Coherent and Incoherent imaging revisited

- White light of low coherence imaging
- Coherent imaging

Coherent Two slits
Slit width = 2 um
Slit separation = 20 um
1 um wavelength

Incoherent Two slits

Convolution
- Point Spread Function is convolved with the object plane intensity distribution to give us the image (Consider 2D PSF and focused case)
Convolution

• Point Spread Function is convolved with the object plane intensity distribution to give us the image (Consider 2D PSF and focused case)

Deconvolution

• Complicated image processing algorithms to guess source from convoluted final image

See homework question about this method
3D or axial PSF

- PSF is a function of distance from the focal plane as well as X and Y in the focal plane

What causes the butterfly wings in the widefield PSF?

Scanning: Laser vs Sample

Sample scanning is done with piezo flexure stages mostly, slow and lower scan range but low optical distortion.

Laser scanning is done by sending the light off-axis, resulting in blurring due to aberrations.
Fluorescence

Fluorescence Resonance Energy Transfer (FRET) Microscopy

Intramolecular Fluorescence Resonance Energy Transfer (FRET)

FRET Detection of in vivo Protein-Protein Interactions

Distance and Energy Transfer Efficiency

Bleaching is a problem

Fluorescence Resonance Energy Transfer (FRET) Microscopy
Confocal Fluorescence microscope

Figure 1. Comparison of widefield (upper row) and laser scanning confocal fluorescence microscopy images (lower row). Note the significant amount of signal in the widefield images from fluorescent structures located outside of the focal plane. (a) and (b) Mouse brain hippocampus thick section treated with primary antibodies to glial fibrillary acidic protein (GFAP; red), neurofilaments H (green), and counterstained with Hoechst 33342 (blue) to highlight nuclei. (c) and (d) Thick section of rat smooth muscle stained with phalloidin conjugated to Alexa Fluor 568 (targeting actin; red), wheat germ agglutinin conjugated to Oregon Green 488 (glycoproteins; green), and counterstained with DRAQ5 (nuclei; blue). (e) and (f) Sunflower pollen grain tetrad autofluorescence.
Figure 6. Lodgepole pine (*Pinus contorta*) pollen grain optical sections. Bulk pollen was mounted in CytoSeal 60 and imaged with a 100x oil immersion objective (no zoom) in 1 micrometer axial steps. Each image in the sequence (1-12) represents the view obtained from steps of 3 micrometers.

Figure 5. Three channel spectral imaging laser scanning microscope confocal scan head with SIM camera laser port. The SIM laser enables simultaneous excitation and imaging of the specimen for photobleaching or photostability experiments. Also illustrated are ports for a visible, ultraviolet, and infrared laser, as well as an arc discharge lamp port for widefield observation.
Further Improvements

**Figure 7.** Three-dimensional volume renders from confocal microscopy optical sections. (a) Autofluorescence in a series of sunflower pollen grain optical sections was combined to produce a realistic view of the exterior surface. (b) Mouse lung tissue thick (16-micrometers) section. (c) Rat brain thick section. These specimens were each labeled with several fluorophores (blue, green, and red fluorescence) and the volume renders were created from a stack of 30-45 optical sections. (d) Autofluorescence in a thin section of fern root.

**Fig. 1.** Schematic diagram of the dual-axes design: $D$, lens diameter; other notation defined in text.

Example: Dual Axis Design
Beyond the Diffraction limit

- Stimulated Emission Depletion of Fluorescence

![Diagram of Confocal, 4Pi, and STED imaging](image)

STED and 4Pi