Biophysics 210: Biological Light Microscopy
Syllabus

Discussion section meets Tuesdays from 1-2:30pm in GH N114
Labs meet Thursday or Friday from 1-4pm GH S252 (NIC)

Week 4: Fluorescence Microscopy

Discussion Section: April 21st

Labs: April 23rd and April 24th

Lectures (watch before discussion section):

- Introduction to Fluorescence Microscopy
- Fluorescent Probes
- Fluorescent Proteins

Reading required for discussion section:

- Shaner, NC., Steinbach, PA., Tsien, RY. 2005. A guide to choosing fluorescent proteins. Nat Methods. 2: 905-9.
- Grimm, JB et al 2015. A general method to improve fluorophores for live-cell and single-molecule microscopy. Nat Methods 12:244-250
- Cranfill, PJ et al 2016. Quantitative assessment of fluorescent proteins. Nat Methods. 13: 557-562

Additional Reading (optional):

- Chroma: Handbook of Optical Filters for Fluorescence Microscopy
- Nikon MicroscopyU: Fluorescence Microscopy
- ZEISS Campus: Introduction to Fluorescence Microscopy
- Spectra viewers: FPbase; Chroma Spectra viewer; Semrock Searchlight; ThermoFisher Fluorescence SpectraViewer
- FPbase: Interactive Chart of FP Properties
- Janelia Fluor Dyes
- Zwier, J.M. et al 2004. Image calibration in fluorescence microscopy. J. Microsc. 216:15–24.
- Waters JC. 2009. Accuracy and precision in quantitative fluorescence microscopy. J Cell Biol. 185(7):1135-48.
- Lavis, LD. 2017. Teaching old dyes new tricks: Biological probes built from fluoresceins and rhodamines. Annual Review of Biochemistry 86:825-843

- Shu X. et al 2009. Mammalian expression of infrared fluorescent proteins engineered from a bacterial phytochrome. Science Vol. 324 no. 5928 pp. 804-807
- Yu D. et al 2014. An improved monomeric infrared fluorescent protein for neuronal and tumour brain imaging. Nat Communications 5:Article 3626.
- Lavis, LD. and Raines, RT. 2008. Bright Ideas for Chemical Biology. ACS Chem. Biol. 3:142-155
- Grimm, JB. and Lavis, LD. 2022. Caveat fluorophore: an insiders' guide to small-molecule fluorescent labels. Nature Methods. 19(2):149-158.
- Sarmento, MJ, and Fernandes, F. 2023. Choosing the Right Fluorescent Probe. Fluorescence Spectroscopy and Microscopy in Biology. Springer Series on Fluroescence, vol 20. Springer, Cham.

Discussion Section Topic: Fluorescent microscopy basics; choosing the right dyes and fluorescent proteins for your microscope and experiment. A laptop may be helpful for this discussion section.

Lab: We will go over the light path of the fluorescent microscope, discuss software control of the microscope, acquire fluorescence images, and measure microscope point spread functions. (GH S252, Nikon Imaging Center)