Laboratory for SCIAN Scientific Image Analysis

BIOMEDICAL NEUROSCIENCE INSTITUTE

High-Throughput Microscopy

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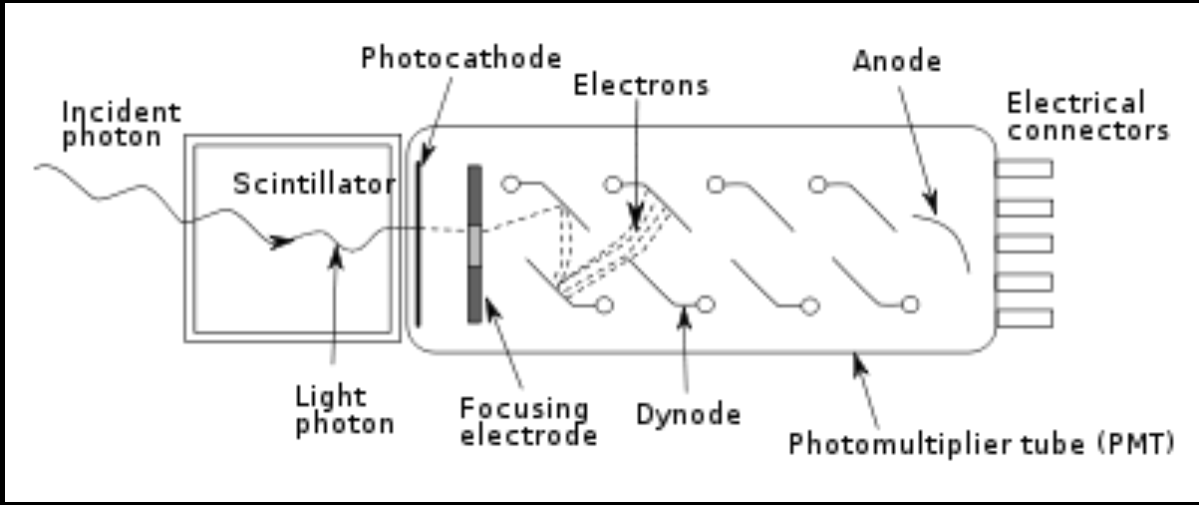
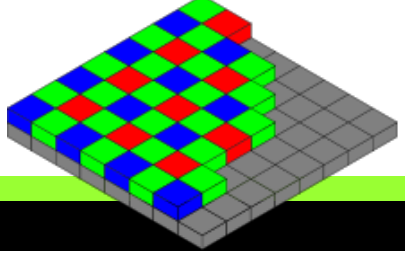
Curso BioFilms 2021

Sensors

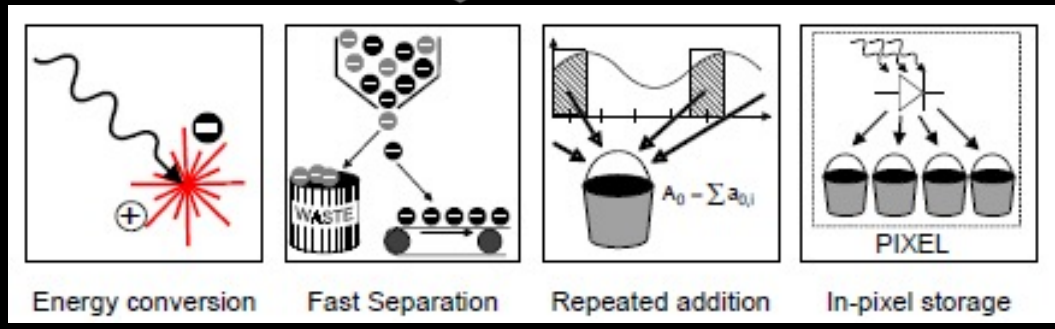
- 1D sensor
  - Photomultiplier
    - Detect photons and amplify the signal (by 100 million times)
    - Sum signal of all detected photons
    - Very sensitive

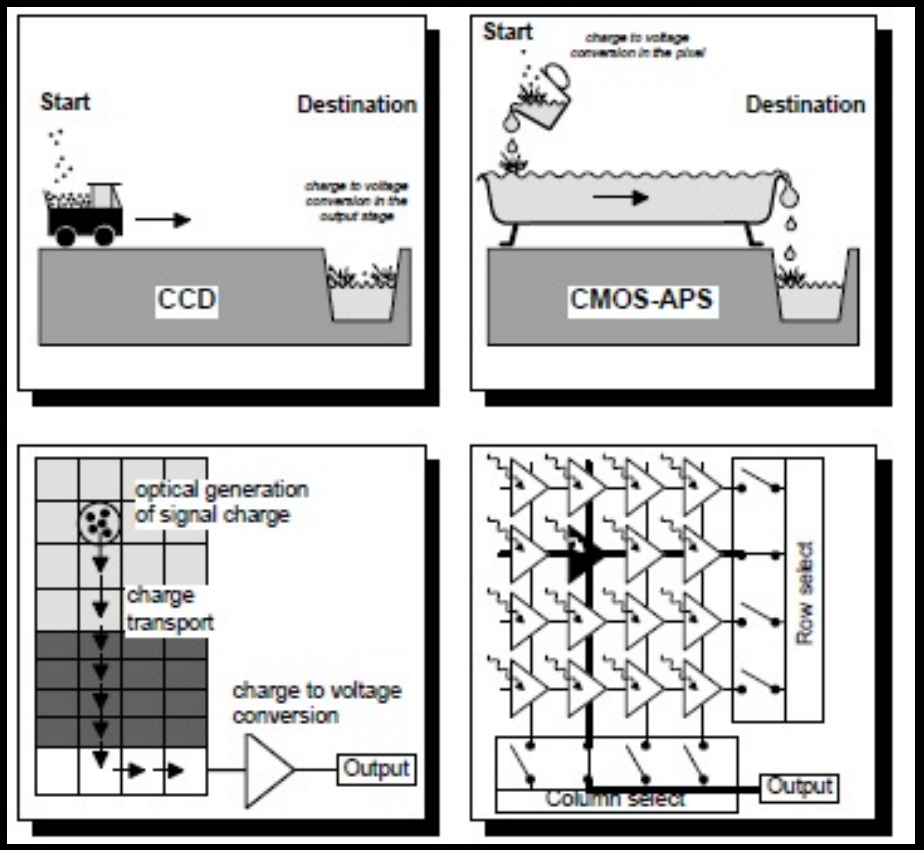


Sensors

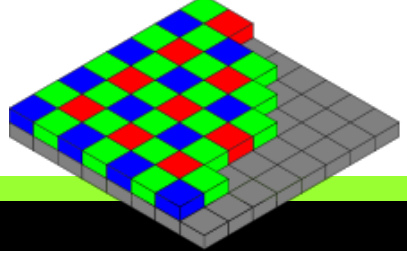


- 2D-Sensor
  - charge-coupled device (CCD)
    - Low noise
    - High power comsuption
    - Need move charges
  - Complementary metal–oxide–semiconductor (CMOS)
    - Moderate noise
    - Low power comsuption
    - Region Of Interest
    - Read directly from pixel storage

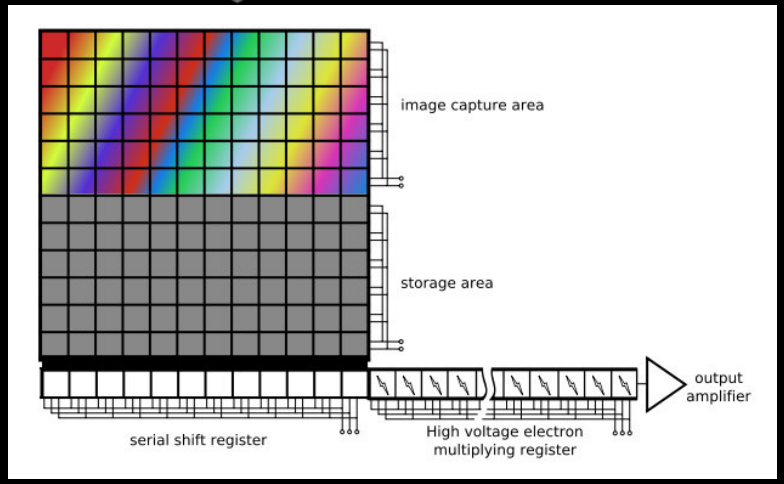




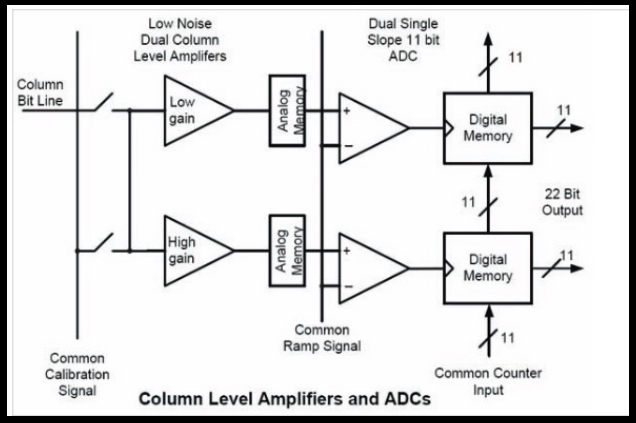
Sensors



- 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)



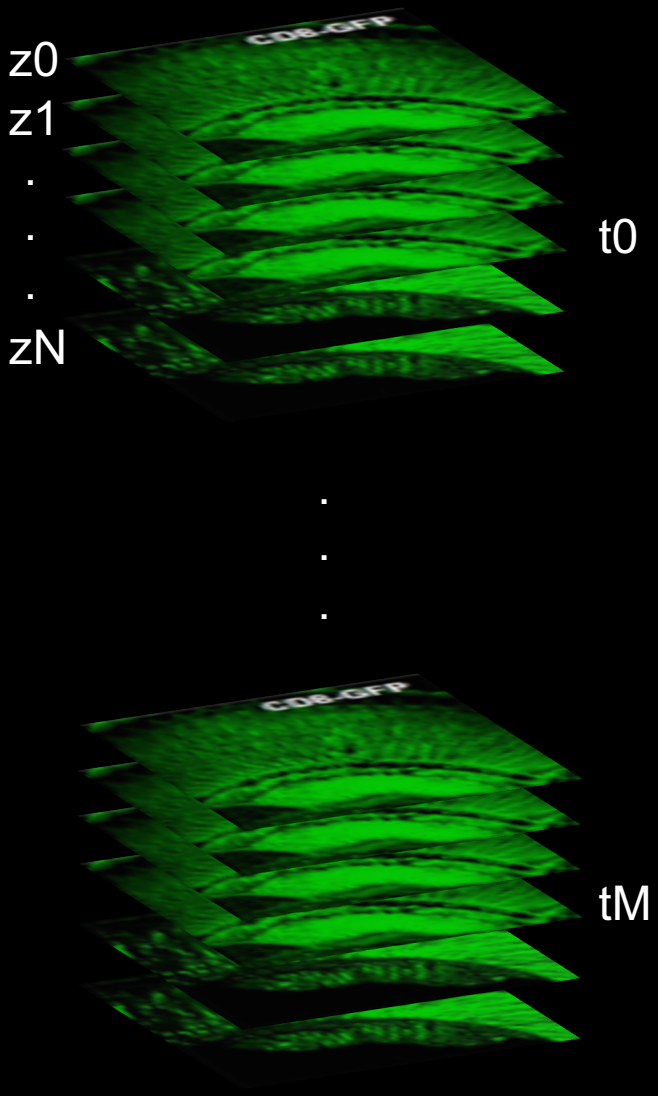
Solid state Electron Multiplying (EM) register to the end of the normal serial register

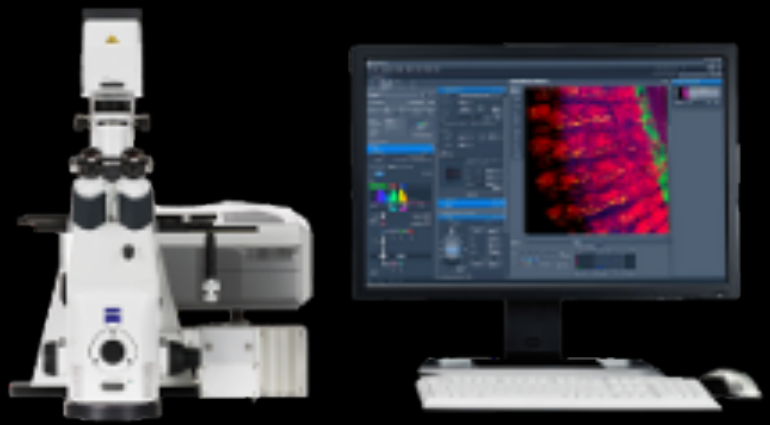


It is a mix between CCD/CMOS

Introduction

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

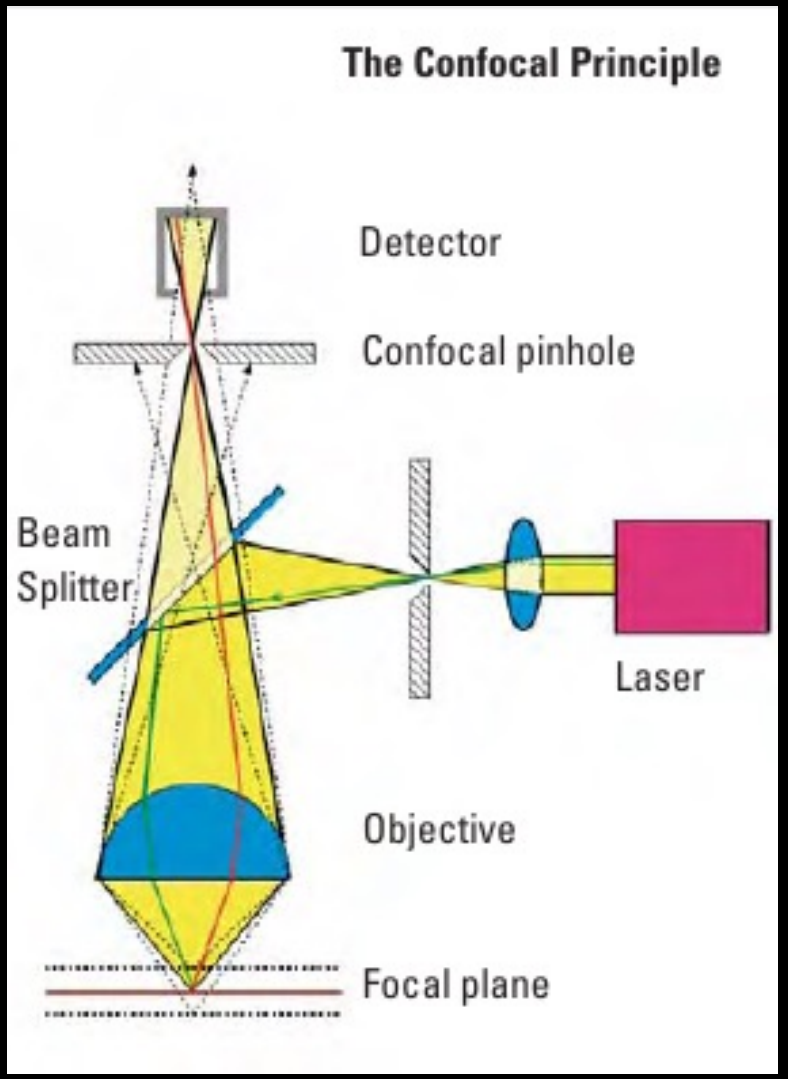




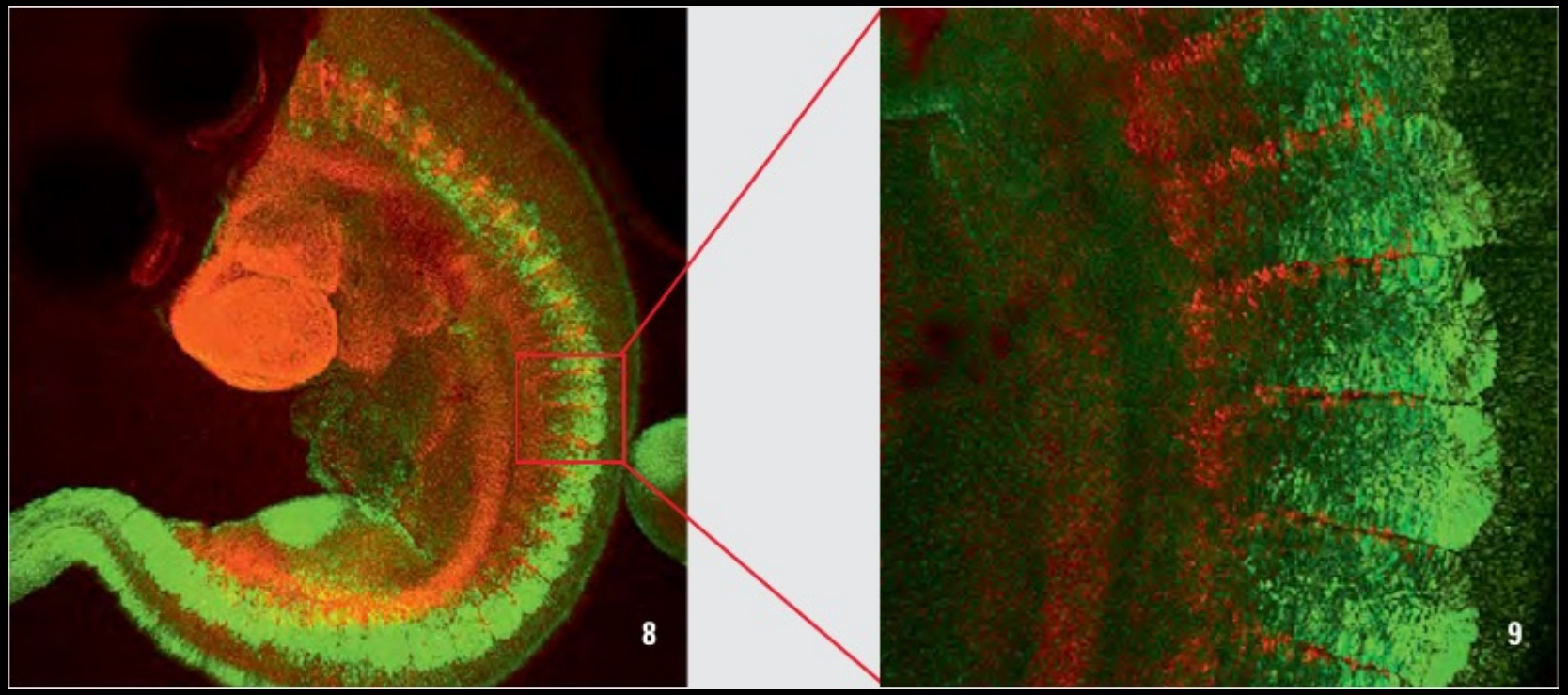
Zeiss LSM 710/780 NLO

Confocal Microscope Macrozoom

- 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



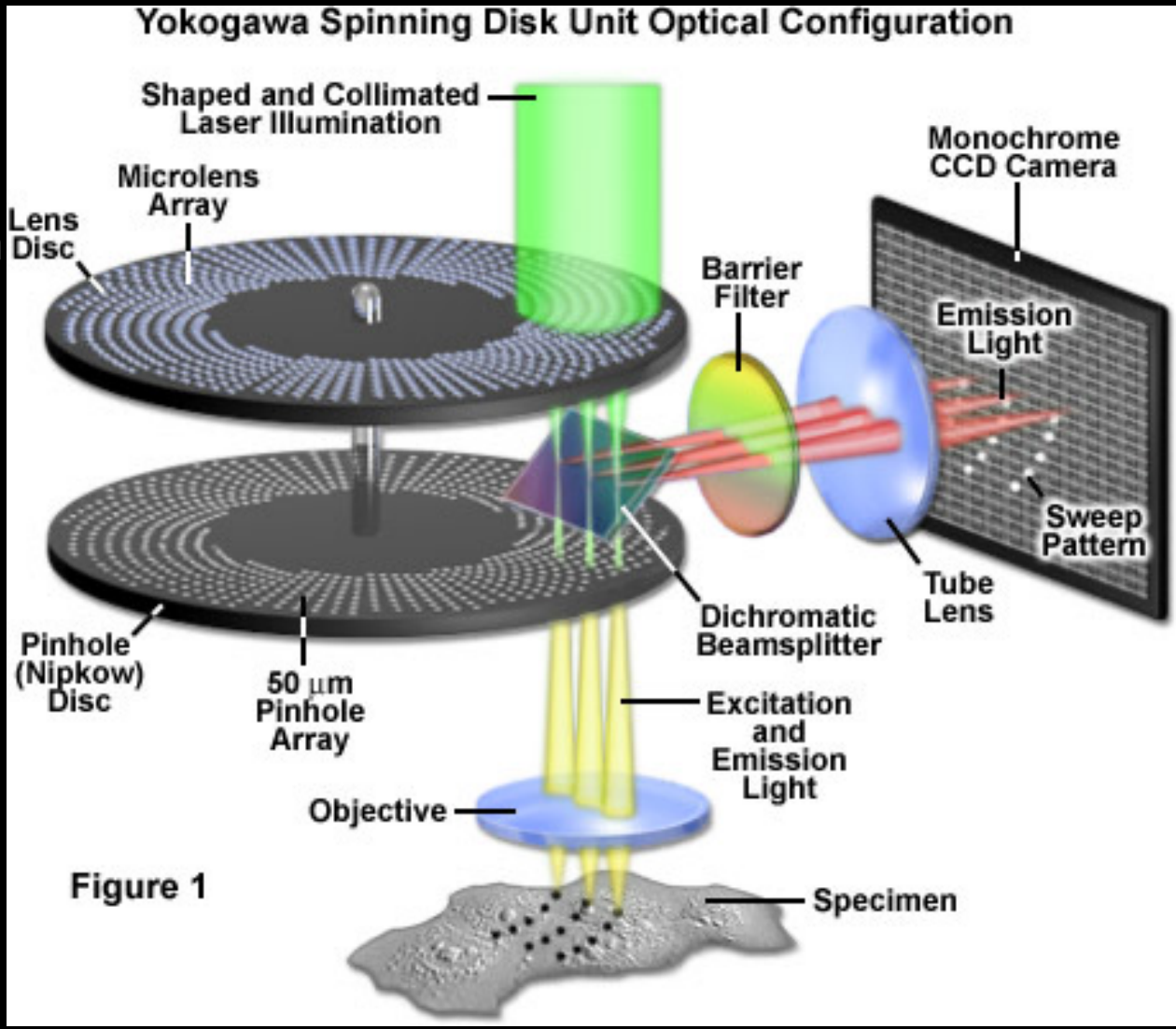
- Image up to 2048x2048
- Z-slices up to 10 nm
- Maximum specimen: 1.5 mm



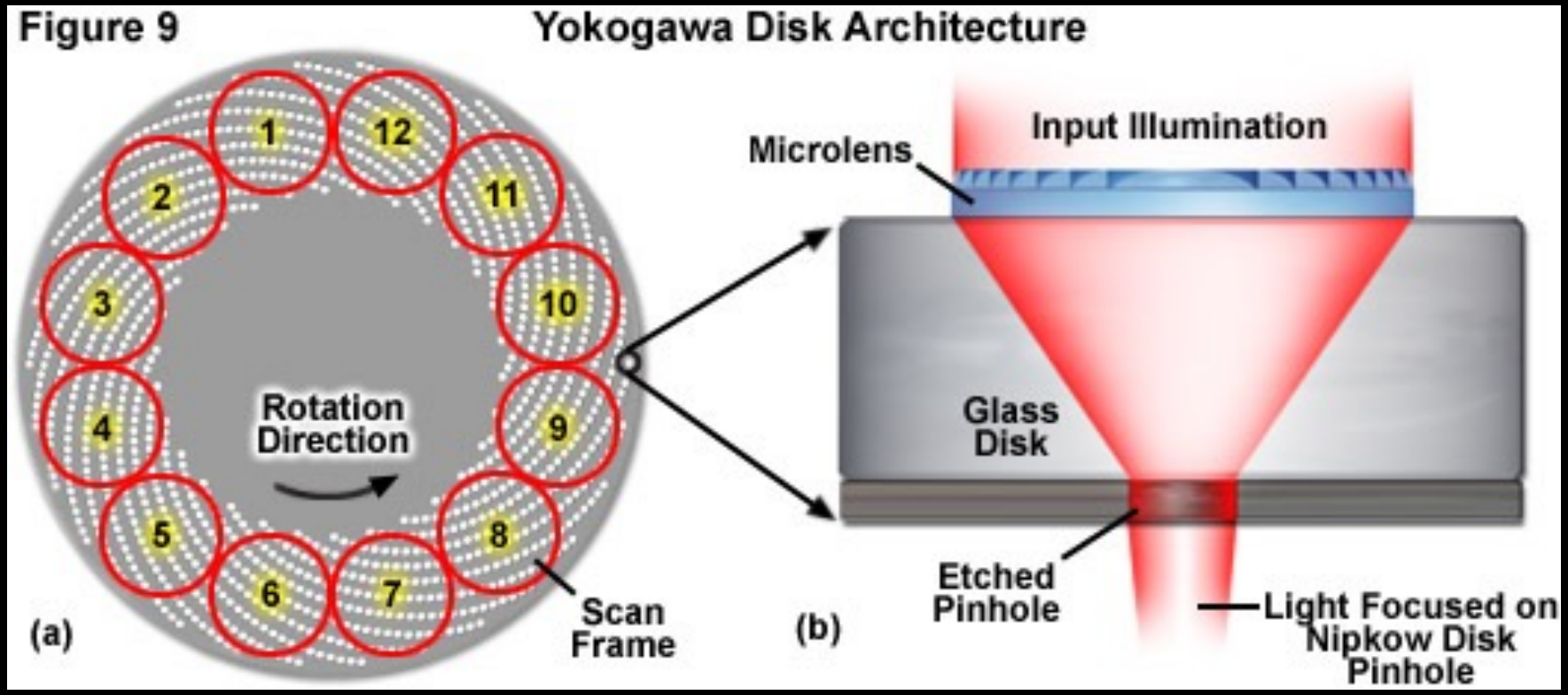
Spinning Disk Confocal Microscope

Zeiss Spinning Disk

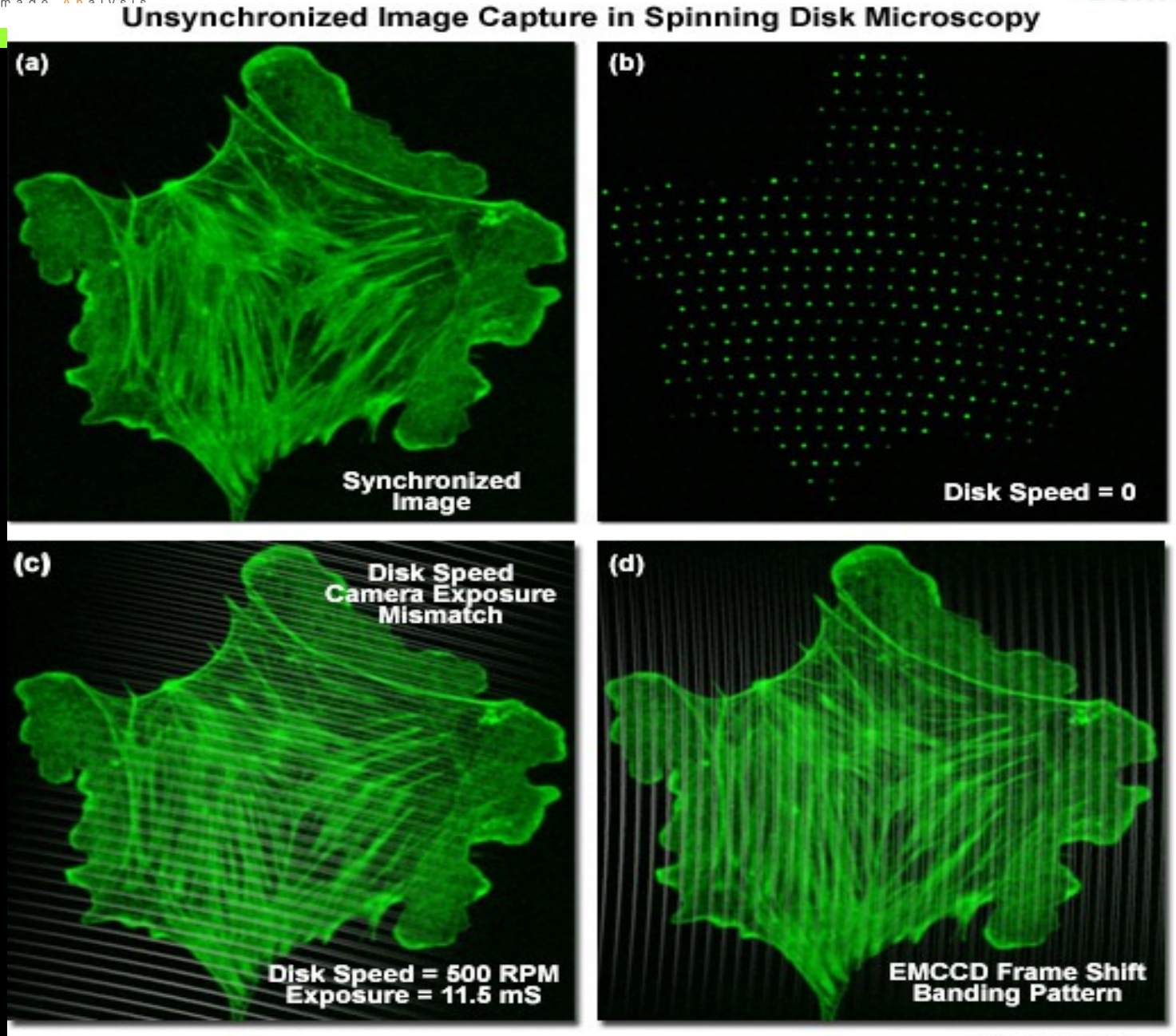
- 2048x2048
- Speed: 30 FPS
- CCD/EMCCD
- Specimen Size: 1 mm
- Scan point by point



Spinning Disk Confocal Microscope



Spinning Disk Confocal Microscope



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

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Spinning Disk Confocal Microscope

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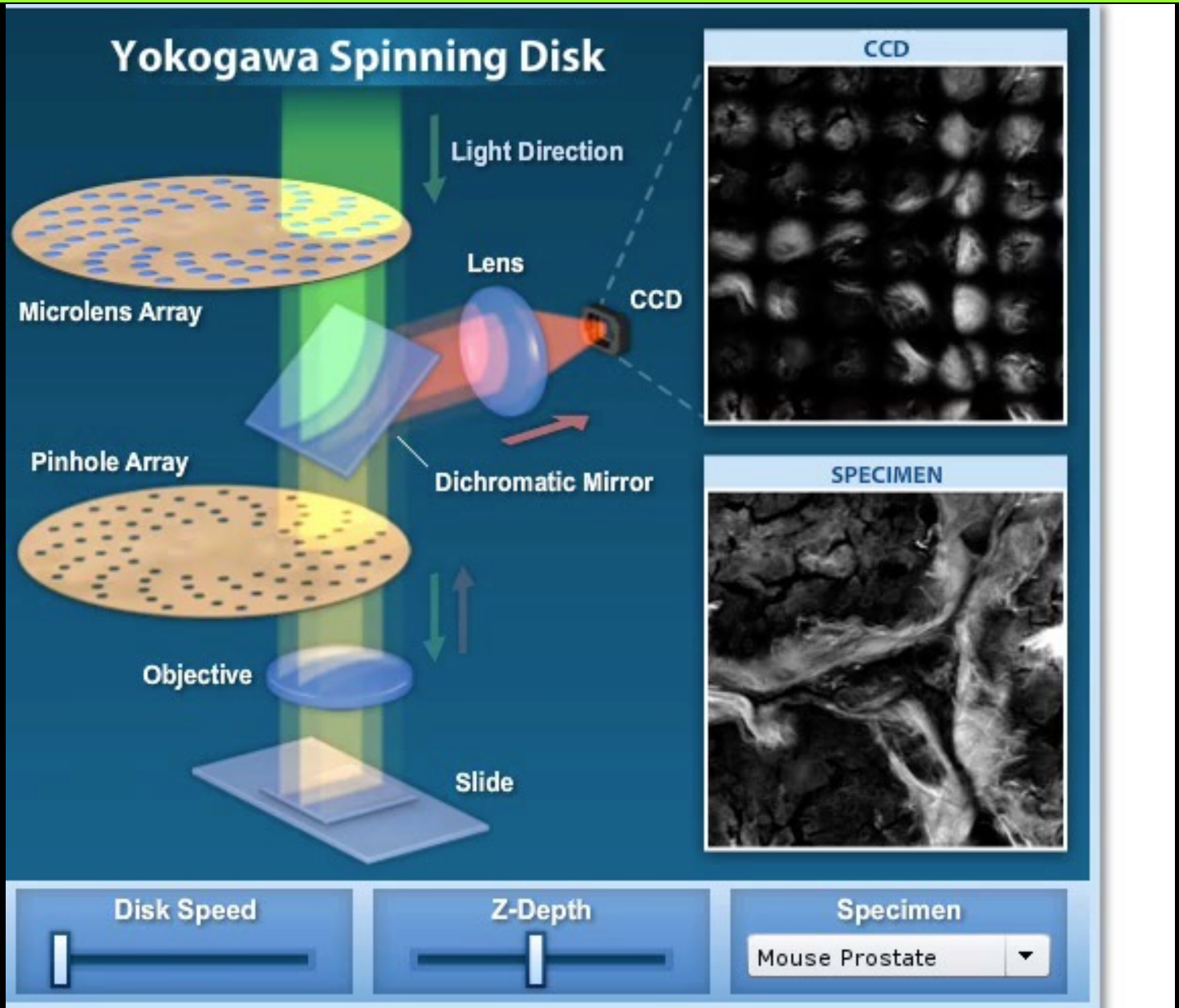


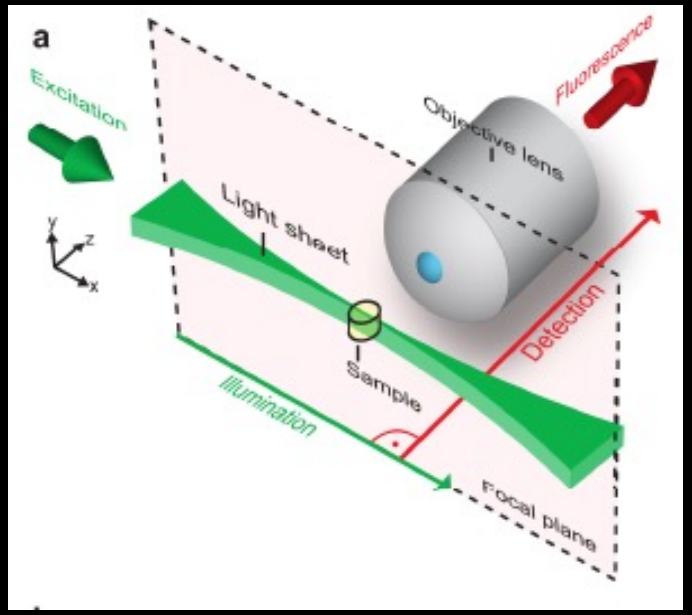
Light Sheet Microscopy

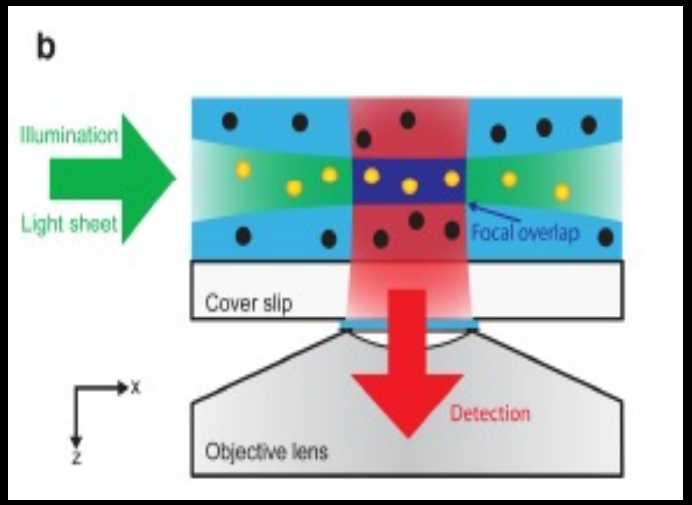
- Light Sheet Microscopy
  - Fluorescence microscopy
  - Optical microscopy (limit of 250 nm)
  - High-resolution microscopy
  - High speed
  - Image thick tissue (> 1 cm)
  - Non-destructive (produce optical sections)
  - Low Photo-toxicity and photo-bleaching
  - Low cost (compared to other microscopes)

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.





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Light Sheet Microscopy

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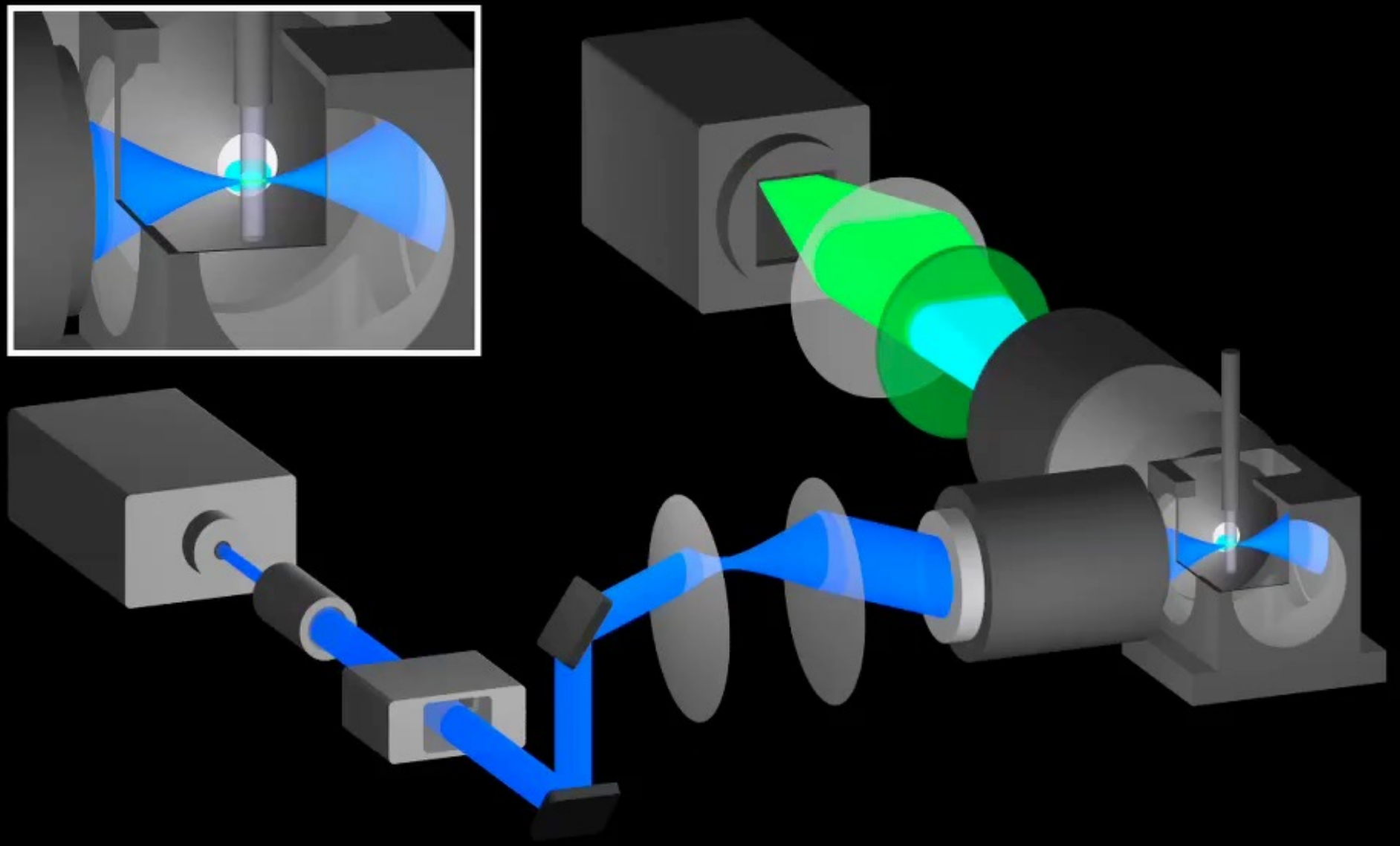


Keller Lab (Janelia Farm)

Light Sheet Microscopy
Comparison with confocal microscope

Confocal Microscopy

One 3D point per scan - Slow

Big part of illuminated

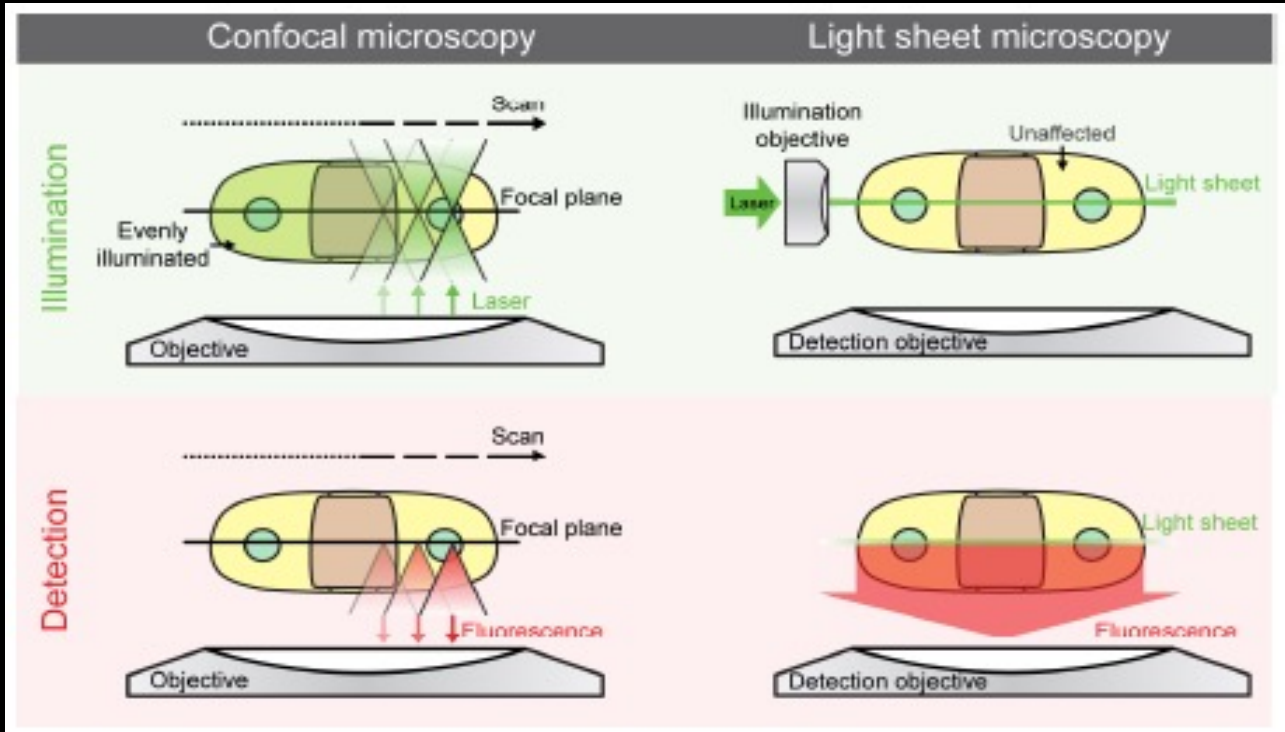
Normal phototoxity and Photobleaching

Light Sheet Microscopy

Complete focal plane – Fast

Only scanned plane illuminated

Reduced photo-toxicity and photo-bleaching



Light Sheet Microscopy
Comparison with other technologies

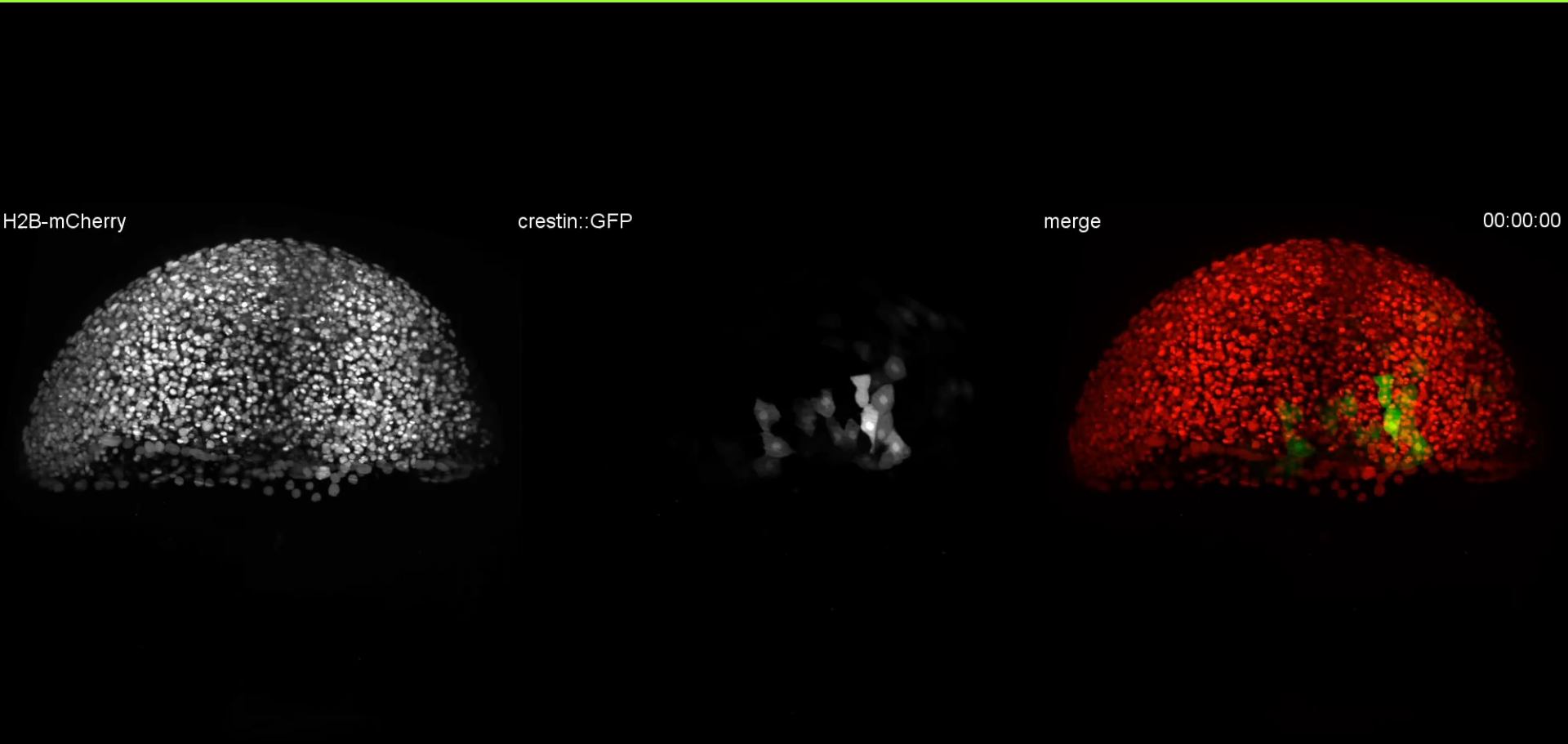
Name	Signal	Resolution	Fluorescent	Size	Imaging Time	Cost ($)	Photobleaching	Citation
Magnetic resonance imaging	Magnetic	mm	No, contrast agent	M	hr	Millions	NA	Lauterbur 1973
Computed tomography	Radioactive	<mm	No, contrast agent	cm	min	Millions	NA	Kalender 2006
Confocal	Laser	<micron	Yes	micron	msec	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]

Light Sheet Microscopy Applications

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

Light Sheet Microscopy Applications



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

Conclusion

- 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.