

Biology 3326G Course Outline (2017-18)

Cell Biology Laboratory

Course Description & Prerequisite Requirements

Biology 3326F/G is a laboratory course which provides hand-on training in current cell biological methods such as animal cell culture, microscopy, cell adhesion, karyotype analysis, PCR, SDS-PAGE, cytochemistry, fluorescence staining of cellular organelles, immunostaining, and bioimaging. An important part of this discovery-based course focuses on application of the mentioned methods to study changes in protein expression and cytoskeleton organization in cells exposed to microenvironmental stress conditions.

Unless you have either the prerequisites for this course or written special permission from your Dean to enroll in it, you may be removed from this course and it will be deleted from your record. This decision may not be appealed. You will receive no adjustment to your fees in the event that you are dropped from a course for failing to have the necessary prerequisites. The prerequisite for this class is Biochemistry 2280A and a minimum mark of 70% in Biology 2383B.

Instructor Information

Dr. Alexander Timoshenko, office: BGS 3032

Office hours: Tuesdays, 10:30 AM to 12:00 (noon) or by appointment.

Tel.: 519-661-2111 ex. 88900, E-mail: atimoshe@uwo.ca.

If you email your instructor, you must use your Western email address and include *Bio3326* in the subject line. Messages from a non-Western account or those that do not include *Bio3326* may be blocked by the university's anti-spam system.

Course Website

Students should check OWL (<http://owl.uwo.ca>) on a regular basis for news and updates. This is the primary method by which information will be disseminated to all students in the class. Students are responsible for checking OWL on a regular basis.

Accessibility

Please contact the course instructor if you require lecture or printed material in an alternate format or if any other arrangements can make this course more accessible to you. You may also wish to contact Services for Students with Disabilities (SSD) at 661-2111 ext. 82147 if you have questions regarding accommodation.

Support Services

Learning-skills counsellors at the Student Development Centre (<http://www.sdc.uwo.ca>) are ready to help you improve your learning skills. They offer presentations on strategies for improving time management, multiple-choice exam preparation, textbook reading, and more. Individual support is offered throughout the Fall/Winter terms in the drop-in Learning Help Centre, and year-round through individual counselling.

Students who are in emotional/mental distress should refer to Mental Health@Western (http://www.health.uwo.ca/mental_health) for a complete list of options about how to obtain help. Additional student-run support services are offered by the USC, <http://westernusc.ca/services>. The website for Registrarial Services is <http://www.registrar.uwo.ca>.

Course Content and Student Learning Outcomes

The work in this course is divided into two modules, outlined below. **The first module** is intended to give students an understanding of and experience with the basic animal cell culture techniques and cell line characterization and identification. **The second module** is based on application of SDS-PAGE and immunofluorescence methods to study protein expression, and remodeling of cytoskeleton systems in cultured cells in response to microenvironmental stress stimuli. The major training objectives are:

Module I. Basics of Animal Cell Culture: • Examining the microscope potential (upright vs inverted; magnification; numerical aperture; resolution; field of view; working distance) • Proper alignment of a bright-field light microscope (Köhler illumination) and a phase contrast microscope • Identification of cells and cellular components on slides and in cell culture flasks • Sterile techniques in cell culture • Harvesting methods for adherent cell lines • Cell counts using hemocytometer • Cell density and confluency • Test for cell viability • Monitoring morphology of animal cells in culture • Animal cell culture contamination • Subculture of a continuous cell line, split ratio • Bioimaging using an inverted microscope • Cell line characterization: growth kinetics • Plotting and analyzing a growth curve, doubling time • Preparation of chromosome spreads from monolayer cultures • Staining procedure for chromosome spreads with Giemsa and DAPI • Karyotype analysis of a cell line • Modal number of chromosomes and heteroploidy • Genetic instability in cell culture • Genomic DNA isolation • PCR assay for identifying cell lines • Agarose gel electrophoresis.

Module II. Cell Stress Biology and Bioimaging: • Preparation of cell lysates using RIPA buffer • Spectrophotometrical determination of protein concentration using Bradford assay • SDS-PAGE • Staining proteins in gels using Coomassie blue • Gel analysis using ImageLab software from Bio-Rad • Fluorescence microscopy • Fluorochromes • Fluorescence microscope operation • Image capture • Direct fluorescence staining of actin microfilaments with phalloidin • Preparation of slides for fluorescence microscopy • Fixation procedure • Monoclonal and polyclonal antibody • Primary and secondary antibody • Indirect immunofluorescence staining • Blocking solution • Observation of microfilament, microtubules and intermediate filament modifications in response to stress stimuli and interpretation of these data • Bioimaging using fluorescence microscope • Nothern Eclipse and ImageJ software.

By the end of the course, successful students will be able to:

- ✓ Demonstrate a working knowledge of animal cell culture techniques and subculture adherent cells.
- ✓ Perform cell line characterization using regular light, inverted and fluorescence microscopy, karyotyping, SDS-PAGE, and PCR assay.
- ✓ Isolate and quantify genomic DNA and proteins in animal cells.
- ✓ Run horizontal and vertical electrophoresis to analyze biological molecules.
- ✓ Prepare chromosome spreads and analyze cell karyotype.
- ✓ Stain the cytoskeleton and cell nucleus using fluorescent probes and antibodies.
- ✓ Operate with inverted and fluorescence microscopes and perform bioimaging of live and stained animal cells.
- ✓ Understand the complexity and nature of the cell cytoskeleton and its remodeling under stress conditions.
- ✓ Analyze data using professional software such as ImageLab and ImageJ.
- ✓ Organize and analyse research projects by formulating hypotheses for each laboratory experiment and providing an interpretation of collected data in laboratory notebooks.
- ✓ Demonstrate competence with reading primary research articles, scientific writing, data assessment, and critical thinking by reporting their experimental results in a form of research paper and poster presentation.
- ✓ Develop good team work habits by working in pairs and discussing the results with lab partners and in the class.
- ✓ Conduct an efficient and independent work with animal/human cell cultures in research laboratories, which practise cell biology and molecular biology methods.

Course Materials

Required materials from the Book Store at Western:

1. Life Sciences Student Lab Notebook (ISBN: 9781930882355, Publisher: Hayden-McNeil)
2. Student laboratory manual “**Biology 3326F/G Cell Biology Laboratory**”, version 2017-18, #M11651

Additional readings (available on reserve in the Taylor Library):

1. Freshney, R.I. (2016) **Culture of animal cells : a manual of basic technique and specialized applications, Seventh Edition**, Willey-Blackwell (Call No. QS525.F885c 2016). This textbook is also available online.
2. Lodish, H., et al. (2016) **Molecular Cell Biology, 8th Edition**, W.H. Freeman and Company, NY (**Chapter 4**, sections 4.1, 4.2; **Chapters: 8**, sections 8.5, 8.6; **Chapters 17, 18**).
3. Alberts, B. et al. (2015) **Molecular Biology of The Cell, 6th Edition**, Garland Science, NY.
4. Knisely, K. (2013) **A Student Handbook for Writing in Biology, 4th Edition**, Sinauer Associates (Call No. QH304.K59 2013). (This textbook is also available in the Book Store at Western).

Lecture notes, lab results, assignments, and grades will be available through OWL.

Tutorial Information

Tutorials will be held on Tuesdays at 9:30 am in BGS-1056. We will discuss the results collected in the teaching lab, statistics, software for data analysis, assignment requirements, and background information for upcoming lab classes.

Weeks	Dates (Tuesdays)	Tutorial topics
Week 1	January 9	Orientation and the first lab overview.
Week 2	January 16	Quantification of adherent animal cells
Week 3	January 23	Culture of animal cells.
Week 4	January 30	Karyotype analysis.
Week 5	February 6	Authentication of animal cell lines.
Week 6	February 13	In class Test #1.
Week 7	February 20	Spring reading week, no classes
Week 8	February 27	Cellular stress responses. Animal cell lysis and protein assay.
Week 9	March 6	SDS-PAGE.
Week 10	March 13	Methods of fluorescence staining in cell biology.
Week 11	March 20	Data analysis, ImageJ.
Week 12	March 27	Poster project.
Week 13	April 3	In-class Test #2.
Week 14	April 10	Student’s questions.

Laboratory Schedule

Laboratory classes (4 h) are held on Wednesdays in B&GS 3077, 1:30-5:30 pm.

Weeks	Dates (Wednesdays)	Laboratory classes
Week 1	January 10	Light microscopy in cell biology
Week 2	January 17	Adherent cells: trypsinization, cell counts, and viability assay
Week 3	January 24	*Subculture adherent cells and bioimaging (a week-long lab)
Week 4	January 31	Cell line characterization by chromosomal staining
Week 5	February 7	Genomic DNA isolation and PCR, ImageLab intro
Week 6	February 14	Agarose gel electrophoresis and gel imaging
Week 7	February 21	Spring reading week, no labs
Week 8	February 28	Quantification of total proteins in animal cell lysates
Week 9	March 7	SDS-PAGE, protein staining and quantification on gels, ImageLab
Week 10	March 14	**Rotation labs: Fluorescence staining of the cytoskeleton and nuclei and bioimaging
Week 11	March 21	
Week 12	March 28	Data analysis and poster project discussion with individual groups
Week 13	April 4	No labs unless more data analysis or bioimaging needed
Week 14	April 11	Poster presentations

IMPORTANT: *Students will be required to monitor their experimental cultures and to capture cell culture images EVERY DAY outside of regular laboratory hours during this week (Thursday January 24 to Tuesday January 30, 2017), including that weekend. **Students will be required to come for ~30 min on the following Thursday or Friday to take pictures of immunostained cells.

Student Responsibilities and General Conduct

All persons working in a laboratory are required to follow the Western University Safety Policy:

1. Laboratory dress code (shirt, long pants, socks and close-toed shoes).
2. Proper lab attire including safety glasses and lab coats.
3. No food or drinks are allowed in the laboratory.
4. Disposable gloves are to be worn only in the laboratory not in the hall.

Time scheduling and equipment:

Throughout this course, you will be required to perform certain tasks at times not scheduled on your timetable. You must be prepared to come in at other times or during the weekend. The equipment you will be using is of research quality and in some instances only one or two items are available. If equipment malfunctions, fails or breaks for any reason, please notify your instructor or TA **as soon as possible** so that steps may be taken immediately to correct the problem. In most cases the equipment can be made operational with little downtime.

Team work:

You will be assigned to work in small groups of usually 2 students per lab station. If you experience difficulty with your arrangement we expect you to talk to the instructor about it.

Evaluation

The overall course grade, out of 100, will be calculated as specified below. Listed next to the respective components are their maximum contributions toward the course grade.

Component	Notes	Value
Writing Centre Appointment	Scheduled by students before the submission of the midterm assignment	1
Class participation	Based on tutorial attendance (1 point) and lab work (5 points)	6
Pre-lab questions	Will be given before some labs (5 min, short answers)	6
Lab notebook	Marked approx. bi-weekly by TAs (4 times in total)	12
Module tests	Two in-class tests (45 min), 20 multiple-choice questions each, February 13 and April 3	20
Cell growth report	Written report due Tuesday, February 6	10
Midterm assignment	Written report due Wednesday, February 28	25
Poster Project	Final presentations in this class, April 11	20

Important Legalities

The university-wide descriptors of the meaning of letter grades outlined as follows were approved by Senate and will be printed on the back of the transcripts:

- A+ 90-100 One could scarcely expect better from a student at this level
- A 80-89 Superior work which is clearly above average
- B 70-79 Good work, meeting all requirements, and eminently satisfactory
- C 60-69 Competent work, meeting requirements
- D 50-59 Fair work, minimally acceptable
- F below 50 Fail

No electronic devices may be in your possession during tests and exams.

It is Faculty of Science policy that a student who chooses to write a test or exam deems themselves fit enough to do so, and the student must accept the mark obtained. Claims of medical, physical, or emotional distress after the fact will not be considered.

Students must write their essays and assignments in their own words. Whenever students take an idea, or a passage from another author, they must acknowledge their debt by using quotation marks where appropriate and by proper referencing such as footnotes or citations. Generally, however, direct quotes are not used in scientific writings. Plagiarism is a major academic offence. Scholastic offences are taken seriously and students are directed to read the appropriate policy, specifically, the definition of what constitutes a Scholastic Offence, at this website:

http://www.uwo.ca/univsec/pdf/academic_policies/appeals/scholastic_discipline_undergrad.pdf.

All required papers may be subject to submission for textual similarity review to the commercial plagiarism detection software under license to the University for the detection of plagiarism. All papers submitted for such checking will be included as source documents in the reference database for the purpose of detecting plagiarism of papers subsequently submitted to the system. Use of the service is subject to the licensing agreement, currently between The University of Western Ontario and Turnitin.com (<http://www.turnitin.com>).

Effective Writing Centre Appointment

Student Development Centre at Western has excellent set of handouts for writing support, which are available at <http://www.sdc.uwo.ca/writing/index.html?handouts> and are strongly advised to use. In addition, as a part of your work on the midterm assignment, you will need to book one-on-one tutoring appointment and visit the Writing Support Centre (Student Development Centre, Room 4130 WSS) with a sample of your midterm paper to have confidential feedback/comments on your writing style and a professional advice how to polish your writing. To book an appointment, call 519-661-3031 or come to the Student Development Centre (4th floor of WSS). During the appointment, do not forget to remind your writing advisor that you are in Biology 3326F/G class to record your visit that contributes 1% toward your final grade in this course. Make sure to book your appointment early, at least two weeks in advance, considering **the assignment deadline (Wednesday, February 28)** for the midterm paper. **Appointments may not be available if you delay in booking for your appointment.**

Class Participation

Class participation includes tutorial attendance (1 point) and lab participation (5 points). Records of tutorial attendance will be taken and you will be responsible to sign up the attendance sheet.

The lab participation will be evaluated by your TAs and the average grade will be submitted to the instructor based on following five grading criteria, each is worth of 1 point:

- 1. Punctuality and readiness** (late to class, no labnotebook, did not read lab notes).
- 2. Lab safety** (lab coat, safety glasses, long pants, socks and close-toed shoes, confine long hairs).
- 3. Performance** (not doing right, what do I do next?, extra time, proper labelling on slides, fail to clean the working station as instructed, accuracy of lab reports).
- 4. Professionalism** (food/drinks in the lab, cell phone, inappropriate behaviour, teamwork).
- 5. Engagement** (proactive contribution, offering ideas, ask appropriate questions).

Pre-lab questions

Six 5 min-quizzes (1 point each) will be given as short-answer questions at the beginning of specific lab classes starting from the second lab to test your readiness to the lab exercises.

Lab Notebook

Complete and accurate record keeping is one of the most important elements in any piece of laboratory work. There are many acceptable forms of record keeping, depending on the nature of the study. In this course, you will be required to keep a bound notebook in the form of a *daily journal*. Your notebook **must** be with you whenever you are in the lab. Here are a few guidelines to keep in mind:

- (a)** Make entries in the notebook **as and when** you do things. Do not transcribe records for the sole purpose of making the book "look neat". It is important to note exactly what you did.
- (b)** The notebook should contain everything that is relevant to your project including literature, methods, protocols, images, etc. Keep notes or copies of your literature searches, relevant papers, ideas for solving problems in your lab notebook.
- (c)** All raw data must be entered in the notebook. There will be some breaks between exercises in the lab; use them to enter all required information in your notebook. Tape in all images you collect.
- (d)** Lab notebooks should be sufficiently complete to allow any laboratory-literate person to duplicate your experiments and analyses. When it comes time to prepare your manuscript and poster presentation, your lab notebook will be your primary source of information.

The lab notebooks will be checked ~bi-weekly. Each time you will submit a copy of your records to TAs for grading. The grades will be averaged and contribute to **12%** of your final grade. Grade zero will be

assigned for each missing submission because we expect that you consistently take care about records in your lab notebook. The following **Lab Notebook Markers** will apply:

1. TOC, pages and entries dated (1 point).
2. Purpose of experiment, hypothesis (1 point).
3. Experimental procedure, protocols (1 point).
4. Raw data, drawings, photos, calculations, etc. (5 points).
5. Interpretation, ideas (2 points).
6. Overall clarity and readability of your records (2 points).

Module tests

Two multiple-choice-question tests (10 points each based on 20 multiple-choice questions format) will be given in class during tutorial time at the end of each lab module. The length of each test is 45 min. Test #1 (Tuesday, February 13) will cover module I (microscopy, animal cell culture, karyotyping, PCR) and Test #2 (Tuesday, April 3) will cover module II (SDS-PAGE, cytoskeleton, fluorescence microscopy, cell staining and bioimaging).

Cell Growth Report and Midterm Assignment

BIO3326 assignments are designed to introduce you to scientific writing and to provide you with proper understanding of formal requirements to research paper manuscripts to be submitted to scientific journals. The assignments will be based on your original experimental results obtained in the module I of the lab course (i.e. growth curve and cell culture, PCR, and karyotype of animal cell lines) and their critical analysis using the literature data. As you will be collecting the raw data in pairs, the assignments are allowed to be prepared by two students working on the same lab station implying that the same grade will be applied to co-authors. We expect that such format will encourage the team work and presentation/discussion of the collected data. Cell Growth Report and Midterm Assignment have different writing formats. **The Cell Growth Report** is a short written assignment, which include the results of the week-long lab (doubling time and cell morphology), their analyses, and must be submitted after that week in tutorial (Tuesday, February 6). **The Midterm Assignment** is organized as a formal manuscript for The Journal of Cell Biology as per the Instructions to Authors of this journal (separate handout) and must be submitted on Wednesday, February 28. Both assignments should be submitted as a hard copy to instructor and as an electronic copy on OWL. Late submissions will be penalized at a rate of 5% per day (including the weekend) until the percentage of the assignment is used up.

Poster Project

BIO3326 posters are group projects of 3-4 students working on the same bench. The posters will be presented by group members using **Prezi** presentation to the class during special poster session on **Wednesday, April 11th**. The poster project will be based on the results of your lab work over the module II including effects of stress on protein expression (SDS-PAGE) and on the cytoskeleton systems of your choice (microfilaments, microtubules, and/or intermediate filaments) in cultured cells. The poster presentations will be your final report in this class. **Please note that the abstract and the PDF file of your poster should be submitted on OWL in advance on Monday, April 9th**. The peer evaluation may change the portion of your poster group mark by 10%. A separate handout provides you with the guidelines and instructions for the preparation and peer evaluation of BIO3326F/G Poster.

Missed Course Components

Attendance in the lab and completion of all lab exercises is mandatory. If you miss a lab class due to a valid reason confirmed by the Dean's office, your average grade will be applied to that class and the case will be dealt with on an individual basis. It is the student's responsibility to make alternative arrangements with their instructor once the accommodation has been approved and the instructor has

been informed. If you do not have the confirmation from Dean's office, there will be 5% deduction from your final mark (i.e. 1 unexcused absence results in a maximum possible final grade of 95%).

Tutorial attendance is mandatory and attendance will be taken. There will be a deduction of 5% from the final tutorial mark for each unexcused absence.

Late submissions of take-home assignment will be penalized at a rate of 5% per day (including the weekend) until the percentage of the assignment is used up.

If you are unable to meet a course requirement due to illness or other serious circumstances, you must provide valid medical or supporting documentation to the Academic Counselling Office of your home faculty as soon as possible.

If you are a Science student, the Academic Counselling Office of the Faculty of Science is located in WSC 140, and can be contacted at scibmsac@uwo.ca. Their website is http://www.uwo.ca/sci/undergrad/academic_counselling/index.html.

A student requiring academic accommodation due to illness must use the Student Medical Certificate (https://studentservices.uwo.ca/secure/medical_document.pdf) when visiting an off-campus medical facility. For further information, please consult the university's medical illness policy at http://www.uwo.ca/univsec/pdf/academic_policies/appeals/accommodation_medical.pdf.

Equal Opportunity and Evaluation Policy

All individuals involved in the offering of Biology 3326F/G were, at one time, students themselves. Accordingly, they thoroughly understand the importance of course grades and the hard work that you will invest into this course. They are there to help you achieve your goals in Biology 3326F/G.

We want you to do well in the course, but we also have to be fair. The university is committed to academic integrity and has high ethical and moral standards. All students will be treated equally and evaluated using the criteria presented in this course outline and their respective weights. The evaluation criteria are based strictly on actual achievement, not on effort or how hard the student tried. Claims of an excellent academic history, of attendance in the course components, or of personal issues (family, relationship, financial, etc) cannot be used to justify a higher grade in the course as they are not criteria for evaluation. There is no extra work available for extra credit or to "make up" another grade.

The requirement for a higher grade in order to, for example, maintain a scholarship, enter a program, or obtain a higher GPA for various reasons, is not a justifiable reason for increasing your grade. If we increased or "bumped" your grade (*i.e.* gave you a grade that you did not legitimately earn), it would be unfair to the other students and also a great disservice to the scholarships and programs who are evaluating all students on the basis of their grades.

Course Feedback

Please do not hesitate to contact your instructor if you have any constructive comments or feedback on any aspect of Biology 3326F/G. We are always trying to improve the course!

This course is supported by the Science Student Donation Fund.

If you are a BSc or BMSc student registered in the Faculty of Science or Schulich School of Medicine and Dentistry, you pay the Science Student Donation Fee. This fee contributes to the Science Student Donation Fund, which is administered by the Science Students' Council (SSC). One or more grants from the Fund have allowed for the purchase of equipment integral to teaching this course. You may opt out of the Fee by the end of September of each academic year by completing paperwork in the Faculty of Science's Academic Counselling Office. For further information on the process of awarding grants from the Fund or how these grants have benefitted undergraduate education in this course, consult the chair of the department or email the Science Students' Council at ssc@uwo.ca.