LMP1006H Cellular Imaging in Pathobiology

January 10

2012

UNIVERSITY of TORONTO
DEPARTMENT OF LABORATORY MEDICINE AND PATHOBIOLOGY

This course explores the powerful intersection of Physics, Biological science, and Imaging technologies. Basic principles of optics such as the nature of light, diffraction, refraction, the nature of lenses, and the design of the light microscope will be covered in this course. We will discuss phase contrast, dark field, interference contrast, and modulation contrast, as well as polarization and fluorescence microscopy. Different types of microscopes and imaging technologies and their use in biological sciences including dissecting, compound, scanning and transmission electron microscopes, positron emission tomography, single photon emission computed tomography, nuclear magnetic resonance imaging, ultrasound, optical imaging, stereology and 3D imaging, optical microscopy, nanoscopy, live cell and whole animal imaging techniques, cytogenetics, X-ray crystallography and imaging in forensic science and their use in diagnostic pathology will be discussed. Some of the lectures will be complemented by laboratory sessions demonstrating these systems. As a result, students will have the opportunity for hands-on experience with state-of-the-art optical, electronic, and digital imaging equipment guided by an experienced staff from the University, hospitals, research facilities, government agencies as well as the industry. This course will focus on the theory, application and implementation of different imaging techniques, and more importantly, on application of biological experimentation relevant to modern biological research or clinical biochemical studies and the common real-life research goal in the industry, hospitals and research laboratories.

Department of Laboratory of Medicine and Pathobiology (LMP), Faculty of Medicine, University of Toronto
Jan 10- Mar 27, 2012

Objective: At the end of this course participants are expected to have acquired knowledge about different types of microscopes and imaging technologies, their functionality and use in biological sciences. The course will provide students with the knowledge and expertise to implement cutting edge microscopic and imaging methods within their own laboratories.

Format: 11 two hours lecture and/or a laboratory session per week and one four hours session.

Course Schedule: January 10 to March 27, 2012, Tuesdays between 10 AM to 12 PM (March 27th →from 9 AM to 1 PM).

Curriculum: Each lecture consists of theory and/or hands on microscopy, imaging instrument, research facility tour, laboratory sessions, and technology or instrument demonstrations.

Prerequisite: No specific courses are required; however, students should have successfully completed advanced courses in molecular biology, cell biology and/or biochemistry. Priority will be given to more senior PhD students.

Evaluation: Four methods of evaluation will be used:

1) **10% Participation** in Lectures and Laboratory Sessions
2) **25% Midterm Test**

The mark will be based on a 40-minute short multiple-choice midterm test (before the course drop-deadline). The exam would be based on the first five lectures.

3) **35% Written Grant Proposal**

The grant proposal will be in IRAP (Industrial Research Assistance Program) Small Project Grant-format. The grant proposal should include at least one imaging technology (max 4000 words). The application will be assessed on the basis of scientific merit and will be reviewed and judged based on: (1) how original the project is, (2) how well the project is planned, (3) how well the proposal budget is developed, and (4) what are the benefits that may result from this project? Course participants are encouraged to seek mentorship from an imaging technology expert for the specific method used in their grant proposal.

4) **30% Oral Presentation**

The mark will be based on oral presentation of the grant application. The presentation will be judged based on: (1) is the message of the presentation concise and well articulated? (2) is the presentation well structured? (3) does the presenter present her own point of view in an appropriate manner? (4) is the response to questions and comments competent, accurate and adequate? (5) is there sufficient evidence presented to support the argument? (6) is there evidence of acceptable critical thinking? (7) is the presentation original or creative in some way? (8) is time keeping managed well?

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Course Schedule

January 10- March 27 2011

Tuesdays 10 AM-12 PM (March 27th → from 9 AM to 1 PM)

Session 1
Jan 10

Dr. Sima Salahshor

Department of Laboratory of Medicine and Pathobiology (LMP), Faculty of Medicine, University of Toronto and Science & Hypothesis Accelerator (ScienceHA) Inc

Title: Introduction to Imaging Technologies

Synopsis: Course content and guidelines for completing the grant application will be discussed.

Dr. William Geddie

University Health Network, Department of Laboratory of Medicine and Pathobiology and Princess Margaret Hospital, Ontario Cancer Institute

Title: Optical microscopy techniques and their application in diagnostic pathology

Synopsis: This 80 minutes lecture will address the ways in which cells and tissues are imaged by either transmitting visible light through them, or reflecting it from their surfaces. Topics to be covered include the basic physics of optical microscopy in its most common form, bright field microscopy, and other ways in which the light microscope can be used to create contrast: polarized light, darkfield, phase, and differential interference contrast microscopy. Each of these topics will be illustrated with examples that illustrate how the development of light microscopy (and concomitant means of creating contrast by staining) revolutionized our concept of the cell, and how light microscopy remains the most essential technique used in diagnostic pathology. Some common problems encountered in microscope set-up, digital imaging, measurement, and preparation of digital photomicrographs for publication will also be presented.

Session 2
Jan 17

Dr. Sergio Grinstein

The Hospital for Sick Children, Department of Biochemistry, U of T

Title: An overview of the fundamentals of fluorescence microscopy

Synopsis: This lecture will cover the basic principles and will describe the equipment necessary to visualize fluorescently labeled specimens (both live and fixed). The topics featured will include the fundamentals of fluorophore absorption/excitation/emission, and microscope and camera optics. The goal is to familiarize students with the theoretical and practical aspects behind fluorescence microscopy, with a focus on cellular imaging.

Paul Paroutis and Michael Woodside

The Hospital for Sick Children, Imaging Facility, McMaster North Annex and TMDT

Title: Basic and advanced live cell imaging: principles and applications

Synopsis: This lecture will focus on four specialized fluorescence microscopy techniques pertaining to live cell imaging. More specifically, techniques such as total internal reflection microscopy (TIR-FM), fluorescence lifetime imaging (FLIM), fluorescence recovery after photobleaching (FRAP) and Forster resonance energy transfer (FRET) will be described, with a

view to establishing quantitative measurements of protein mobility, protein-protein interactions and vesicle fusion.

Session 3
Jan 24

Dr. Jeff Lee
Department of Laboratory Medicine and Pathobiology, University of Toronto

Title: X-ray crystallography: principles and applications
Synopsis: X-ray crystallography has become the most common method to obtain three-dimensional structures of proteins and protein–protein complexes. This lecture will briefly describe the fundamentals of X-ray crystallography and its applications in medical research. Topics to be covered include background to structural biology and the techniques involved in structure determination. In addition, students will learn to critically examine crystal structures deposited in the Protein Data Bank. Finally, the importance of protein structures to drug development and biomedical research will be illustrated with real life examples.

Session 4
Jan 31

Dr. Rita Kandel and Douglas Holmyard
Mount Sinai Hospital, Department of Pathology and Laboratory Medicine and Advanced Bioimaging Centre

Title: Principle of Scanning (SEM) and Transmission Electron Microscopy (TEM)
Synopsis: General principles underlying electron microscope and differences between scanning and transmission microscopes will be discussed.

Session 5
Feb 7

Dr. Mary Ann George and Dr. Mary Shago
Cytogenomics Laboratory, Department of Paediatric Lab Medicine, and Cytogenomics & Genome Resources Facility, The Centre for Applied Genomics, The Hospital for sick Children

Title: Cytogenomic technologies to investigate genomic organization and structural alterations
Synopsis: This lecture will briefly introduce the field of cytogenetics and molecular cytogenetics and then outline the current major technologies that are used to detect both intra and interchromosomal rearrangements. These include methods used for bright field microscopy such as G-banding, C-banding as well as those used for fluorescence microscopy. There will be a focus on Fluorescence in situ Hybridization (FISH) applications including interphase and metaphase FISH, Spectral Karyotyping (SKY) and whole chromosome painting. The most appropriate choice of technology for validation, detection or characterization of specific chromosomal alterations will be discussed. Illustrative cases or projects from research and clinical settings will be presented.

Session 6- Mid Term Exam
Feb 14

Dr. Sima Salahshor
University of Toronto, Faculty of Medicine, Department of Laboratory of Medicine and Pathobiology and ScienceHA, Inc.

Mid-term Exam & Grant Proposal Application Review

Session 7
Feb 21

Dr. Brian Wilson
Department of Medical Biophysics, Ontario Cancer Research, Princess Margaret Hospital

Title: Advanced and Emerging Optical Microscopy Techniques
Synopsis: This lecture will cover some of the newest advances in optical microscopy that are likely to become important in studying normal and pathological cell structure and function. These include advanced confocal and hyperspectral techniques, super-resolution microscopy ('nanoscopy'), Raman microscopy, and the use of targeted molecular and nanoparticle-based optical reporters. The status, potential advantages and current limitations of each technique will be considered.

James Jonkman
Department of Medical Biophysics, Ontario Cancer Research, Princess Margaret Hospital

Title: Advanced Confocal Microscopy Applications
Synopsis: This laboratory session will introduce students to the advanced capabilities of both laser-scanning and spinning-disk confocal microscopes. Optical sectioning of thick specimens, and real-time confocal imaging will be demonstrated on several samples. As well, spectral imaging will be applied to show how separation of overlapping fluorescence spectra can be unmixed.

Session 8
Feb 28

Dr. Bojana Stefanovic
Sunnybrook Health Sciences Centre, Imaging Research, Sunnybrook Research Institute
Title: In vivo imaging of brain hemodynamics

Synopsis: This lecture will present the physical basis of 2 imaging modalities for imaging of cerebral hemodynamics in preclinical models- two photon laser scanning microscopy and functional magnetic resonance imaging. The source of contrast, signal equation, system hardware, and basic tradeoffs of respective modalities will be presented; the experimental design described, and sample images shown.

Fady Hanna, MHSc.
Huron Technologies International, Inc

Title: Advanced Techniques for 3D Image Scanning, Visualization and Analysis
Synopsis: The lecture focused on the advantages of digital slide scanning as well as benefits and drawbacks of traditional confocal fluorescence, and an overview of multi-photon microscopy theory. It also reviews how advanced digital slide scanner improves on the drawbacks of traditional confocal fluorescence by minimizing photo-bleaching through large field-of-view, large strip widths, point-scanning techniques and acquisition of up to 4 fluorophores simultaneously.

Dr. Isabelle Aubert
Sunnybrook Health Sciences Centre, Imaging Research, Sunnybrook Research Institute

Title: It's your data: Make it count, Make it shine. Introduction to unbiased stereology and virtual slices
Synopsis: This lecture will provide an introduction to a set of methods designed to rigorously quantify and present imaging data. Stereology is used to quantify the size, length, volume and number of objects (i.e. cells). Two-dimensional (2D) and three-dimensional (3D) virtual slide acquisition, analysis and presentation will also be discussed.

Session 9
March 6

Dr. Catherine Theodoropoulos
VisualSonics, Inc
STTARR Facility, Toronto Medical Discovery Tower

Title: High-frequency ultrasound and photoacoustic imaging for preclinical research
Synopsis: The lecture will focus on the research applications of high-frequency ultrasound and photoacoustic imaging for preclinical research. Participants will learn and see how these technologies work in a laboratory setting and understand the basics of the workflow and software quantification tools. Specific examples will be presented in the fields of cancer and cardiovascular research.

Session 10
March 13

Dr. Jeff Lee
Department of Laboratory Medicine and Pathobiology, University of Toronto

Title: Practical Protein Crystallography
Synopsis: Structural biology of proteins yield a wealth of molecular information unobtainable through other methods. Students will be shown basic, modern protein purification techniques. In addition, participants will have hands-on training in growing protein crystals, processing X-ray diffraction data and building a protein amino acid sequence into previously determined electron density. This laboratory session focuses on the major techniques used to determine the 3D structure of proteins and is designed for biologists who are new to macromolecular crystallography, but may want to employ it in their own work. No prior experience in structural biology is required.

Session 11
March 20

Proposal and Project Specific Laboratory session
Each student works on her/his own project in a research facility/team of choice.

Session 12- Final Exam: Proposal Presentation from 9:00 AM-1:00 PM
March 27

Dr. Sima Salahshor
Department of Laboratory of Medicine and Pathobiology, Faculty of Medicine, University of Toronto and Science & Hypothesis Accelerator (ScienceHA), Inc.

Final Evaluation: Grant proposal submission and oral presentation of the small grant application.