

## Biophysics 210: Biological Light Microscopy Syllabus

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

Labs meet Thursday from 1-4pm GH N114

### Week 8: Digital Images and Image Analysis

**Goals:** Understand how information is encoded in digital images, what the bit depth of an image means, how images must be transformed for printing or display on a screen, and how RGB and monochrome images differ. Learn about basic image analysis operations. Use Fiji/ImageJ for executing basic image analysis operations.

**Discussion Section:** May 19<sup>th</sup>

**Labs:** May 21<sup>th</sup> and May 22<sup>th</sup>

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

- [Introduction to Digital Images](#)
- [Introduction to Bioimage Analysis](#)

**Reading required for discussion section:**

- Waters, JC (2009) [Accuracy and precision in quantitative fluorescence microscopy](#). J Cell Biol. 185:1135-1148
- Pawley, J (2000) [The 39 steps: A cautionary tale of quantitative 3D fluorescence microscopy](#). BioTechniques 28(5):884-886
- Cromeey, DW (2013) [Digital Images Are Data: And Should Be Treated as Such](#). Methods Mol. Biol. 931:1-27

**Additional Reading** (optional):

*General*

- [Dealing with Imaging Data](#)
- [Image Analysis](#) (more in-depth video about using filters)
- [Metadata in Bioimaging](#) (Part of [Bioimage Analysis Course](#) on iBiology)
- Wait, EC et al (2020) [Hypothesis-driven quantitative fluorescence microscopy- the importance of reverse-thinking in experimental design](#). J Cell Sci. 133:jcs250027
- Jost, APT and Water, JC (2019) [Designing a rigorous microscopy experiment: Validating methods and avoiding bias](#). J Cell Biol 218:1452-1466
- Belthangady, C and Royer, LA (2019) [Applications, promises, and pitfalls of deep learning for fluorescence image reconstruction](#). Nature Methods 16:1215-1225

### *Open-Source Software Tutorials/Resources*

- [The Scientific Community Image Forum \(Image SC\)](#)
  - Forum for over 50 open access software platforms including ImageJ/Fiji, Cell Profiler, and napari. If you have an image analysis question this is the place to search for answers and post questions.
- [Fiji](#)
- [Cell Profiler](#); also see [Cell Profiler tutorials](#) and [Cell Profiler Blog](#)
- [napari](#); also see [napari tutorials](#), [napari-hub](#) for sharing plugins, and [GitHub](#) repository
- [Peter Bankhead: Introduction to Bioimage Analysis](#)- Excellent primer for image analysis
- COBA: Center for Open Bioimage Analysis: [YouTube](#) channel with tutorials for ImageJ and Cell Profiler
- Biolmaging North America: [Training & Education Resources](#), Multiple great resources linked here.
- [I2K meeting](#): From Images to Knowledge; links to all the talks and workshops from this year can be found on the website

### *Papers on Useful Tools*

- Bray, MA. et al (2016) [Cell painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes](#). Nature Protocols 11:1757-1774
- Ljosa V and Carpenter AE (2009) [Introduction to the quantitative analysis of two-dimensional fluorescence microscopy images for cell-based screening](#). PLoS Comput Biol 5(12):e1000603
- Lucas, AM et al (2021) [Open-source deep-learning software for bioimage segmentation](#). Mol Biol Cell 32:823-829
- Bolte, S and Cordelieres, FP (2010) [A guided tour into subcellular colocalization analysis in light microscopy](#). J of Microscopy 224:213-232
- Sommer, C and Gerlich, DW (2013) [Machine learning in cell biology- teaching computers to recognize phenotypes](#). J Cell Sci 126:5529-5539.
- Arganda-Carreras, I and Andrey, P (2017) [Designing Image Analysis Pipelines in Light Microscopy: A Rational Approach](#). In: Markaki Y., Harz H. (eds) Light Microscopy. Methods in Molecular Biology, vol 1563. Humana Press, New York, NY

### *Reporting, Reproducibility, and Ethics*

[Nature Methods Dec 2021](#) was an issue dedicated to imaging reproducibility discussing both how to keep your reporting accurate as well as tools that can be used! We are only highlighting a subset of references here.

- Llopis, PM et al (2021) [Best practices and tools for reporting reproducible fluorescence microscopy methods](#). Nat Methods 18:1463-1476
- Swedlow, JR et al (2021) [A global view of standards for open image data formats and repositories](#). Nat Methods 18:1440-1446
- Boehm, U et al (2021) [QUAREP-LiMi: a community endeavor to advance quality assessment and reproducibility in light microscopy](#). Nat Methods 18:1423-1426
- Moore, et al (2021) [OME-NGFF: a next-generation file format for expanding bioimaging data-access strategies](#). Nat Methods 18:1496-1498

### *Other papers on reproducibility and ethics*

- Lee, JY and Kitaoka M (2018) [A beginner's guide to rigor and reproducibility in fluorescence imaging experiments](#). Mol Bio of the Cell <https://doi.org/10.1091/mbc.E17-05-0276>
- Aaron, J and Chew TL (2021) [A guide to accurate reporting in digital image processing – can anyone reproduce your quantitative analysis?](#) J Cell Sci 134:jcs254151
- Heddleston, JM et al (2021) [A guide to accurate reporting in digital image acquisition – can anyone replicate your microscopy data?](#) J Cell Sci 134:jcs254144
- Laine, RF et al (2021) [Avoiding a replication crisis in deep-learning-based bioimage analysis](#). Nat Methods 18:1136-1144
- North, AJ (2006) [Seeing is believing? A beginners' guide to practical pitfalls in image acquisition](#). J Cell Biol 172:9-18.
- Cromey, DW (2010) [Avoiding Twisted Pixels: Ethical Guidelines for the Appropriate Use and Manipulation of Scientific Digital Images](#). Sci Eng Ethics 16:639-667
- Pearson, H (2007) [The good, the bad and the ugly](#). Nature 447:138-140
- Pearson, H (2005) [Image manipulation: CSI: cell biology](#). Nature 434:952-953

**Discussion Section Topic:** In the discussion section we will cover file formats, image analysis basics, common issues encountered, and how acquisition parameters impact your results.

**Lab:** In the lab we will work through a series of image analysis techniques and problems using the open-source Fiji distribution of ImageJ. Please install Fiji from <http://fiji.sc/Fiji> to your laptop before lab section and bring your laptop if you can. A link to the images for these exercises will be sent out with the lab section write up.