

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 227

Week 9: Super-resolution Microscopy

Goals: Understand the most common super-resolution methods: Structured Illumination (SIM), Localization Microscopy (STORM, PALM, etc.), Airyscan Confocal Microscopy, Expansion Microscopy and Stimulated Emission Depletion Microscopy (STED).

Lecture and Discussion Section: May 26th

Labs: May 27th and 28th GH 227 and GH S252 (start in GH227)

In class overview lecture by Nico Stuurman

Optional Lectures:

- [Super-Resolution: Overview and Stimulated Emission Depletion \(STED\) Microscopy](#)
- [Super-Resolution: Localization Microscopy](#)
- [Super-Resolution: Structured Illumination Microscopy \(SIM\)](#)

Required Reading:

- Lambert, TJ and Waters, JC (2016) [Navigating challenges in the application of superresolution microscopy](#). Journal of Cell Biology jcb.201610011
DOI <https://doi.org/10.1083/jcb.201610011>
- Jacquemet, G et al (2020) [The cell biologist's guide to super-resolution microscopy](#). J Cell Science 133:jcs240713

Additional Reading (optional):

- [Cell Size and Scale](#)
- Valli, J et al (2021) [Seeing beyond the limit: A guide to choosing the right super-resolution microscopy technique](#). J Biol Chem 297:100791
- Liu, S et al (2022) [Super-Resolution Microscopy for Structural Cell Biology](#). Annual Review of Biophysics 51:301-326
- Wang, L et al (2019) [Small-Molecule Fluorescent Probes for Live-Cell Super-Resolution Microscopy](#). J Am Chem Soc 141:2770-2781
- Tillberg, PW and Chen, F (2019) [Expansion Microscopy: Scalable and convenient super-resolution microscopy](#). Ann Rev Cell and Dev Biol 35:683-701
- Shi, X et al (2021) [Label-retention expansion microscopy](#). J Cell Biol 220:e202105067
- Nieves, DJ and Baker, MAB (2021) [Pushing the super-resolution limit: recent improvements in microscopy below the diffraction limit](#). Biochem Soc Trans 49:431-439

- Wegel, E et al. (2016) [Imaging cellular structures in super-resolution with SIM, STED and Localisation Microscopy: A practical comparison](#). Scientific Reports 6:27290 doi:10.1038/srep27290
- Ball, G et al (2015) [SIMcheck: a Toolbox for successful super-resolution structured illumination microscopy](#). Scientific Reports 5:15915, doi:10.1038/srep15915
 - SIMcheck is available as an ImageJ plugin
- Huff, J (2015) [The Airyscan detector from Zeiss: confocal imaging with improved signal-to-noise ratio and super-resolution](#). Nature Methods 12: doi:10.1038/nmeth.f.388
- Fornasiero, EF and Opazo, F (2015) [Super-resolution imaging for cell biologists](#). BioEssays, 37:436-451
- Demmerle, J et al (2017) [Strategic and practical guidelines for successful structured illumination microscopy](#). Nature Protocols 12:988-1010
- von Diezmann, A et al (2017) [Three-dimensional localization of single molecules for super-resolution imaging and single-particle tracking](#). Chem. Rev. 117:7244-7275
- Whelan, DR and Bell, TDM (2015) [Super-resolution single-molecule localization microscopy: Tricks of the trade](#). J. Phys. Chem. Lett. 6:374-382
- Blom, H and Widengren, J (2017) [Stimulated Emission Depletion Microscopy](#). Chem Rev 117:7377-7427
- Vicidomini, G et al (2018) [STED super-resolved microscopy](#). Nature Methods 15:173-182
- Whelan, DR and Bell, TD (2015) [Image artifacts in Single Molecule Localization Microscopy: why optimization of sample preparation protocols matters](#). Sci Rep 5, 7924
- Schermelleh, L et al. (2010) [A guide to super-resolution fluorescence microscopy](#). J Cell Biology 190:165-175
- Nosov, G et al (2020) [The Decade of Super-Resolution Microscopy of the Presynapse](#). Front Synaptic Neurosci, <https://doi.org/10.3389/fnsyn.2020.00032>
- Dempsey, GT et al. (2011) [Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging](#). Nat. Methods 8, 1027–36
- Huang, B., Babcock, H. & Zhuang, X. (2010) [Breaking the diffraction barrier: super-resolution imaging of cells](#). Cell 143, 1047–58
- Halpern, AR, et al (2015) [Point by point: An introductory guide to sample preparation for single-molecule, super-resolution fluorescence microscopy](#). Curr. Protoc. Chem. Biol. 7:103-120. doi: 10.1002/9780470559277.ch140241
- Huang, B., Bates, M and Zhuang, X. (2009) [Super-resolution fluorescence microscopy](#). Annual Review of Biochemistry 78:993-1016.
- Puig, DL et al. (2016) [Tips and tricks for artifact-free PFA-based fixation of the actin cytoskeleton and its regulatory proteins for single molecule localization super-resolution microscopy](#). Protocol Exchange doi:10.1038/protex.2016.042

Microscopy Matters Blog Posts on Super-Resolution Imaging:

- [Overview of techniques at Janelia Farms](#)
- [Probes for Single Molecule Localization Microscopy](#)
- [Structured-Illumination Microscopy: What types of samples are not suitable and why](#)

Discussion Section Topic: We will discuss the mechanisms by which the various super-resolution techniques provide resolution beyond the diffraction limit, the sample requirements for each technique, and their strengths and weaknesses.

Lab: Expansion microscopy demo with Mark Pownall in the teaching lab 2nd floor Genentech Hall