BIOL 595S: Microscopy of macromolecules
Offered: Spring semester, weeks 8-15
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Credit: 2
Course Description: The purpose of this course is to give a student a rudimentary introduction to high-resolution electron microscopy of macromolecules. Upon completion of the course, a student should be able to examine negatively stained samples and to record publication-quality images. Though some training in cryo-electron microscopy will be given, students will not be sufficiently proficient in this technology without advanced training.

BIOL 595R is a prerequisite for this course. Consent of instructor is required to enroll. The course will follow a combination lecture/demonstration format. The course will last for 8 weeks and will, for the most part, be scheduled on an individual basis. Lecture and lab times to be arranged. Location to be arranged.

Course Syllabus:
Lecture/Demonstration Topics:

Topic 1 Course introduction

Topic 2 Introduction to the operation of the Philips EM410 and EM420

a) Introduction to the column
b) Introduction to the controls on the EM410
c) Cool down procedure for the anticontaminators (EM 420)
d) Insertion of sample
e) Filament saturation
f) Alignment of the Philips EM410
g) Initial sample examination

Topic 3 Focusing and astigmatism correction

a) Demonstration on the Philips CM200 on the intensified TV camera and on the CCD camera

i) Use of holes in a holey carbon film
ii) Use of carbon grain at high magnification
iii) Use of CCD-generated FFTs for judging the quality of images

(1) Level of defocus
(2) Astigmatism
(3) Drift
(4) Limits of resolution
(5) Introduction to CTF concepts as it relates to microscope operation

b) Focusing and astigmatism correction on the Philips EM410

Topic 4 Carbon support film preparation

a) Formvar and holey formvar film preparation
b) Carbon coating of grids and completion of films
c) Mica carbon film technique

Topic 5 Negative staining of macromolecules

a) Procedure
b) Examination of possible artifacts

Topic 6. Photography and film processing, examination of the results

Topic 7 Introduction to low dose microscopy on the Philips EM420

Topic 8 Operation of the Philips EM420 under low dose conditions

Topic 9 Demonstration of plunge freezing of particulate specimens and sample insertion into the microscope

Topic 10 Demonstration of low dose examination of frozen-hydrated specimens and data recording

a) Examination for proper ice thickness
b) Identifying sample at low and intermediate magnifications
c) Examination of artifacts

Topic 11 Introduction to digital dark room techniques

a) Digitizing films
c) Digital printing of EM films

Evaluation:

1) Demonstrated competence in procedures in Topics 1 through 3 (15% of grade). Individual examinations will be given before spring break.

2) Demonstrated competence in production of carbon and holey carbon films (15% of grade). Individual examinations will be given around the fifth week.

3) Demonstrated competence in performing low dose techniques (15% of grade). Individual examinations will be given around the 8^{th} week.

4) Class project described below. Point 1 of the project will be worth 40% of the grade and point 2 will be worth 15%.

Class Project:

Each student will need to turn in the following at the end of the semester:

1) At least 2 complete focal series on film of carbon holes and carbon grain. Submitting more than 2 series will allow us to better judge your abilities.
   a) One series should be at no more than 50,000X magnification and should have a carbon hole in the image.
   b) One series should be at 100,000X magnification or higher and have a carbon hole that is small enough to be entirely in the image. Focusing and astigmatism correction should preferably be done on the carbon. The hole can be used as an aid in checking

the quality of the image after film processing.
c) Micrographs should be properly stigmated.
d) Each micrograph series should have one image that is 200nm underfocus, one at near Gaussian focus, and one at 200nm overfocus.
e) A small written description should be given of each image indicating your assessment of the quality of the image, paying particular attention to the assessment of the level of focus and the degree of astigmatism in the image.
f) One series should be digitized and printed on glossy, photo-quality inkjet paper.

2) The micrograph and an inkjet print of a negatively stained specimen recorded at 30,000X to 50,000X magnification. A small write-up should also be given of this describing the quality of the image and the quality of the preparation technique.