

Biophysics 210: Biological Light Microscopy Syllabus

Discussion section meets Tuesdays from 1-2:30pm GH N114

Labs meet Thursday or Friday from 1-4pm GH S252 (CALM)

Week 5: Optical Sectioning: Confocal Microscopy, Multi-Photon and TIRF

Goals: Understand the different mechanisms by which confocal microscopy, two-photon microscopy, and TIRF microscopy produce optical sectioning. Understand the trade-offs involved in these techniques and the kinds of samples that each technique is most appropriate for.

Discussion Section: April 28th

Labs: April 30th and May 1st

Lectures (watch before discussion section):

- [Optical Sectioning and Confocal Microscopy](#)
- [Total Internal Reflection Fluorescence Microscopy](#)
- [Two Photon Microscopy](#)

Additional Reading (optional):

- [MicroscopyU: Confocal Basics](#)
- [MicroscopyU: TIRF Microscopy](#)
- [MicroscopyU: Multiphoton microscopy](#)
- Bayguinov, PO et al. (2018) [Modern Laser Scanning Confocal Microscopy](#). Current Protocols in Cytometry 85(1):e39
- Jonkman. J et al. (2020) [Tutorial: guidance for quantitative confocal microscopy](#). Nature Protocols 15, pages1585–1611
- Reilly W.M., Obara C.J. (2021) [Advances in Confocal Microscopy and Selected Applications](#). In: Brzostowski J., Sohn H. (eds) Confocal Microscopy. Methods in Molecular Biology, vol 2304. Humana, New York, NY.
- Poulter NS et al. (2015) [The Physical Basis of Total Internal Reflection Fluorescence \(TIRF\) Microscopy and Its Cellular Applications](#). In: Verveer P. (eds) Advanced Fluorescence Microscopy. Methods in Molecular Biology (Methods and Protocols), vol 1251. Humana Press, New York, NY
- Pallen, S. et al. (2021) [Advances in nonlinear optical microscopy techniques for in vivo and in vitro neuroimaging](#). Biophysical Reviews, 13, 1199-1217
- Oreopoulos, J et al. (2014) [Spinning-disk confocal microscopy: present technology and future trends](#). Methods in Cell Biology 123:153-175

- JM Murray et al. (2007) [Evaluating performance in three-dimensional fluorescence microscopy](#). J. Microsc. 228:390-405
- Darius Koester (2021) [Total Internal Reflection Fluorescence \(TIRF\) fundamentals](#). (webinar)
- Mattheyses, AL. et al. (2010) [Imaging with total internal reflection fluorescence microscopy for the cell biologist](#). J Cell Sci. 123:3621-3628
- Sancataldo, G., Barrera, O., Vetri, V. (2022). [Two-Photon Imaging](#). In: Nechyporuk-Zloy, V. (eds) Principles of Light Microscopy: From Basic to Advanced . Springer, Cham. https://doi.org/10.1007/978-3-031-04477-9_9
- Karel Svoboda (2022) [The past, present and future of calcium imaging to spy on neurons in their native habitat](#). (webinar)
- Lina Streich (2021) [High-resolution deep brain imaging using adaptive optics three-photon microscopy](#) (webinar)

Discussion Section Topic: In the lab you will be introduced to laser-scanning confocal microscopy, spinning disk confocal microscopy, two-photon microscopy and TIRF microscopy. For the discussion section we will talk about the different techniques, important factors that impact the quality of the images, and how to choose between techniques.

Lab: Introduction to Laser-scanning confocal microscopy, spinning disk confocal microscopy, and TIRF (CALM) and walk over to the CVRI to see the Two photon in the LCA core.