

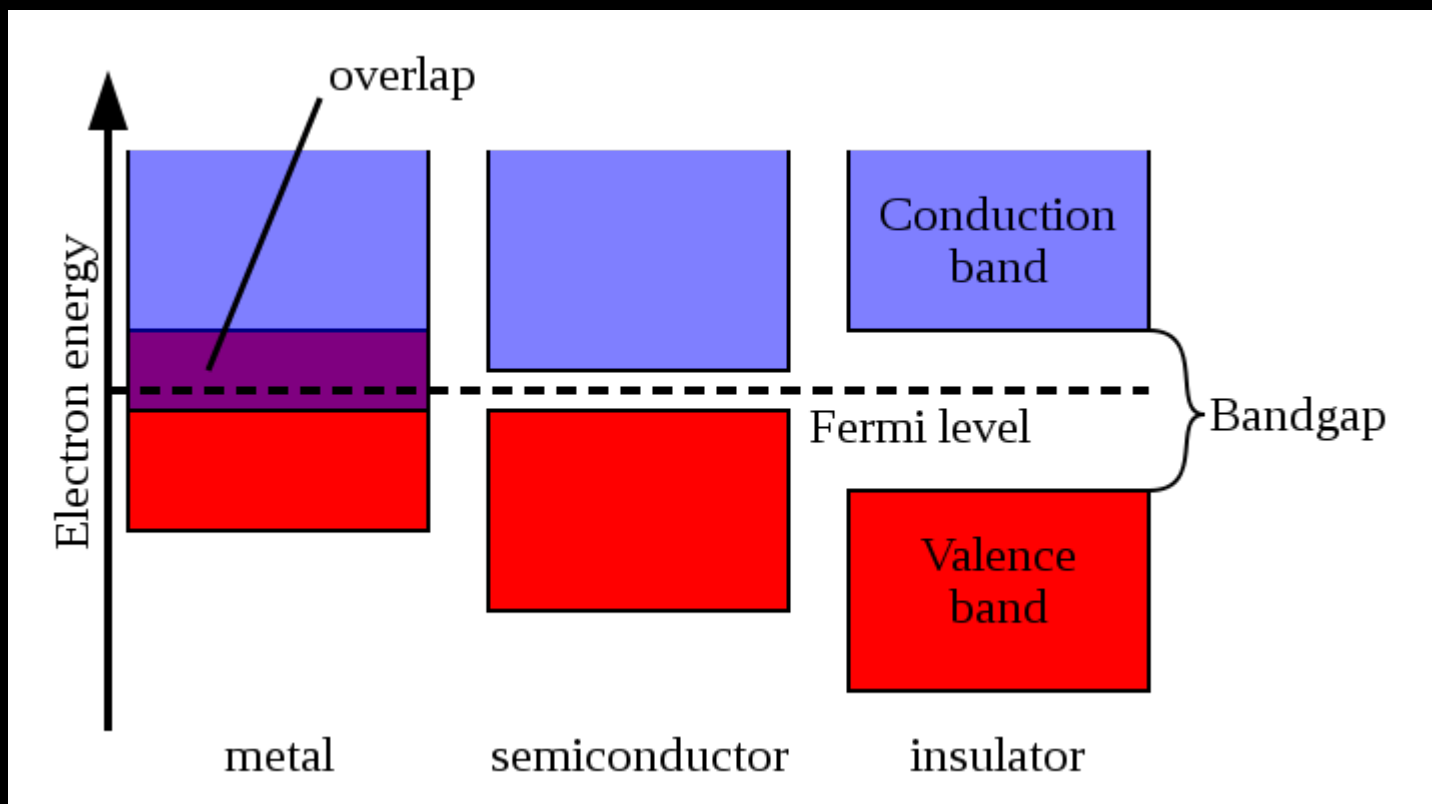
High-Throughput Microscopy

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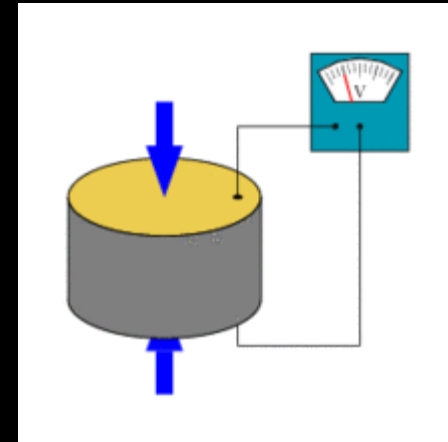
- Metal, Semiconductor, Insulator



- 1D Sensor

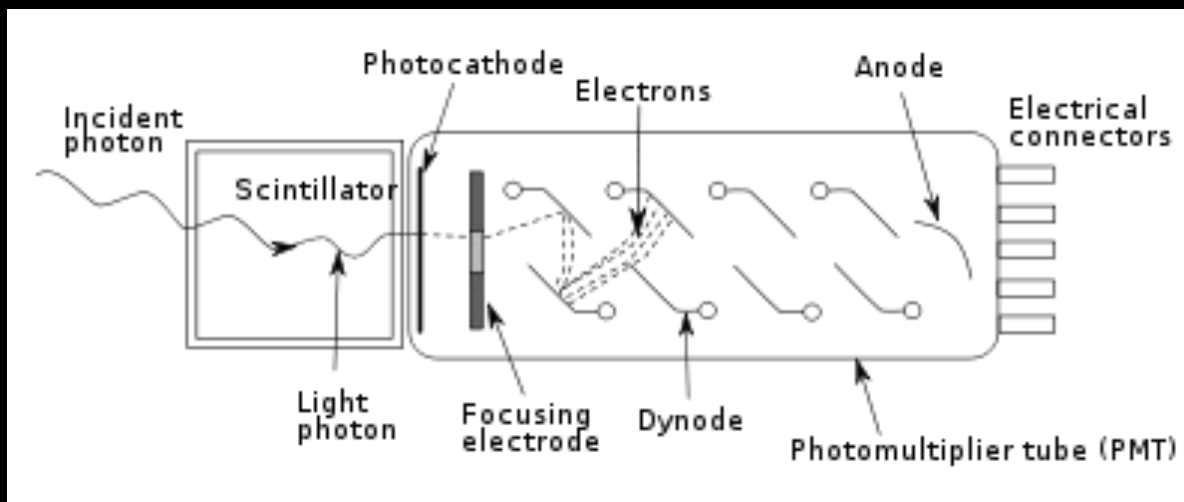
- Piezoelectric

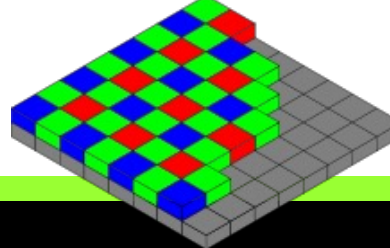
- Measure mechanical motion
 - Generate a voltage when deformed
 - mechanical stress causes the charge separation in the individual atoms of the material
 - Eg. Audio, autofocus objective



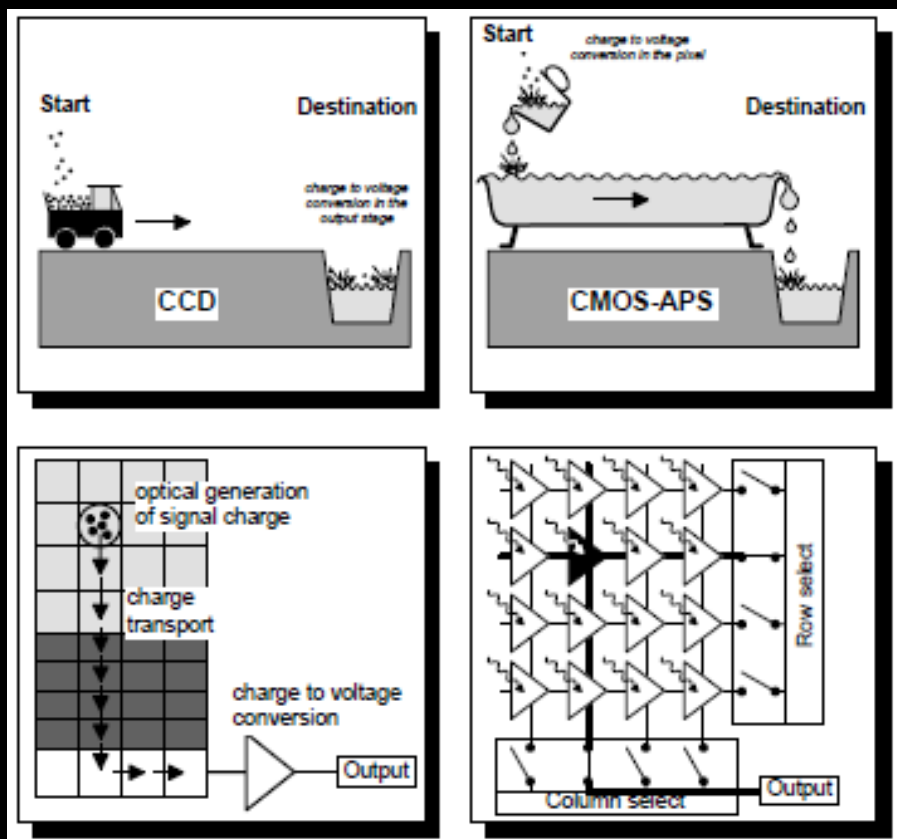
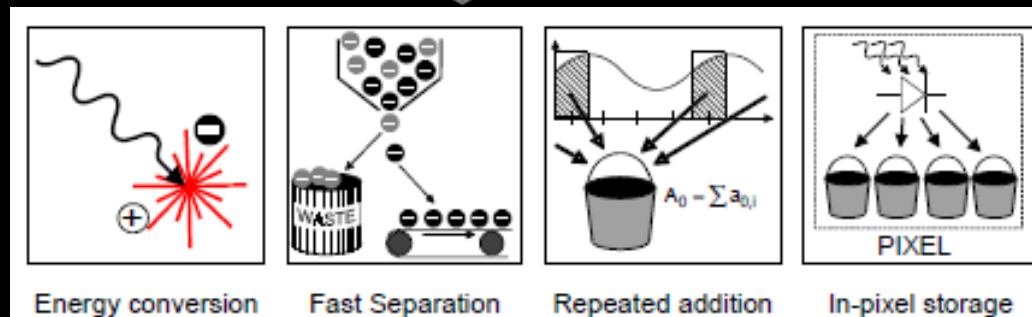
Principle	Strain Sensitivity [V/ μ^*]	Threshold [μ^*]	Span to threshold ratio
Piezoelectric	5.0	0.00001	100,000,000
Piezoresistive	0.0001	0.0001	2,500,000
Inductive	0.001	0.0005	2,000,000
Capacitive	0.005	0.0001	750,000

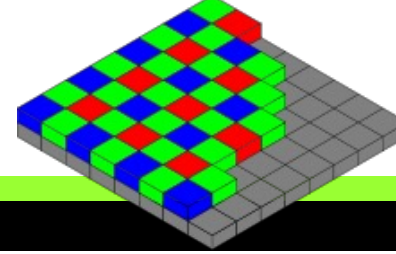
- 1D sensor
 - Photomultiplier
 - Detect photons and amplify the signal (by 100 million times)
 - Sum signal of all detected photons
 - Very sensitive



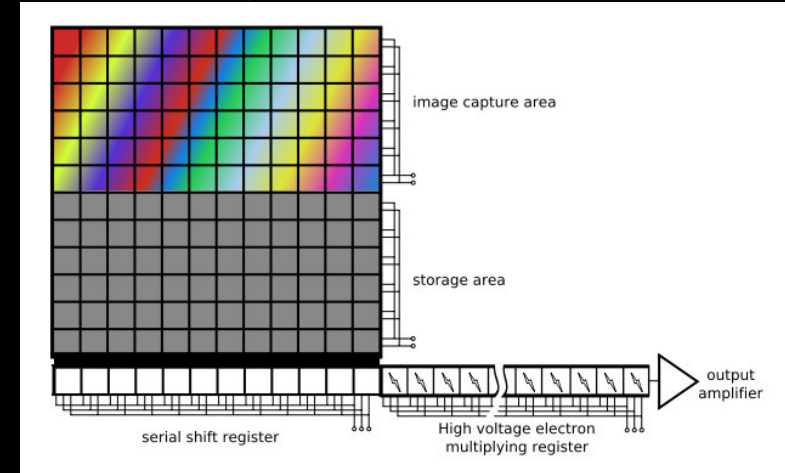


- 2D-Sensor
 - charge-coupled device (CCD)
 - Low noise
 - High power consumption
 - Need move charges
 - Complementary metal–oxide–semiconductor (CMOS)
 - Moderate noise
 - Low power consumption
 - Region Of Interest
 - Read directly from pixel storage

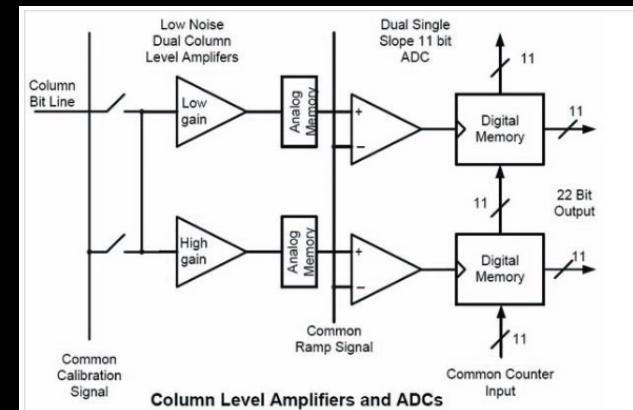




- 2D-Sensor
 - Electron multiplying charge-coupled device (EMCCD)
 - Very low noise
 - High and broad QE
 - Single Photon Sensitive
 - Good dynamic range possible
 - Fast or slow readout
 - Scientific complementary metal–oxide–semiconductor (sCMOS)
 - Extremely low noise
 - Rapid frame rates
 - Wide dynamic range
 - High quantum efficiency (QE)

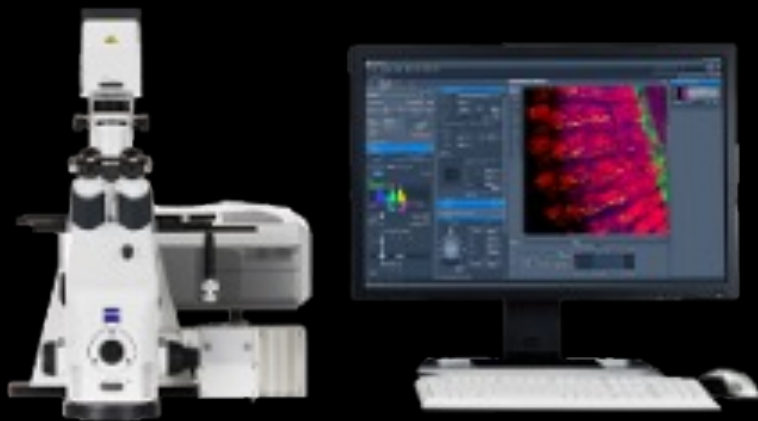


Solid state Electron Multiplying (EM) register to the end of the normal serial register

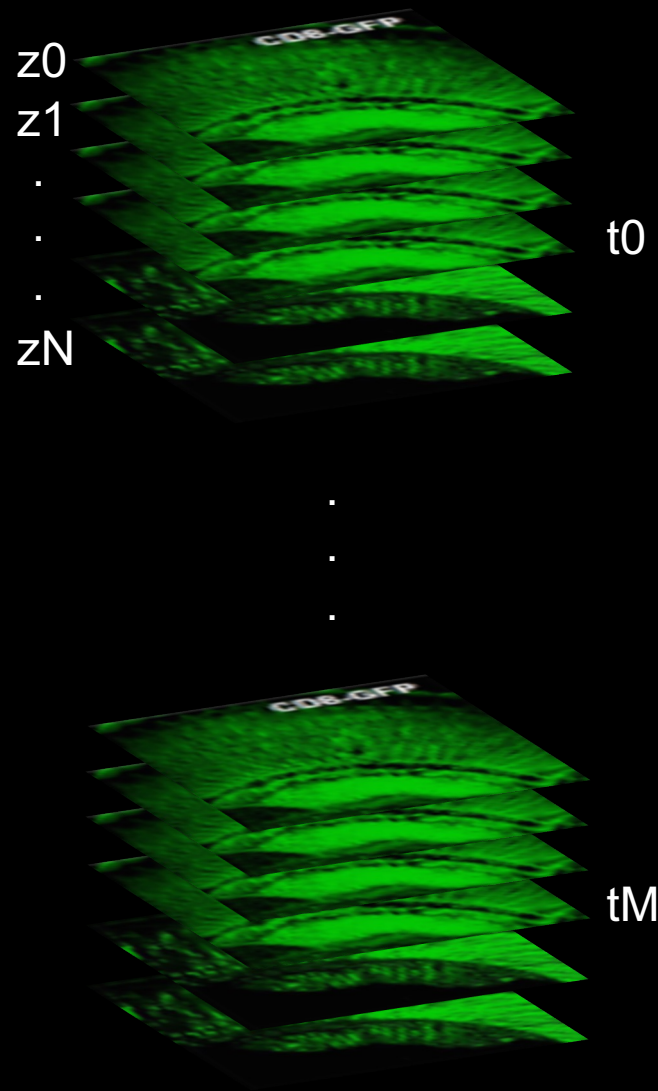


It is a mix between CCD/CMOS

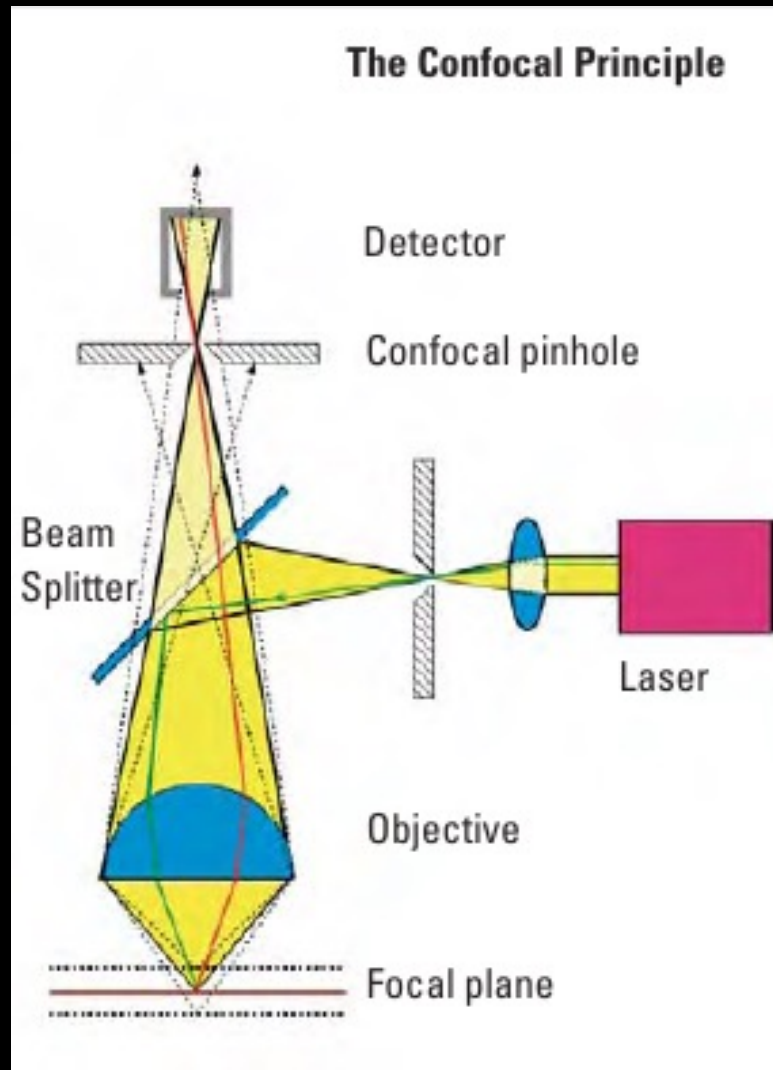
- High-Throughput Microscopy
 - Normally 3D Microscopy
 - Big size of image files
 - Big number of z-slices
(an image stack)
 - Big number of time stacks



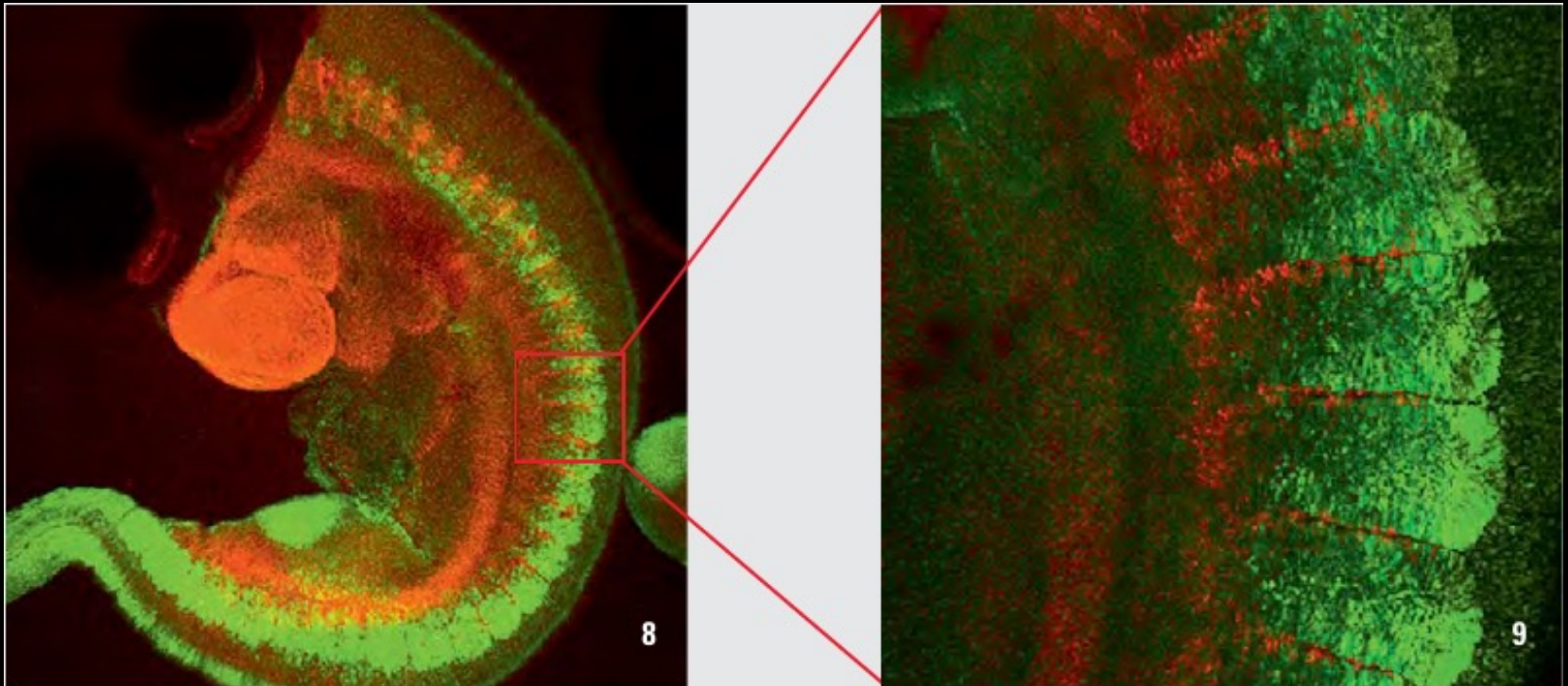
Zeiss LSM 710/780 NLO



- Motorized zoom: 1x – 16x
- Resolution: 128x128 until 2048x2048
- Speed: 6.0 FPS at 128x128 to 0.36 FPS at 2048x2048
- Photo-Multiplier
- Scan point by point



- Image up to 2048x2048
- Z-slices up to 10 nm
- Maximum specimen: 1.5 mm



Zeiss Spinning Disk

- 2048x2048
- Speed: 30 FPS
- CCD/EMCCD
- Specimen Size: 1 mm
- Scan point by point

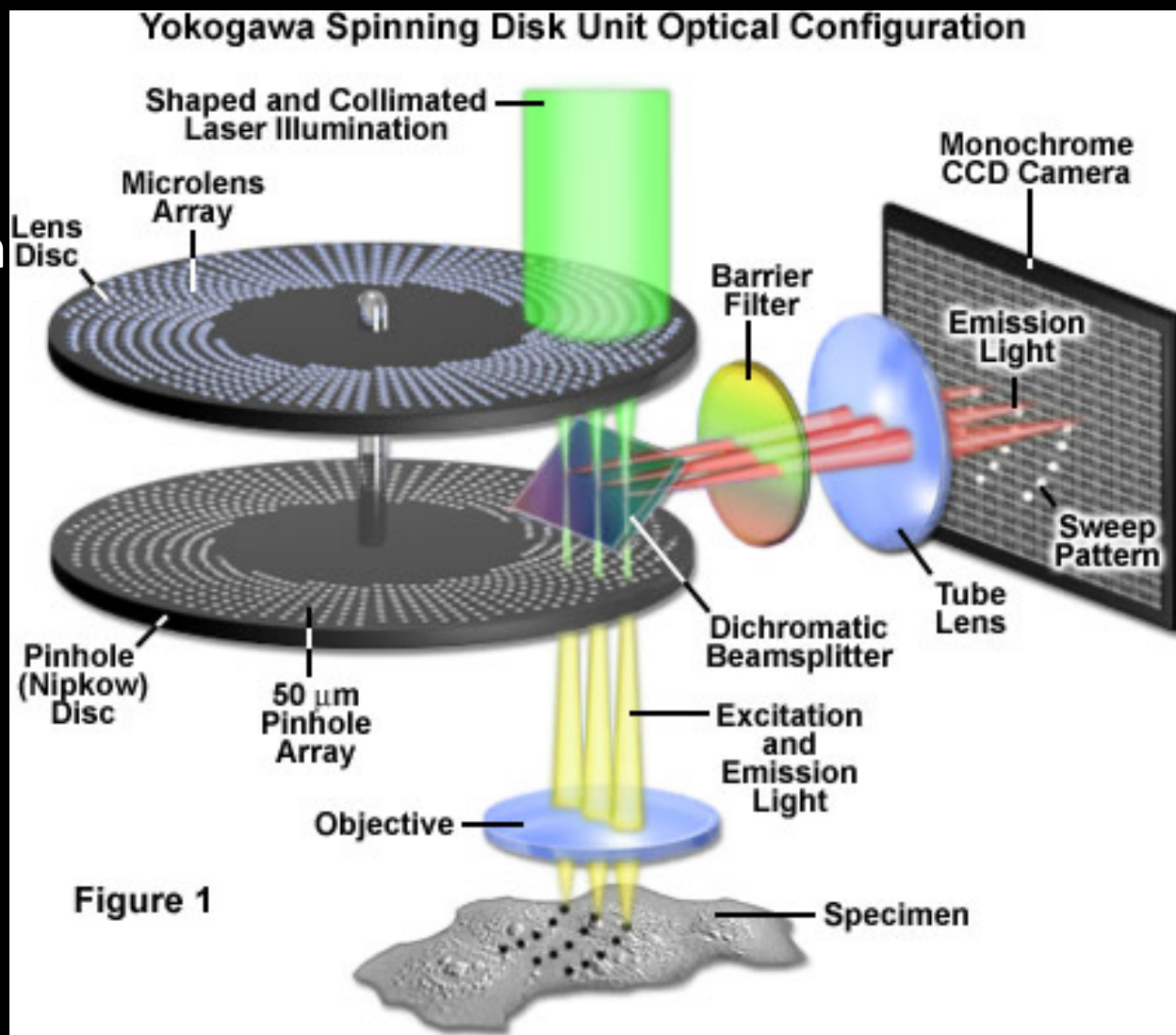
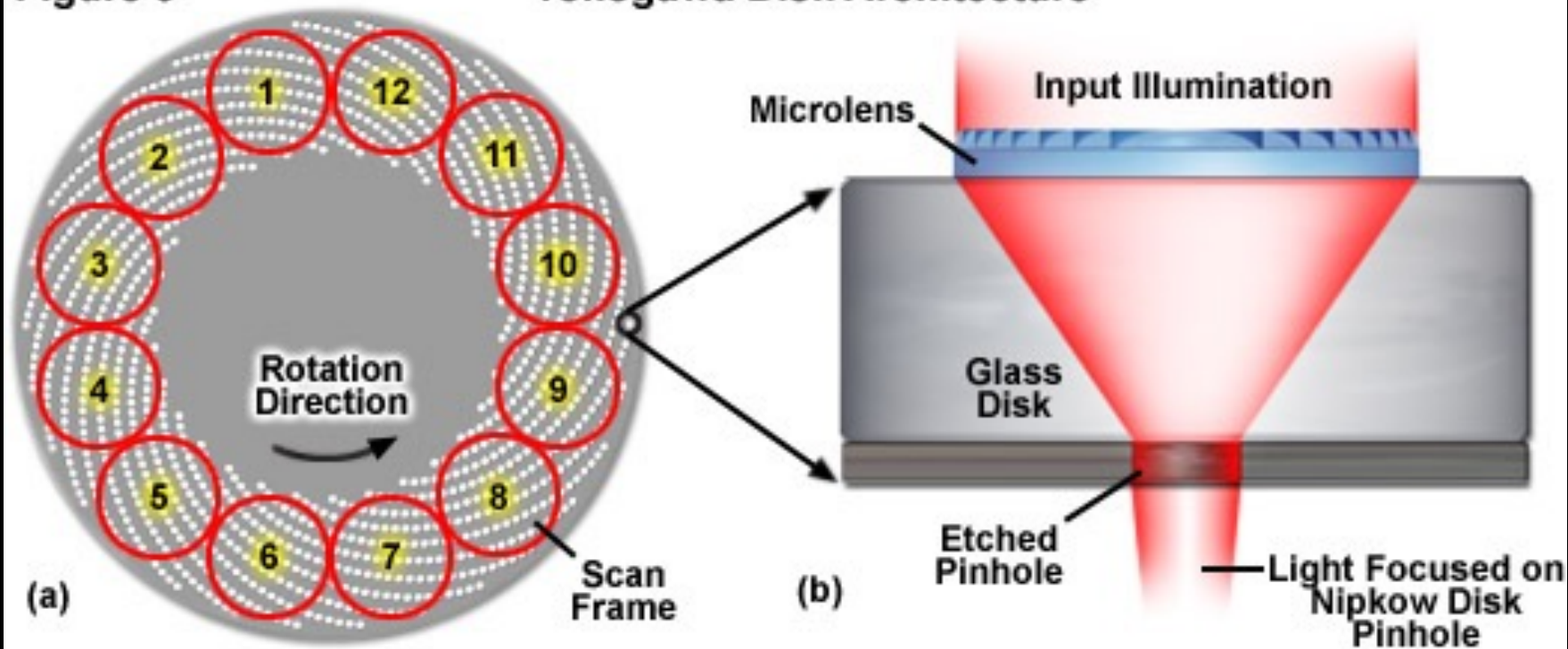
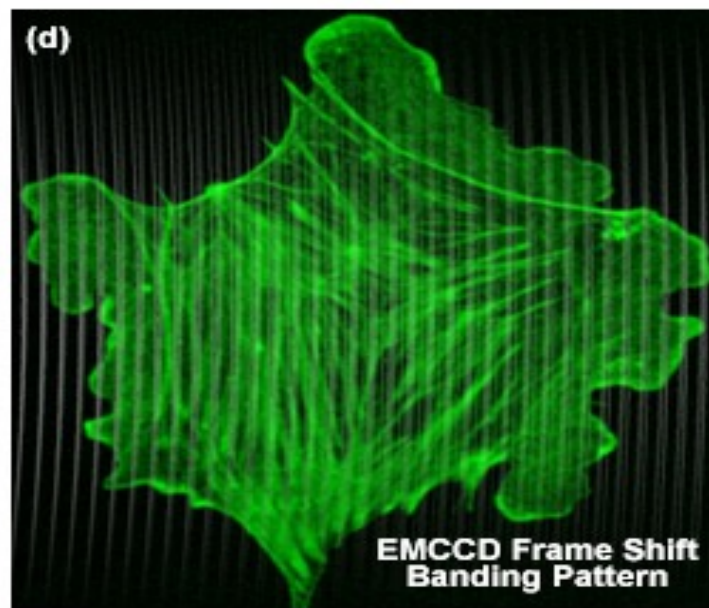
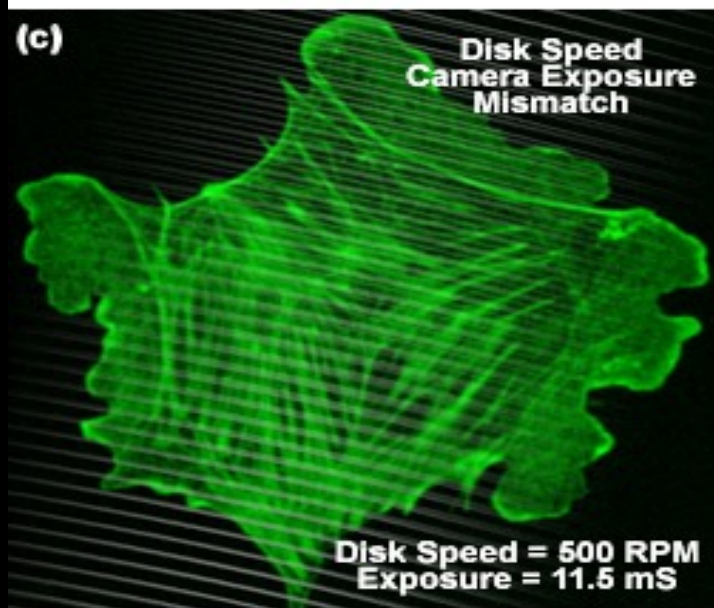
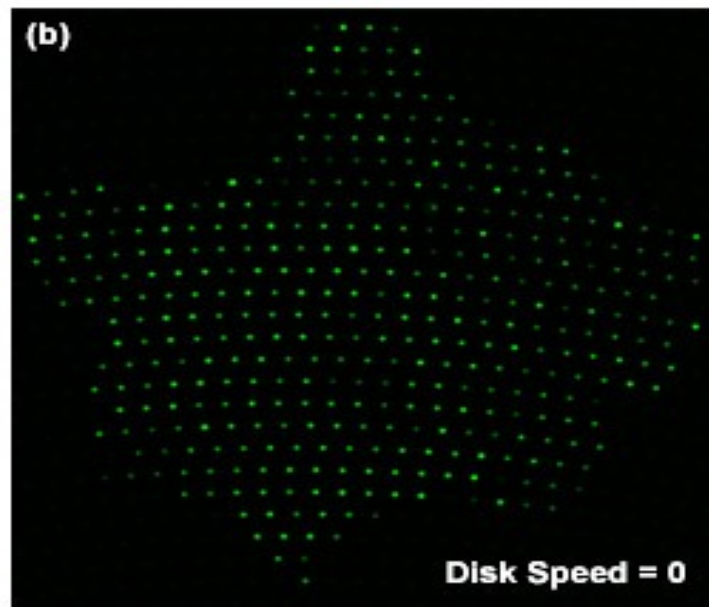
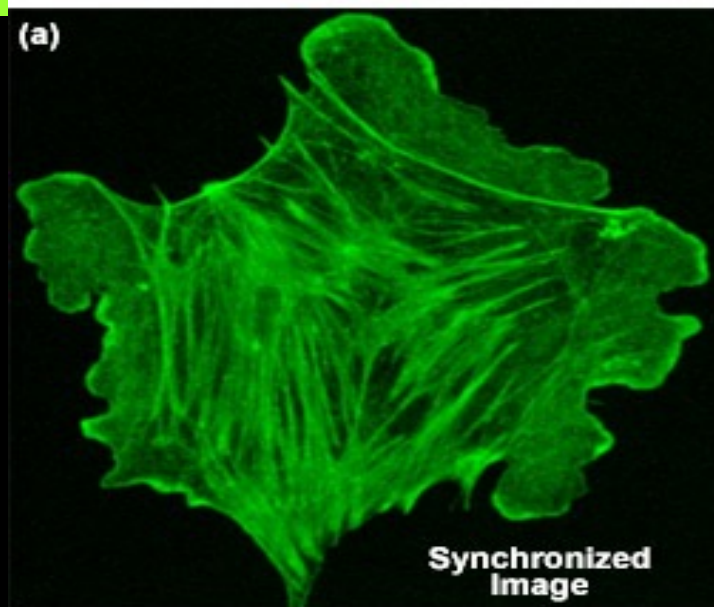


Figure 9

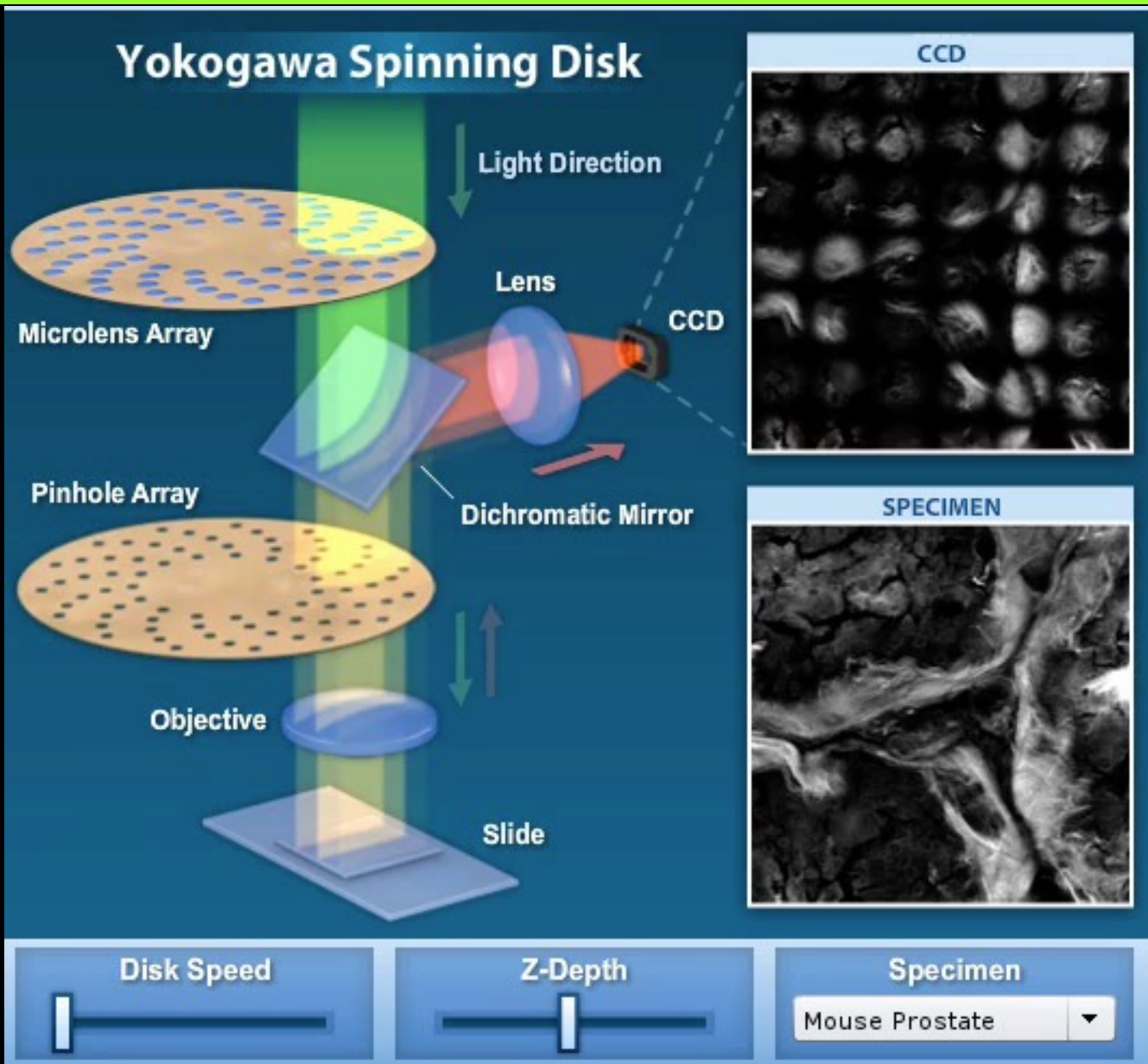
Yokogawa Disk Architecture



Unsynchronized Image Capture in Spinning Disk Microscopy



<http://zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html>



- Light Sheet Microscopy
 - Fluorescence microscopy
 - Optical microscopy (limit of 250 nm)
 - High-resolution microscopy
 - High speed
 - Image thick tissue (> 1 cm)
 - Non-destructive (produce optical sections)
 - Low Photo-toxicity and photo-bleaching
 - Low cost (compared to other microscopes)

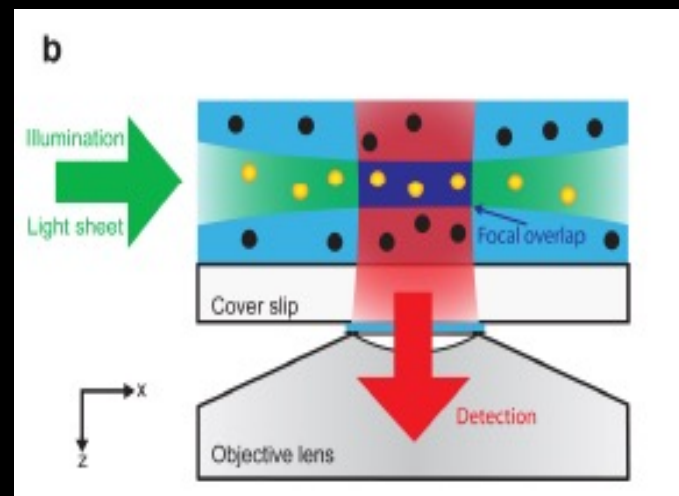
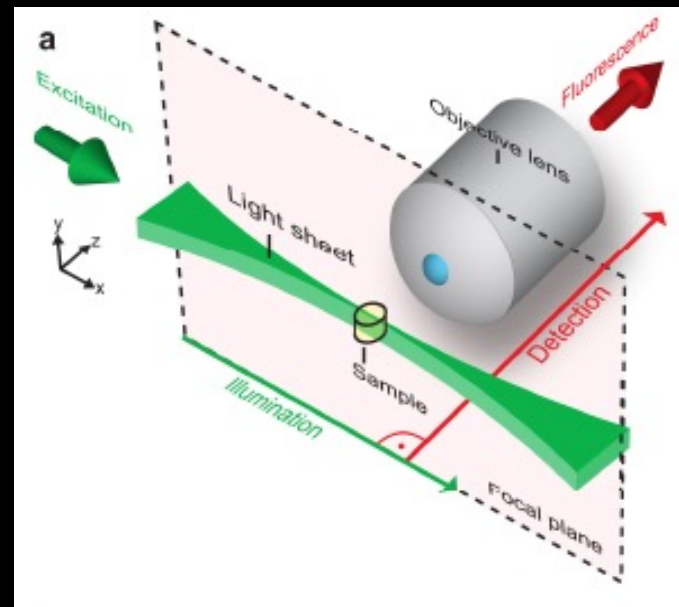


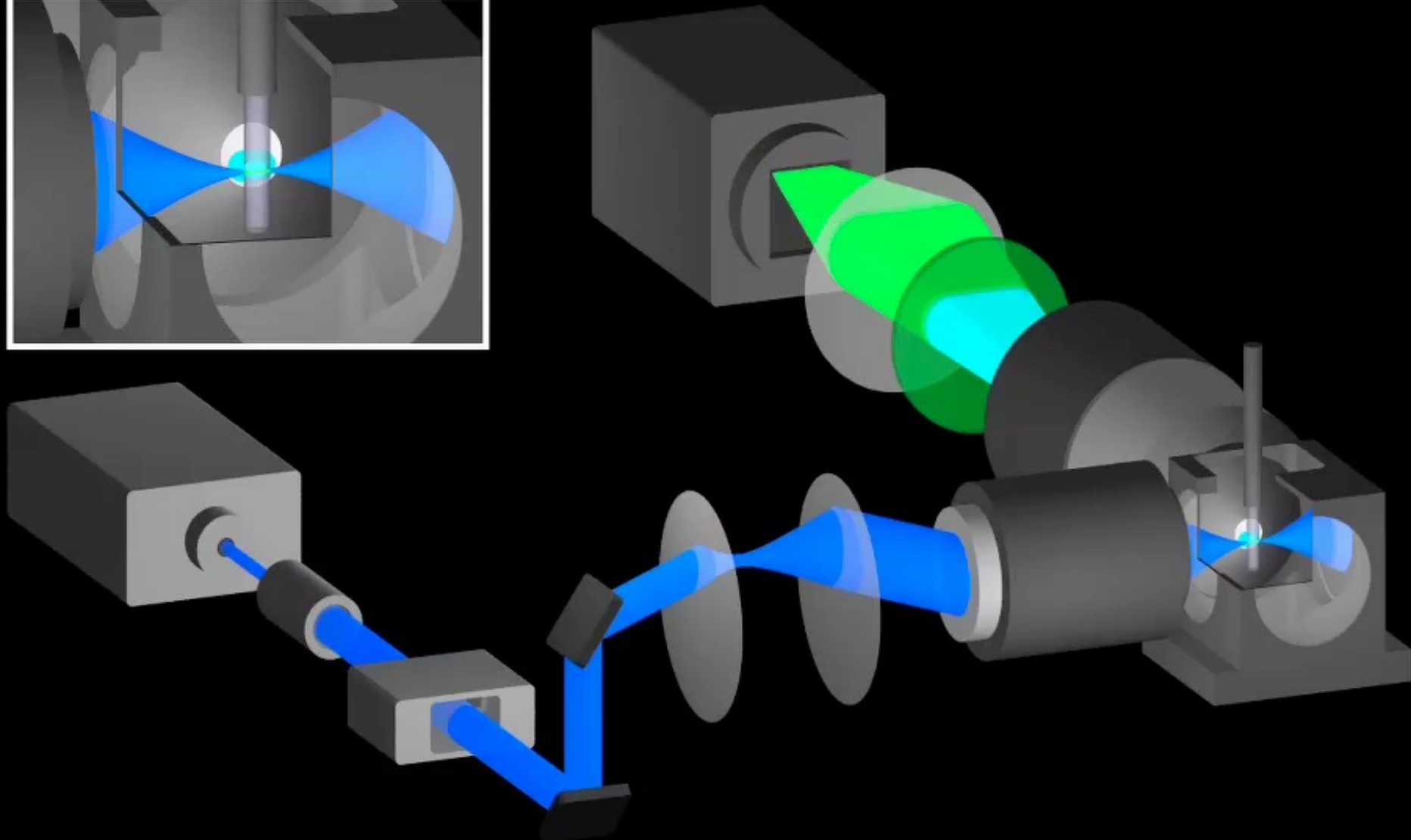
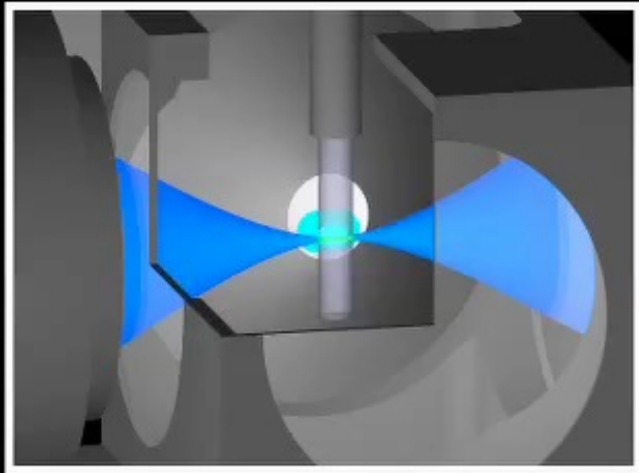
Zeiss Lightsheet Z1

Light Sheet Microscopy

How it Works?

- Use only a light sheet to activate fluophores.
- Excitation light is perpendicular to the detection objective.
- Objective lens is used to collect fluorecense.
- No out-of-focus fluorecense contributes in the measurement.





Keller Lab (Janelia Farm)

Light Sheet Microscopy

Comparison with confocal microscope

Confocal Microscopy

One 3D point per scan - Slow

Big part of illuminated

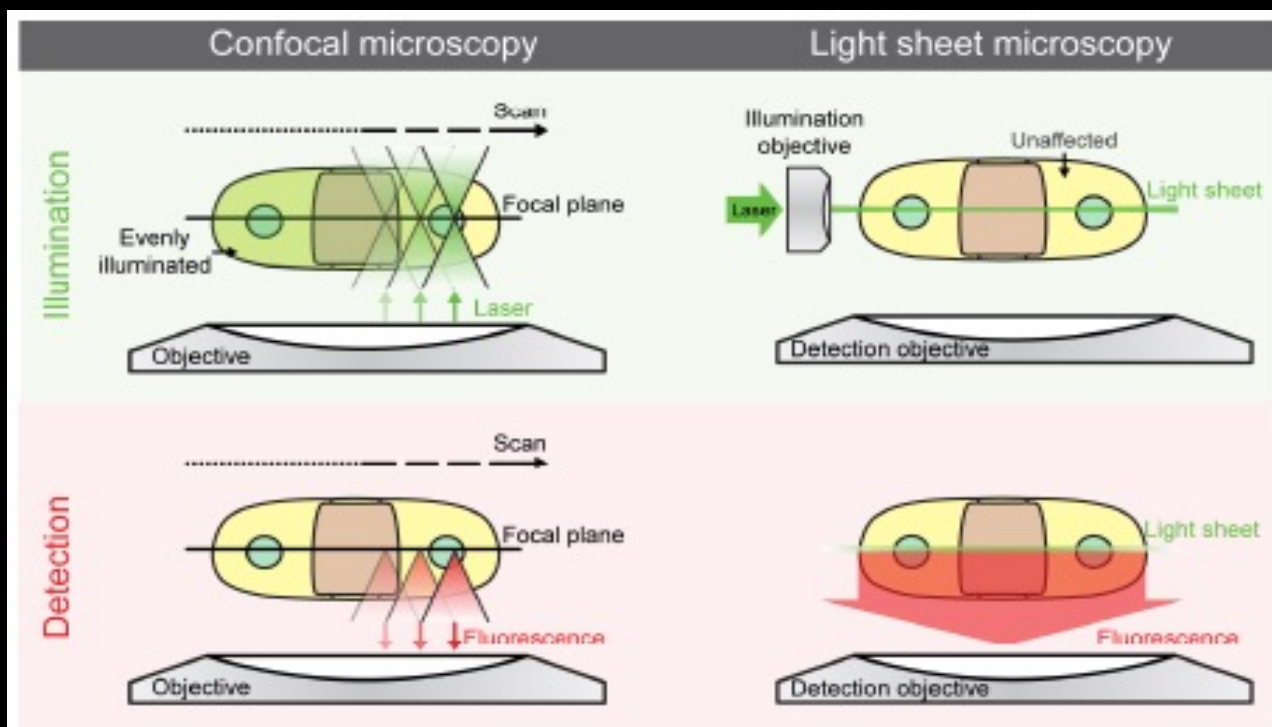
Normal phototoxicity and Photobleaching

Light Sheet Microscopy

Complete focal plane – Fast

Only scanned plane illuminated

Reduced photo-toxicity and photo-bleaching



Light Sheet Microscopy

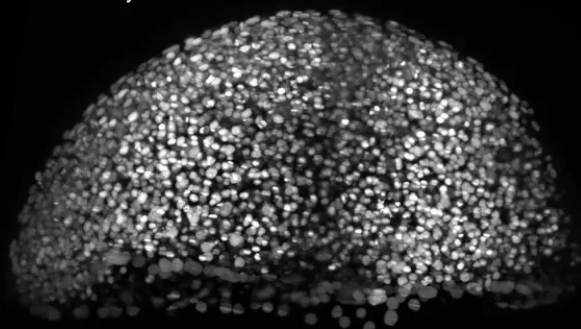
Comparison with other technologies

Name	Signal	Resolution	Fluorescent	Size	Imaging Time	Cost (\$)	Photobleaching	Citation
Magnetic resonance imaging	Magnetic	mm	No, contrast agent	M	hr	Millions	NA	Lauterbur 1973
Computed tomography	Radioactive	<mm	No, contrast agent	cm	min	Millions	NA	Kalender 2006
Confocal	Laser	<micron	Yes	micron	msec	200,000	Yes	Minsky 1961
2-Photon	Laser	<micron	Yes	mm	msec	500,000	Less	Denk et al. 1990
Light sheet fluorescence microscopy	Laser	micron	Yes	>cm	msec	30,000	Least	Voie et al. 1993

[Santi, JHC, 2011]

- Single molecule tracking
- Observing specimen in vitro, in vivo and in toto
- Observation of Embryos (Medaka, Drosophila melanogaster, mouse)
- Observation of big specimens (Mouse brain, inner ear, zebrafish)

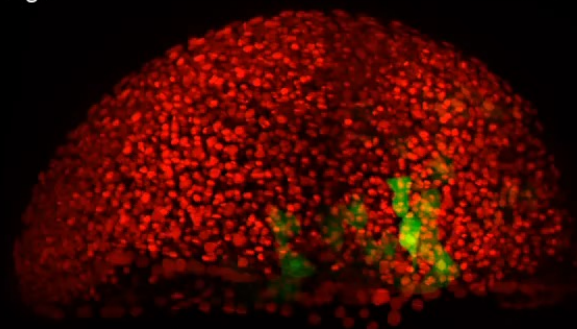
H2B-mCherry



crestin::GFP



merge



00:00:00

Reconstruction of zebrafish by scanned light sheet at Keller at. El.
E. Pulgar Unpublished data

- Macrozoom: Low signal and multi-spectral, slow but precise and sensitive.
- Spinning Disk: Fast and precise for small specimen
- Light Sheet Microscope: Fast, big specimen and low photo-bleaching and photo-toxicity.

