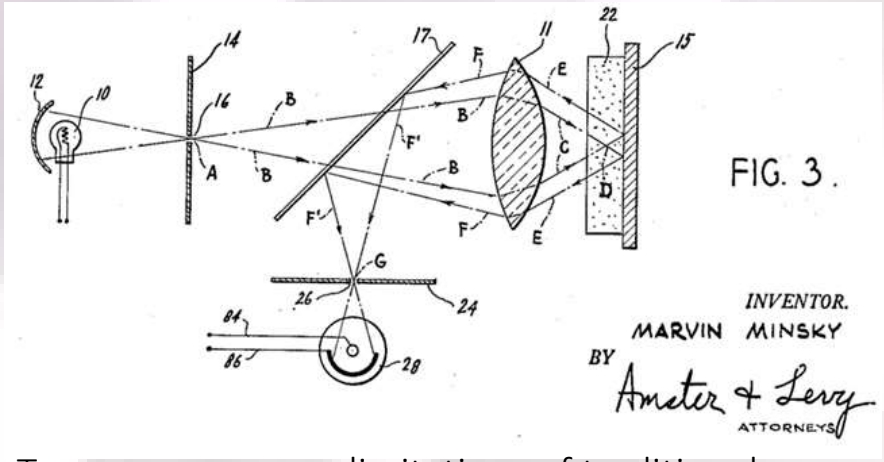
Principle of Confocal Microscopy and its applications

Confocal Microscopy

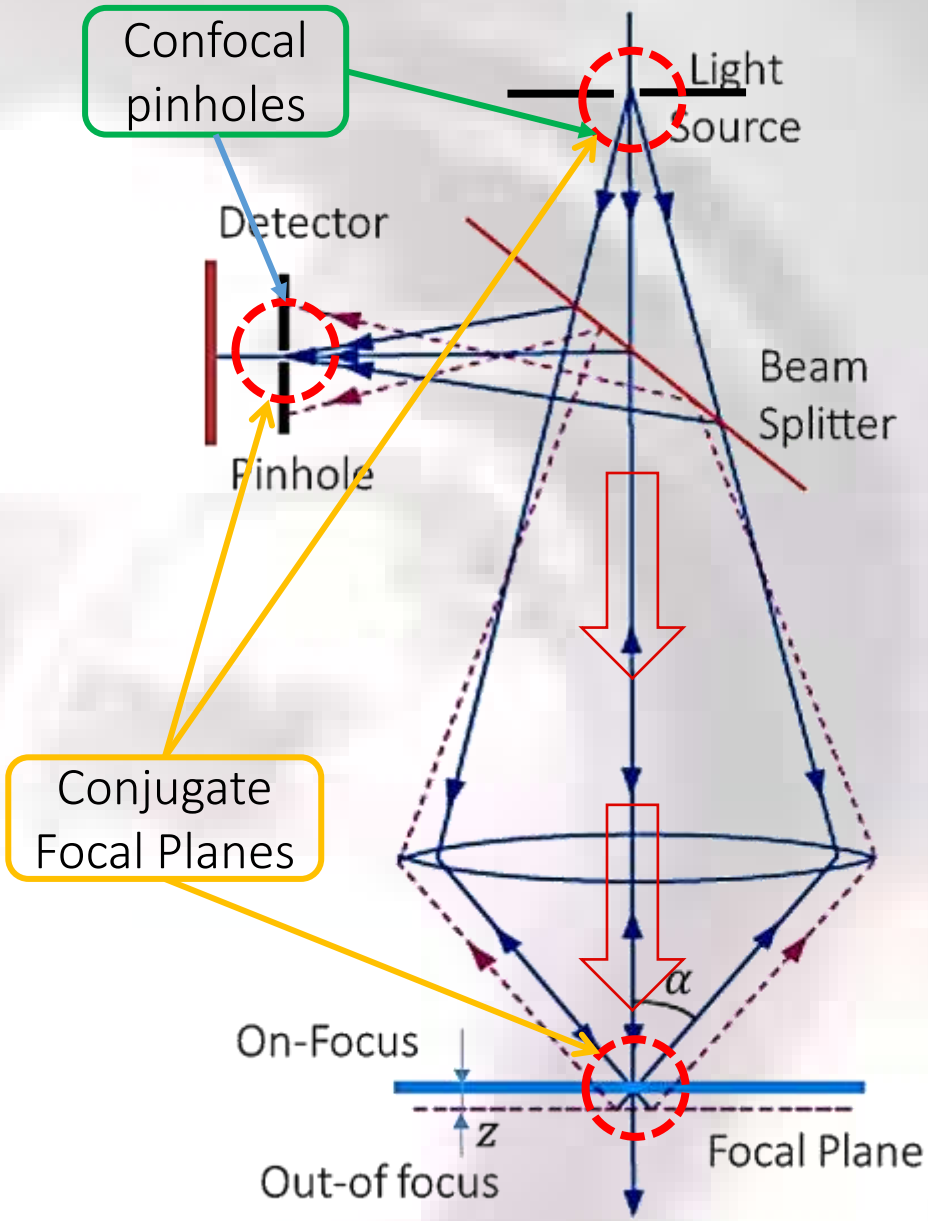


To overcome some limitations of traditional wide-field fluorescence microscopes.

Confocal Microscopy is now frequently known as

- **confocal laser scanning microscopy (CLSM)**
- **laser confocal scanning microscopy (LCSM),**

is an optical imaging technique for increasing optical resolution and contrast of a micrograph by means of using a spatial pinhole to block out-of-focus light in image formation. (From wiki)



Concept of Confocal Microscopy

DOI: 10.1016/S1369-7021(02)05329-4

Confocal Microscopy-Spatial Resolution

For a Confocal Microscope, the pinhole in front of sensor should be smaller than the diameter of Airy diffraction image formed by the object lens. The image of Airy diffraction pattern would be trimmed to region around the center.

Resolution of a optical microscope

$$\text{Lateral} = \begin{cases} \dfrac{\lambda}{2 \times \text{NA}}, \text{Abbe Equ.} \\ \dfrac{0.61\lambda}{\text{NA}}, \text{Rayleigh limit} \\ \dfrac{1.22\lambda}{NA_{obj} + NA_{Cond}}, \end{cases}$$

$$\text{Depth} = \frac{2\lambda}{NA^2}, Abbe$$

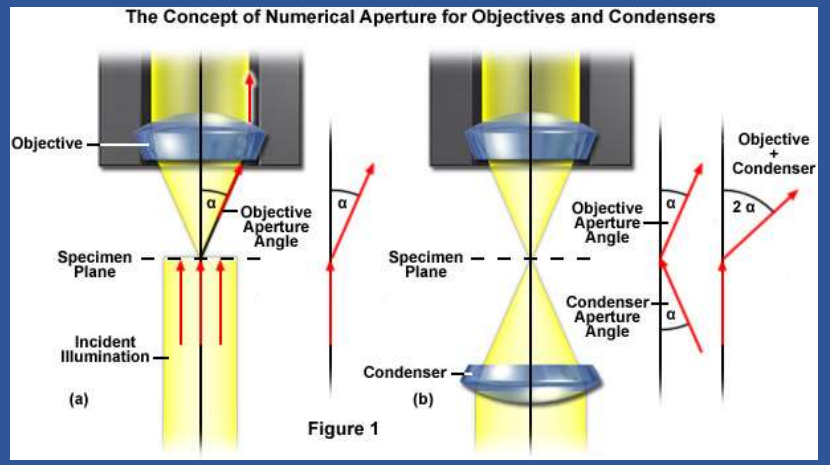


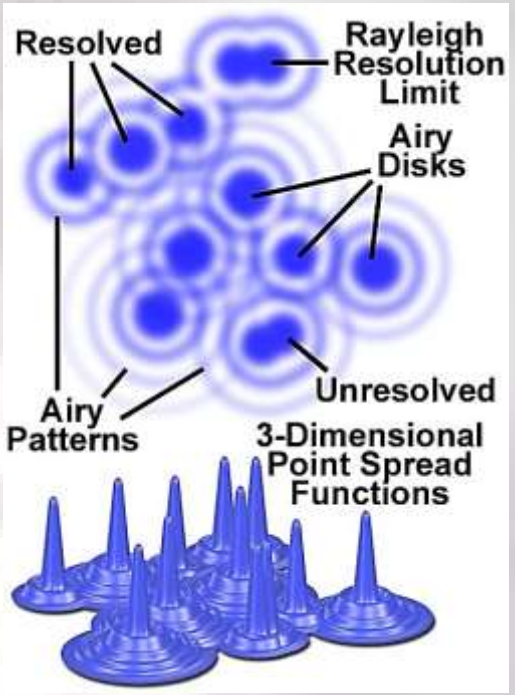
Figure 1

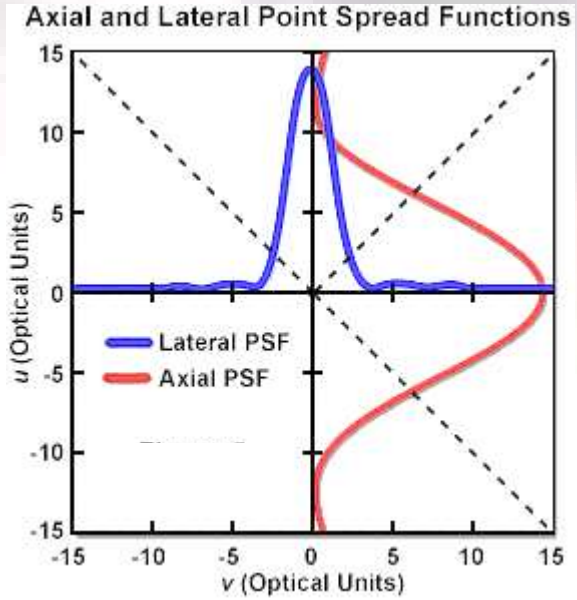
Resolution of a optical microscope

$$\text{Lateral Resolution} = 0.37\lambda/NA$$

$$\text{Depth Resolution} = \begin{cases} \sqrt{\left(\dfrac{0.88\lambda}{1-\sqrt{1-NA^2}}\right)^2 + \left(\dfrac{\sqrt{2}d}{NA}\right)^2}, \text{Geometrical–optical confocality} \\ \dfrac{0.64\lambda}{1-\sqrt{1-NA^2}}, \text{Wave–optical confocality} \end{cases}$$

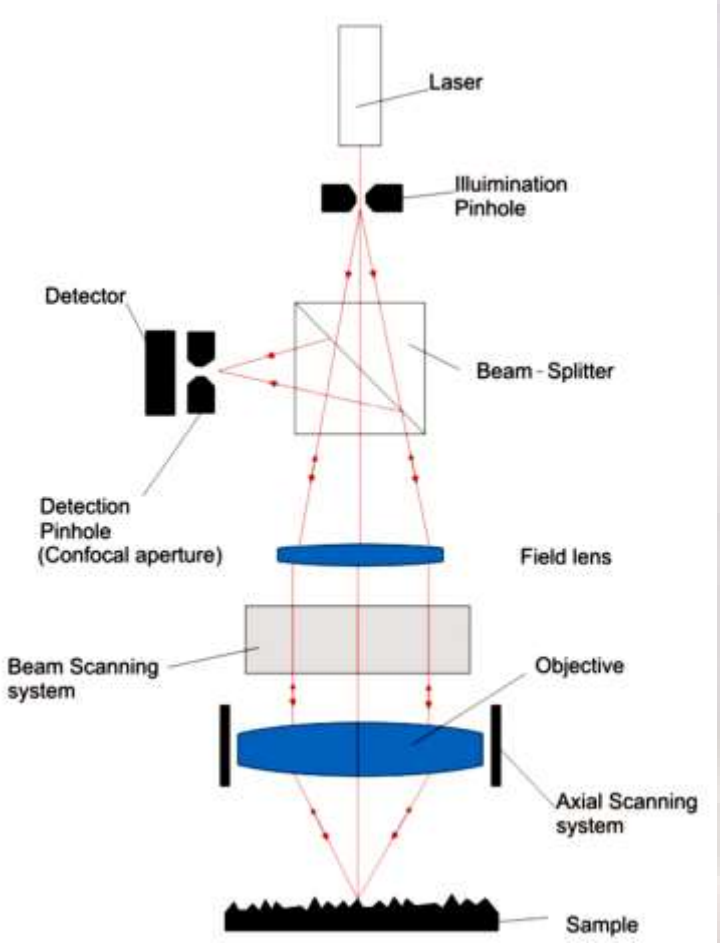
DOI 10.1007/978-3-642-12012-1



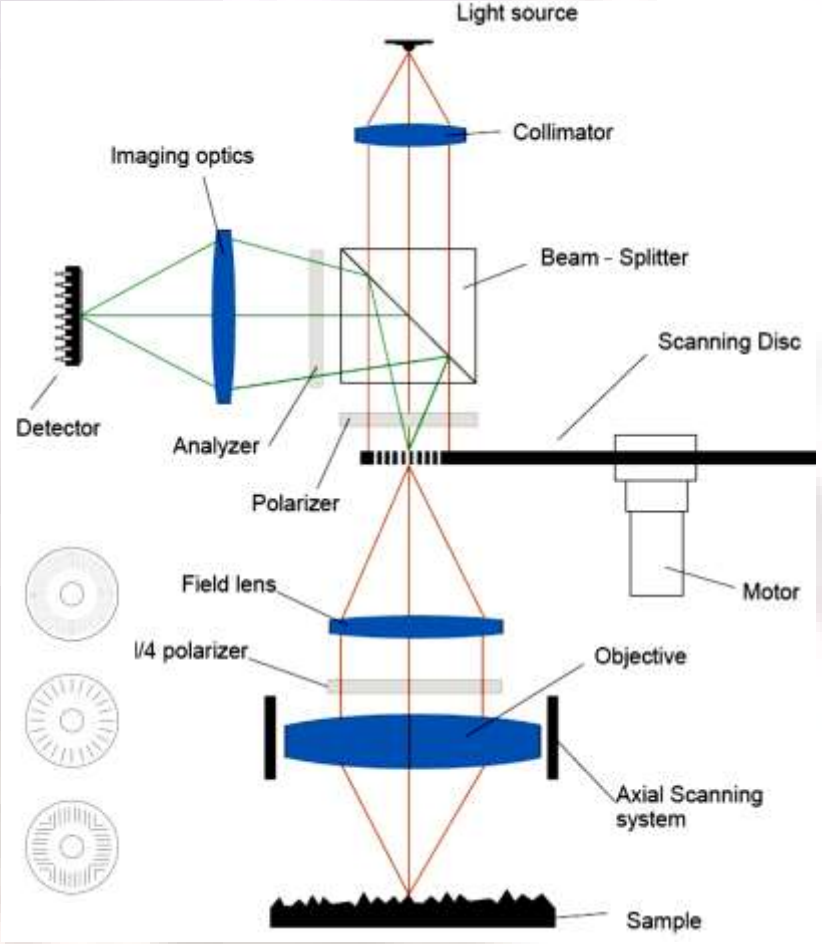


https://www.olympus-lifescience.com/en/microscope-resource/primer/techniques/confocal/signaltonoise/

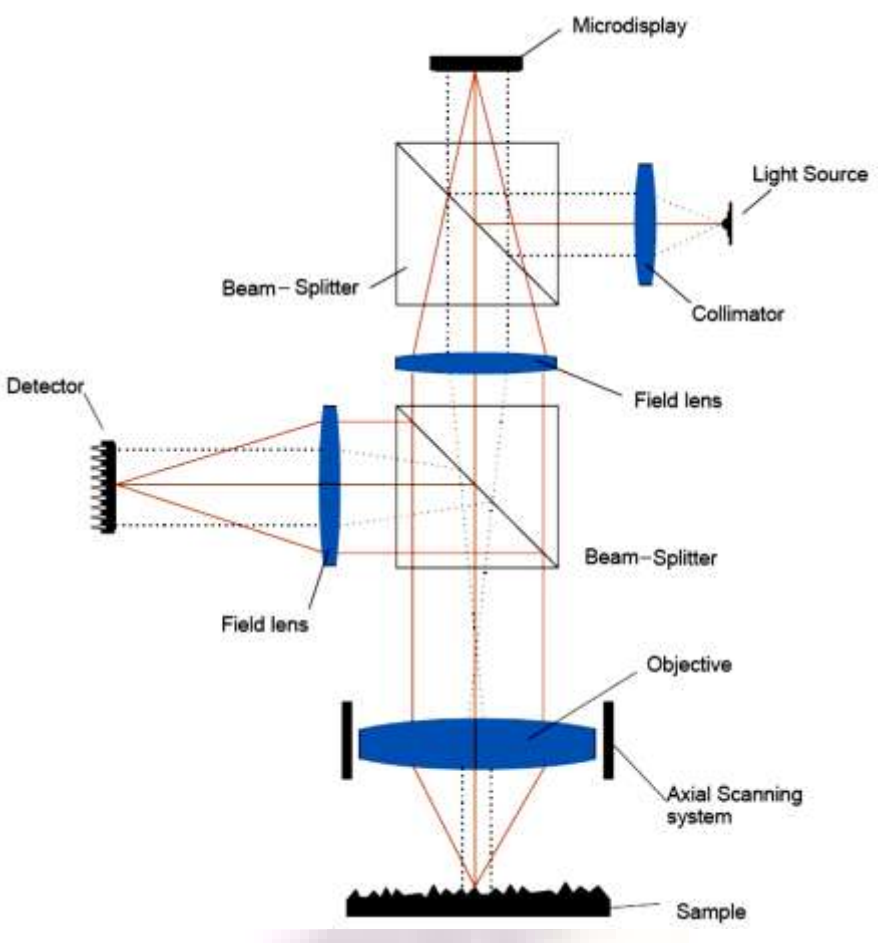
Types of Confocal Microscopes



Laser Scanning Confocal Microscope



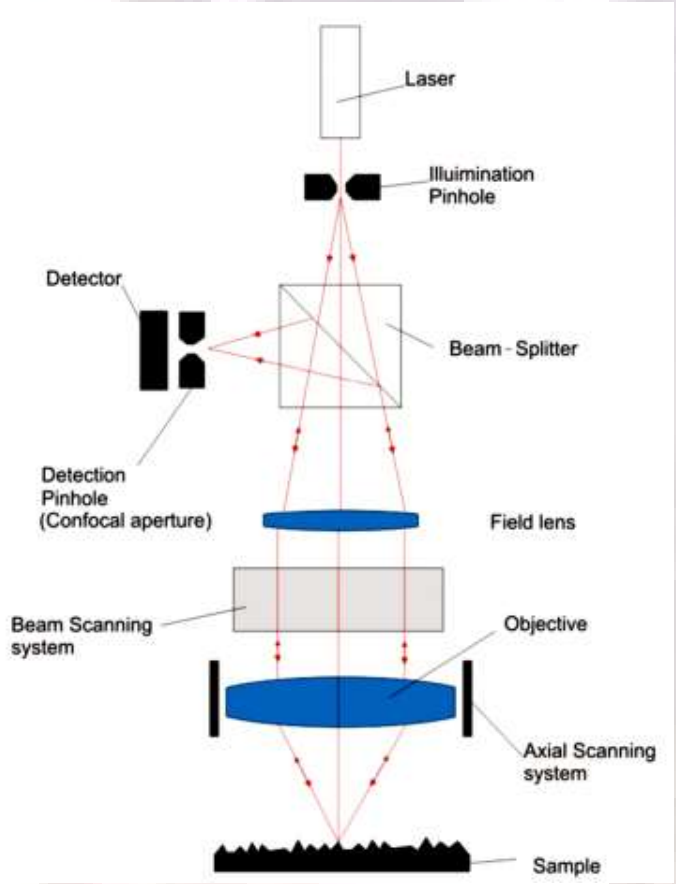
Disc Scanning Confocal Microscope



Programmable Array Scanning Confocal Microscope

Laser Scanning Confocal Microscope-

Scanning Mechanisms



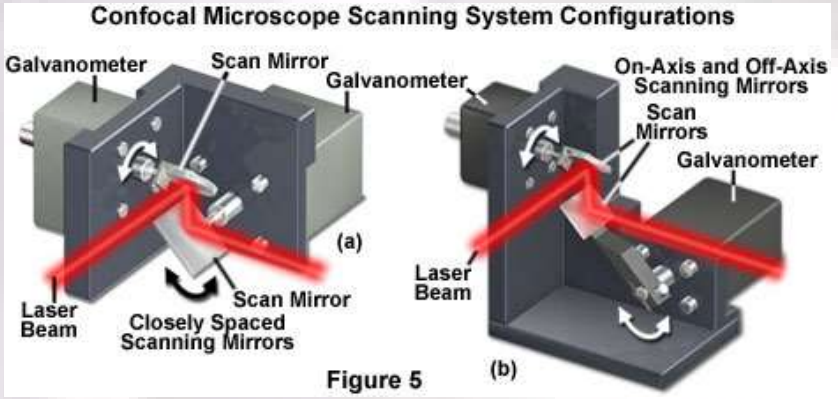


Figure 5

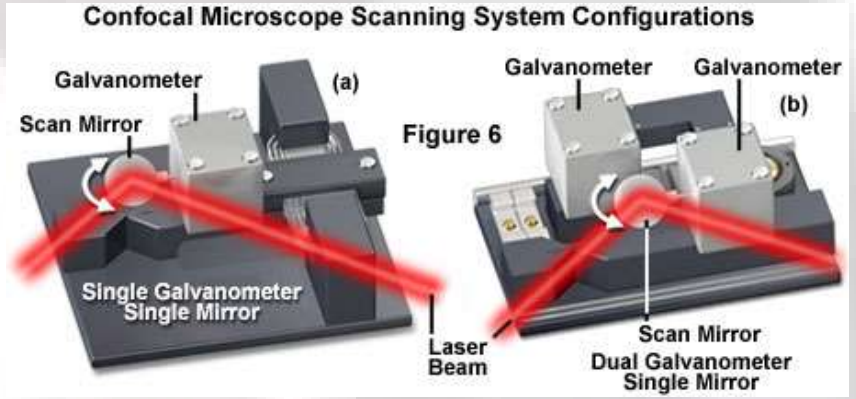
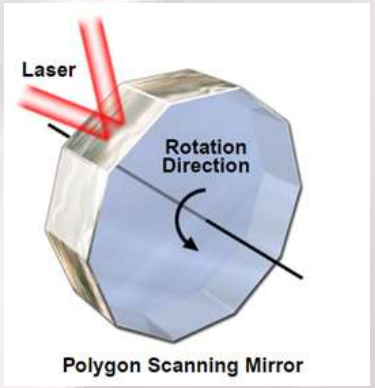
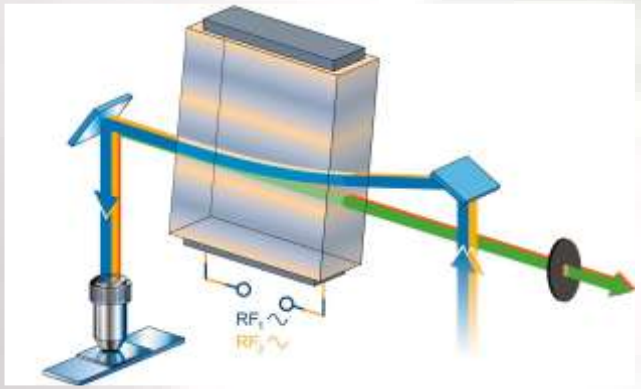
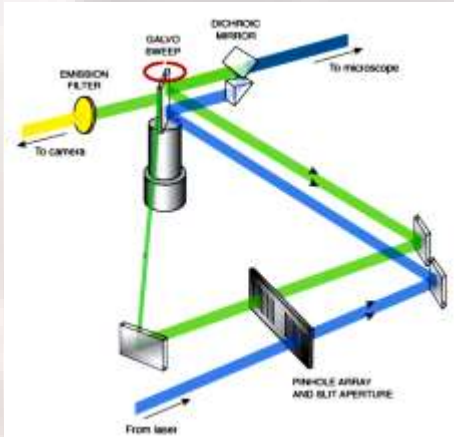


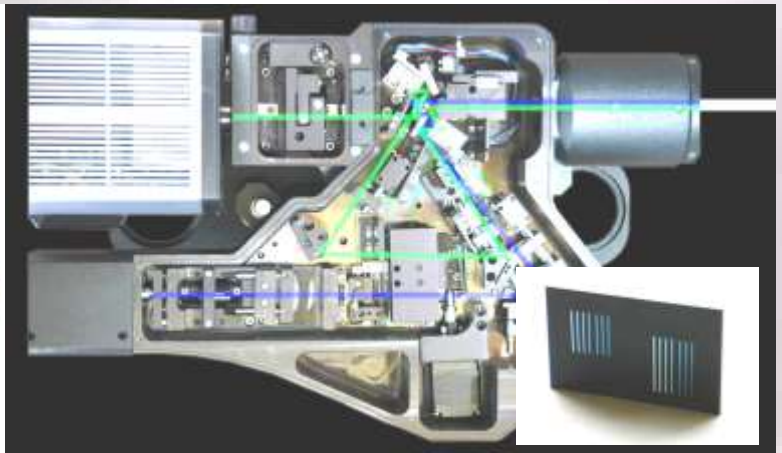
Figure 6



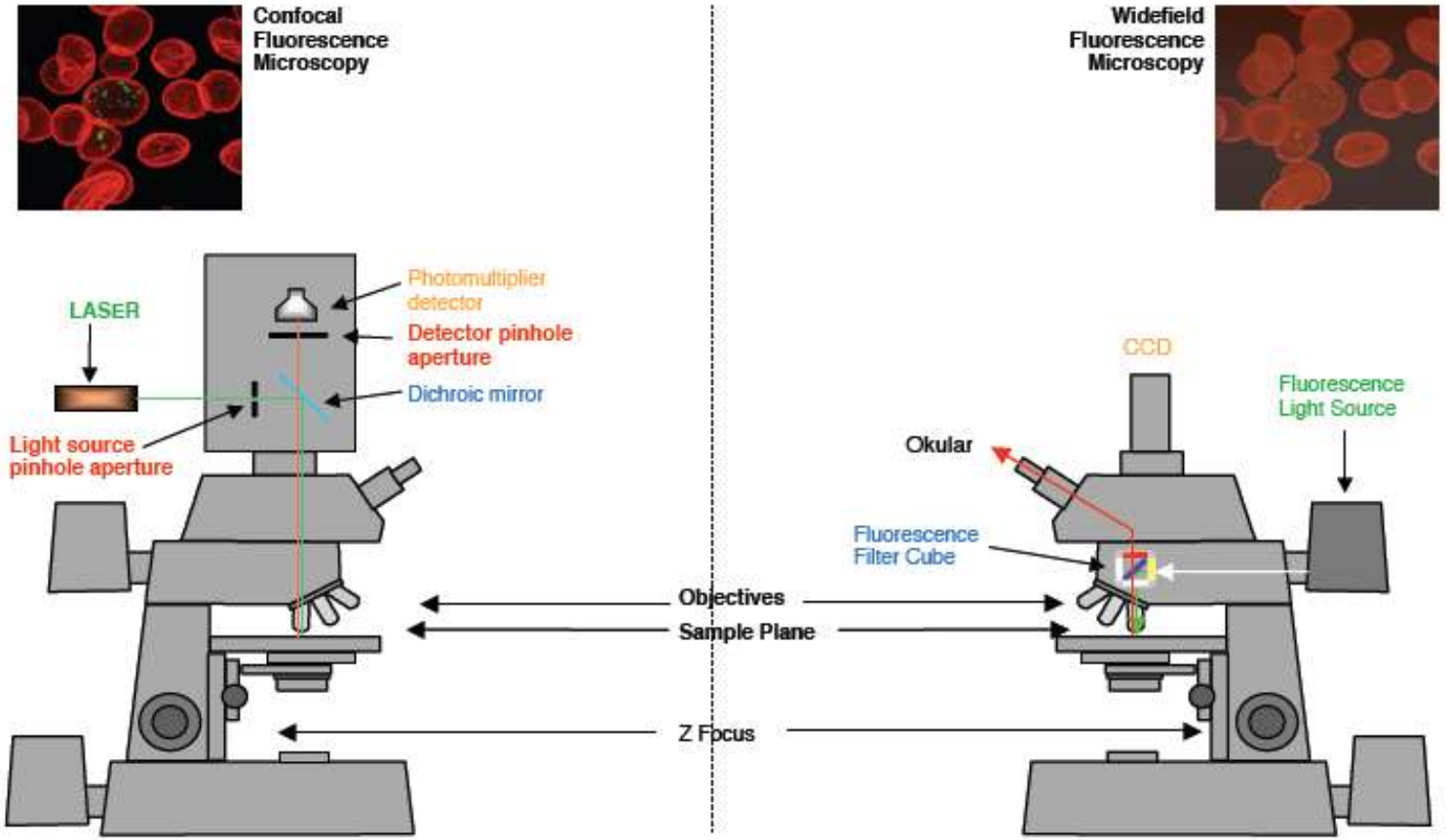
Polygon Scanning Mirror

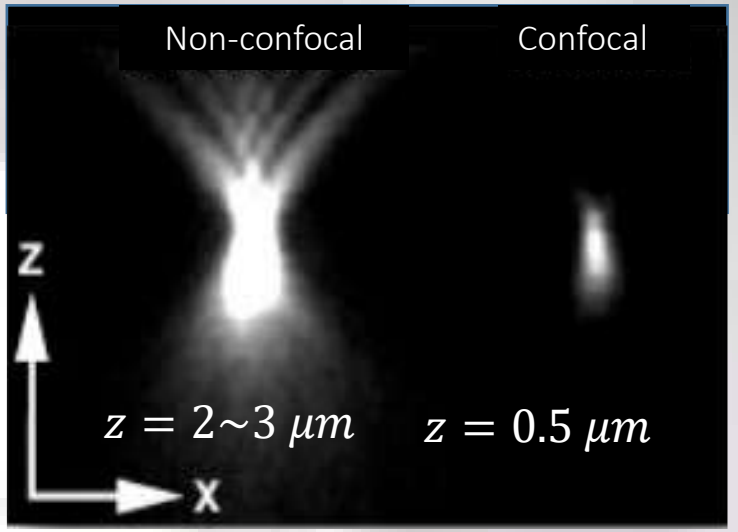




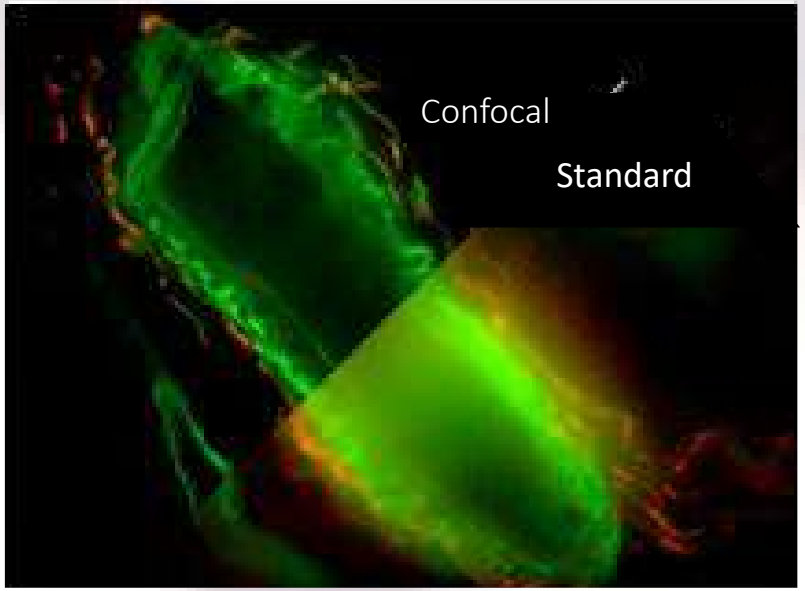


Confocal Fluorescence Microscopy

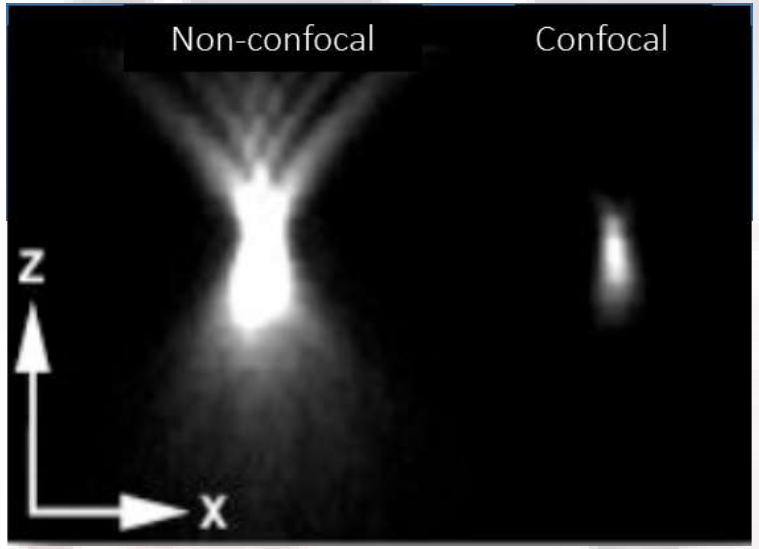


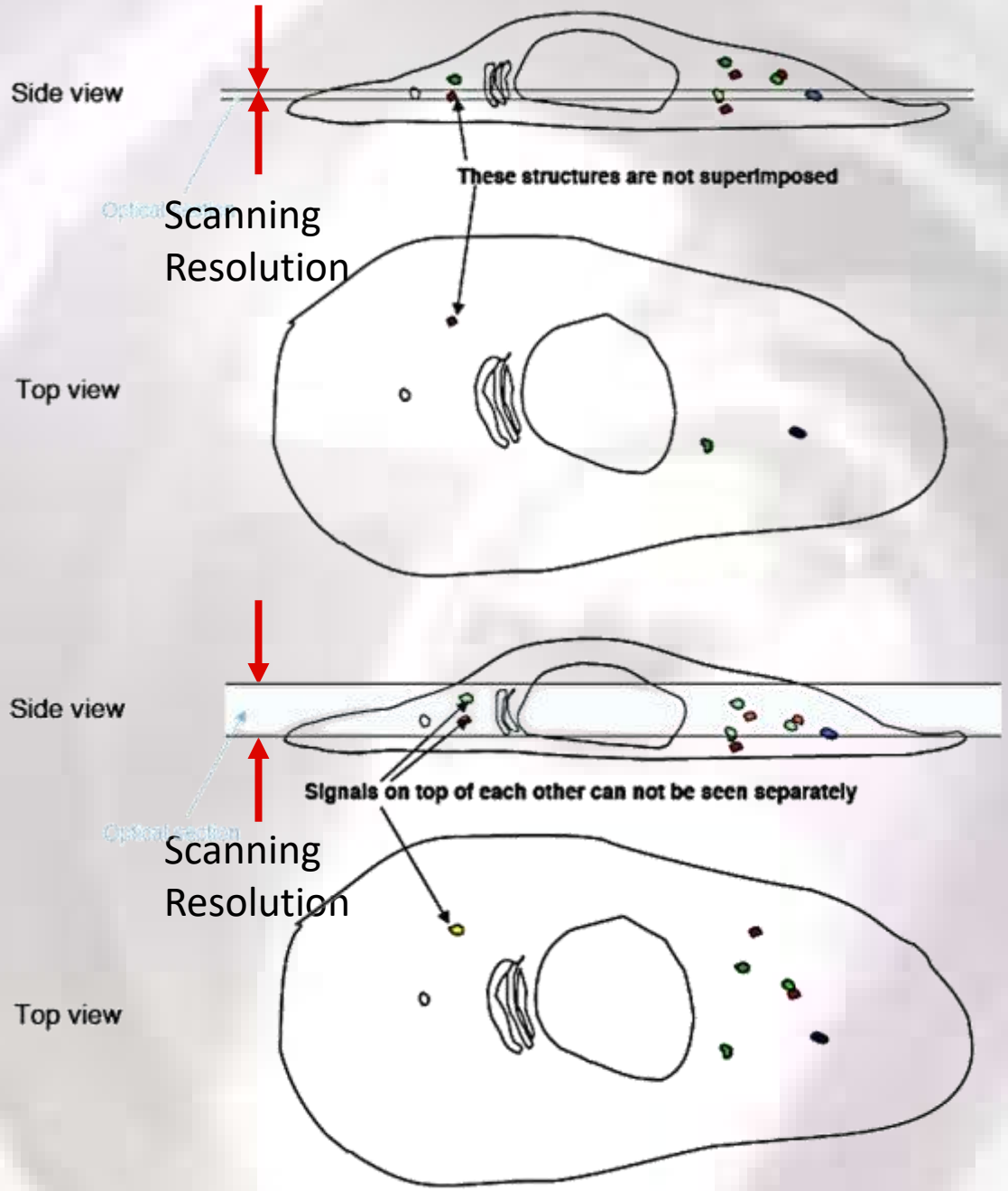


Confocal Microscopy:
higher z-resolution and reduced out-of-focus-blur

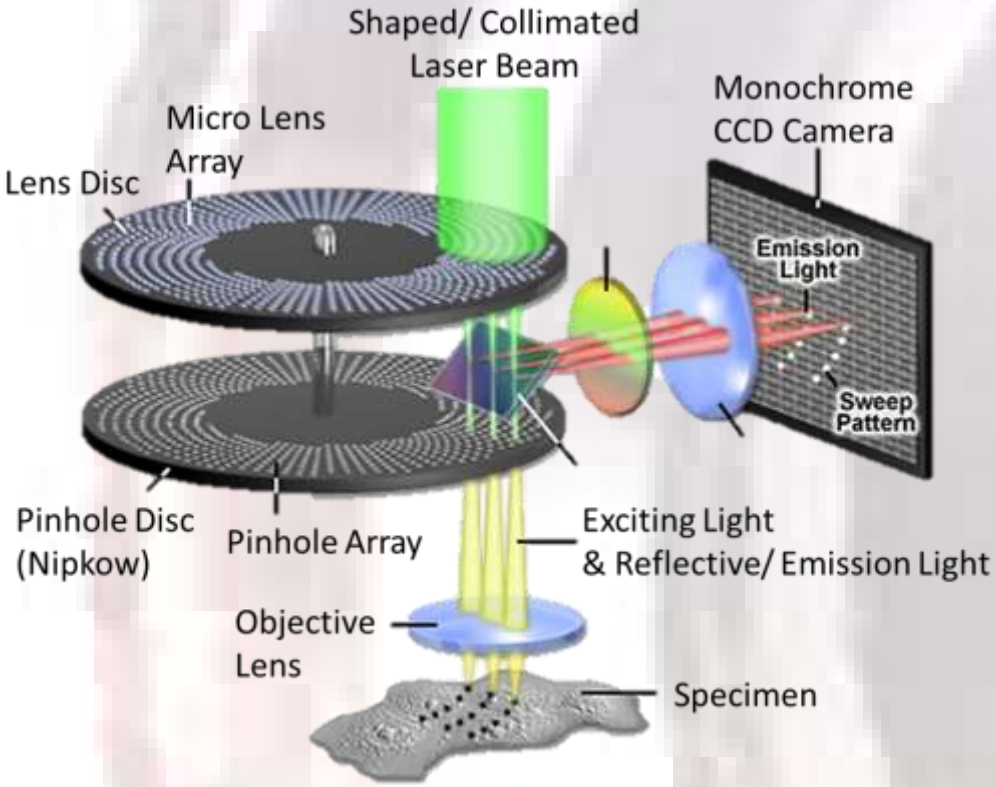


Optical Section





Spinning disk confocal



Yokogawa Spinning Disk Scanning Unit

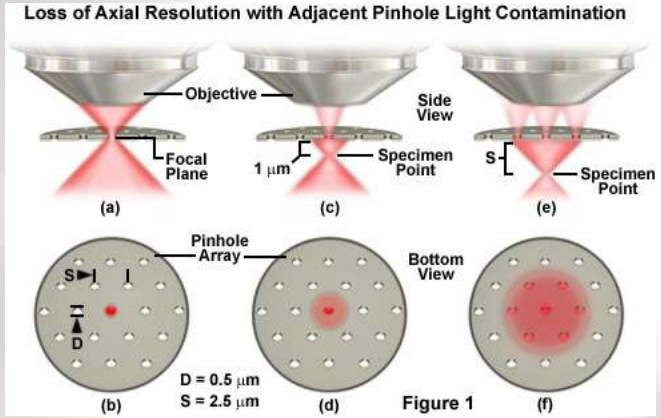
Pros:

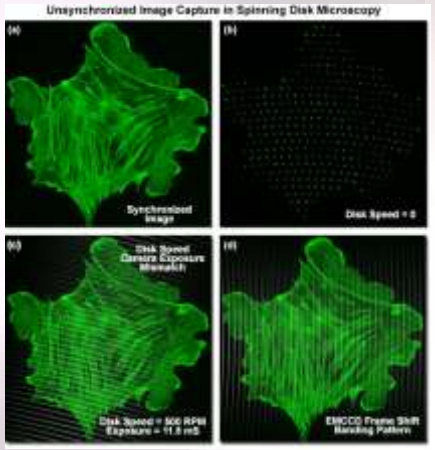
- Accelerating the scanning process
- Fast – multiple points are illuminated at once
- Photon efficient – high QE of CCD
- Gentler on live samples – usually lower laser power

Cons:

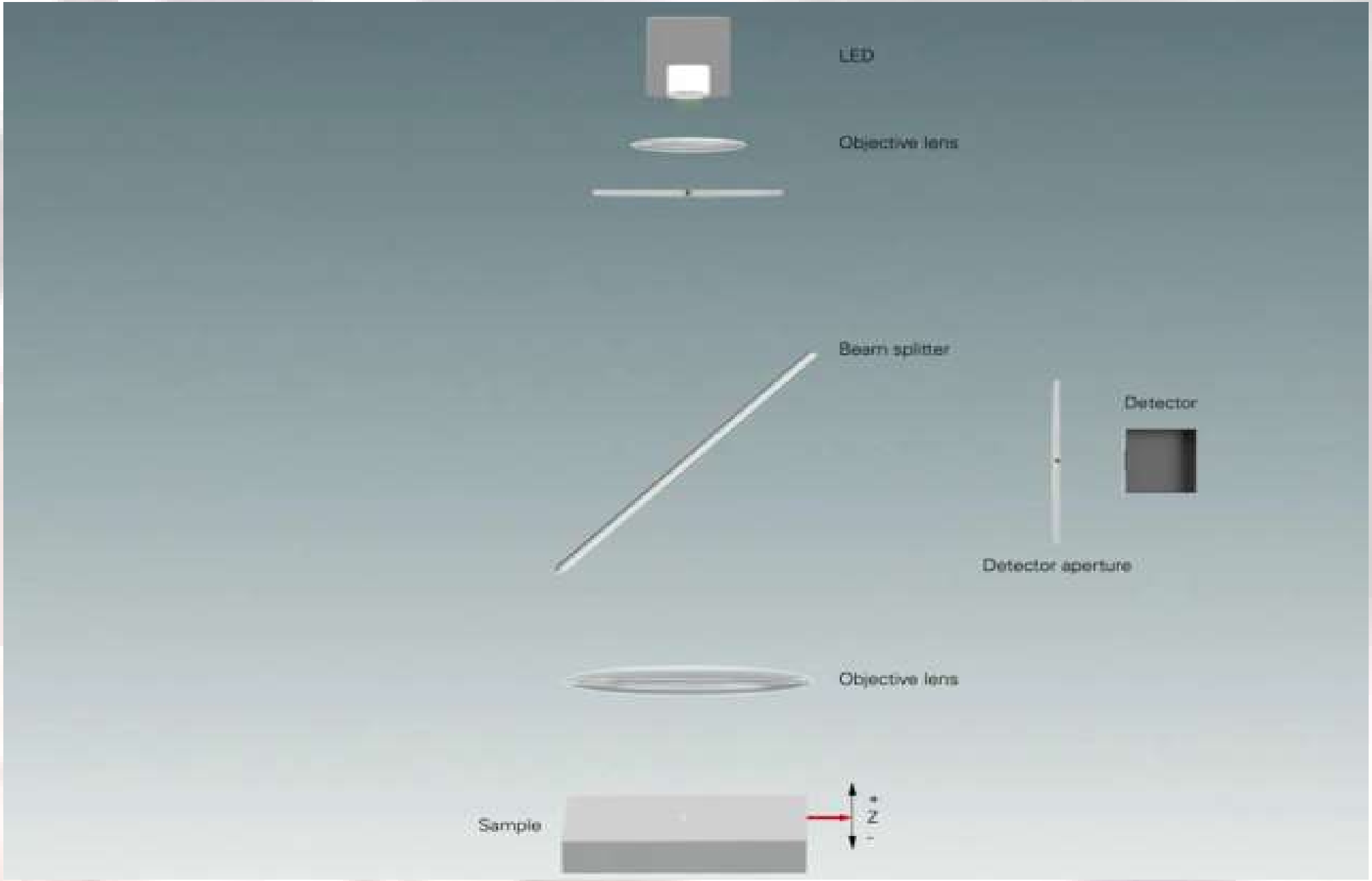
- multiple fluorophore colors might not be generated because of PMT is replaced by CCD sensor
- Cross talk problem would degrade depth resolution
- Fixed pinhole – except in swept-field
- Small field of view (usually)

CCD and the pinhole disk must be synchronized

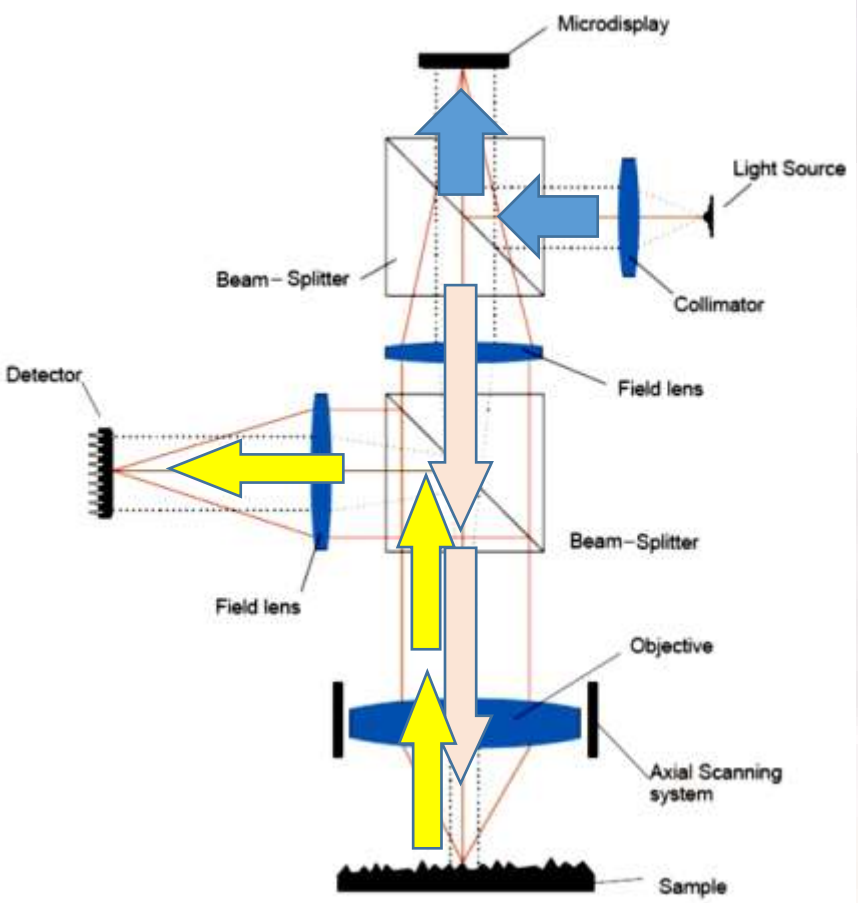


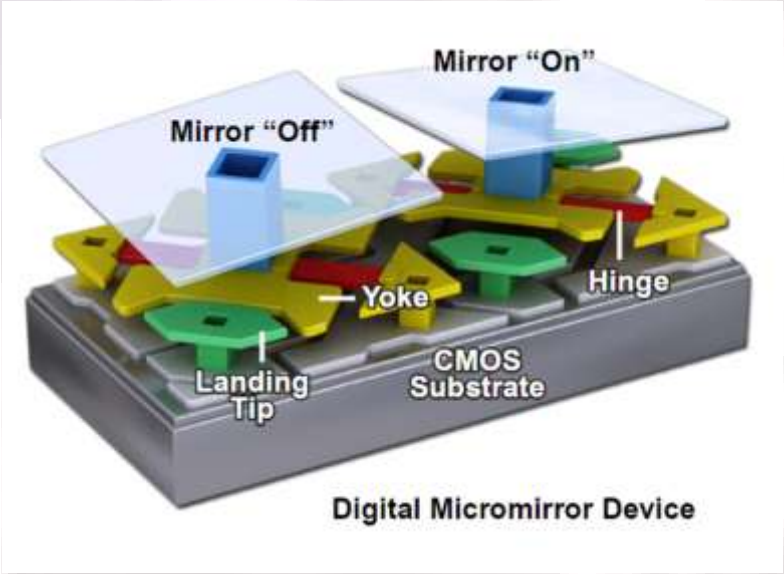


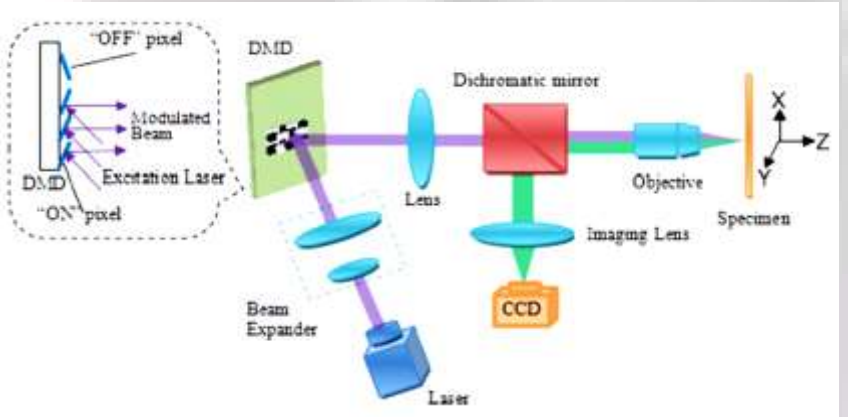
http://zeiss-campus.magnet.fsu.edu/tutorials/spinningdisk


LED
Objective lens
Beam splitter
Detector
Detector aperture
Objective lens
Sample
+
Z
-

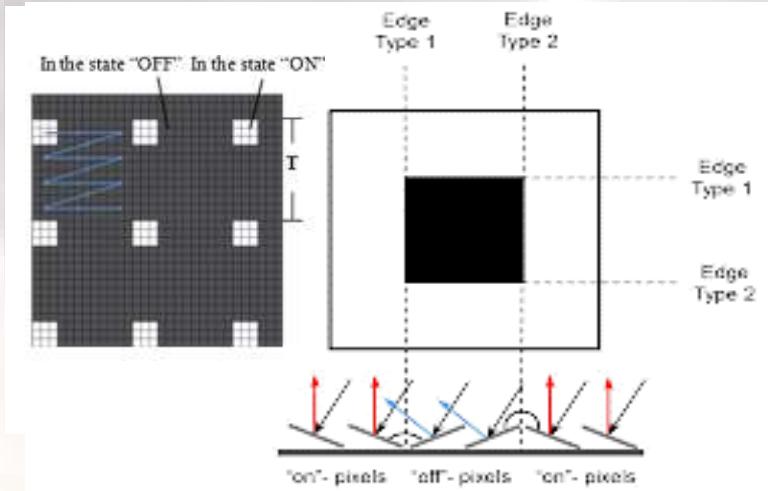
Programmable Array Scanning Confocal Microscope

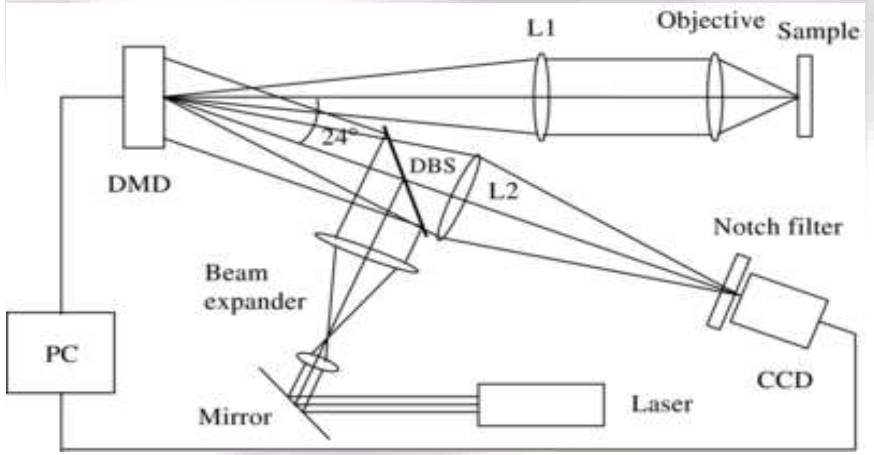




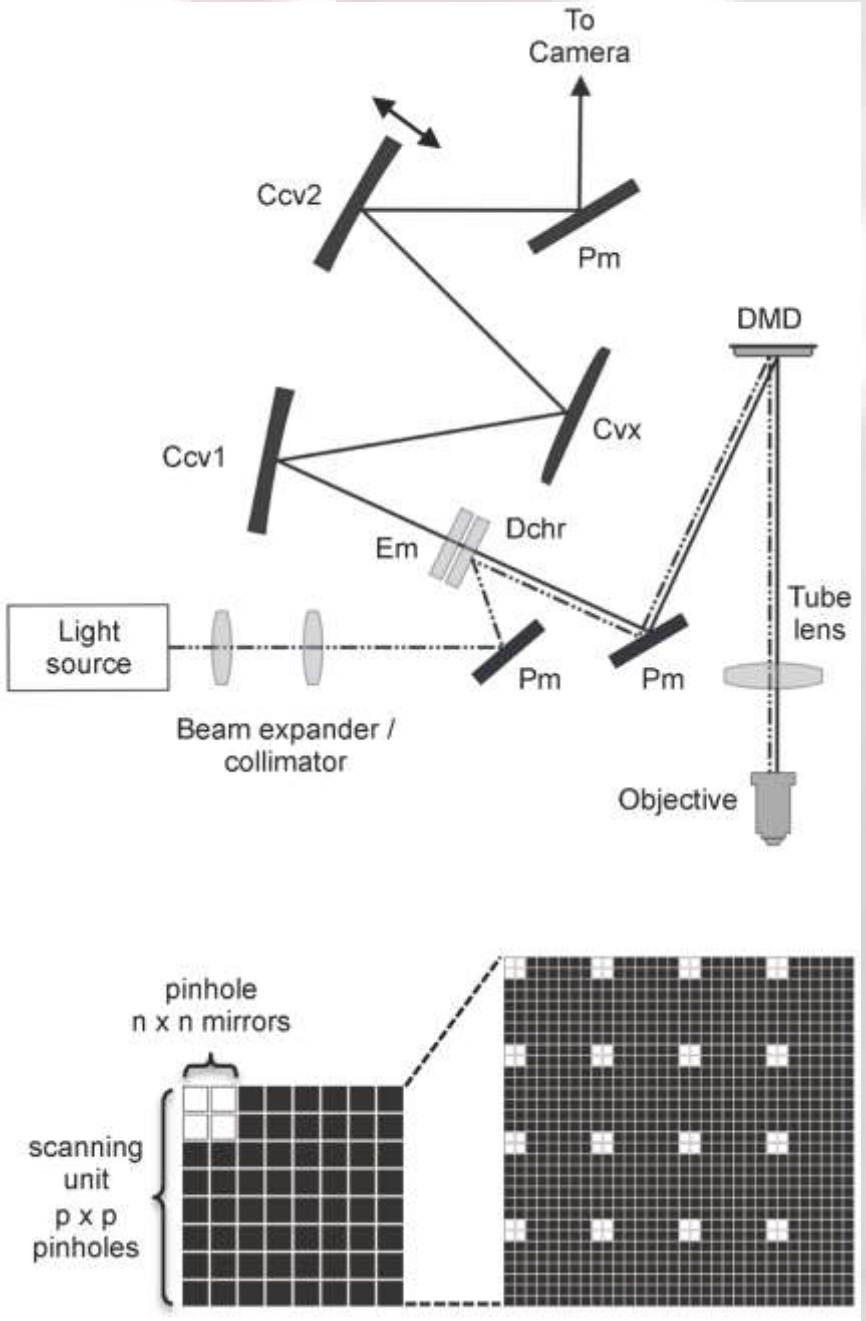


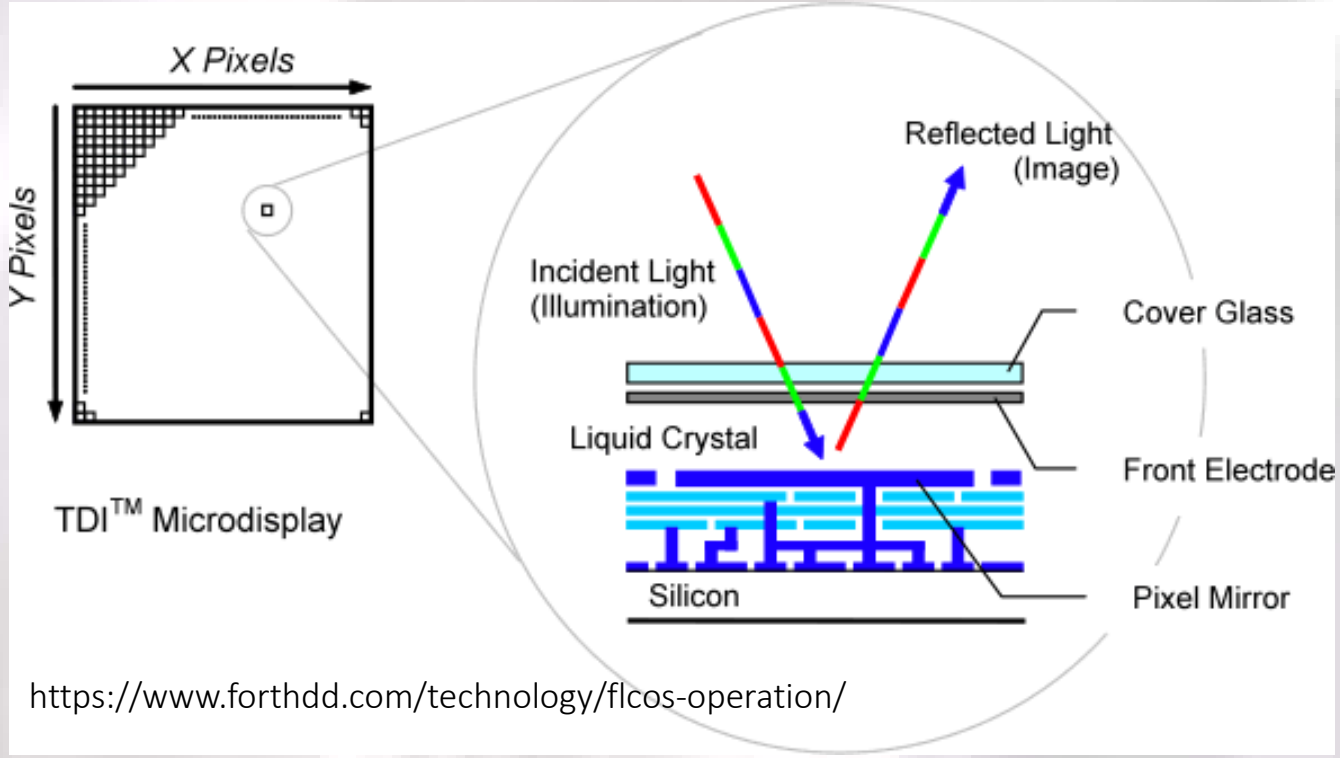
SCANNING VOL. 38, 234–239 (2016)





DMD(Digital micromirror device)



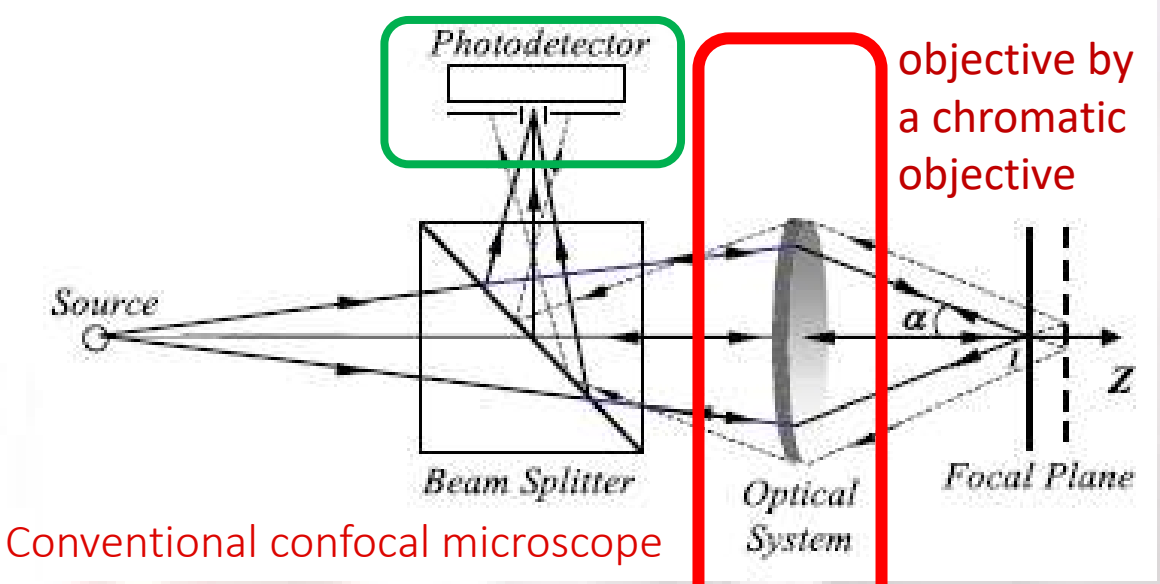


https://www.forthdd.com/technology/flcos-operation/

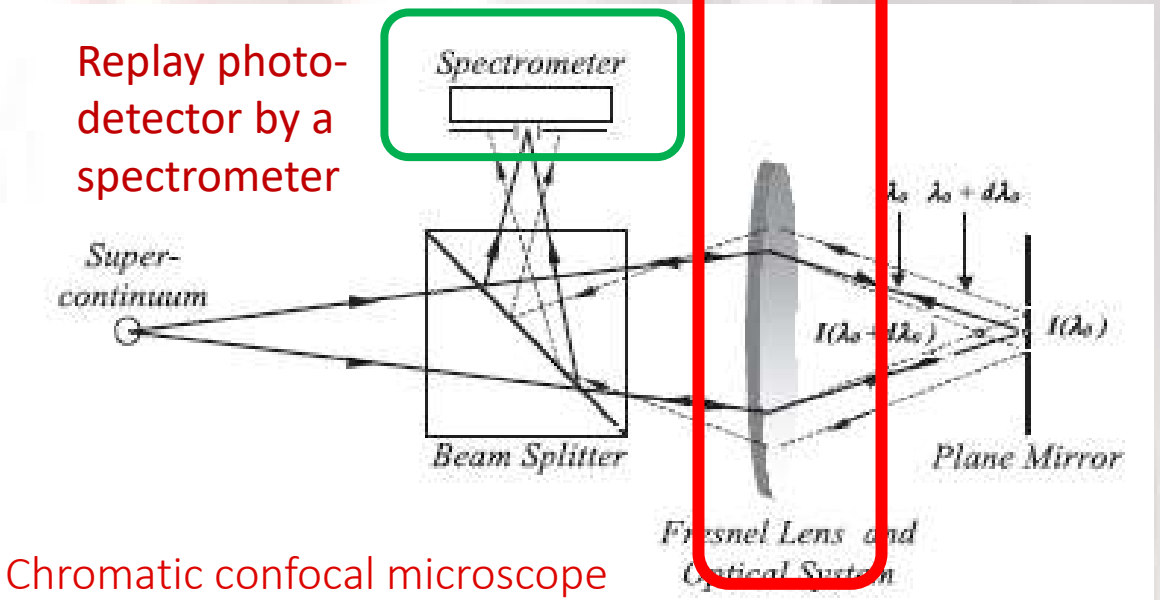
- Switching Speed of the ferroelectric liquid crystal is 40 μs
- FLCos device
  - —CMOS substrate/ individual pixels as mirror
  - —Cover front glass coted with a transparent electrode
  - —middle layer is the ferroelectric liquid crystal
- On/Off by switching polarization angle:
  zero angle polarization state is manufactured as the black point of the device, while the 90º polarization state is the white point

https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0043942.g001

Chromatic (dispersion) confocal microscopy (CDCM)



Conventional confocal microscope



Chromatic confocal microscope

doi:10.1088/1464-4258/10/10/104028

Lateral Scanning Mechanism

- Laser Scanning Confocal Microscope
- Disc Scanning Confocal Microscope
- Programmable Array Scanning Confocal Microscope

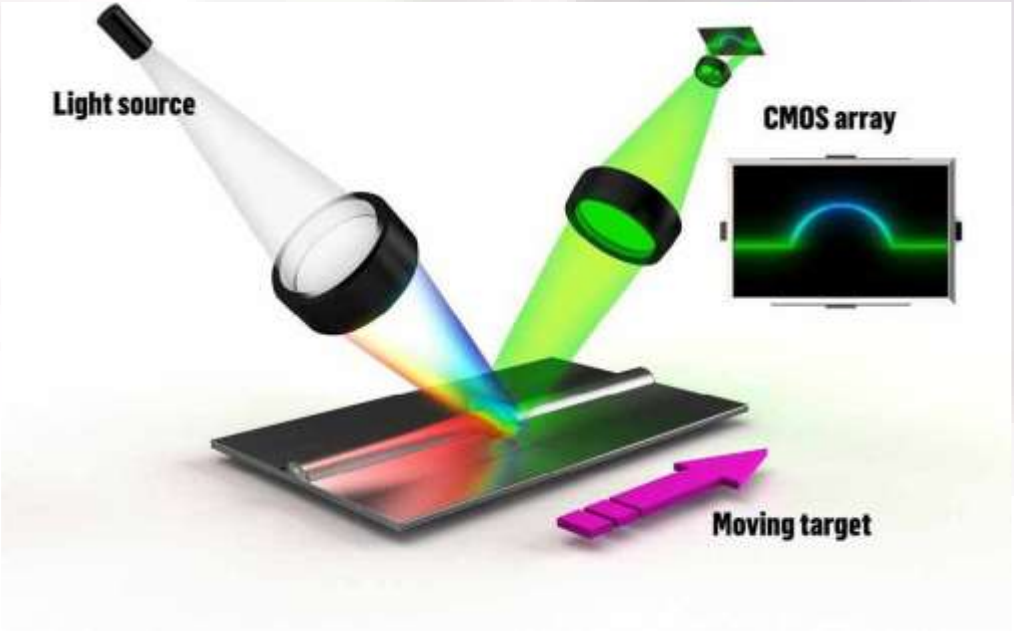
Vertical Scanning—Optical Section

- Moving the Objective lens

Vertical Scanning is replaced by Chromatic Aberration → the optical head entirely static, without any spurious vibration generated by an internal mechanism

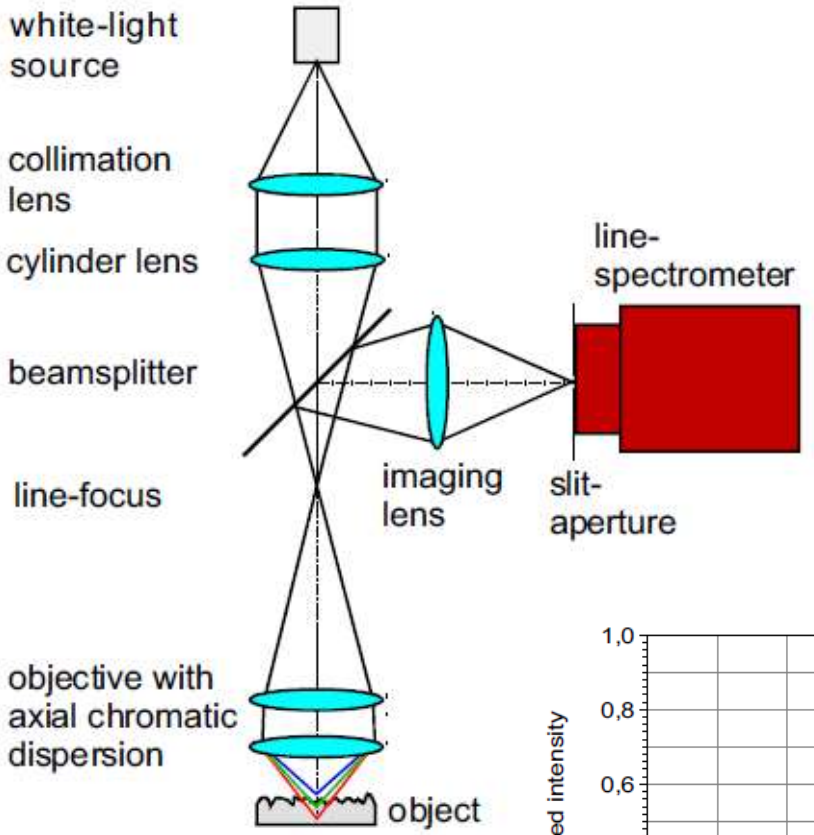
Recalling Chromatic Aberration, different wavelength would focus at different focal point along the optical axis

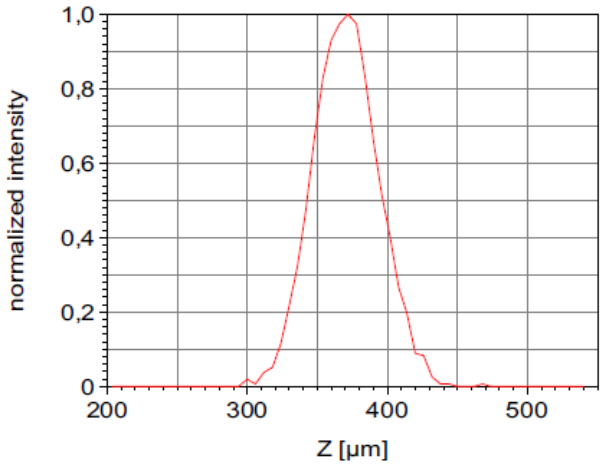
Line-type chromatic confocal sensors



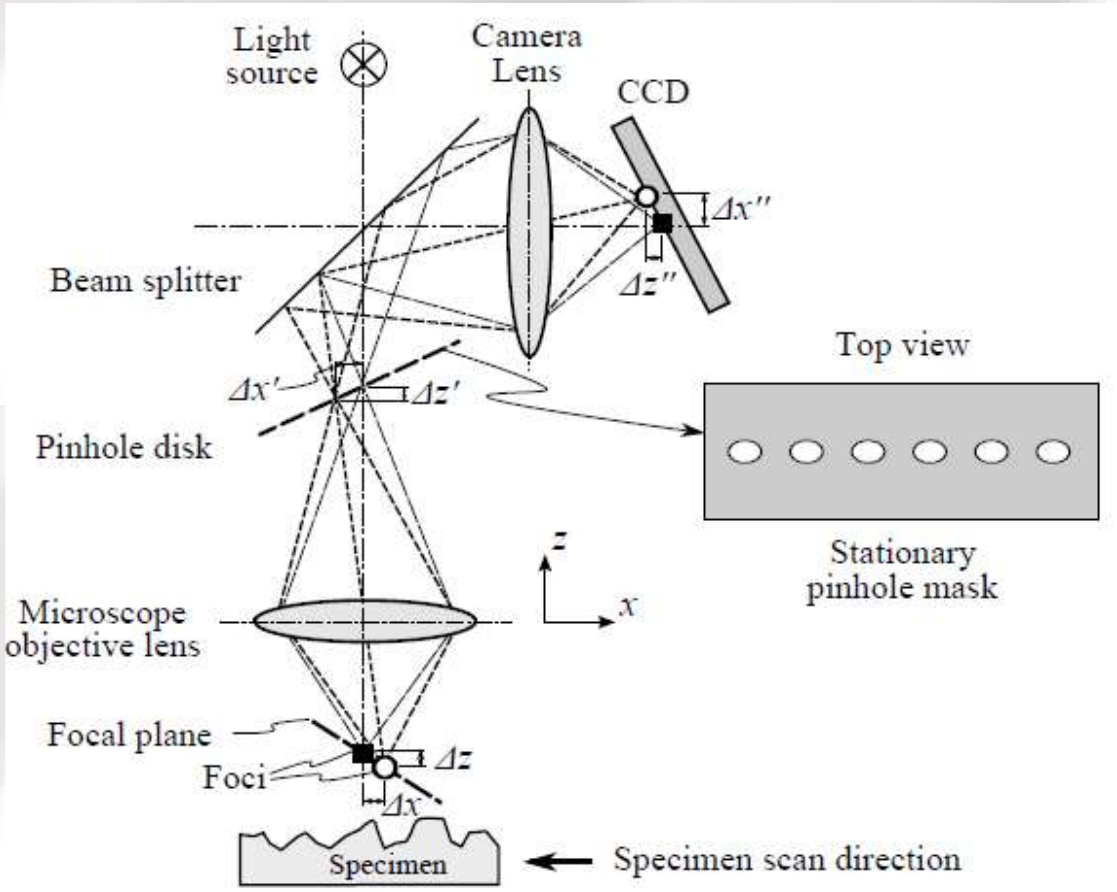
https://www.focalspec.com/wp-content/files/LCI_animation_14112018.mp4

Line-type chromatic confocal sensors



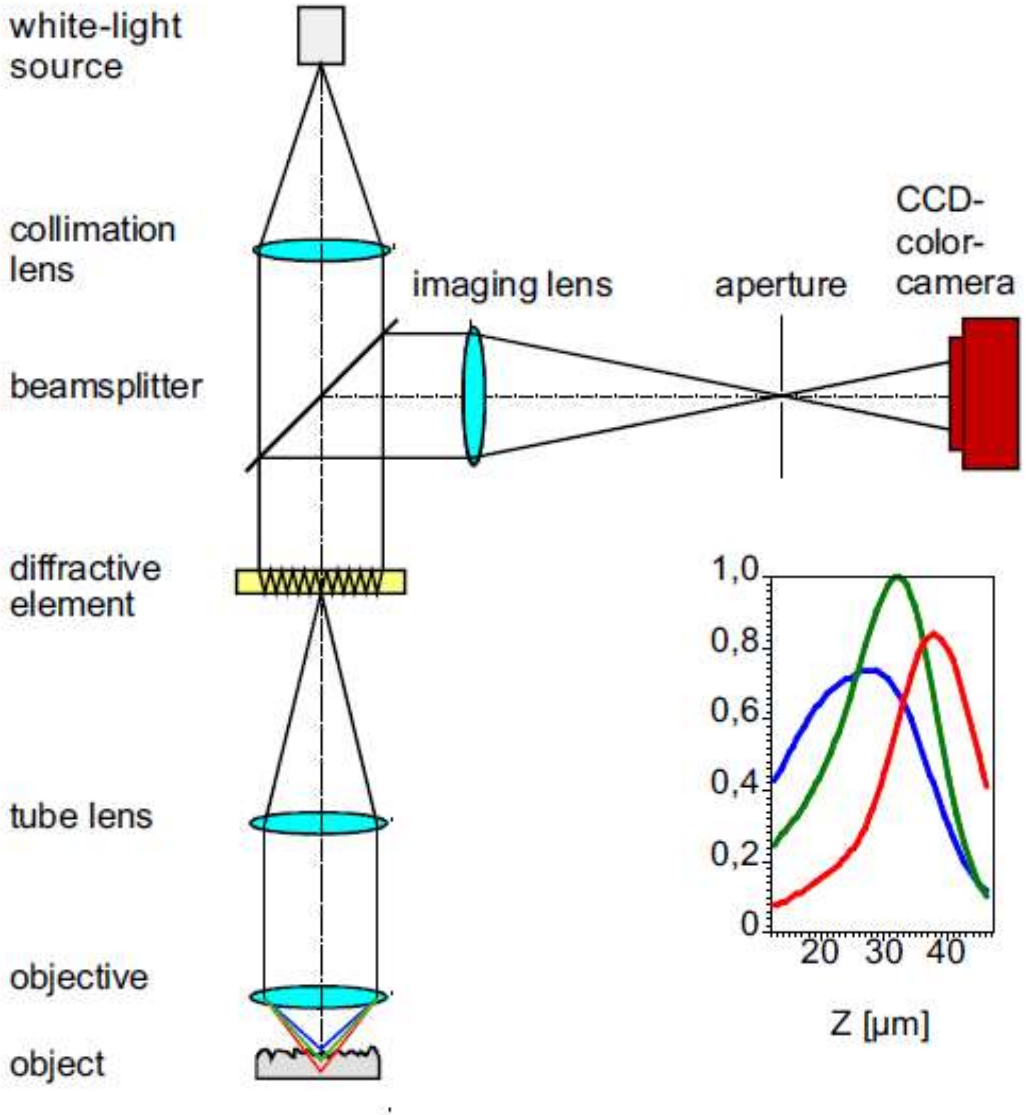


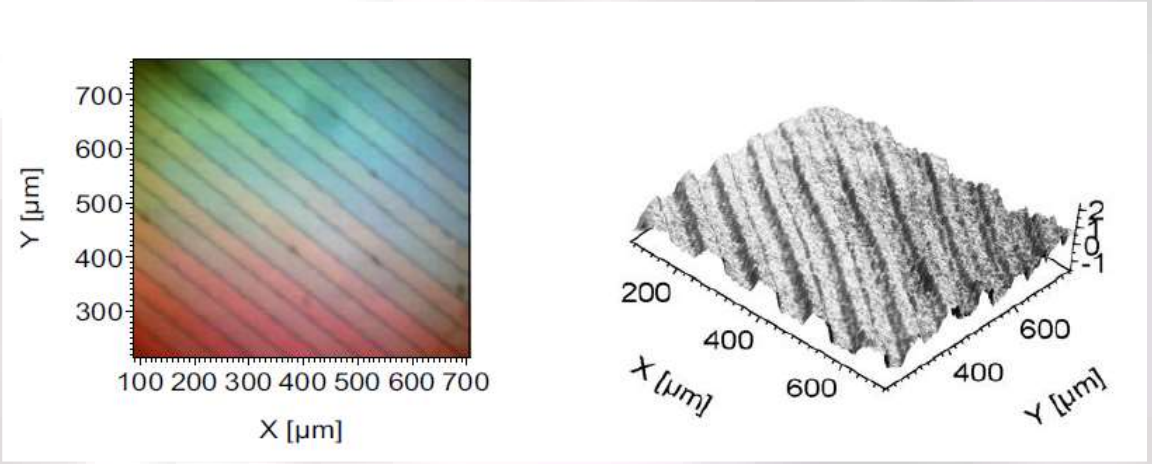
Chromatic confocal detection for high speed micro-topography measurements

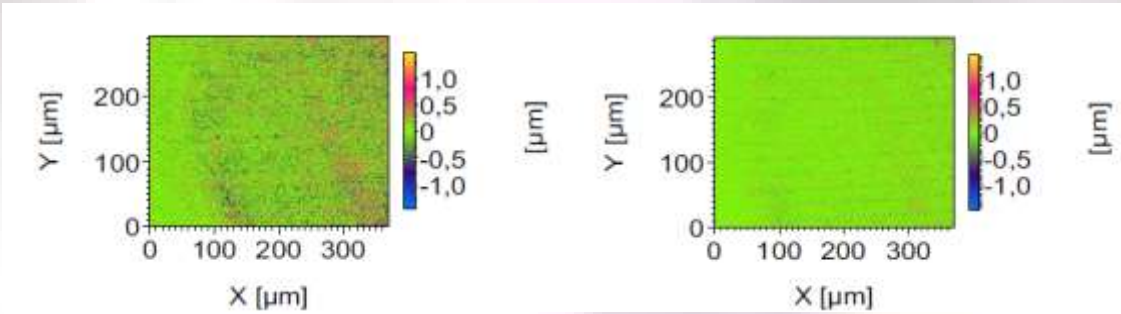


The tilted focal plane is formed from the tilted pinhole mask by an objective lens. To create a parallel depth scanning scheme, the specimen is scanned through the stationary stack of diffraction foci in a stepwise fashion or in a continuous approach

Area Type Chromatic Confocal Microscope







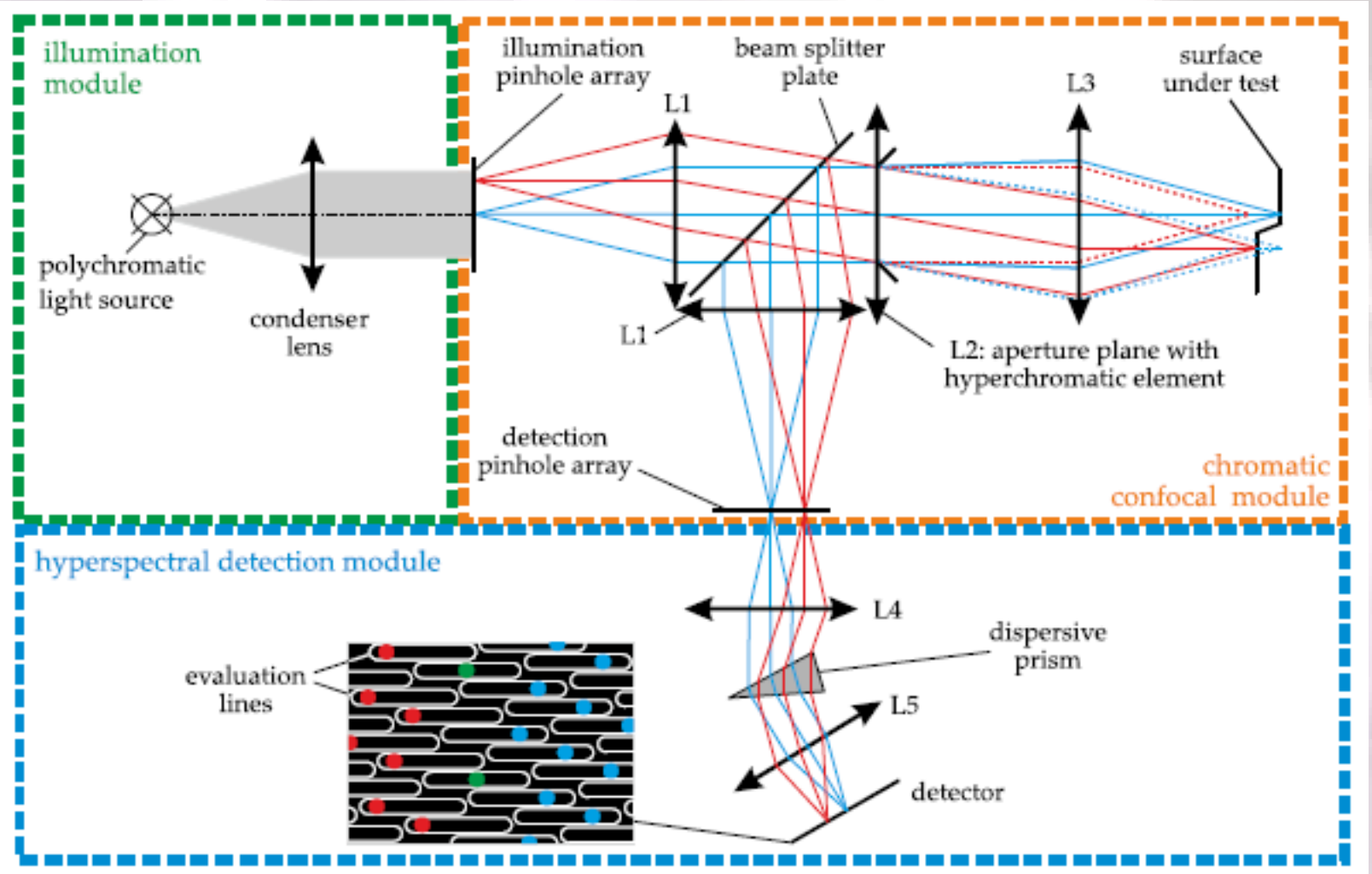
One Shot

Area Method

Chromatic confocal detection for high speed micro-topography measurements

Chromatic confocal matrix sensor

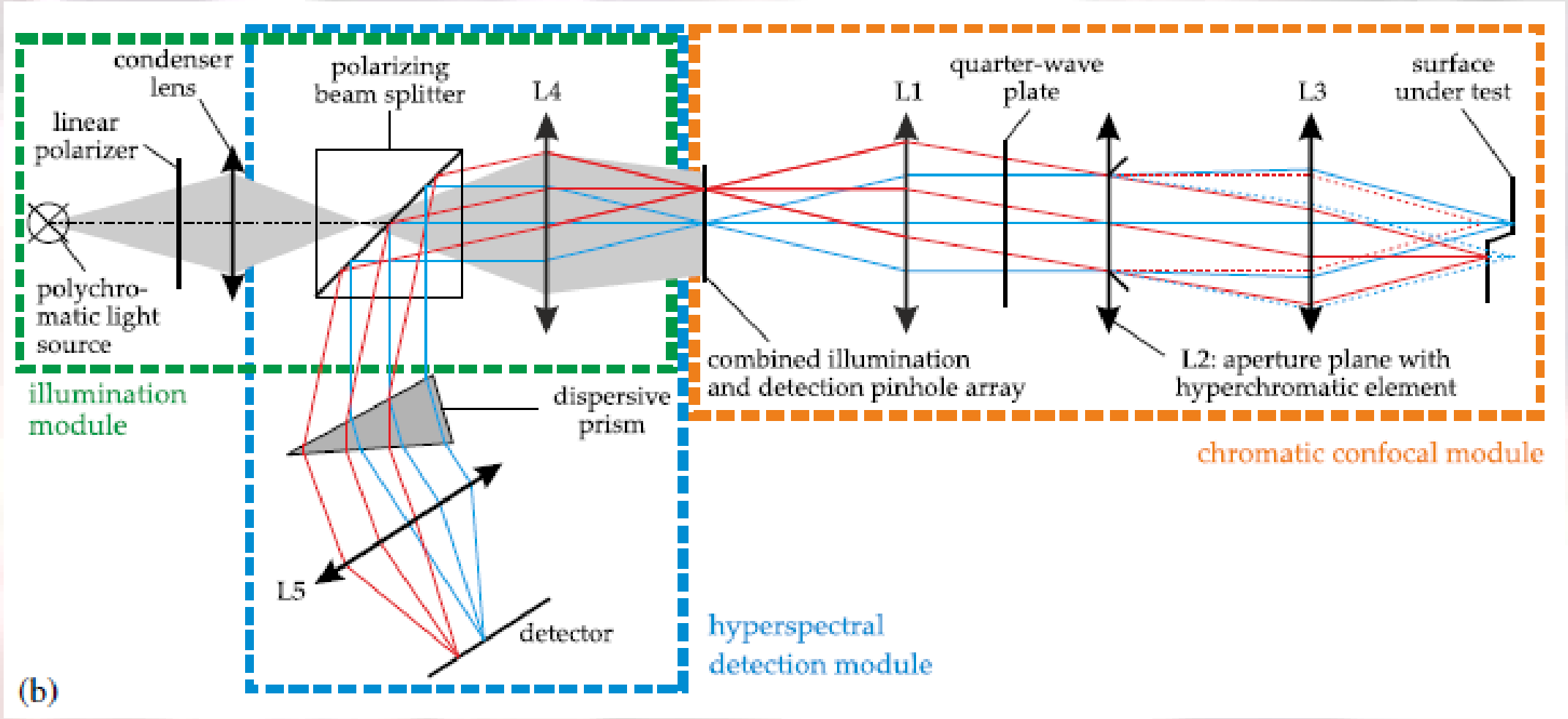
Separate illumination and detection pinholes

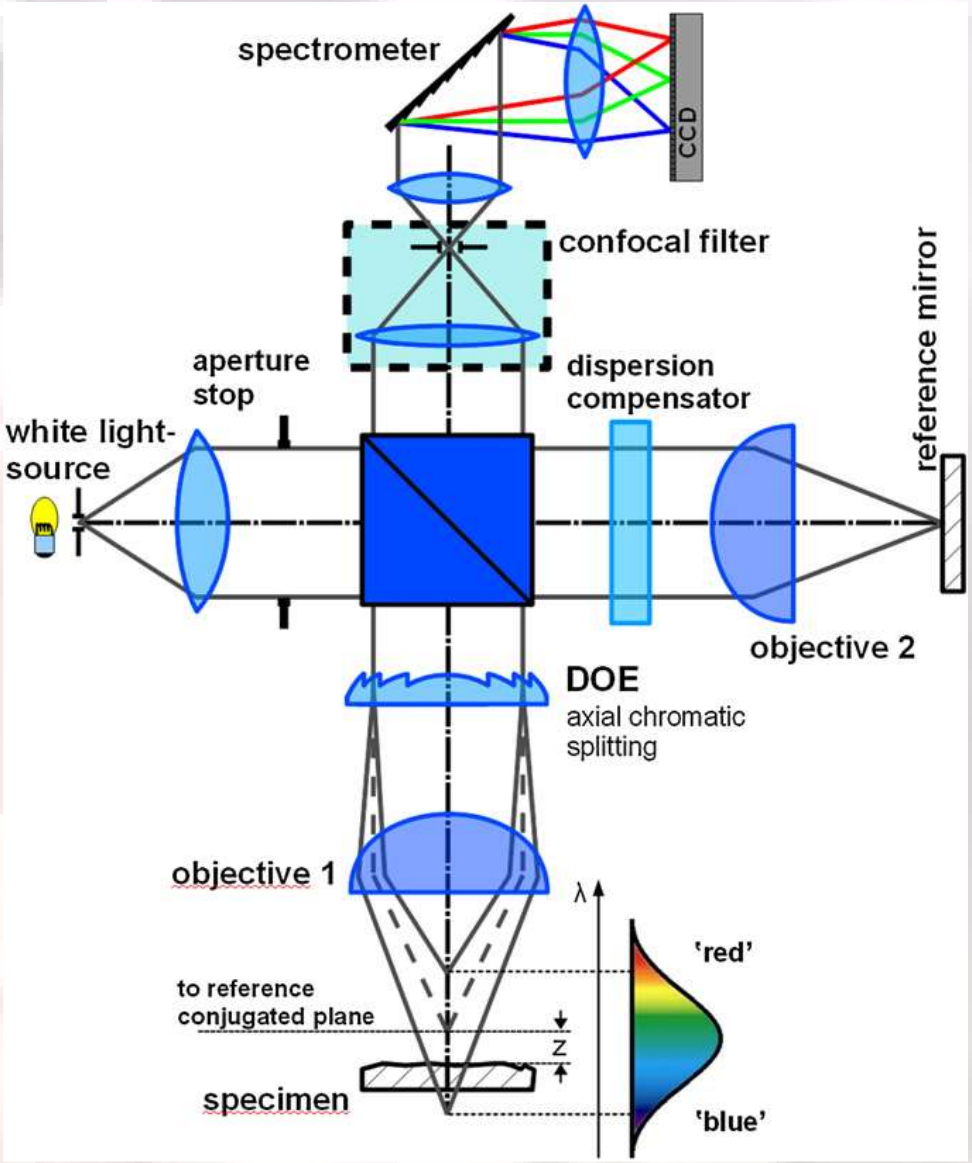


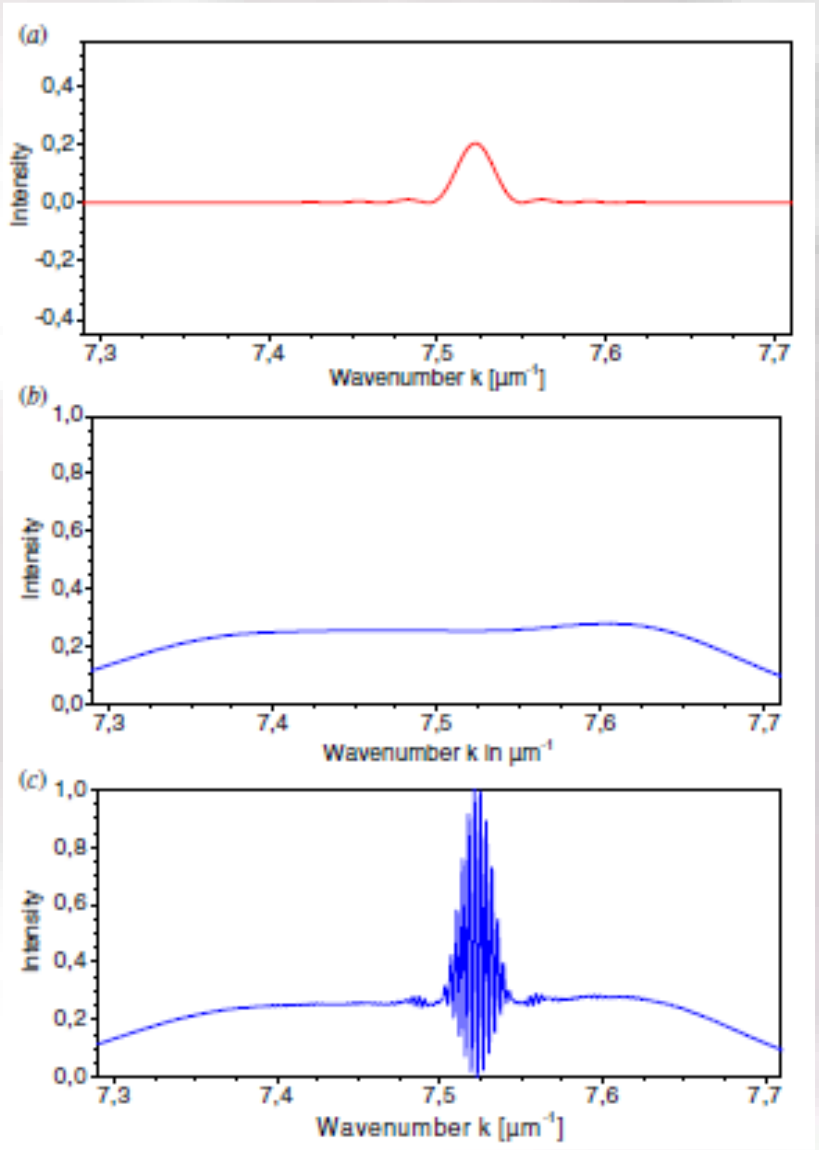
http://dx.doi.org/10.1364/AO.53.007634
http://dx.doi.org/10.1364/AO.54.004927

Chromatic confocal matrix sensor

Single pinhole array used in double pass

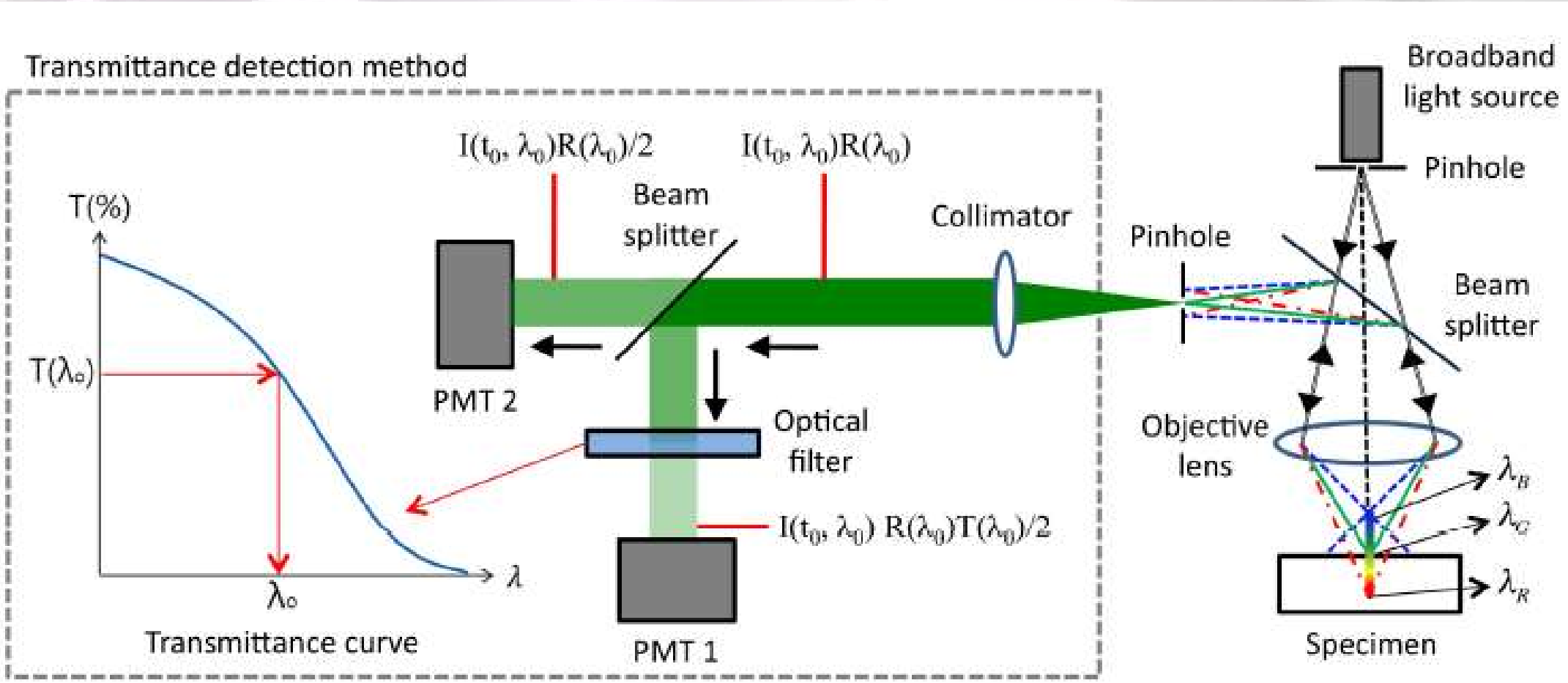






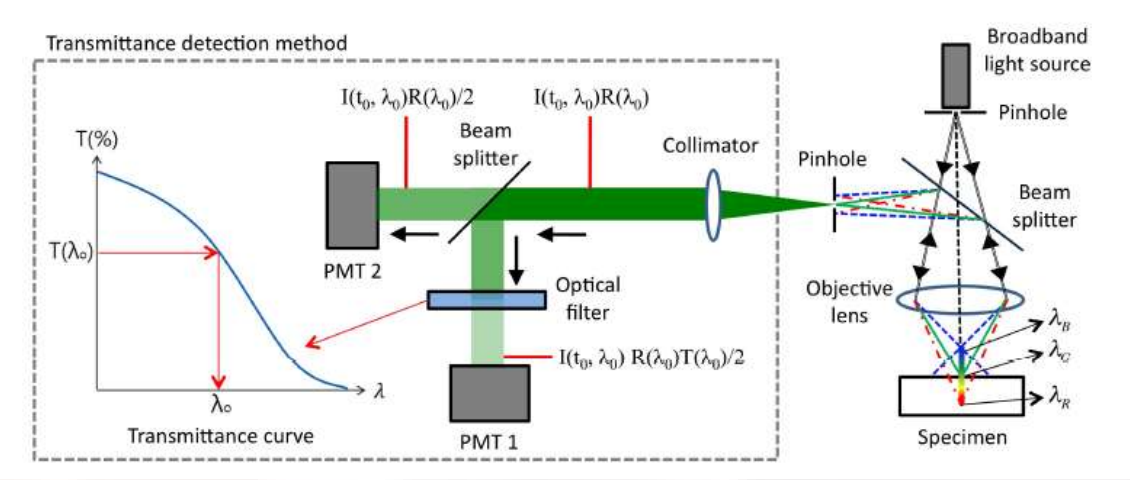
doi:10.1088/0957-0233/23/5/054009

Dual Sensors

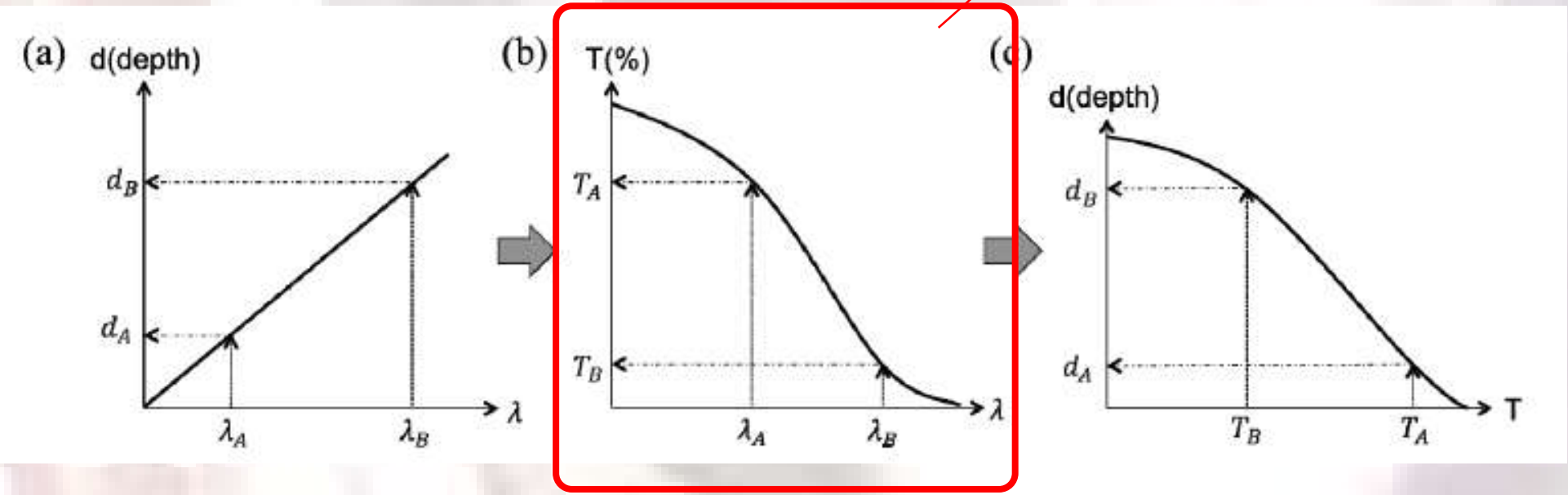


https://doi.org/10.1364/OE.21.006286

Dual Sensors

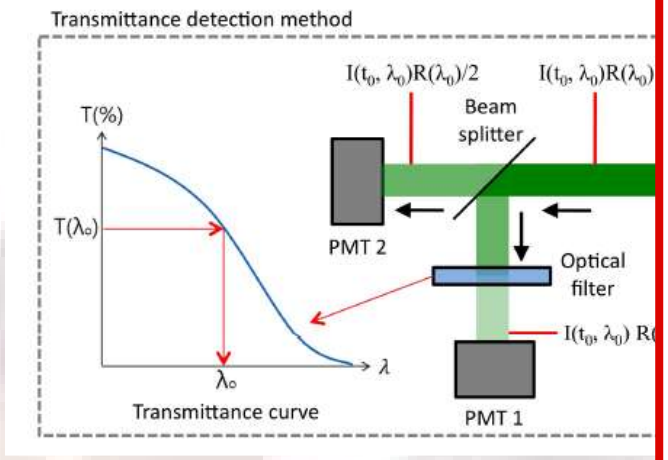


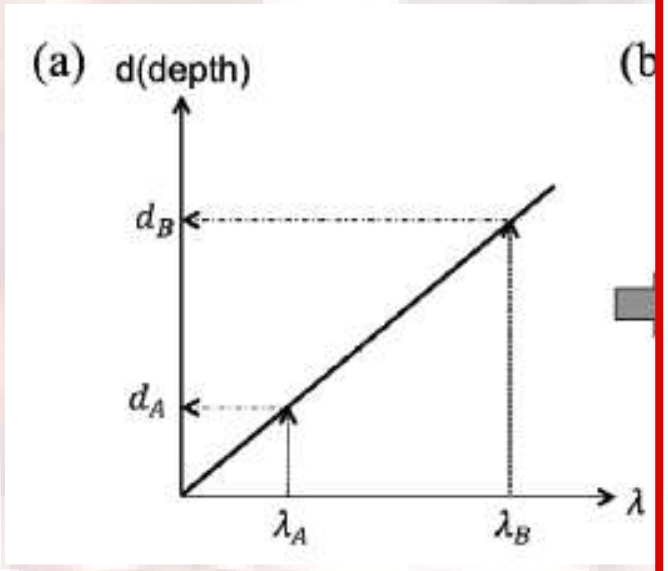
Determined by Ratio of Light Intensity
Detected at PMT1 and PMT2
In front of PMT1, there is a optical filter placed
In front of the sensor

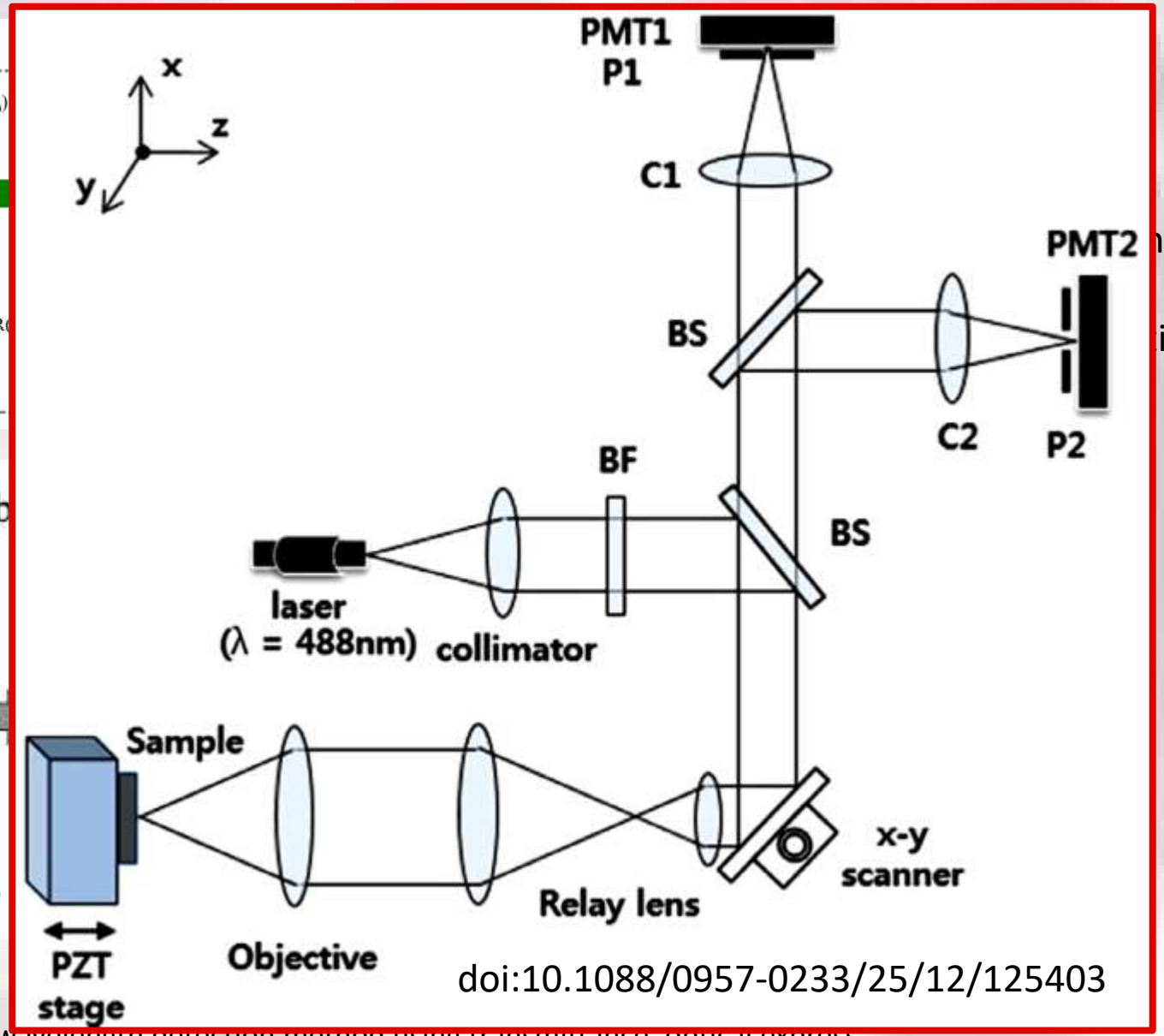


Chromatic confocal microscopy with a novel wavelength detection method using transmittance, optical express

Dual Sensors



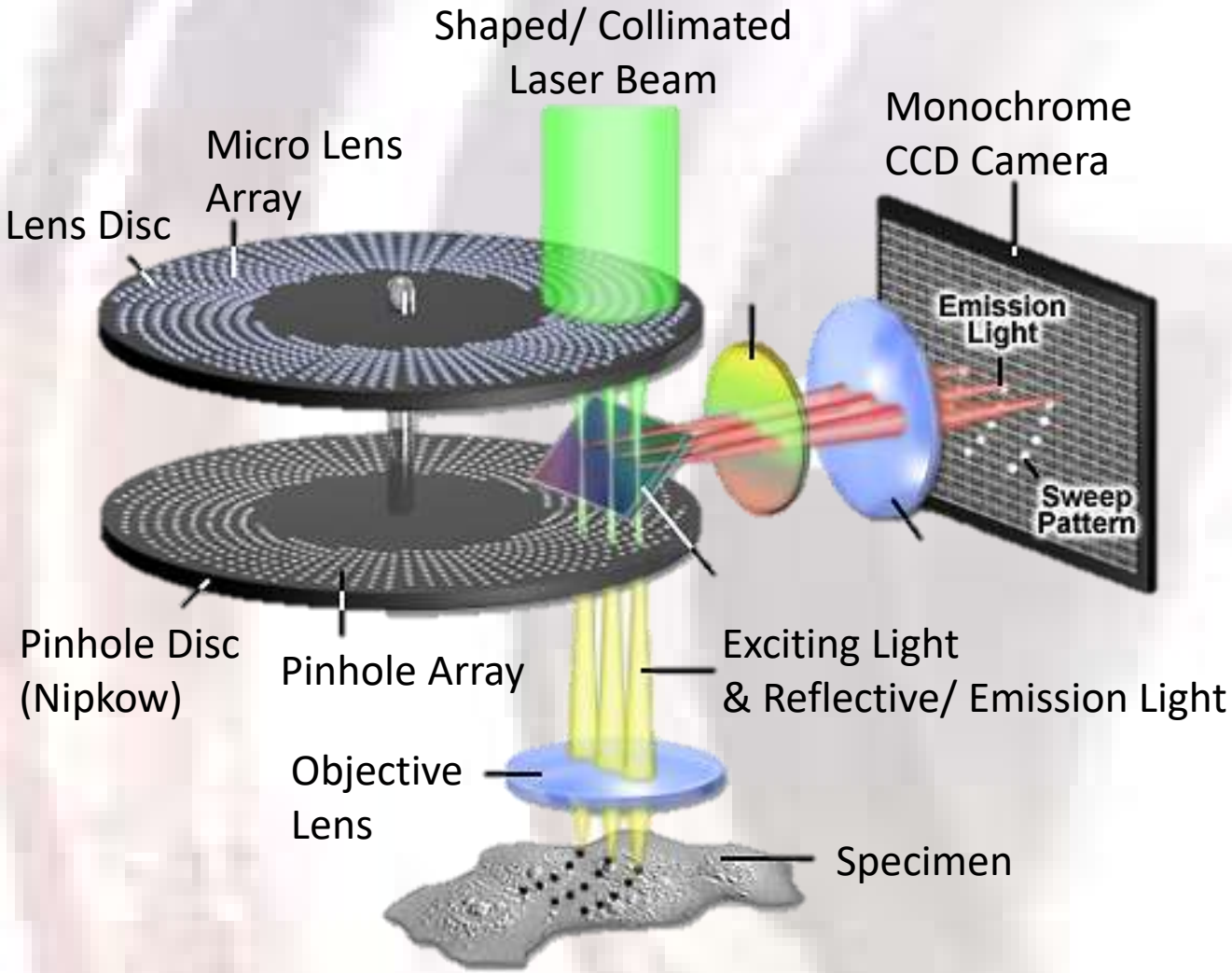




ıtensity

ical filter placed

Chromatic confocal microscopy with a novel wavelength detection method using transmittance, optical express


Shaped/ Collimated
Laser Beam
Micro Lens
Array
Lens Disc
Monochrome
CCD Camera
Emission
Light
Sweep
Pattern
Pinhole Disc
(Nipkow)
Pinhole Array
Exciting Light
& Reflective/ Emission Light
Objective
Lens
Specimen