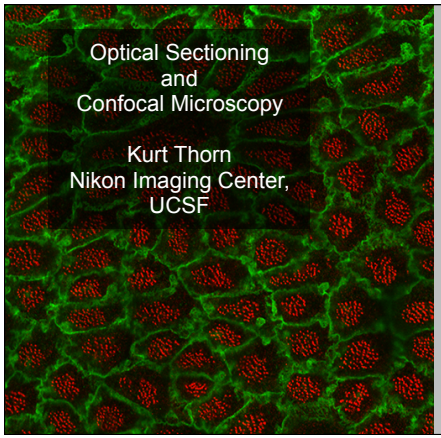
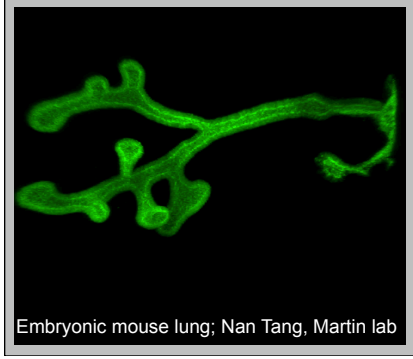
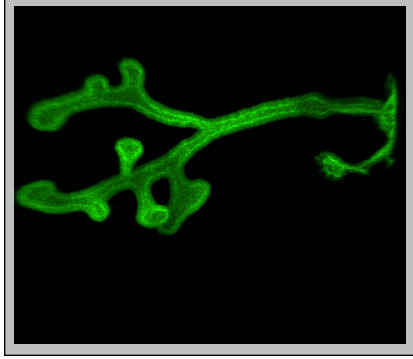


Optical sectioning and 3D reconstruction

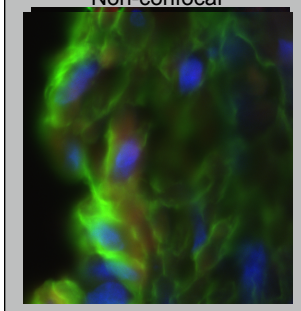


Embryonic mouse lung; Nan Tang, Martin lab

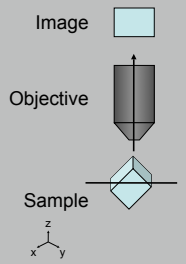
Optical sectioning and 3D reconstruction



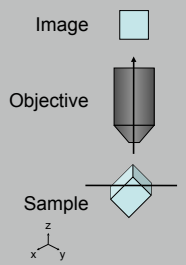
Confocal
Non-confocal



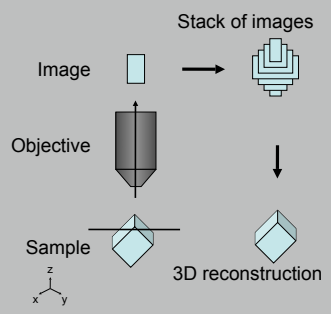
Optical Sectioning and 3D reconstruction



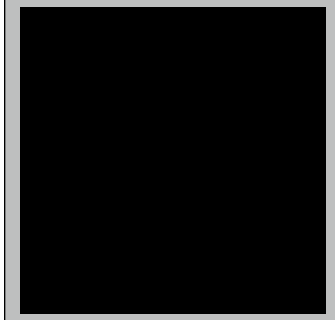
Optical Sectioning and 3D reconstruction



Optical Sectioning and 3D reconstruction

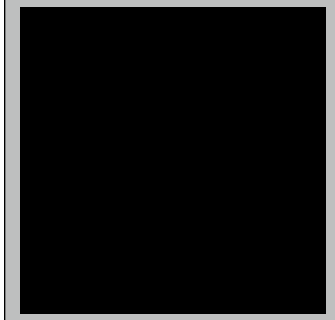


Optical Sectioning and 3D reconstruction



C. elegans with two different sensory neurons expressing GFP, DsRed; 85 Z slices, 250 nm apart

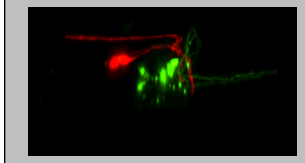
Optical Sectioning and 3D reconstruction



C. elegans with two different sensory neurons expressing GFP, DsRed; 85 Z slices, 250 nm apart

Optical Sectioning and 3D reconstruction

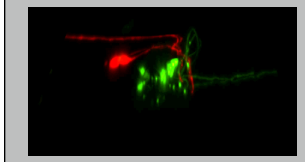
3D reconstruction



C. elegans with two different sensory neurons
expressing GFP, DsRed; 85 Z slices, 250 nm apart

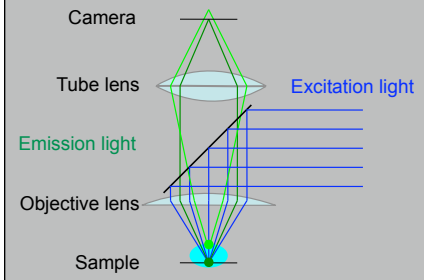
Optical Sectioning and 3D reconstruction

3D reconstruction



C. elegans with two different sensory neurons
expressing GFP, DsRed; 85 Z slices, 250 nm apart

Fluorescence Illumination of a single point



Problem – fluorescence is emitted along
entire illuminated cone, not just at focus

The Confocal Microscope

Use a pinhole to block out-of-focus light

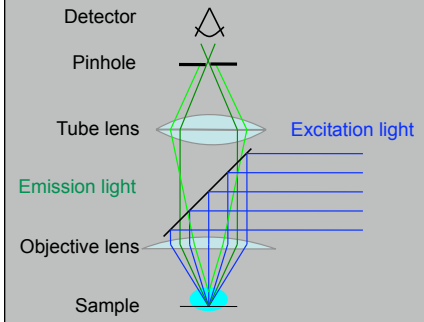
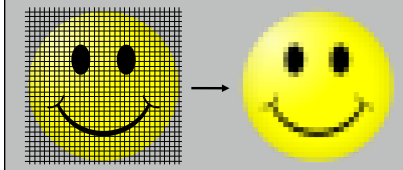


Image by raster scanning

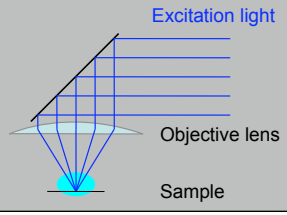
Create image by scanning laser point-by-point over sample and recording intensity at each spot.



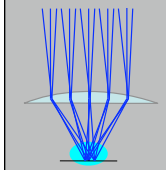
Light sources

Excitation light must be focused to a diffraction limited spot

Enter the laser:
Perfectly collimated and high power



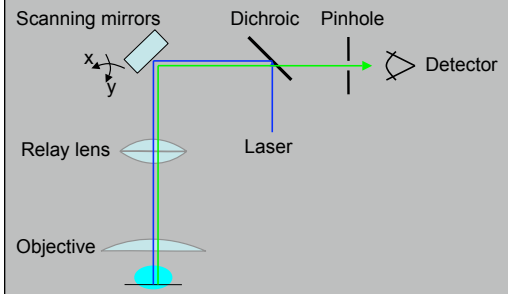
Scanning



Changing entrance angle of illumination moves illumination spot on sample

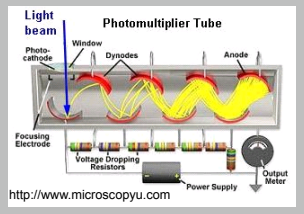
The emission spot moves, so we have to make sure pinhole is coincident with it

Confocal optical path

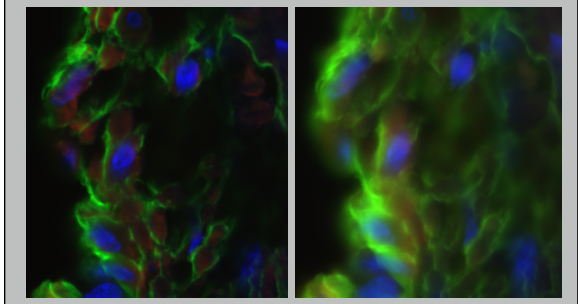


Detectors - photomultiplier tube (PMT)

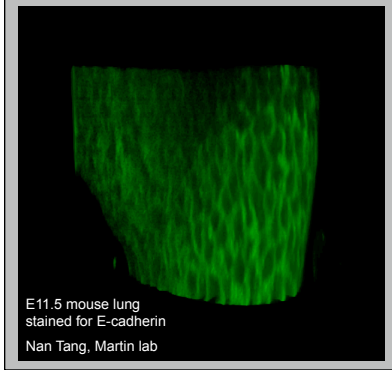
- Must be fast – confocal beam spends only a few μs on each pixel



Confocal Non-confocal

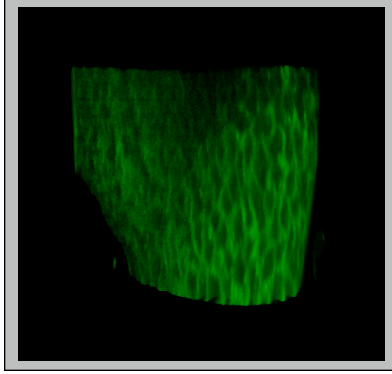


20 μm rat intestine section recorded
with 60x / 1.4NA objective



E11.5 mouse lung
stained for E-cadherin

Nan Tang, Martin lab



Confocal microscope drawbacks

Scans excitation spot point-by-point to build up image

Slow (~1 sec to acquire an image)

Low light efficiency (due to use of PMT as detector)

Solution:
Use multiple pinholes and a camera

A Solution: Spinning Disk Confocal

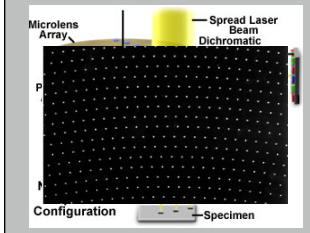
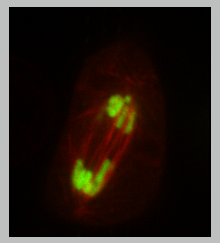


Image with many pinholes at once, so fast

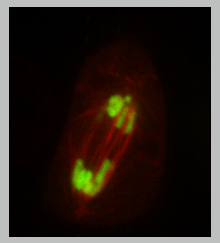
Use CCD as detector, so high light efficiency

Spinning Disk Examples



Drosophila S2 cell expressing
GFP-H2B and mCherry-tubulin
(Nico Stuurman and Ron Vale)

Spinning Disk Examples



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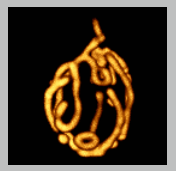
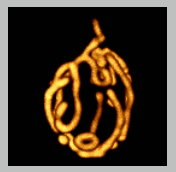
Spinning Disk Examples



S. cerevisiae expressing a
mitochondrially targeted
RFP, Susanne Rafelski,
Marshall lab

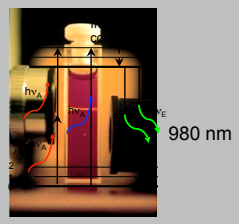
Spinning Disk Examples



S. cerevisiae expressing a
mitochondrially targeted
RFP, Susanne Rafelski,
Marshall lab

Multi-photon excitation

Use two photons to do the work of one



Brad Amos, MRC, Cambridge

Multi-photon excitation does not excite out-of-focus light, so you can get rid of pinhole

When to use confocal?