

Biophysics 210: Biological Light Microscopy Syllabus

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

Week 7: Measuring Cellular Processes; Fluorescent Biosensors

Goals: Understand methods for measuring dynamical processes in cells, both for tracking motion of biomolecules and for following chemical changes in cells using biosensors.

Discussion Section: May 12th

Labs: May 14th and 15th

Lectures (watch before discussion section):

- [Fluorescent Protein Indicators](#)
- [Measuring Dynamics: Photobleaching and Photoactivation](#)
- [Förster Resonance Energy Transfer \(FRET\) Microscopy](#)
- [Fluorescent dye based indicators development](#)
 - Development of a tag-able dye-based Ca indicator from about min 10 to about min 25
- [Intro to Fluorescence Lifetime Imaging \(FLIM\)](#)

Required Additional Reading:

- Okabe et al (2012) [Intracellular temperature mapping with a fluorescent polymeric thermometer and Fluorescence Lifetime Imaging microscopy](#). Nature Communications
- Pages 1293-1297 (skipping Box 1 on page 1295), page 1302 “time resolution” paragraph of: Inada et al (2019) [Temperature imaging using a cationic linear fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy](#). Nature Protocols.
- Miller E.W. (2016) [Small molecule fluorescent voltage indicators for studying membrane potential](#)
- Hayward R. F. *et al* (2024) [Magnetic control of the brightness of fluorescent proteins](#)

Additional Reading (optional):

- To, TL et al (2015). [Rationally designed fluorogenic protease reporter visualizes spatiotemporal dynamics of apoptosis in vivo](#). Proceedings of the National Academy of Sciences, 112(11), 3338-3343.

- Specht, EA et al (2017) [A Critical and Comparative Review of Fluorescent Tools for Live-Cell Imaging](#). Annual Review of Physiology 79:93-117
- Bajar, BT et al (2016) [A Guide to Fluorescent Protein FRET Pairs](#). Sensors 16:1488
- Lippincott-Schwartz, J et al (2003). [Photobleaching and photoactivation: following protein dynamics in living cells](#). *Nature Cell Biology*, S7-14.
- Deo, C et al (2021) [The HaloTag as a general Scaffold for far-red tunable chemigenetic indicators](#). Nature Chemical Biology
- Deo, C and Lavis, LD (2018) [Synthetic and genetically encoded fluorescent neural activity indicators](#). Current Opinion in Neurobiology
- McKinney, SA et al. (2009). [A bright and photostable photoconvertible fluorescent protein](#). *Nature methods*, 6(2), 131-133.
- Berney, C., & Danuser, G. (2003). [FRET or no FRET: a quantitative comparison](#). *Biophysical journal*, 84(6), 3992-4010.
- Chen, T. W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., ... & Kim, D. S. (2013). [Ultrasensitive fluorescent proteins for imaging neuronal activity](#). *Nature*, 499(7458), 295-300.
- Giakoumakis N.N., Rapsomaniki M.A., Lygerou Z. (2017) [Analysis of Protein Kinetics Using Fluorescence Recovery After Photobleaching \(FRAP\)](#). In: Markaki Y., Harz H. (eds) Light Microscopy. Methods in Molecular Biology, vol 1563. Humana Press, New York, NY
- Wagner, TR and Rothbauer, U (2021) [Nanobodies-Little helpers unravelling intracellular signaling](#). Free Radical Biology and Medicine
- iBiology Video: [Measuring Dynamics: Fluorescent Speckle Microscopy](#)
- Salmon, E. D., & Waterman, C. M. (2011). [How we discovered fluorescent speckle microscopy](#). *Molecular biology of the cell*, 22(21), 3940-3942.
- Danuser, G., & Waterman-Storer, C. M. (2006). [Quantitative fluorescent speckle microscopy of cytoskeleton dynamics](#). *Annu. Rev. Biophys. Biomol. Struct.*, 35, 361-387.
- Hochreiter, B et al. (2015) [Fluorescent Proteins as Genetically Encoded FRET Biosensors in Life Sciences](#). Sensors 15:26281-26314

Discussion Section Topic: We will discuss FRAP / FLIP / photoactivation and other techniques for tracking movement as well as different strategies for biosensor design and their strengths and weaknesses.

Lab: We will explore how different fluorescence biosensors and microscopy techniques can be used to measure dynamic cellular processes by comparing voltage, calcium, molecular interactions, and environmental responses across multiple systems.

