preparation will be evaluated during the check-out (e.g. dehydration followed by CPD). All basic checkouts **must be attempted before the end of 5 March 2020**! Significant late penalty point deductions will be applied after this date. After passing this check-out, students can operate the SEM independently, and they may work in the laboratory (including the microscope) on nights and weekends provided this privilege is not abused.

Oral Proficiency Exams (100 pts): Each student will orally articulate and demonstrate any laboratory procedures learned during the class including basic and advanced microscope use during the weeks of April 13 - 17 and 20 - 24. After the basic check-out exam, students are expected to ask questions of the instructor based on the lab demonstrations and their independent use leading to portfolio images so that they are "expert" by the time they take this 100 point exam.

Research projects (Graduate Students): BIOL 6450 (100 pts). Since the SEM represents a tool for acquiring high quality research data, students must propose a research topic that can be studied using the equipment and procedures learned in the course. Students will research the literature and take preliminary photographs of any specimens that fit into a scientifically valid study. Graduate students will give a 10 min PowerPoint presentation, on their proposed research project during a lecture period TBA but near March 1st. A final 20 min PowerPoint in the format of a scientific talk and containing images from the SEM, will be presented to the class on April 29, 2020 during the lecture period. The PowerPoint presentation given by the graduate student will have a title slide and an abstract slide with full abstract at the beginning of the presentation (not used in presentation), and a slide with fully cited references at the end of the presentation. The Ppt presentation must be given to Dr. Goddard well in advance of the presentation (electronically) so that notes / handouts can be distributed to students in the class.

Attendance: Students who miss class (lecture or laboratory) will lose points toward their final grade. Don't miss class.

Midterm Date: 5 March 2020; last day to drop without penalty is 12 March 2020

FERPA: The Family Educational Rights and Privacy Act (FERPA) prohibits the posting of grades by social security number or in any manner personally identifiable to the individual student. Grades will not be posted by social security number or by name. No grades can be given over the telephone, as positive identification cannot be made by this manner.

Students with Disabilities: Students requesting classroom accommodations or modifications because of a documented disability should contact the professor of this course and the Access Office for Students with Disabilities located in Farber Hall. The Access Office phone numbers are 245-2498 (voice) and 219-1285 (tty), and 375-5871 (Video Phone).

Grading: The final grades will be based on a percentage of your cumulative points relative to the total points possible: Guaranteed grade distribution is as follows (Max. pts = 650 for BIOL 4450; 750 for BIOL 6450):

A = 90-100%	Points available: **BIOL 4450**:		Points available: **BIOL 6450**:	
B = 80-89%	Lecture Exams:	300 pts	Lecture Exams:	300 pts
C = 70-79%	Basic Check-Out:	50	Basic Check-Out:	50
D = 60-69%	Oral Proficiency Exam:	100	Oral Proficiency Exam:	100
F = ≤ 59%	Lab Image Portfolio:	200	Lab Image Portfolio:	200
	Total:	**650 pts**	Research Proposal	25
			Research Presentation:	75 pts
			Total:	**750 pts**

Tentative EXAM SCHEDULE:

Exam 1: Monday, 17 February 2020
Exam 2: Monday, 30 March 2020
Exam 3: Monday, 4 May 2020

Final Exam Period: **Wednesday-May 6; 12:30 – 2:30 p.m.** (Currently there is no final exam scheduled for this course. The final exam period may be used for the last regular lecture exam or graduate student research presentations should any scheduling conflicts arise in the tentative course schedule listed below.

It is expected that both the students and instructor will abide by the University policy on academic integrity found in the Student Code of Conduct:

General Course Objectives [Relevance to University General Education Outcomes listed as ***VSU#***, for Biology undergraduate educational outcomes as ***BIOL #***, and for Biology Masters educational outcomes as ***MS #***]**:**

At the end of the course, each student will be able to:

1. Operate all instruments pertaining to SEM preparation (CPD, Sputter Coater, etc.) [VSU #3, BIOL #1, MS #1]
2. Operate the SEM proficiently and safely in all modes of operation. [VSU #3, BIOL #1, MS #1]
3. Analyze elemental characteristics of various samples [VSU #3, BIOL #1, MS #1]
4. Use image analysis software to make simple measurements of digital images. [VSU #7, BIOL #1, MS #1]
5. Understand what types of samples are amenable to SEM examination under different modes of operation. [VSU #5, BIOL #3, MS #1]
6. Identify the basic types of data that the SEM can produce and how that data can be interpreted and analyzed. [VSU #7, BIOL #1, MS #1]
7. Identify topical content standards that can be addressed with an SEM study. [VSU #5, BIOL #1& 3, MS #1]
8. Additional for Graduate Course 6450:
    - Develop a good scientific question that leads naturally to a good experimental design that is carried through to a written paper in the format of a scientific journal. [MS #2]
9. Present image data in a written portfolio of required images generated throughout the course. [VSU #7, BIOL #1, MS #2]
10. Present an oral PowerPoint presentation to the class of a research proposal to study a biological problem with SEM. [VSU #4&7, BIOL #1, MS #1&2

Tentative Lecture and Laboratory Schedule: Note: Final portfolio requirements will be available in the first week of class but changes may be made to these requirements during the progression of the course.

Lecture:			Laboratory:	
Week	**Date:**	***Topic :***	**Day**	**Exercise**
1.	13 Jan.	Introduction and history of microscopy,	13 Jan.	Introduction to & Safety in the Microscopy Laboratory; Collection, Fixation and preparation of specimens for SEM – Preparation of fixatives and buffers
	15 Jan	Biological Specimen Preparation, Fixation	15 Jan	Dehydration and CPD
2.	20 Jan.	MLK Holiday: No Class	20 Jan.	MLK Holiday: No Class
	22 Jan.	**Biological Preparation Techniques**	22 Jan.	Mounting Specimens; Operation of the Denton Desk V sputter coater.
3.	27 Jan.	Magnification vs. resolution; Specimen-e- beam interactions; Resolution and contrast	27 Jan.	Basic Operation of the SEM (Part 1): Cold vs. Warm start principles, Specimen exchange, turning on the microscope. Safety procedures
	29 Jan.		29 Jan.	" – second half of class.
4.	3 Feb.	Magnification vs. resolution; Specimen-e- beam interactions; Resolution and contrast Electron Guns, Lenses, vacuum systems, SEM Modes of Operation Illumination Systems and Aberrations, Magnification, Resolution, and Depth of Field	3 Feb.	Basic Operation of the SEM (Part 2): Selection of Accelerating Voltage, Spot size, mechanical stage controls. Course and Fine Stage Manipulations; Orientation of your sample for critical imaging.
	5 Feb.		**5 Feb.**	" – second half of class.
5.	**10 Feb.**		**10 Feb.**	Basic Operation of the SEM (Part 3): Optimization of resolution, depth of field, and signal to noise ratios, etc. Building a portfolio File Management on Lab Computer (Lacie Drive)
	12 Feb.	Applications of LM, TEM, and SEM, Illumination sources (photons vs. accelerated electrons), Lens systems	12 Feb.	" – second half of class.
6.	17 Feb.	**Lecture Exam #1**	17 Feb.	Selection of Detectors for different sample composition; BEI imaging; Carbon Coating
	19 Feb.	SEM Imaging Processes SEM Signal Detectors	19 Feb.	" – second half of class. **Portfolio Requirements 1 – 3 due!**
7.	24 Feb.	SEM Contrast Formation and Image Quality Other Contrast Mechanisms	24 Feb.	**Basic Checkout Lab Exams**
	26 Feb.		26 Feb.	**Basic Checkout Lab Exams**
8.	2 Mar.	**Grad Student Research Proposals Due**	2 Mar.	Portfolio prep.
	4 Mar.	High Resolution Imaging and Signal Processing	4 Mar.	" – second half of class.
9.	9 Mar.	Microscopy of Non-Conducting Specimens	9 March	Advanced Operation: Low Voltage SEM & Low Vacuum SEM
	11 Mar.		11 March	" – second half of class. **Portfolio Requirements 4-13 due!**
10.	16 Mar.	**Spring Break. No Classes**	16 March	**Spring Break. No Classes**
	18 Mar.		18 March	
11.	23 Mar.	Low Voltage Microscopy	23 March	Advanced Operation: Energy Dispersive X-ray analysis
	25 Mar.	Variable Pressure SEM and Environmental SEM.	25 March	" – second half of class.

12.	30 Mar.	**Lecture Exam #2**	30 March	Advanced Operation: Energy Dispersive X-ray analysis
	1 April		1 April	" – second half of class.
13.	6 April	High-Resolution Microscopy methods	6 April	Independent Use and Practice
	8 April		8 April	Image Artifacts; Measuring Image data with ImageSys.; Photoshop and maintenance of image data
14.	**13 April**	Final Check-out Exams – possibly no lecture…	**13 April**	**Final Checkout / Oral exams**
	15 April		**15 April**	**Final Checkout / Oral exams**
15.	20 April	Analytical SEM: Qualitative X-ray Analysis with EDS and WDS	20 April	Final Preparation of Portfolio's
	22 April		22 April	**Final Portfolio due on Friday! 5 p.m.**
16.	27 April	Stereomicroscopy. Image Processing	27 April	
	29 April	Graduate PPTs	29 April	Lab Clean-Up (Required!)
17.	**4 May**	**Lecture Exam #3**	**4 May**	
	6 May	**Final Exam Period 2:45 – 4:45 pm** Only if needed		

Check out procedure for use of the JEOL 6480 SEM

1. Student will have a minimum of 8h logged time on the SEM.
2. Student will demonstrate proper sample handling techniques and use of sample preparation instruments (e.g. CPD, sputter coater, carbon coater).
    a. Students are provided with an FAA fixed sample of *Drosophila virilus*. The sample is stored in FAA.
    b. Students should wash and dehydrate their sample.
    c. Students will critical point dry, mount and coat their sample with Au/Pd for checkout.
    d. During checkout students will be asked to acquire a focused image of their sample in the orientations and magnifications that will correlate with Dr. Goddard's reference sample.
3. User will demonstrate proper sign in using the electronic log book.
4. User will demonstrate proper sample exchange including all safety protocols.
5. User will demonstrate how to acquire an image of their sample.
6. User will be able to demonstrate differences in signal acquisition for different materials using variable accelerating voltage.
7. User will be able to demonstrate proper change of final aperture, centering, and use of different apertures for different image characteristics
8. User will be able to properly and safely demonstrate different image characteristics resulting from changing working distance.
9. User will be able to adjust the microscope parameters to acquire a low magnification image with increased depth-of-field.
10. User will be able to optimize microscope parameters to acquire a sharply focused, high-resolution image at high magnification (ca. 50 -100,000 x).
11. User will demonstrate safe stage manipulations (tilt, rotate) at short working distances.
12. User will be able to competently acquire a digital photograph of an optimal image of interest for any working parameters.
13. User will exercise proper clean methods in data removal from the microscope so as to prevent the spread of any computer viruses to the SEM computers.
14. User will demonstrate shutdown procedures to a standby setting and/or a full shutdown of the instrument.

Advanced procedures not included in check-out but requiring further training:

LV SEM (Low vacuum SEM)
BSE signal detection.
EDS element analysis and mapping