Biophysics 210: Biological Light Microscopy
Kurt Thorn
Syllabus

Discussion section meets Tuesdays from 1-2:30pm in MH2100
Labs meet Thursday or Friday from 2-5pm (location varies)

**Week 8: 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 19th
**Labs:** May 21st and 22nd

**Lectures** (watch before discussion section):

- Measuring Dynamics: Photobleaching and Photoactivation
- Measuring Dynamics: Fluorescent Speckle Microscopy
- Förster Resonance Energy Transfer (FRET) Microscopy
- Fluorescent Protein Indicators

**Required Additional Reading:**

- To, T. L., Piggott, B. J., Makhijani, K., Yu, D., Jan, Y. N., & Shu, X. (2015). Rationally designed fluorogenic protease reporter visualizes spatiotemporal dynamics of apoptosis in vivo. *Proceedings of the National Academy of Sciences*, *112*(11), 3338-3343.

**Additional Reading** (optional):

- Lippincott-Schwartz, J., Altan-Bonnet, N., & Patterson, G. H. (2003). Photobleaching and photoactivation: following protein dynamics in living cells.*Nature Cell Biology*, S7-14.
- McKinney, S. A., Murphy, C. S., Hazelwood, K. L., Davidson, M. W., & Looger, L. L. (2009). A bright and photostable photoconvertible fluorescent protein.*Nature methods*, *6*(2), 131-133.
- 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.
- 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.

**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 demonstrate $Ca^{++}$ imaging using GCaMP and photoactivation and tracking of mEos2.