Biophysics 210: Biological Light Microscopy
Discussion Section 6: Optical Sectioning: Deconvolution and Light Sheet
Tuesdays 1-2:30pm
Location: Genentech Hall Rm N114

1. What is the purpose of deconvolution? Name 3 practical issues that can impact your deconvolution results?

2. There are several strategies that can be used for deconvolution. In general terms, what does nearest-neighbor deconvolution do?

3. When moving to 3D deconvolution, Fourier transforms can be used to simplify the convolution to multiplication. What does the addition of a Wiener filter accomplish when dealing with 3D data sets? What changes with the use of iterative algorithms for 3D deconvolution in comparison to the simpler Wiener filter method?

4. What are the advantages to light sheet imaging when compared to spinning disk confocal? What are the advantages when compared to laser-scanning confocal?

5. What determines the optical sectioning when imaging with a light sheet? How is this controlled with the optics of forming the light sheet and what has been done to improve it?

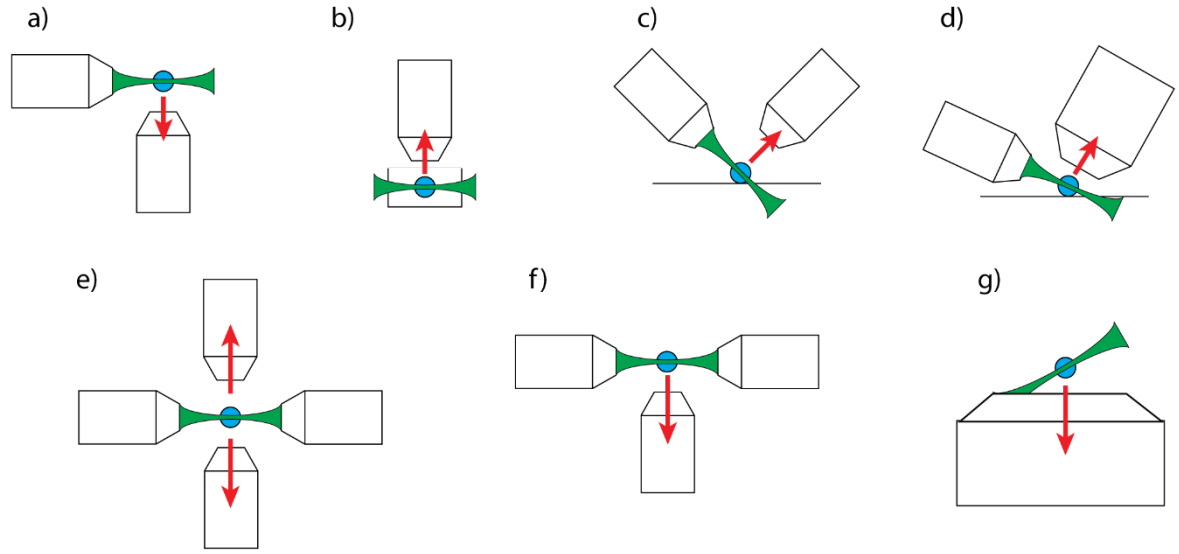
6. What are important practical considerations when deciding on which light sheet configuration to use? What are common artifacts that can appear in light sheet imaging?

7. Match the geometry drawn below with the light sheet method from the list. Identify which configurations utilize a Gaussian light sheet, a Bessel beam light sheet, or a dynamic light sheet (hint: more than one type of light sheet is possible). For each method list which of the following samples can be imaged; large cleared organs, zebrafish, Drosophila, tissue culture cells.

Light Sheet Methods:	Geometry	Sheet Type	Samples
SPIM			
mSPIM			
MuVi-SPIM			
iSPIM/diSPIM			
SOLS			
Ultramicroscope			
Lattice Light Sheet			

Light Sheet Geometry:

Green = Light Sheet
Blue = Sample
Red Arrow = Detection Path



8. How does structured illumination improve resolution beyond the diffraction limit? What role do Moiré patterns and frequency mixing play?

9. How does SIM achieve optical sectioning compared to confocal and light sheet microscopy? What are the limitations in thick or scattering samples?

12. How does iSIM differ from traditional SIM in implementation? What enables its higher temporal resolution? Why is it often described as a hybrid between confocal and SIM?

13. Image reconstruction in SIM:
    Why is computational reconstruction required in SIM?
    What artifacts can arise from reconstruction errors (e.g., pattern mismatch, noise, motion)?

10. Think about the methods in relationship to each other. Fill out the table with the general knowledge of each microscope. How do they compare in each category?
    Note: for light sheet assume a Single Objective Light sheet optimized for imaging cells

	Laser scanning Confocal	Spinning Disk Confocal	Light Sheet (SOLS)	SIM
Resolution				
Optical Sectioning				
Acquisition speed				
Live cell Compatibility				
Post processing?				