BIOL5070 - Fall semester 2018

Practical Aspects of Light Microscopy in the Biological Sciences
Instructor: Prof. Ammasi Periasamy
ap3t@virginia.edu
http://www.kcci.virginia.edu/people/profile/ap3t
Course location: PLSB 030, Monday-Wednesday, 12-2pm
Credit: 3
Prerequisites: Instructor Permission (Graduate students and Fourth year undergraduate students)

Brief Syllabus
This is a hands on training course on various light microscopy techniques (theory & laboratory) including Phase contrast, DIC, wide-field, confocal, Multiphoton, resonance energy transfer (FRET), fluorescence lifetime imaging (FLIM), spectral imaging, total internal reflection microscopy (TIRF), Fluorescence recovery after photobleaching (FRAP), light sheet, etc. Biological applications are discussed for each microscopy techniques. The grading is by participation in the class, assignments, presentations, multiple choice questions test, one-on-one discussion & training, assembling and aligning the microscopy, and the final test.
Final Test: the student has to complete a project based on a light microscopic techniques/Image analysis and poster presentation of their project results. The expectation is that the student should be working in a microscopy related project in a lab so that he/she can complete the final project to pass the course.

1. Basics of Microscopy
Light, Geometrical Optics, refractive index, absorption, reflection, refraction, diffraction, Abbe's theory.

History of Microscopy, Lenses, Components, Light sources, Condenser and Field Diaphragm actions, Illuminating Light Paths, Koehler illumination.
Phase Contrast, Polarized Light. Birefringence. Polarization Microscopy, Differential Interference Contrast (Nomarski) Microscopy. How to select and adjust the microscopy systems for various biological investigations.
Discussions and Laboratory training/demo.

3. Detectors used in the microscopy systems
Charge coupled devices (CCD) cameras, front and back illuminated chip, frame and line transfer cameras, photomultiplier tubes (PMTs), alkali and multialkali PMTs, conventional and avalanche photodiodes, quantum efficiency, dark current, photon shot noise, how to select and adjust the detectors for various biological investigations.
Discussions and Laboratory training/demo.

4. Basics of Fluorescence
Jablonski energy level diagram, absorption, emission, quantifying absorption properties, triplet state, quenching, Stokes shift, quenching and non-radiative relaxation, intersystem crossing and phosphorescence, quantum yield, environmental influence on fluorescence properties, lifetime, photobleaching, fluorescence collection efficiency.
Discussions and Laboratory training/demo.

5. Fluorescence Microscopy
Various fluorescence microscopy techniques and its configuration, including Wide-field (arc lamp and laser based), Digital Deconvolution, Confocal Laser Scanning Microscopy, Multiphoton Microscopy, Total Internal reflection fluorescence (TIRF) microscopy, Fluorescence lifetime imaging (FLIM), Fluorescence resonance energy transfer (FRET).
Discussions and Laboratory training/demo.

6. Fluorophores, labeling and targeting the biological components
Types of fluorophores, fluorescent probe conjugation to antibodies, green fluorescent proteins (GFPs), spectral characteristics of fluorophores, selection of fluoropohores, loading and labeling of live cells. Discussions.

7. Digital Imaging and image processing
What's pixel, gray level intensity, bits and bytes, digital resolution (8-bit vs 16 bits), saturation, noises in digital images, commercial software for image manipulation, adobe photoshop, and illustrator. How to use and adjust the image acquisition and processing for various biological investigations. Group Discussions and Laboratory training/demo.

8. Advanced microscopy techniques
Fluorescence correlation spectroscopy (FCS). Fluorescence recovery after photobleaching (FRAP), Second and Third Harmonic Generation (SHG & THG) Microscopy, Bioluminescence Imaging, super resolution microscopy (PALM, STED, SIM), Raman and CARS microscopy. How to select and work with different microscopy systems for various biological investigations.
Discussions.

9. Case study
Lectures given by various investigators using the microscopy techniques for their biological or engineering applications.

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References: (The first 4 books are for basics, available in the Biol/Psych library, Other books are for advanced microscopy techniques)

1. Murphy, D.B. and Davidson, M.W. Fundamentals of Light Microscopy and Electronic Imaging, 2nd Edition. John Wiley & Sons, NY, 2013.
2. Foster, B. Optimizing Light Microscopy for Biological and Clinical Laboratories, Kendall/Hunt Publishing Company, American Society for Clinical Laboratory Science, 1997.
3. Shinya Inoue and K. Spring, Video Microscopy. 2nd Edition. 1998, Plenum Press, NY.
4. Pawley, J.B., *(ed.) Handbook of Biological Confocal Microscopy, 2nd edition*. 1995, New York: Plenum Press.
5. Guy Cox, Optical Imaging Techniques in Cell Biology, CRC Press, 2007.
6. Lakowicz, J.R., Principles of Fluorescence Spectroscopy. 2nd ed. 1999: Plenum Press, New York.
7. Periasamy, A. and Day, R.N. (eds.) Molecular Imaging: FRET Microscopy and Spectroscopy, 2005: New York: Oxford University Press.
8. Periasamy, A. and Clegg, R.M. FLIM Microscopy in Biology and Medicine, CRC Press, 2010.