Department of Physiology
PHGY 560 - Light Microscopy for the Life Sciences
Winter 2022 (3 credits)

Time: Mondays & Wednesdays from 10:35 a.m. – 12:25 p.m.
Place: McIntyre Rm1131

Course Coordinator: Dr. C.M. Brown, claire.brown@mcgill.ca
Course Lecturers: Dr. Joel Ryan

Course Description

Introduction to optics, light microscopy imaging and data analysis for life scientists. The course starts with how light and matter interact and ends with a review of the latest technologies in the field. Lectures will be conducted in the new microscopy teaching lab so topics will be reinforced with hands-on activities. A critical review and presentation of a primary research article by pairs of students will round out the course.

Light Matter Interactions and Image Formation: The light spectrum, reflection and refraction, interference, diffraction, polarization; basic lenses; Abbé theory of image formation.

Optical Light Path Components and Alignment: Upright and inverted microscope transmitted light optical train, image and aperture planes, infinity correction, Köhler illumination and alignment.

Resolution, Airy Disk, Diffraction Patterns, Resolution, Nyquist: Airy discs, resolution, colour, numerical aperture. Nyquist sampling and resolution. Objective lenses and optical aberrations. Diffraction videos. Diffraction pattern in the microscope.

Objective Lenses: Objective lens labelling, depth of field, field curvature, working distance, spherical aberrations, chromatic aberrations, specialized lenses, other distortions.

Contrasting Techniques #1: Brightfield microscopy, colour imaging, phase contrast microscopy and darkfield.

Contrasting Techniques #2, Fluorescent Dyes: Polarization and differential interference contrast (DIC). Brightfield case studies. Introduction to fluorescent dyes, absorption, quantum yield and stability.

Fluorescent dyes & Fluorescent Proteins: Organic dyes, organelle probes, fluorescent proteins. Exercise to find two similar fluorescent organic dyes and compare them (absorbance, QE, stability). Find two similar fluorescent proteins and compare them.

The Fluorescence Microscope & Light Sources: The fluorescence microscope, optical filters and mirrors. Use searchlight application to find information about fluorescent cubes and learn to read spectral properties. Light sources - tungsten, mercury, metal halide, LED.

Cameras: Interline-CCD, sCMOS, colour and EM-CCD cameras, talked about how to calculate the pixel size on the camera based on the optics on the microscope. Difference between physical pixel size and pixel size in the images. Examples in FIJI/ImageJ.

Three Colour Wide Field Imaging: Compare different methods with different light sources, three cubes versus triple cube, monochrome versus colour camera. Hands on activity with searchlight application.

3D Optical Sectioning Microscopy #1: Wide-field deconvolution, confocal laser scanning confocal microscopy (CLSM), PMTs, HyD, spectral detectors, lasers, compare wide-field and CLSM. Multi-photon microscopy.

3D Optical Sectioning Microscopy #2: Spinning disk confocal microscopy (SDCM), Light Sheet Microscopy, tissue clearing. Total Internal Reflection Fluorescence (TIRF) microscopy.

Case Studies and Review:
Discussion and comparison of 3D imaging techniques.

Digital Images and Image Processing: Pixels, voxels, bit-depth, dynamic range, binning, histograms, colour balance, image display, look up tables (LUTs), file formats, image display, brightness, contrast, gamma, background corrections, shading corrections.

Study Break February 28 – March 4, 2022

Digital Images and Image Processing: Image processing filters, segmentation, masks, scale bars, annotations, time stamps, montages. Does an image represent the sample?

Quantitative Image Analysis: Quantitative imaging. Hands-on activity - measure cell intensities using FIJI/ImageJ, take images of actin and DAPI. Save images for making a figure.

Making a Figure for Publication: Make a figure with the images and the cell data collected earlier in the course using images and graphing in prism. How many cells, how many images, statistics, box plots, t-test. Super plots of experimental replicates.

Live Cell Imaging: Illumination overhead and protocols for optimal live cell imaging.

Live Cell Imaging Exploring the Data: Comparison of live cell data from different modalities.

Nanoscopy (Super-resolution): Single molecule localization (PALM, STORM), structured illumination microscopy (SIM), stimulated emission depletion microscopy (STED).

Nanoscopy (Super-resolution): Fluorescent probes for nanoscopy. 3D, multi-colour and live cell nanoscopy. Super-resolution case scenarios and discussion.

Measuring Dynamics: Cell tracking, photo-bleaching, photo-activation, ratiometric imaging.

Measuring Interactions: Co-localization, fluorescence resonant energy transfer (FRET), fluorescence cross correlation, proximity ligation assay (PLA).

April 6, 2022 – April 11, 2022
Student Seminars

Student Evaluation:

Class participation will be evaluated based on class attendance and participation in class discussions. Students will have quizzes and assignments to do throughout the course. Quizzes will be based on content from a subset of lectures, but some content may build on earlier lessons. Student groups of two will choose a journal article from those provided by the course coordinator and present a constructive critique of the work as a team seminar. Seminar evaluations will be based on the oral presentation, how questions are handled during and following the presentation. Each student will independently write a term paper about the same article and be assessed individually. The final exam will be open book and will be based on a choice of several case studies followed by research and a report of the experimental design from sample preparation to image acquisition, image analysis and figures that would be presented.

Class Participation	10%

Quizzes and Assignments	40%
Seminar	10%
Term Paper	10%
Final Take Home Assignment	30%
Total	**100%**

SEMINAR PAPER: The written term paper is due on **Monday April 11, 2022** at **11:59pm** and must be submitted as a **"WORD"** document through myCourses. A template for the paper will be provided.

NOTE:

If you miss writing the class test(s), you must submit supporting documentation within 2 weeks of the class test date. If documentation is not provided, you will receive a **zero** on the class test(s). No deferred exam will be offered for missing class tests.

Grading:

The Department of Physiology will **NOT** revise/upgrade marks except on sound academilc grounds. Once computed, the marks in this course will **NOT** be altered/increased arbitrarily. Decimal points will be "rounded off" as follows: if the final aggregate mark is computed to be 79.5%, the mark will be reported as 80% (an A-); a final aggregate mark of 79.4% will be reported as 79% (a B+). These marks are **FINAL and NON-negotiable**.

Optional course materials:

Websites:
https://www.leica-microsystems.com/science-lab/
http://www.microscopyu.com/
http://www.olympusmicro.com/index.html
http://zeiss-campus.magnet.fsu.edu/
http://micro.magnet.fsu.edu/primer/index.html

BOOKS:

Fundamentals of Light Microscopy and Electronic Imaging, 2nd Edition Douglas B. Murphy, Michael W. Davidson, ISBN: 978-0-471-69214-0, 552 pages, October 2012, Wiley-Blackwell

Handbook of Biological Confocal Microscopy Hardcover – Jun 2 2006 by James Pawley (Editor), Hardcover: 988 pages, Publisher: Springer; 3rd ed. 2006 edition (June 2 2006), Language: English, ISBN-10: 038725921X, ISBN-13: 978-0387259215

Articles (Dr. Brown):

1. Mubaid, F., Brown, C.M. (2017) "Less is More: Longer exposure times with low light intensity is less photo-toxic". Microscopy Today, November Issue, 1-8.
2. Deagle, R.C., Wee, T.E., Brown, C.M. (2017) "Reproducibility in light microscopy: Maintenance, standards and SOPs." Int. J. Biochem. Cell Biol., 89: 120-124.
3. Jonkman, J., Brown, C.M. (2015) "Any Way You Slice It—A Comparison of Confocal Microscopy Techniques" J. Biomolecular Tech., 26(2).
4. Jonkman, J., Brown, C.M., Cole, R.W. (2014) "Quantitative Confocal Microscopy: Beyond a Pretty Picture." Methods Cell Biol. 123: 113-134.
5. Lee, J.S., Wee, T.-L., Brown, C.M. (2014) "Calibration of Wide-field Deconvolution Microscopy for Quantitative Fluorescence Imaging." J. Biomolecular Tech. 25(1): 31-40.
6. Rodic, S., Brown, C.M. (2014) "High UV Excitation Intensity Induces Photo-conversion of DAPI During Wide-Field Microscopy." McGill Science Undergraduate Research Journal 9 (1).
7. Broussard, J. A., Lin, Rappaz, B., Webb, D. J., Brown, C.M. (2013) "Fluorescence Resonance Energy Transfer Microscopy as Demonstrated by Measuring the Activation of the Serine/Threonine Kinase Akt" Nat. Proto., 8(2):265-81.
8. Lacoste, J., Young, K. and Brown, C.M., (2013) "Live-cell Migration and Adhesion Turnover Assays", Cell Imaging Techniques, Volume 2, Editor Dr. Douglas Taatjes. Humana Press. Methods Mol. Biol. 931:61-84.
9. Webb, D. J. and Brown, C.M., (2013), "Epi-fluorescence Microscopy", Cell Imaging Techniques, Volume 2, Editor Dr. Douglas Taatjes. Humana Press. Methods Mol. Biol. 931:29-59.
10. Aswani, K., Jinadasa, T., Brown, C.M., (2012) "Fluorescence microscopy Light Sources." Microscopy Today, 20(4), 22-28.
11. Cole, R.W., Jinadasa, T., Brown, C.M., (2011) "Measuring and Interpreting Point Spread Functions to Determine Confocal Microscope Resolution and Ensure Quality Control." Nat. Protoc. 6, 1929-1941.
12. Lacoste, J., Vining, C., Zuo, D., Spurmanis, A., Brown, C.M., (2012) "Optimal Conditions for Live Cell Microscopy and Raster Image Correlation Spectroscopy (RICS)." Chapter in Annual Reviews in Fluorescence 2010, Editor Chris D. Geddes, 269-310.
13. Frigault, M. M., Lacoste, J., Swift, J. L. and Brown, C.M. (2009). "Live-cell microscopy-tips and tools." J. Cell Sci. 122, 753-67.
14. Brown, C.M. (2007). Fluorescence microscopy--avoiding the pitfalls. J Cell Sci. 120:1703-5.
