Charles Chevalier's Horizontal Microscope (circa 1834)

neuro technology studio

Visual Microscopy Workshop Series

Principles of Confocal Microscopy

Lai Ding
BWH NeuroTechnology Studio

Theory defines the limit

Resolution (r) = 0.61 λ /N.A.

Lateral resolution

Resolution(z) = 2 λ • n / N.A.^{2}

Axial Resolution

Resolution

Objective/ N.A.	x-y	z
10x/0.3	1000nm	11.4um
25x/0.8	400nm	2.4um
40x/1.3	230nm	0.9um
63x/1.4	214nm	0.8um
100x/1.4	214nm	0.8um

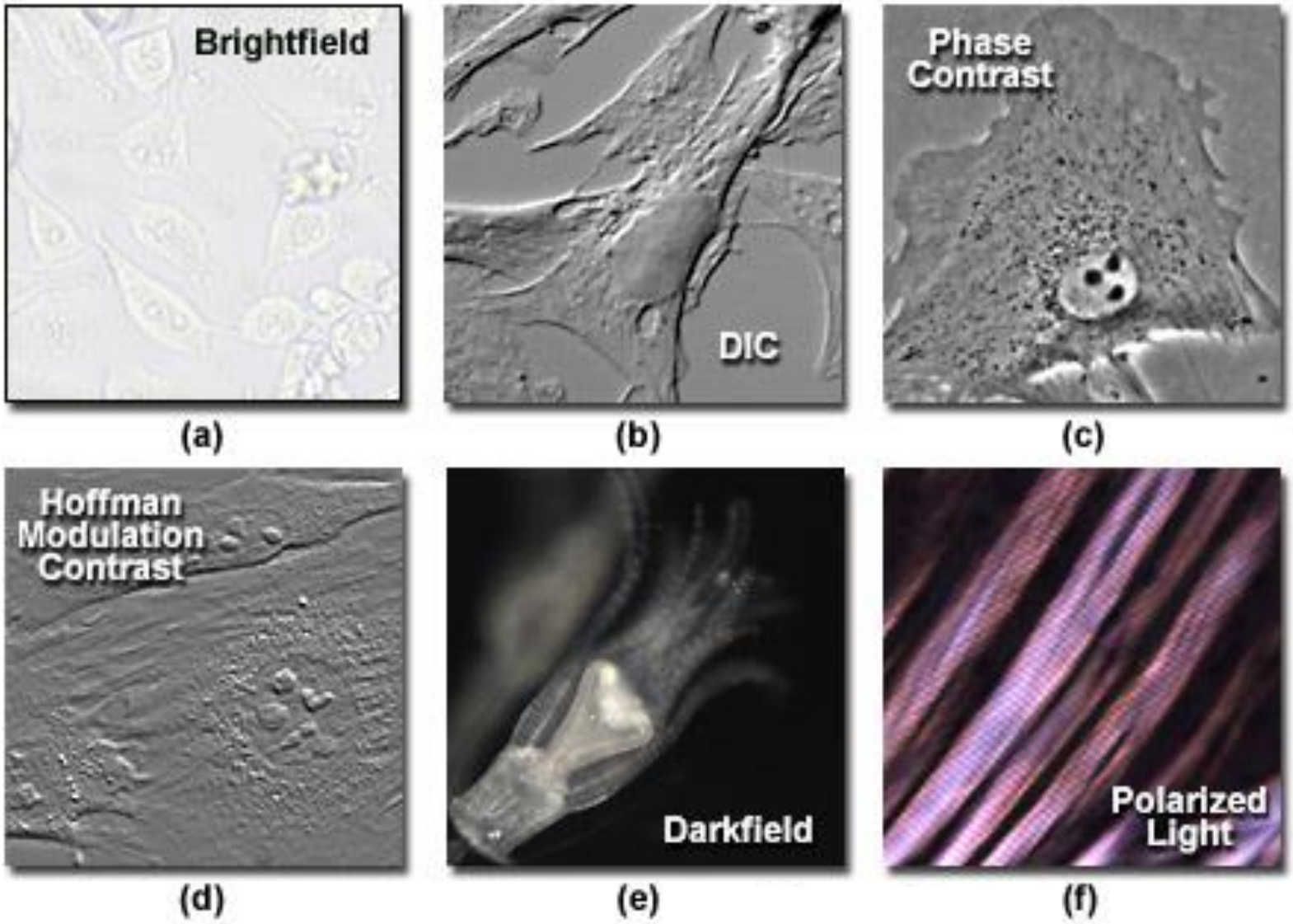
$R_{x-y}=0.61\ \lambda / N.A$

$R_z=2\ \lambda \bullet n / N.A^2$

Mission Accomplished?

Contrast

Transmitted Light Techniques in Live-Cell Imaging

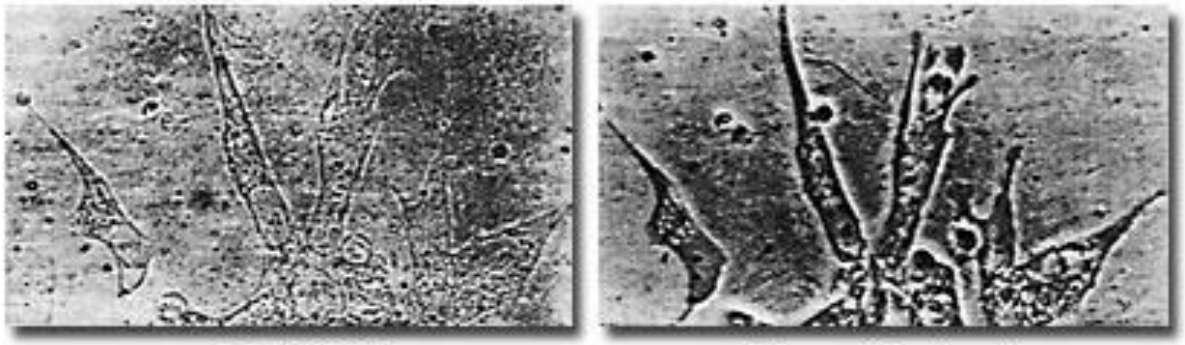


Frits Zernike
1888-1966

Nobel Prize in Physics 1953

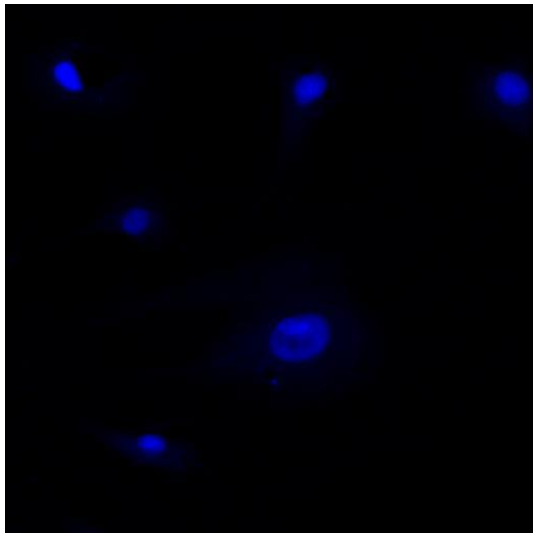
"for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope"

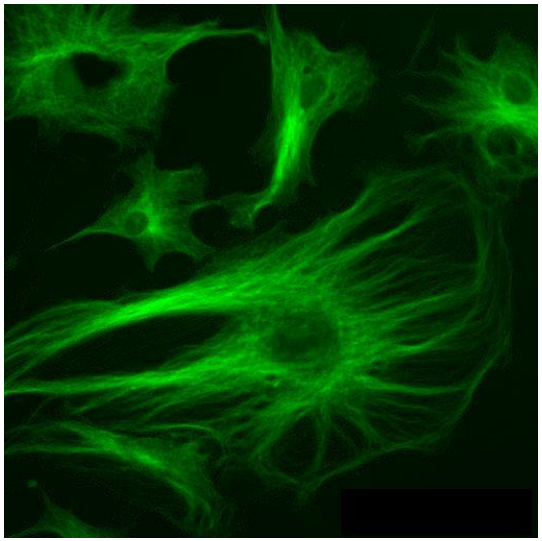
Original Phase Contrast Photomicrographs of Human Cells

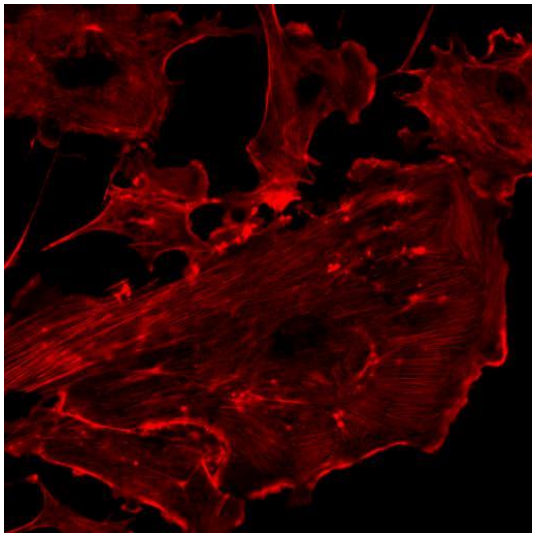


Brightfield **Phase Contrast**

FLUORESCENCE



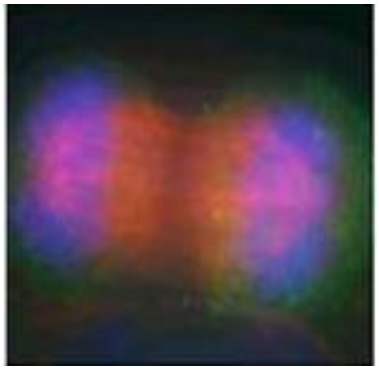
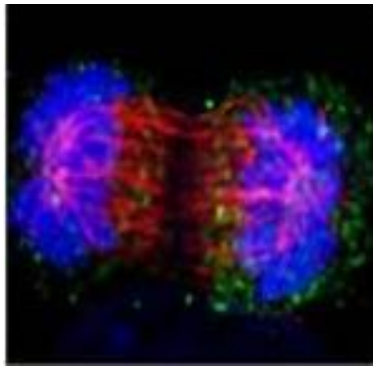


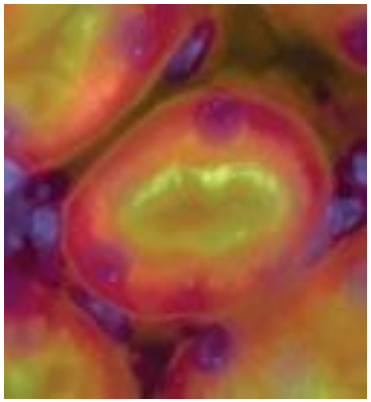
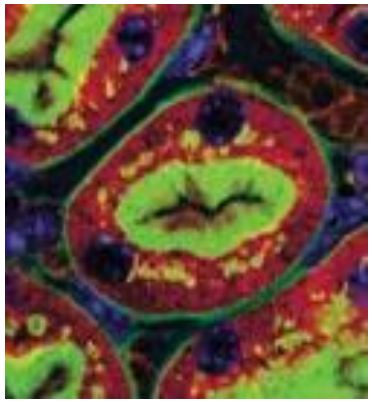
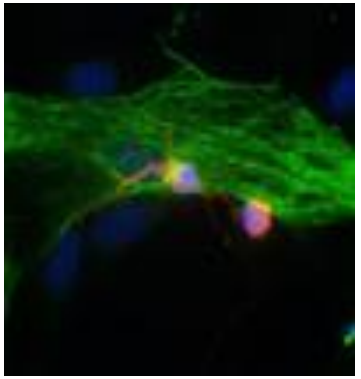
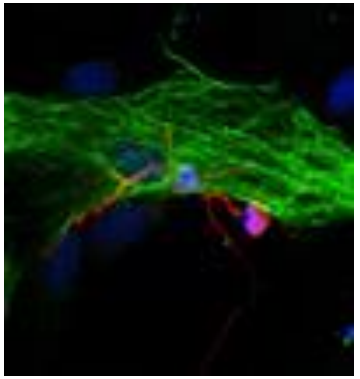


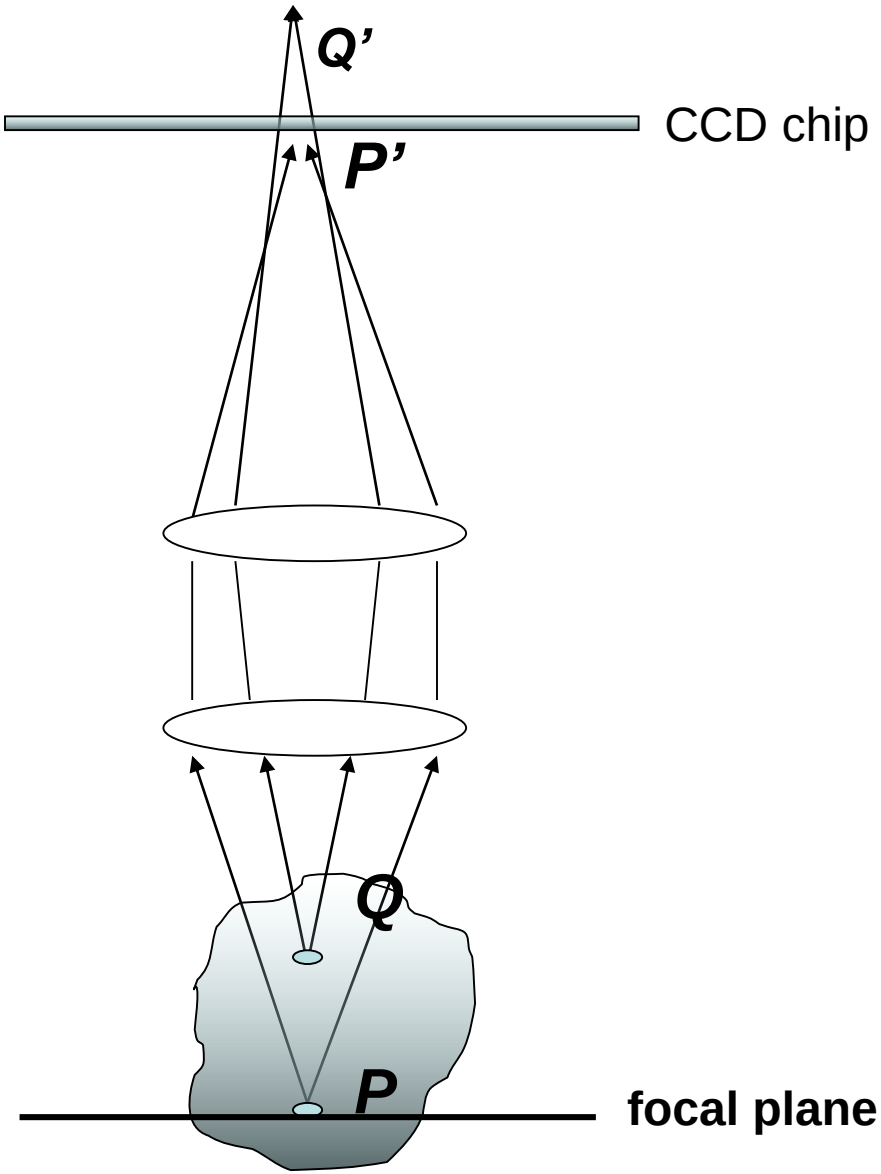
bovine pulmonary artery endothelial cells

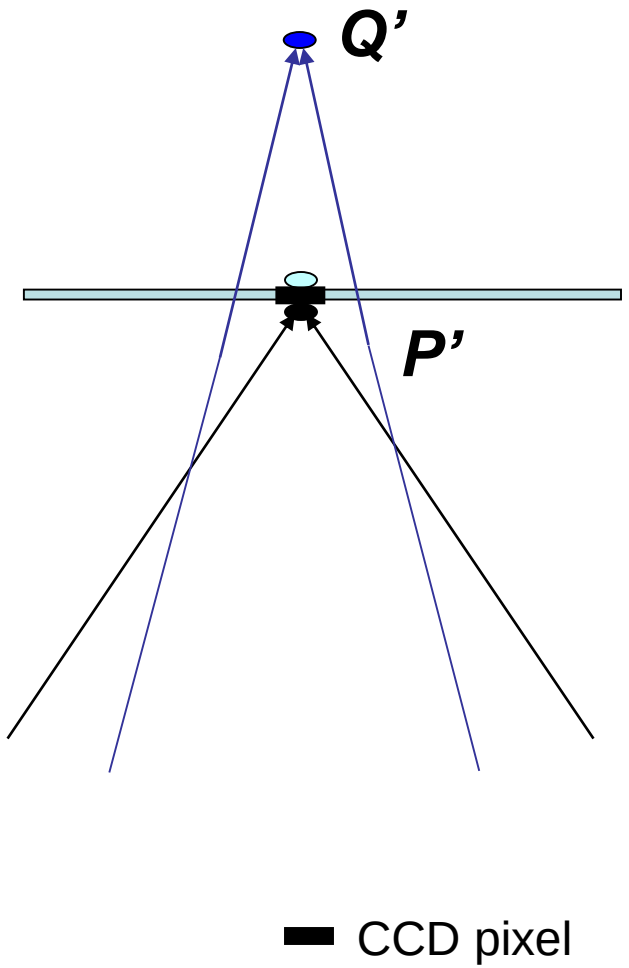
Nucleus	Tubulin	F-actin
DAPI	BODYPI	Texas-Red

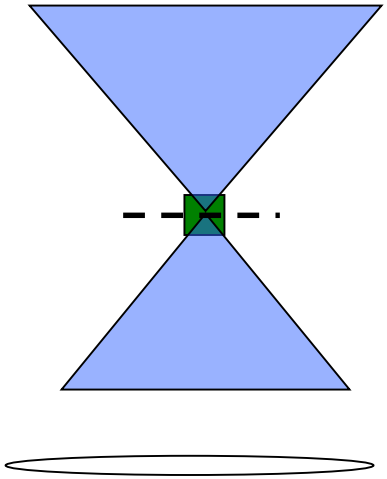
Mission Accomplished Now?

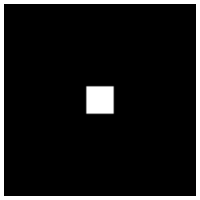



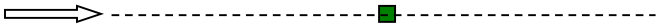







Q'
CCD chip
P'
Q
P
focal plane

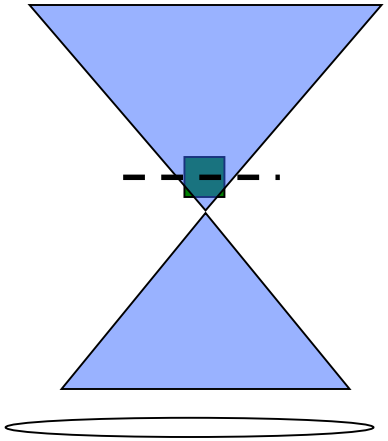

Q'
P'
CCD pixel







When sample move in z-direction



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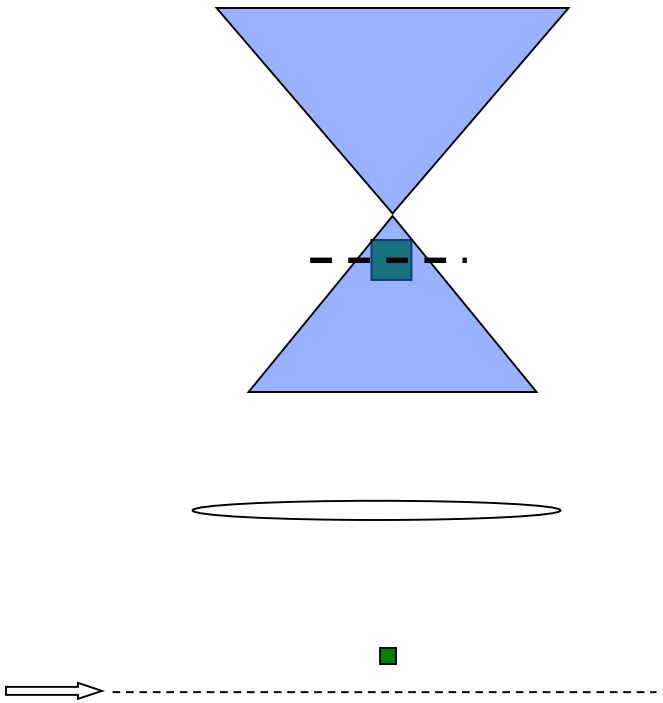
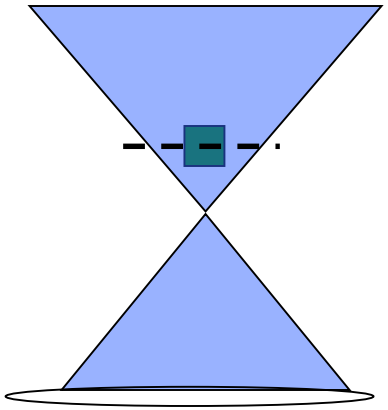
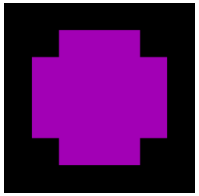
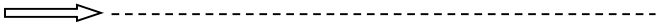


When sample move in z-direction







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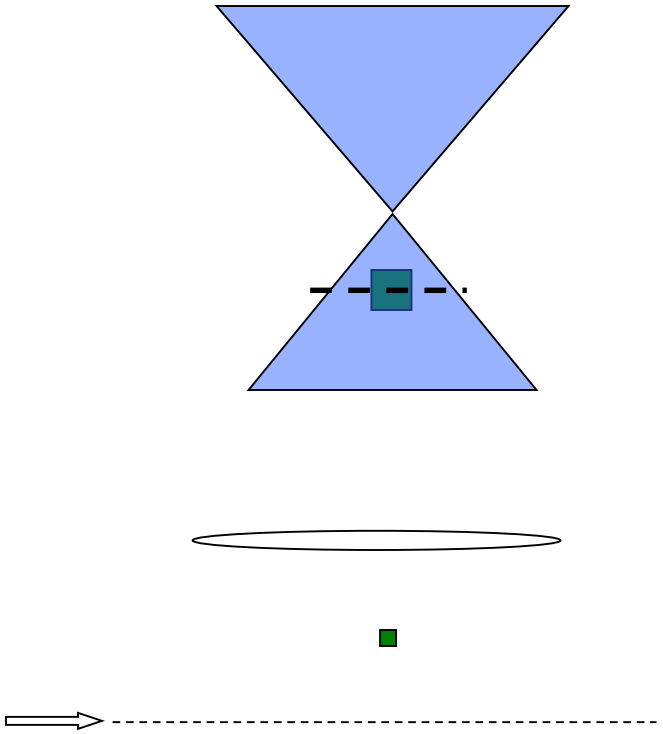
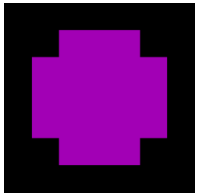
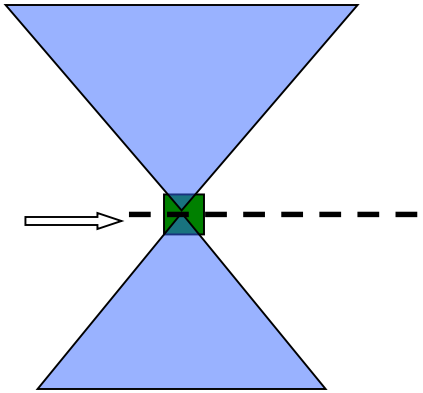
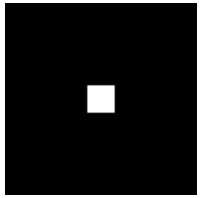
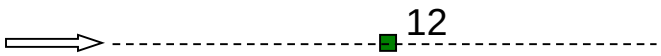




When sample move in z-direction

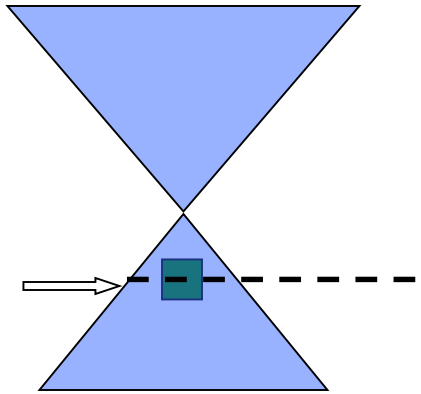


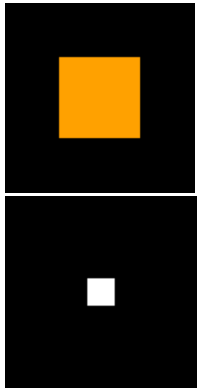


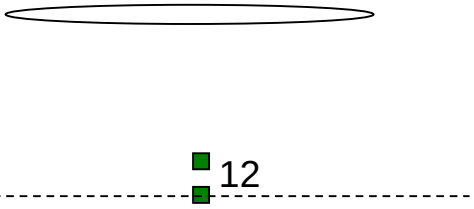


sample

Multiple fluorescence locations in z-direction

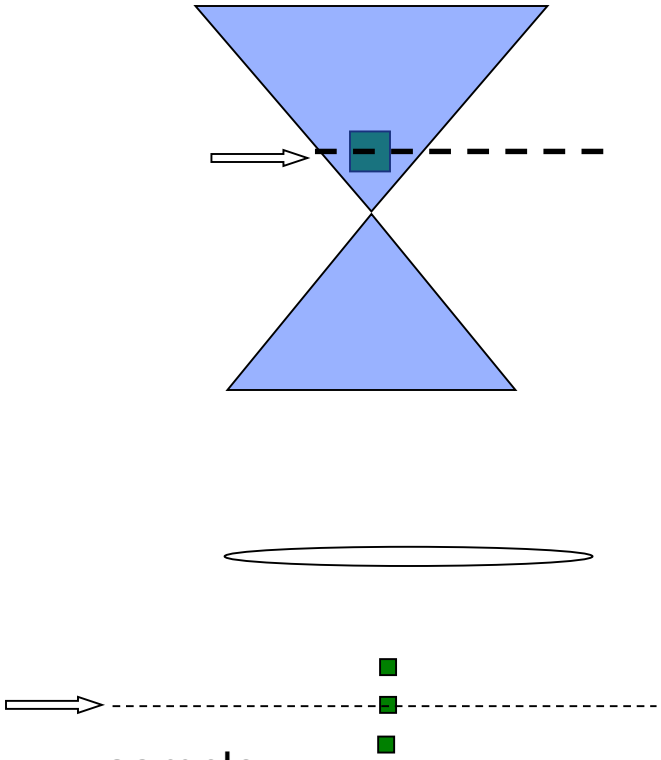


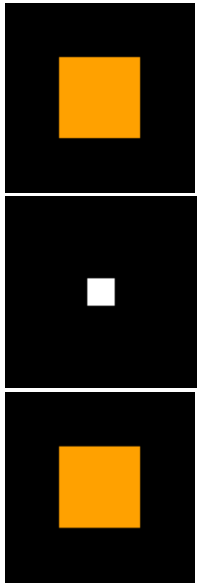


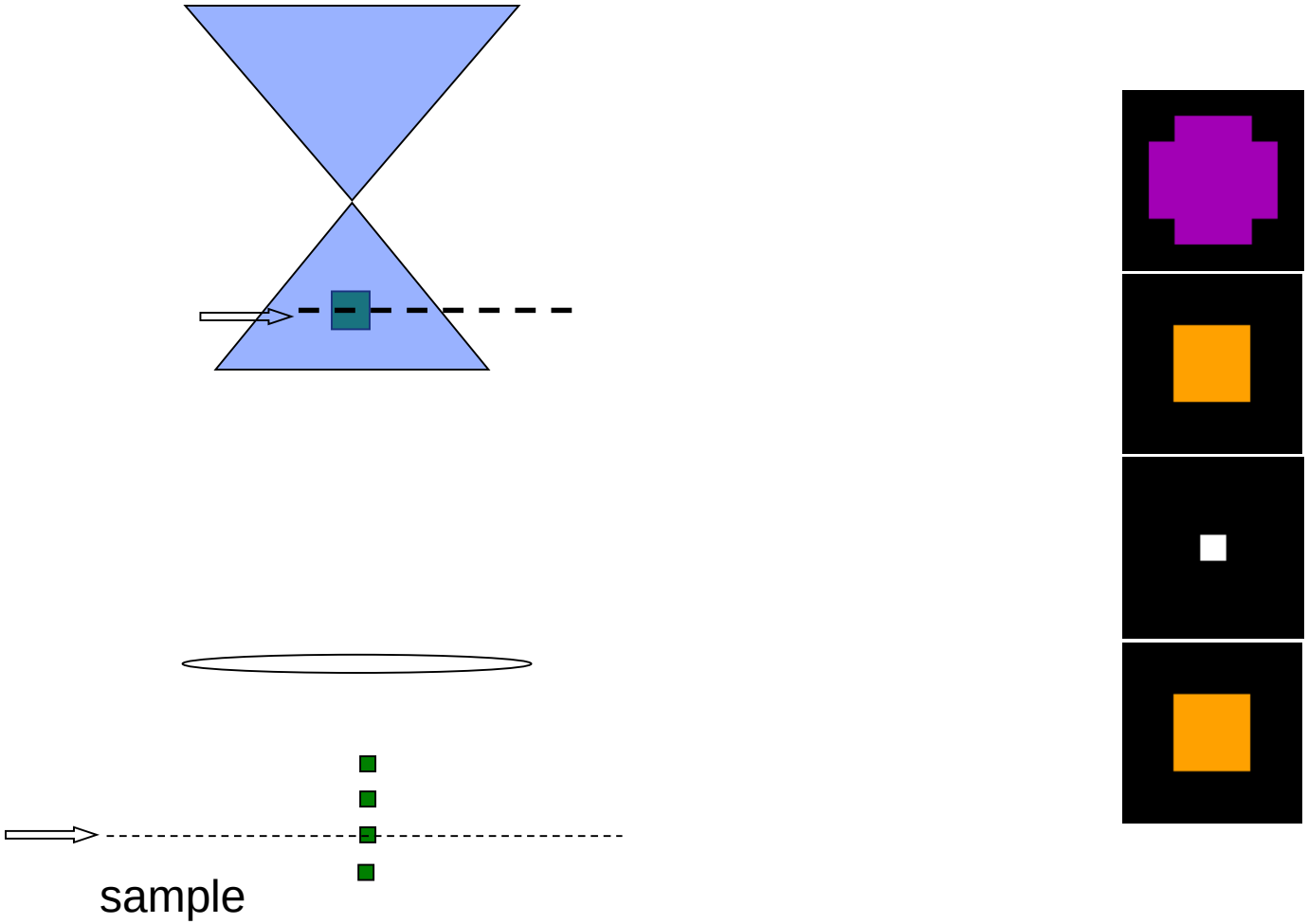


sample

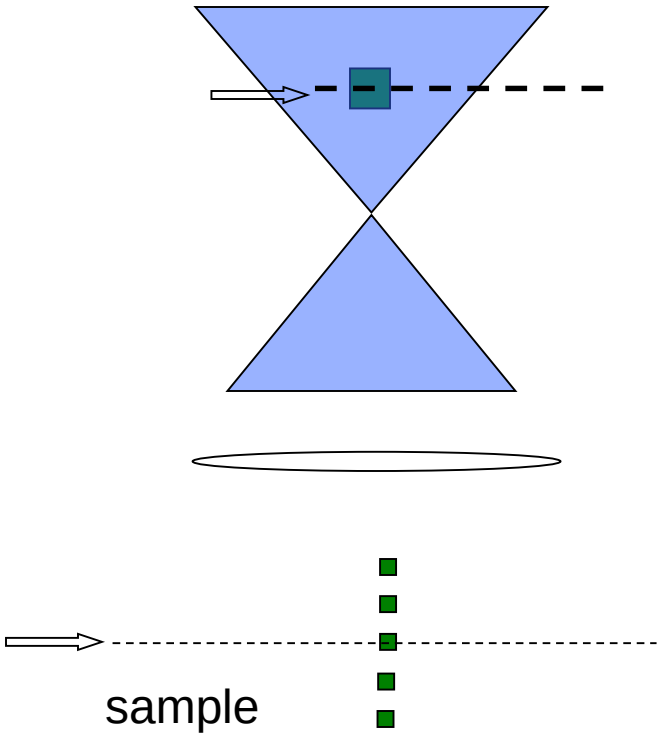
camera stays

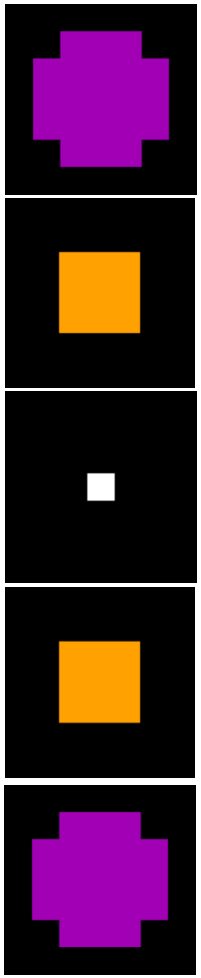

sample

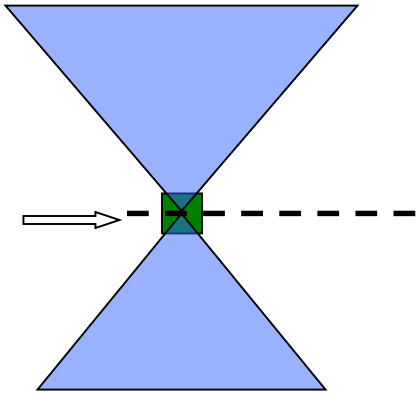


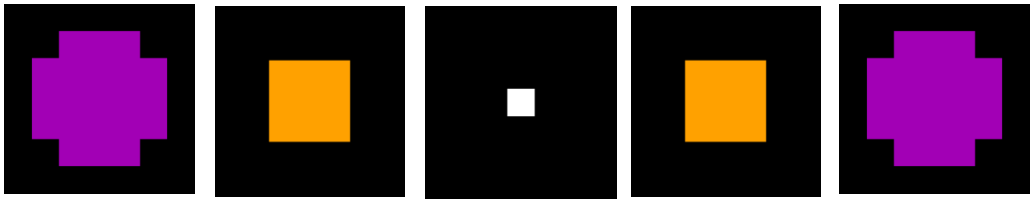


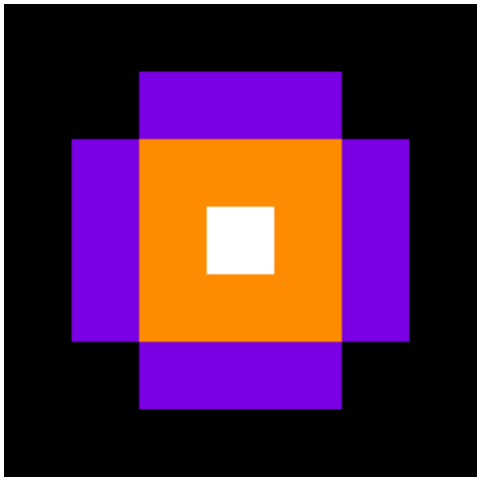
camera stays

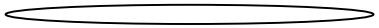

sample

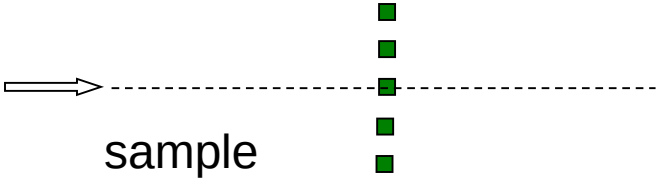








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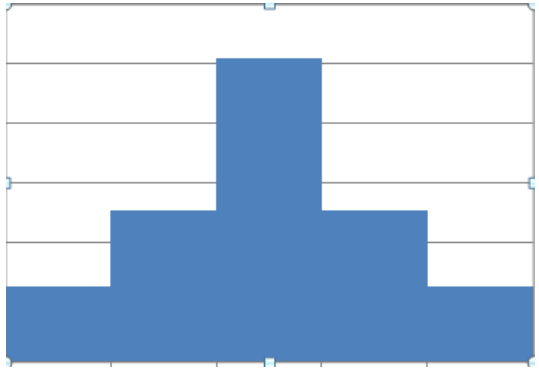
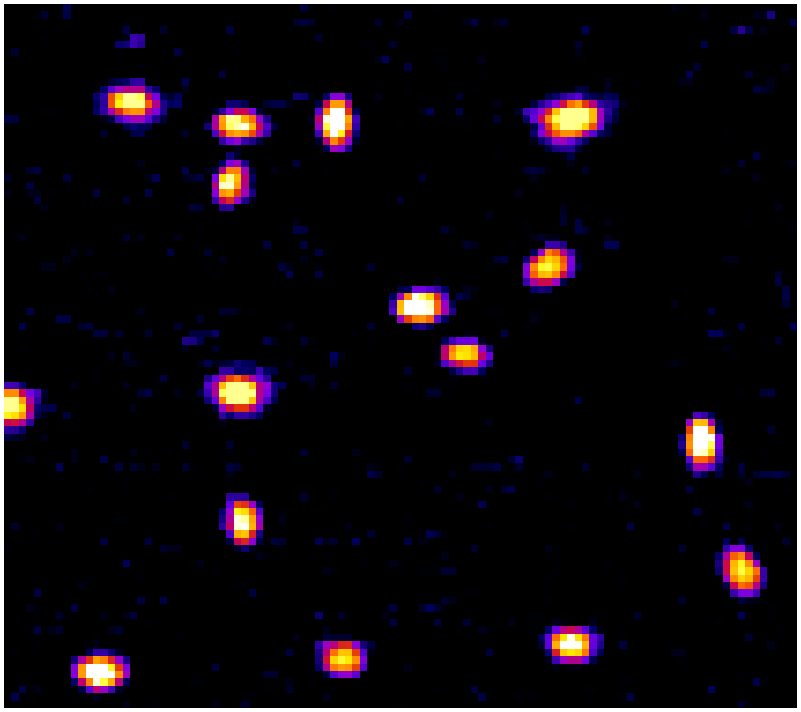
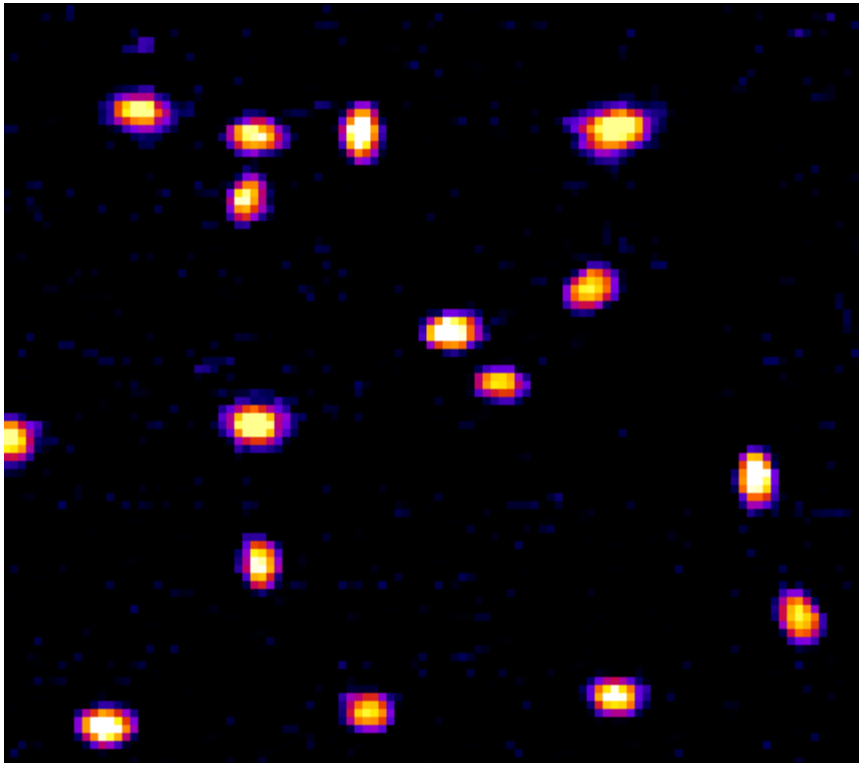
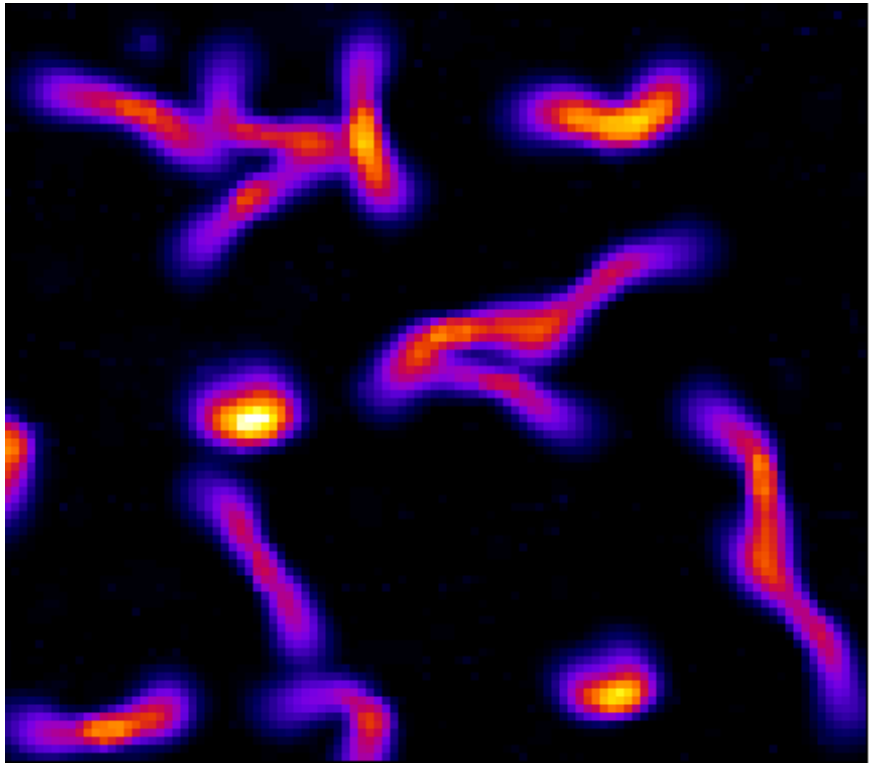
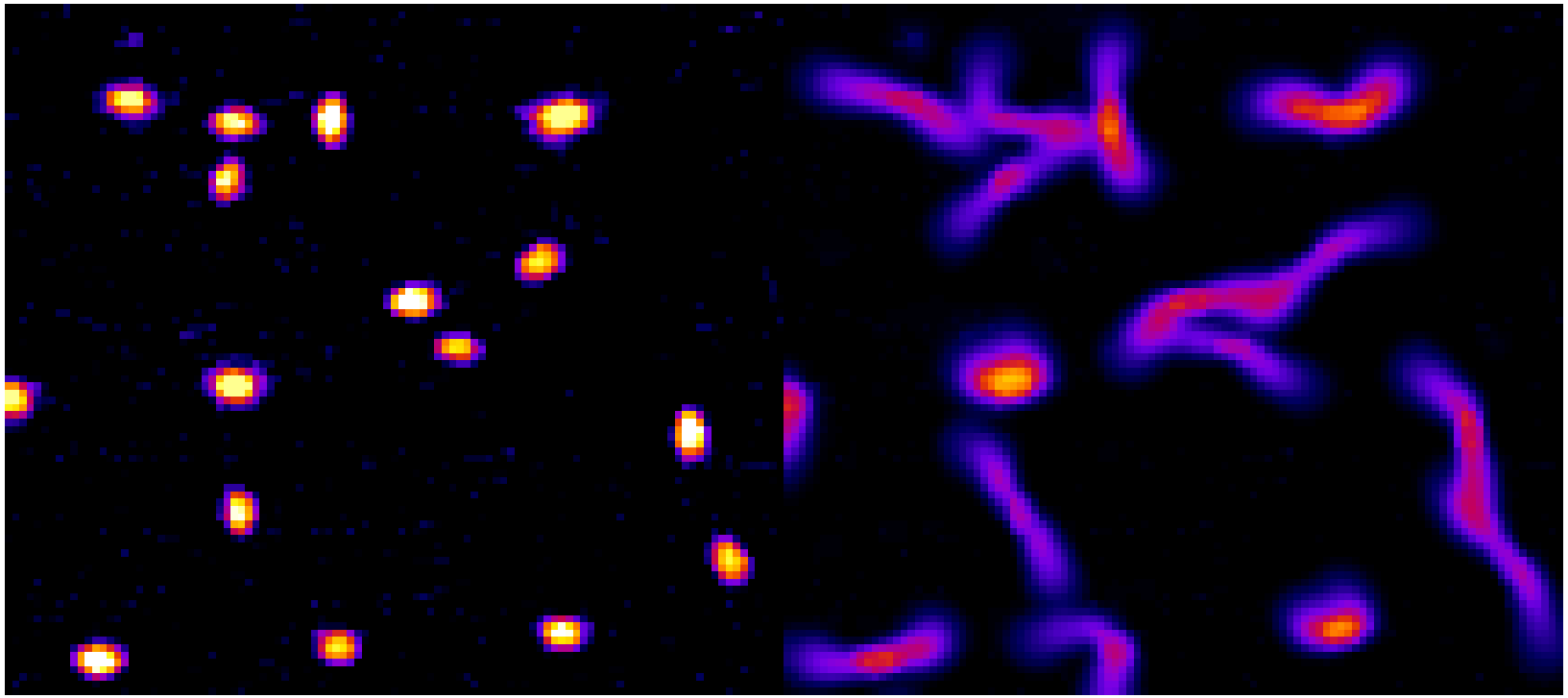


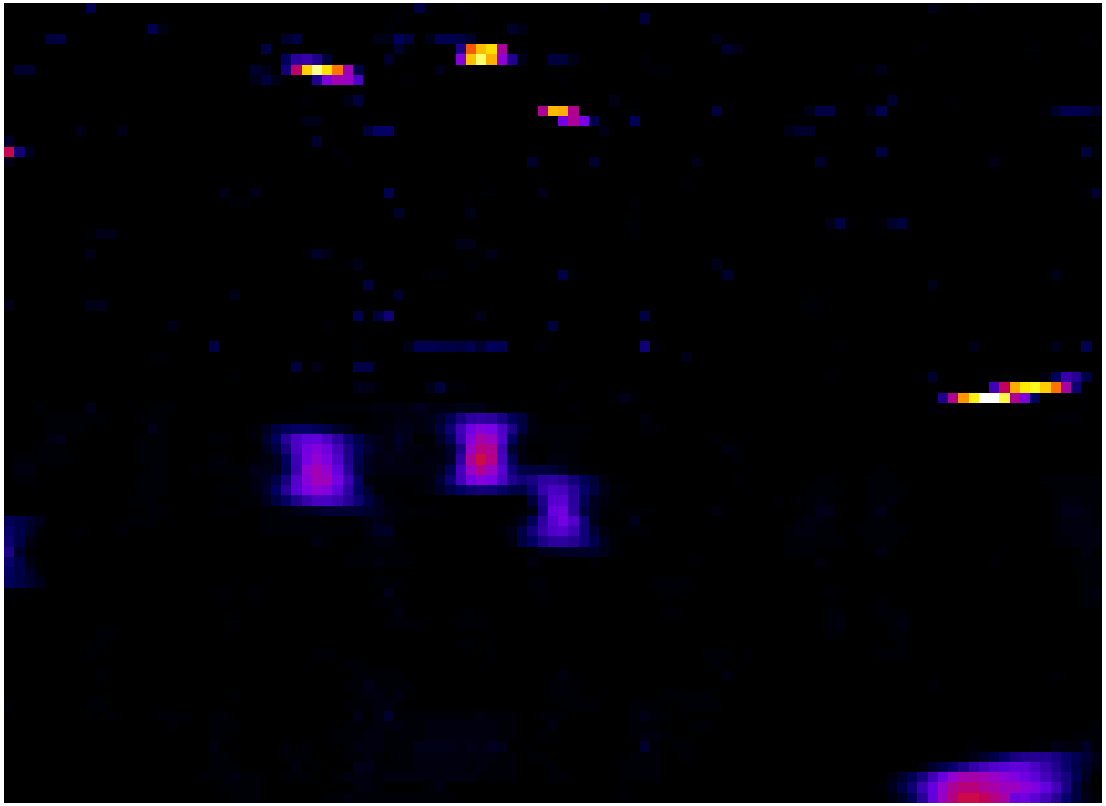


artificial Z-stack image

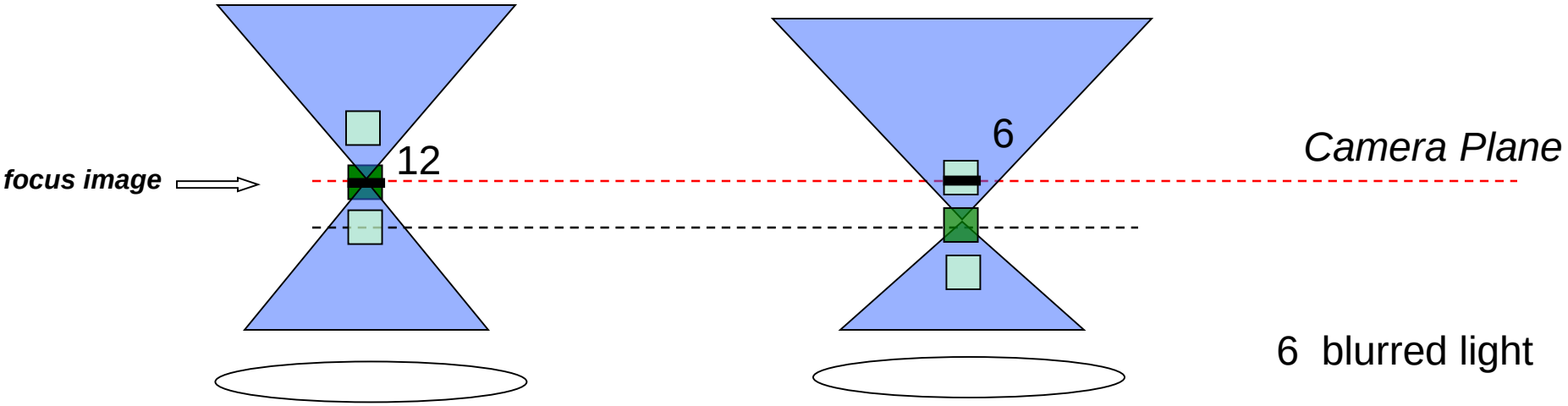


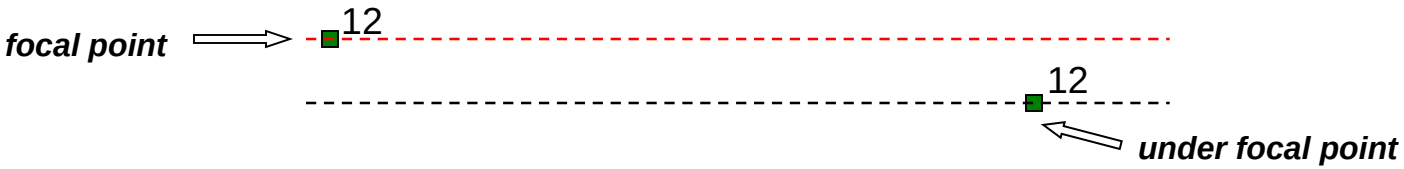


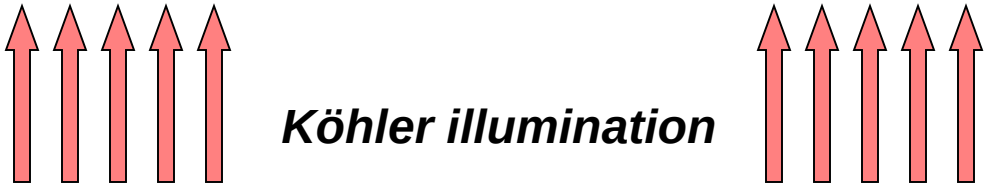


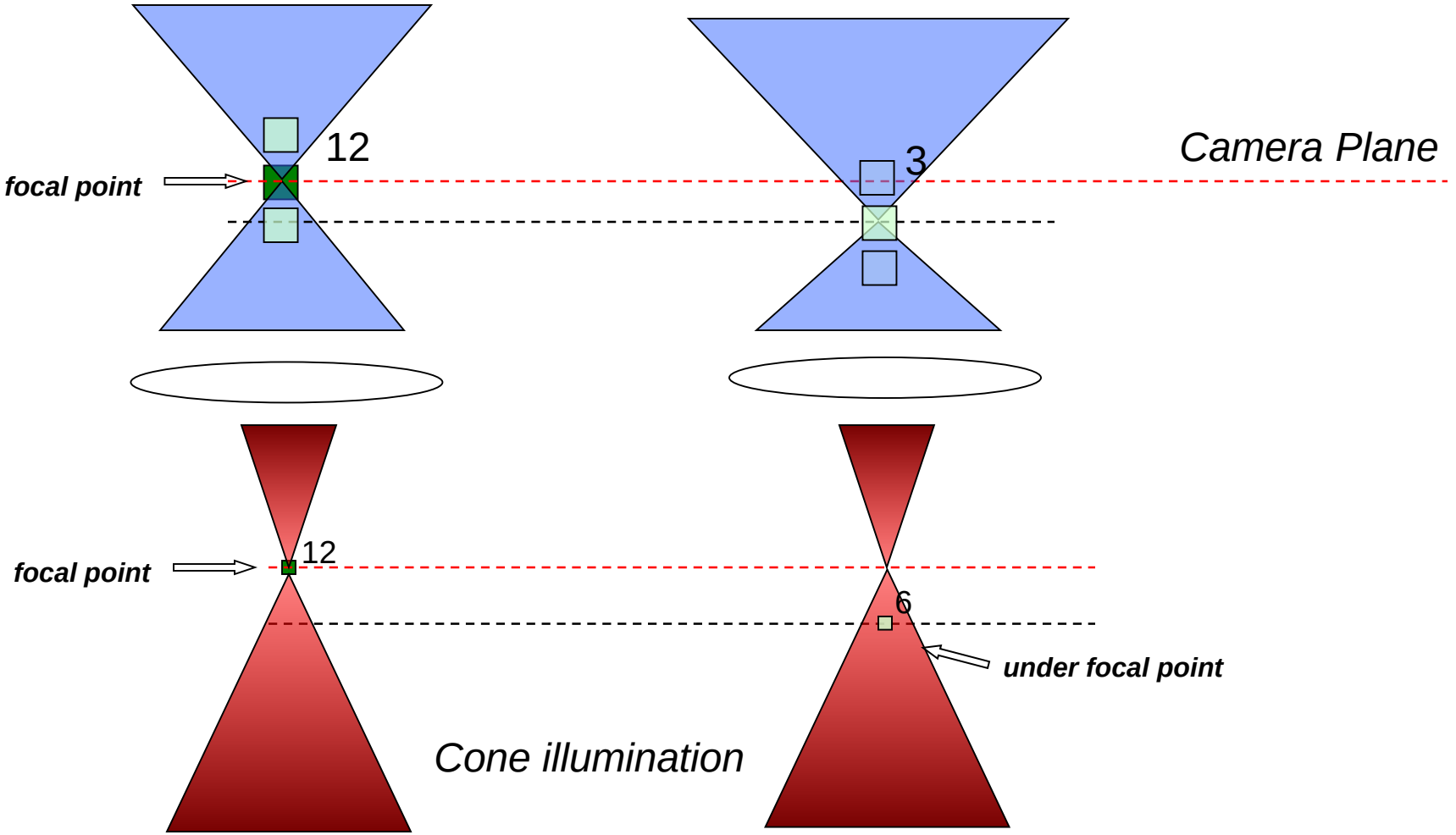


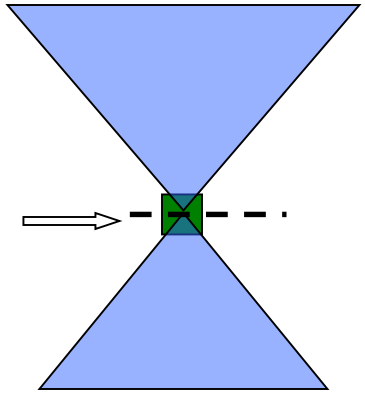
XZ plane of the stack

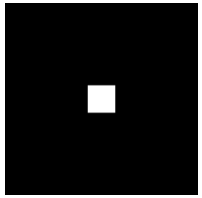

focus image
12
6
Camera Plane
6 blurred light

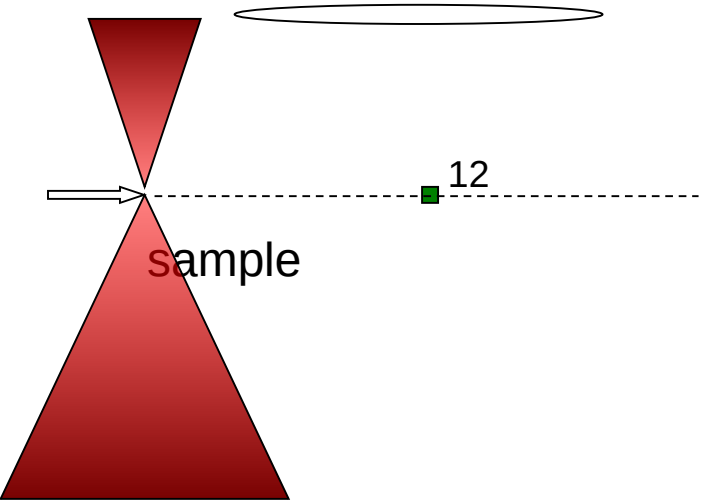

focal point
12
12
under focal point

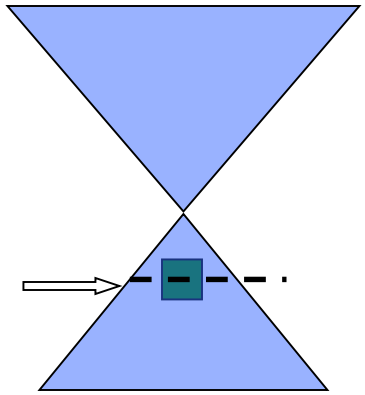

Köhler illumination

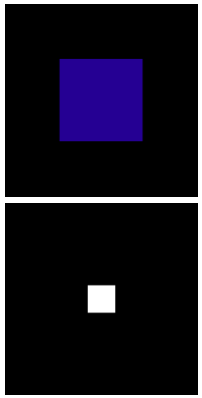

12
3
Camera Plane
focal point
focal point
12
6
under focal point
Cone illumination

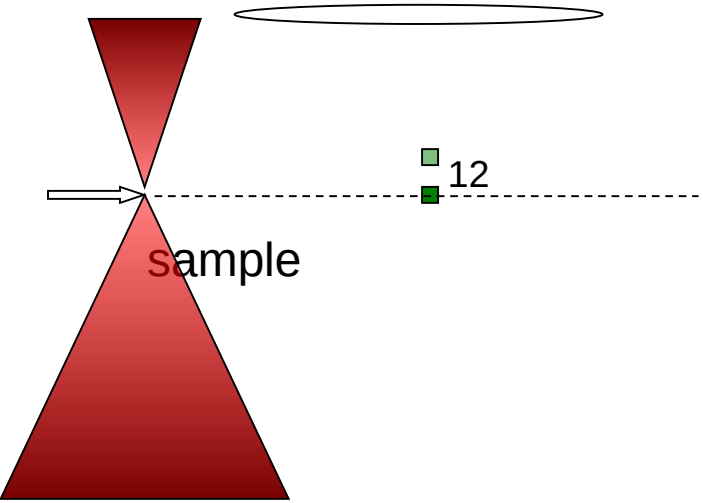


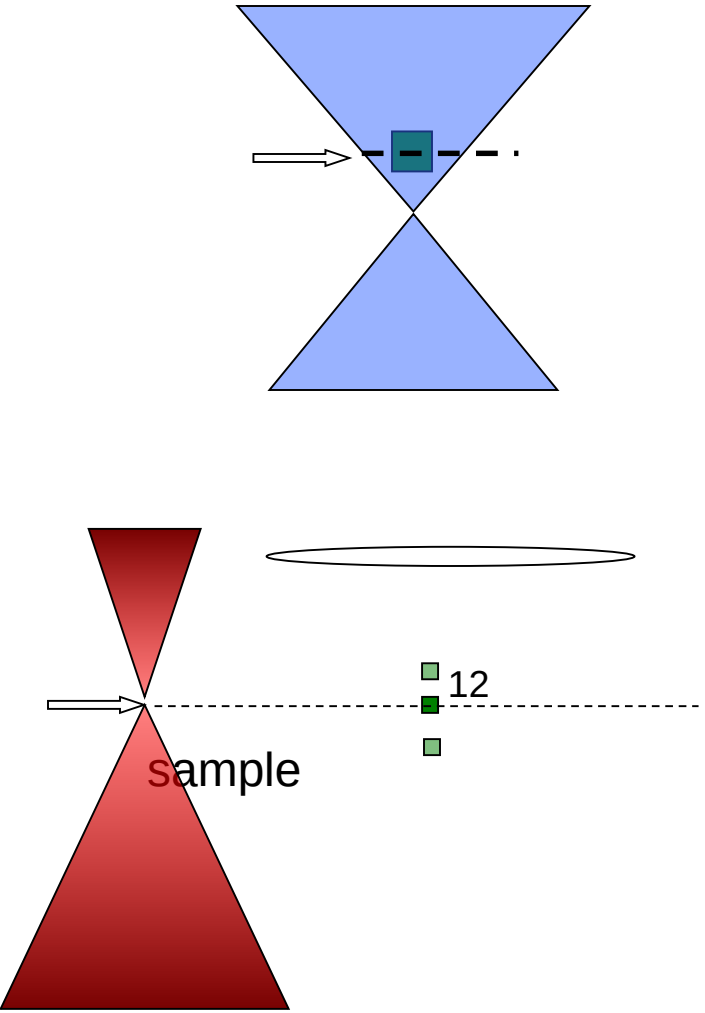


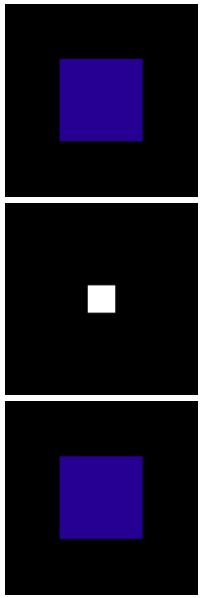

12
sample

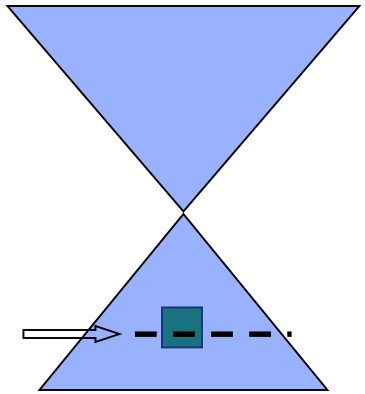


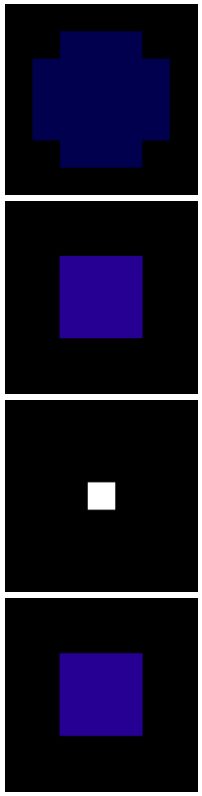


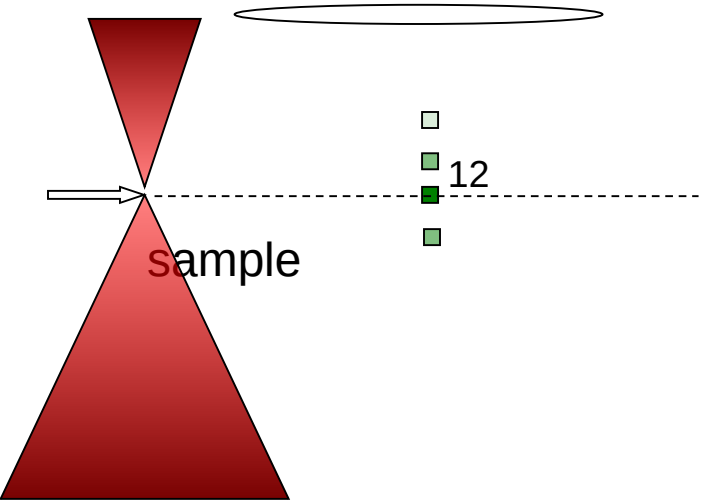

12
sample


12
sample





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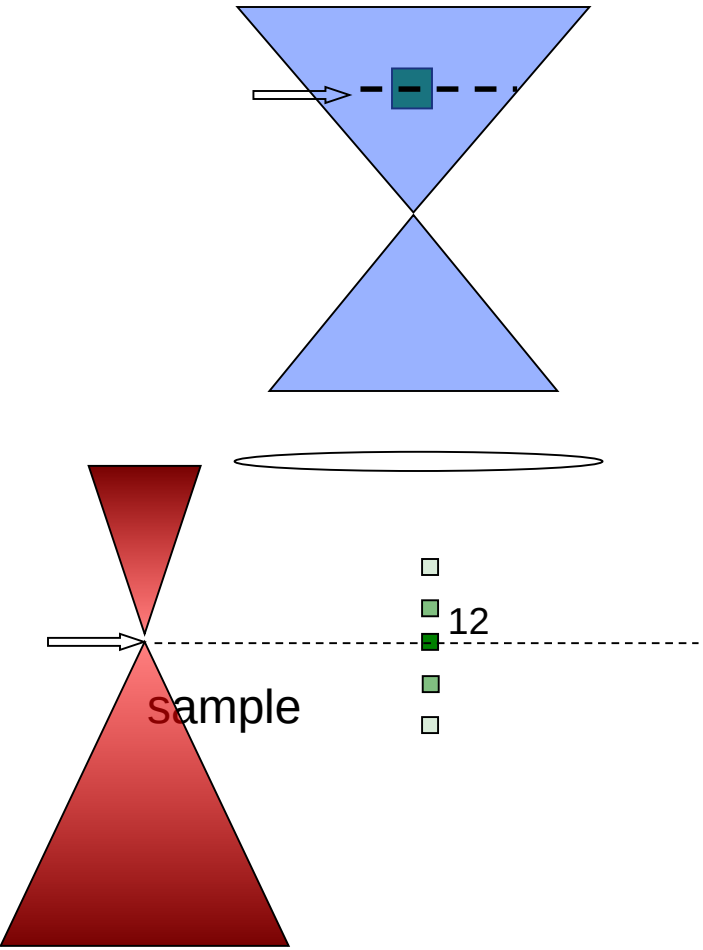
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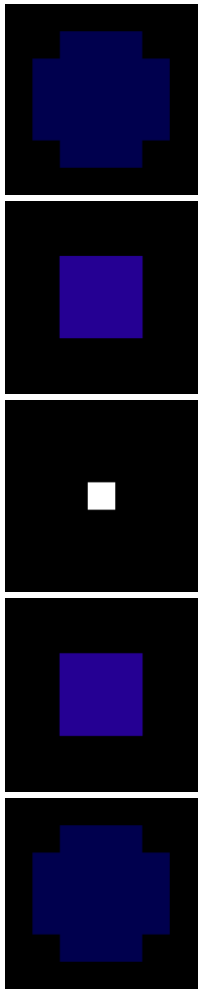

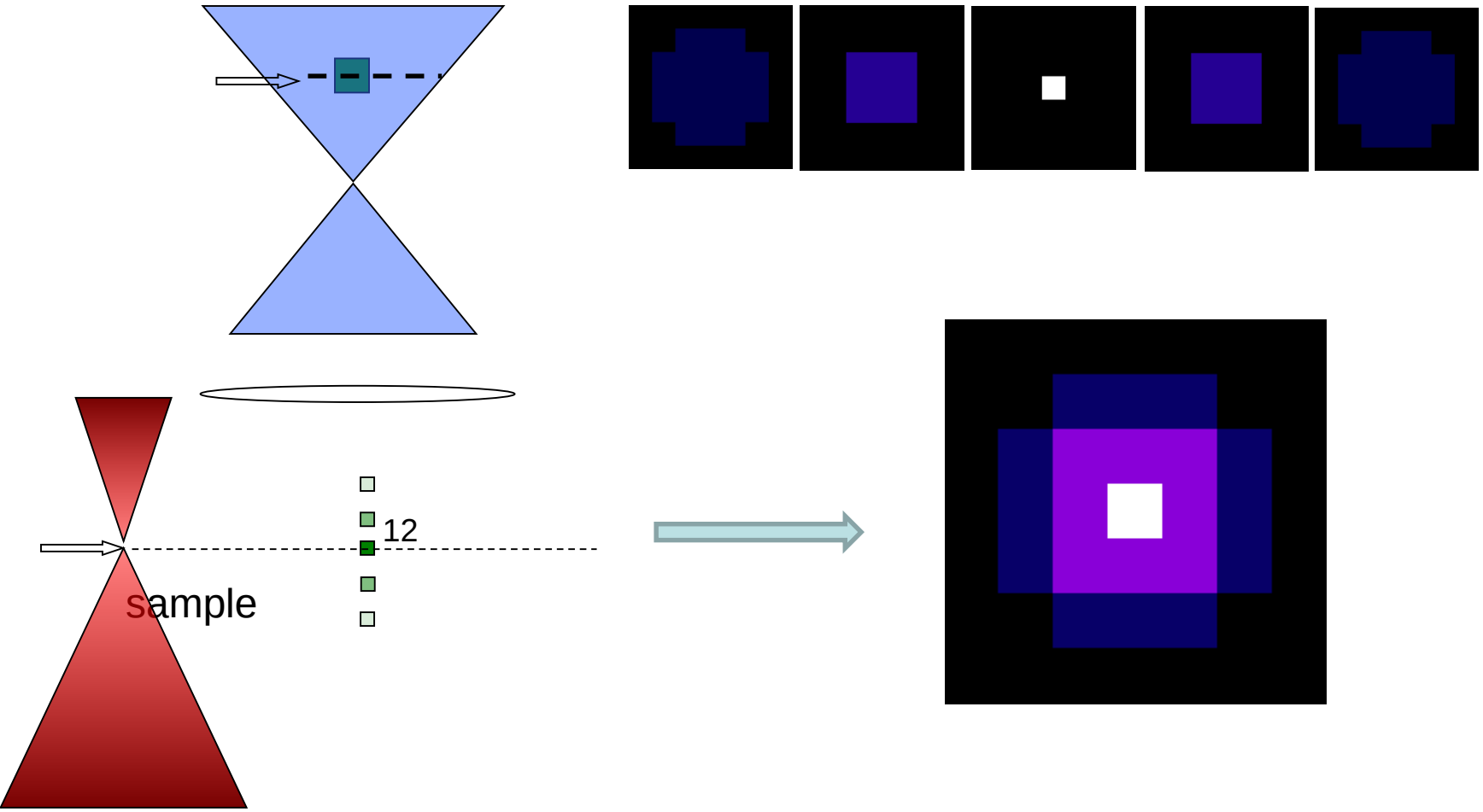
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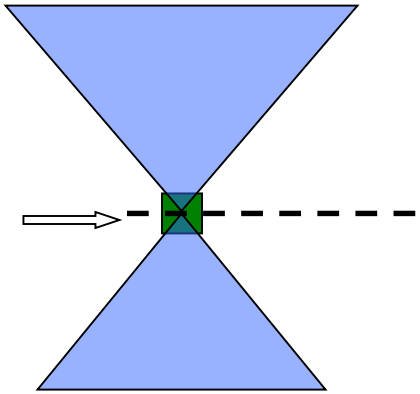
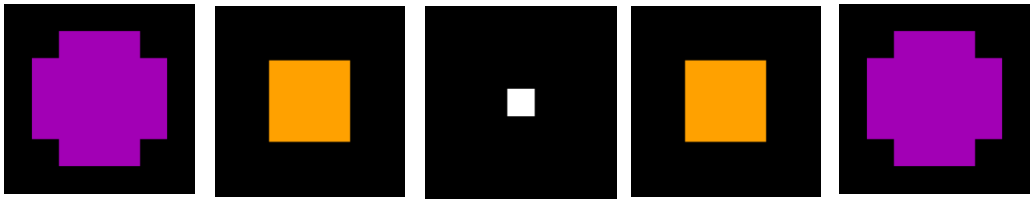
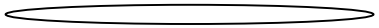
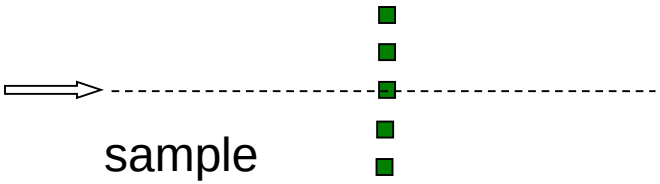
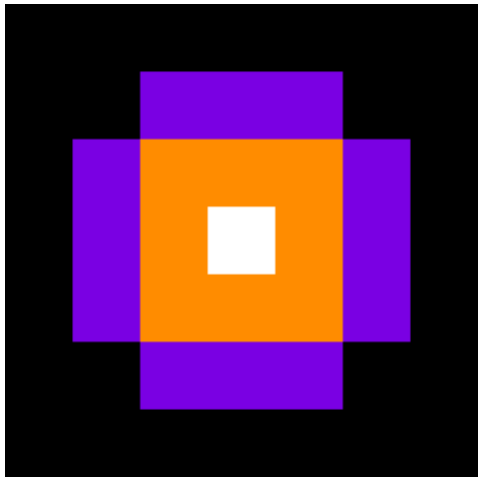
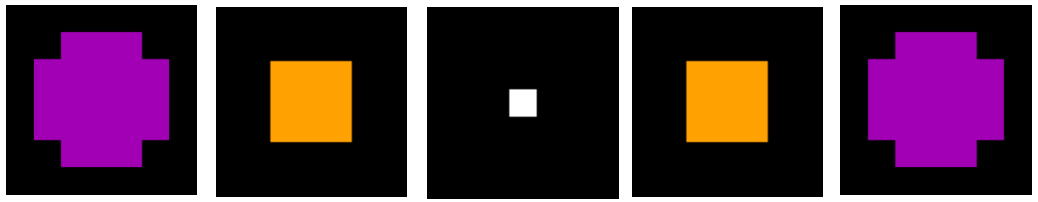




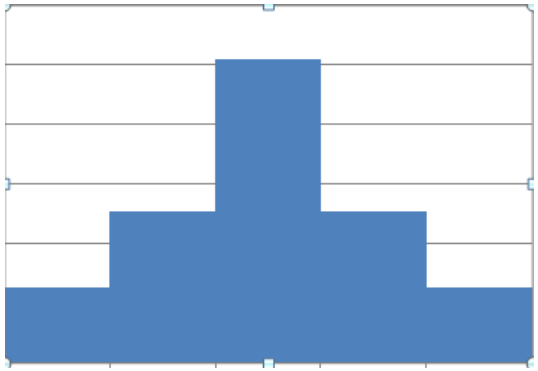



sample

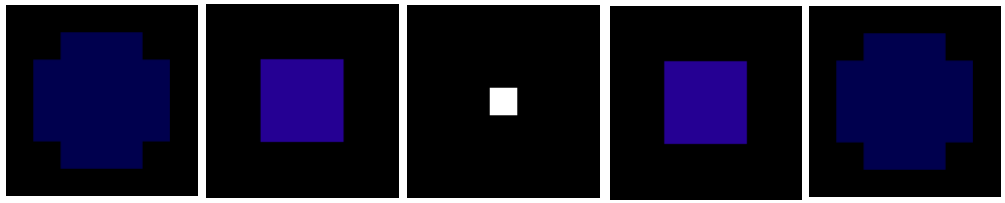




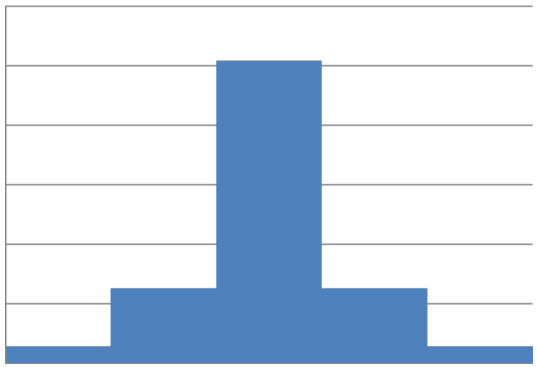
widefield



Central pixel intensity by z

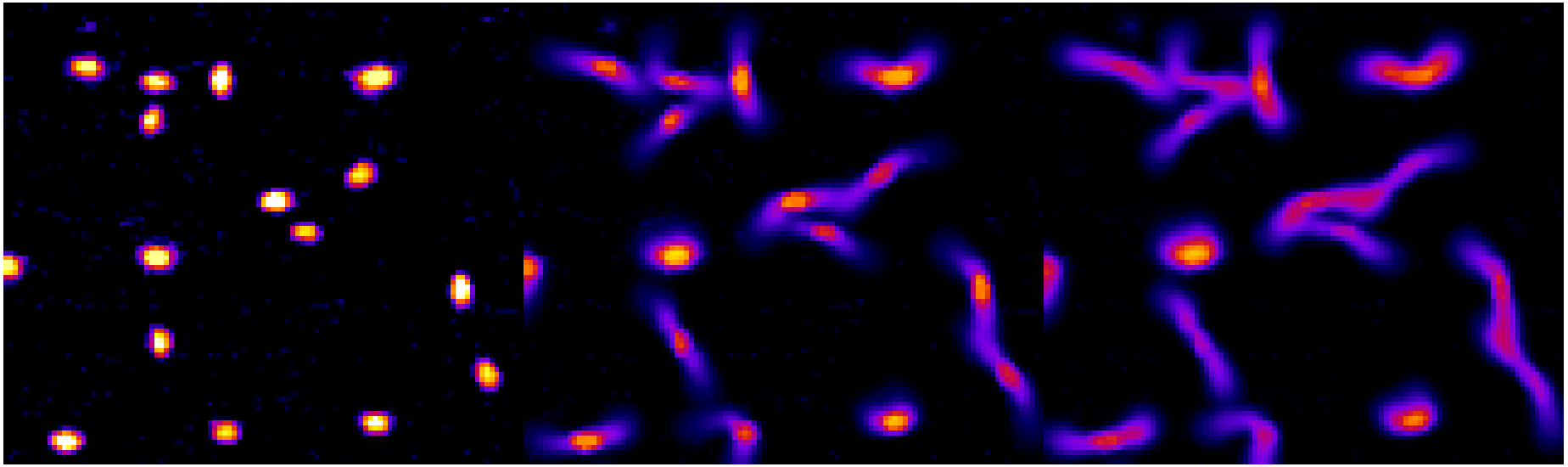


confocal



Central pixel intensity by z

XY plane

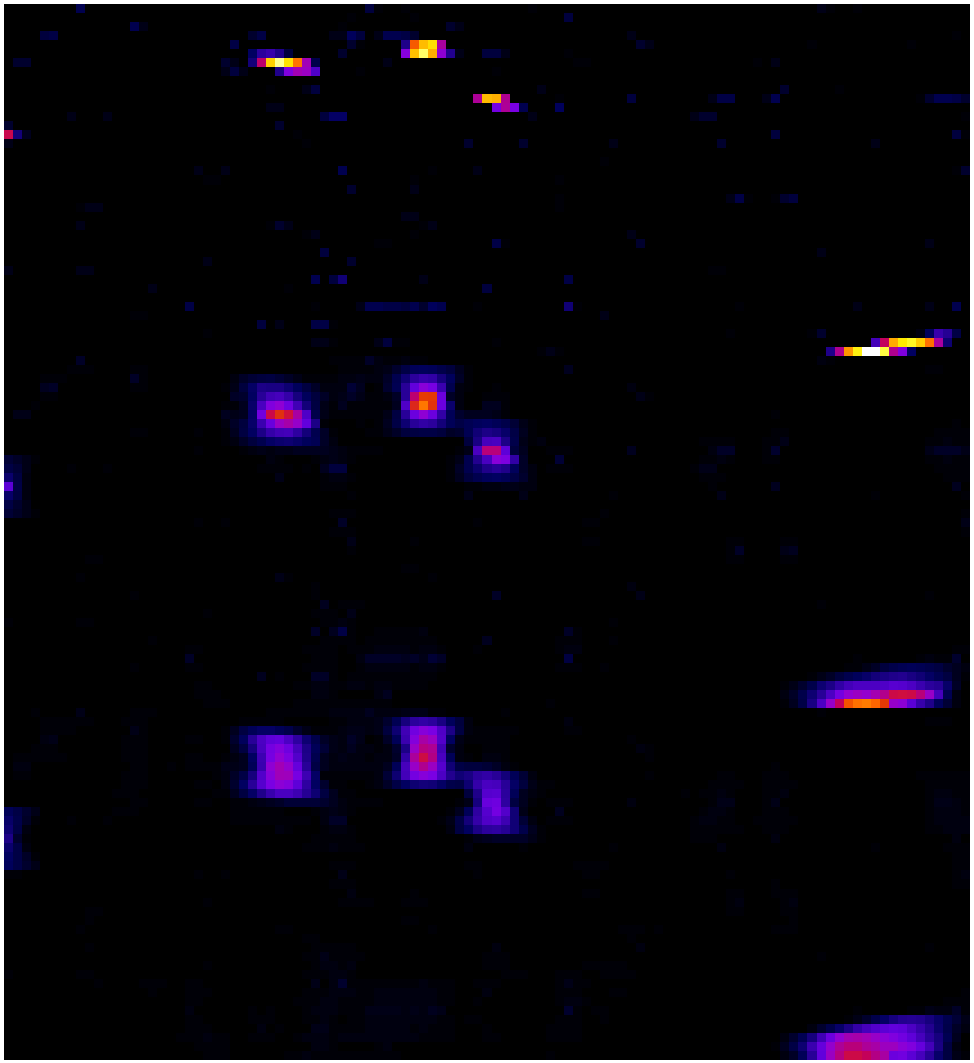


Raw

Confocal

WF

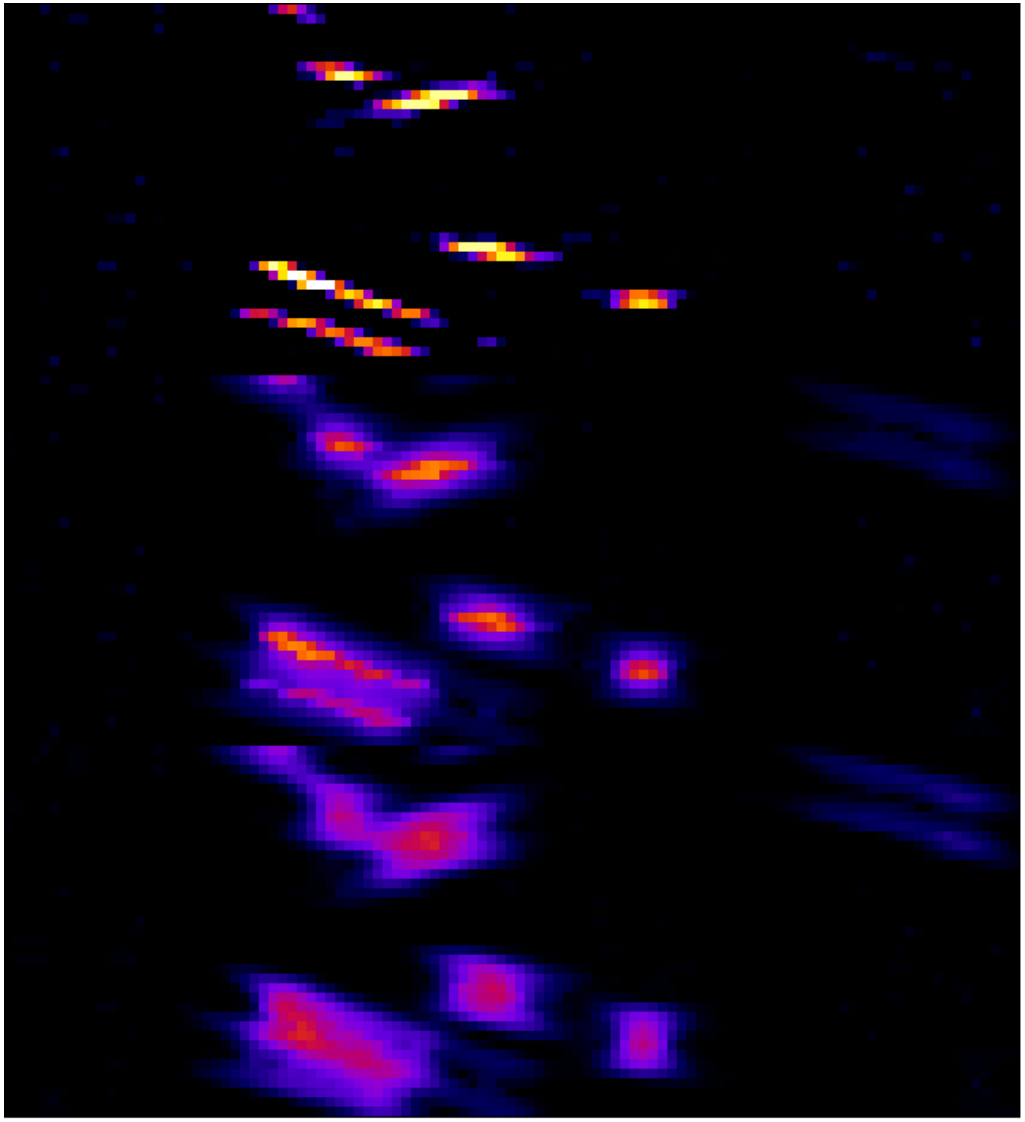
XZ plane

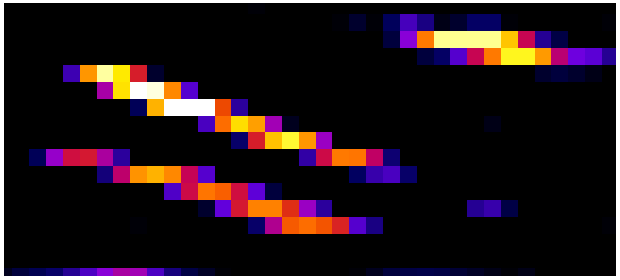


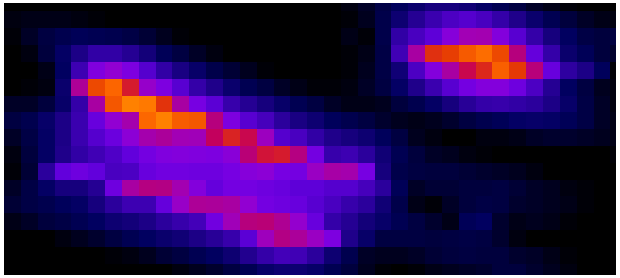
Raw

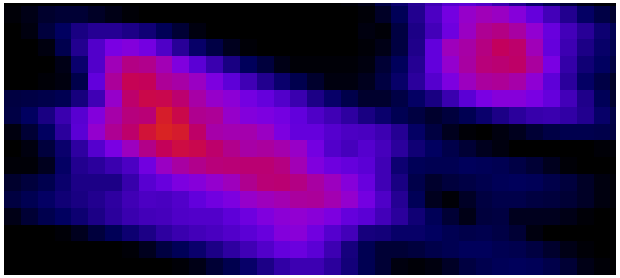
Confocal

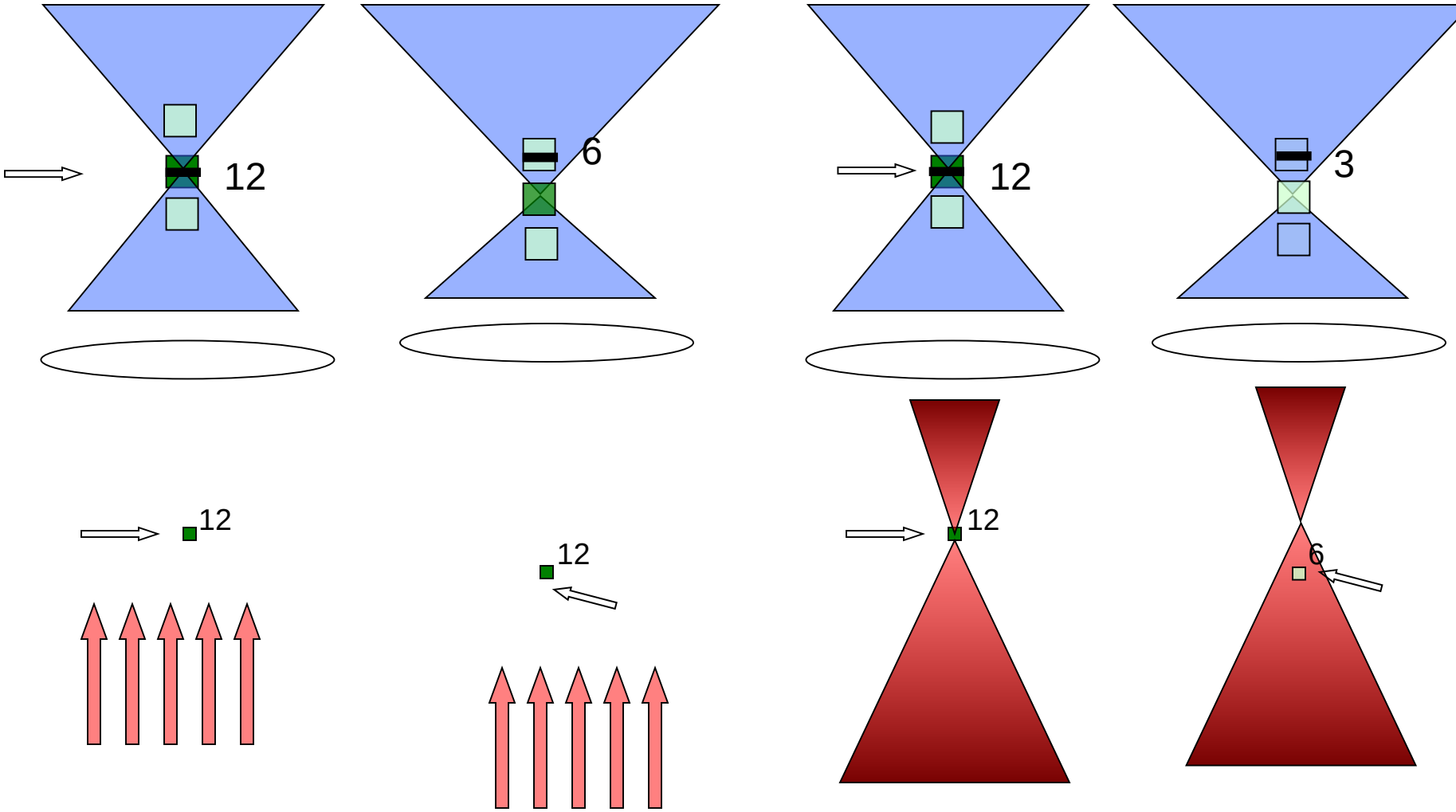
WF







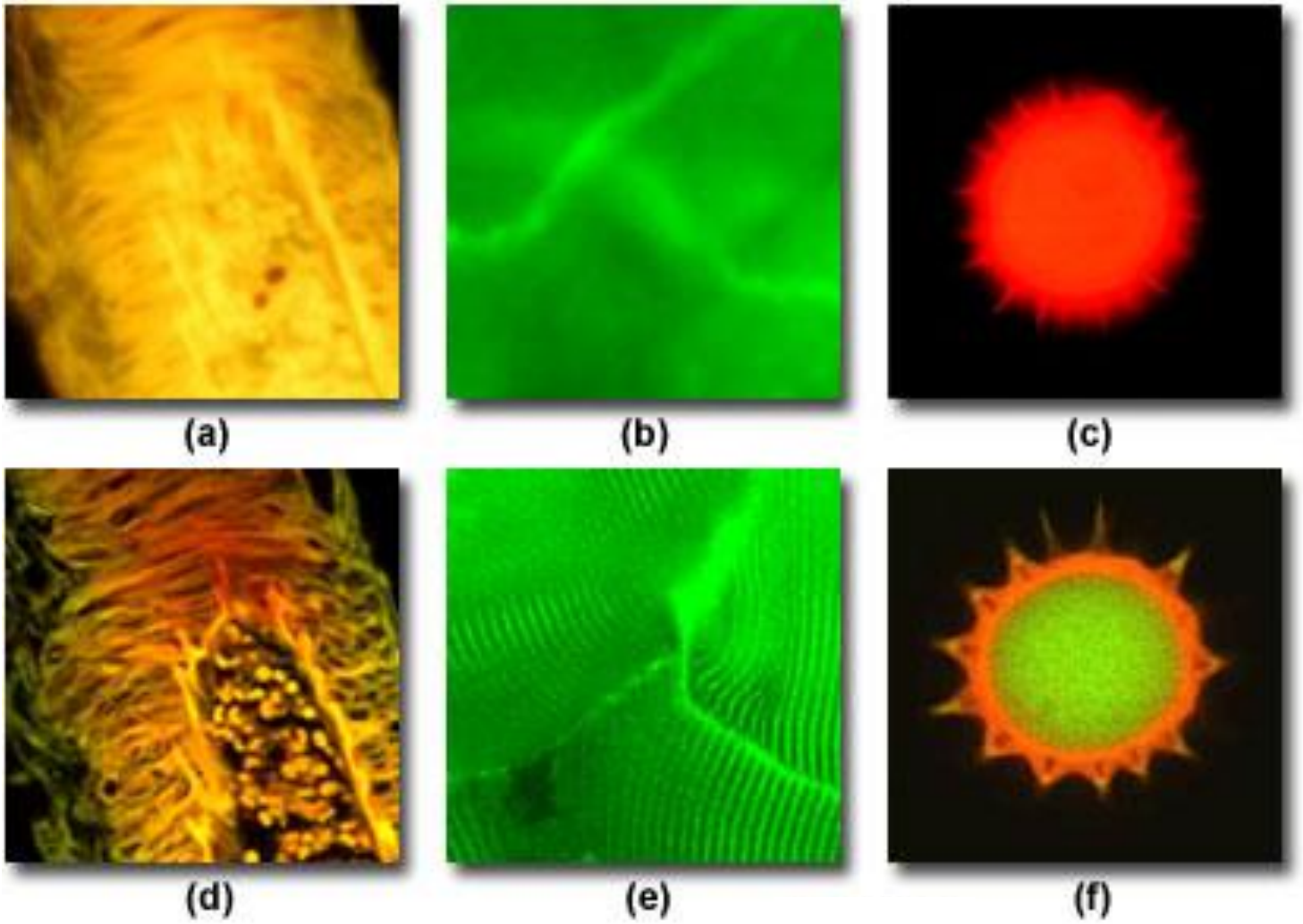




The two-cone structure is the key to increase contrast

Demo on Zeiss LSM 710 open pinhole and pinhole at 1 airy unit
On pollen sample 40x oil

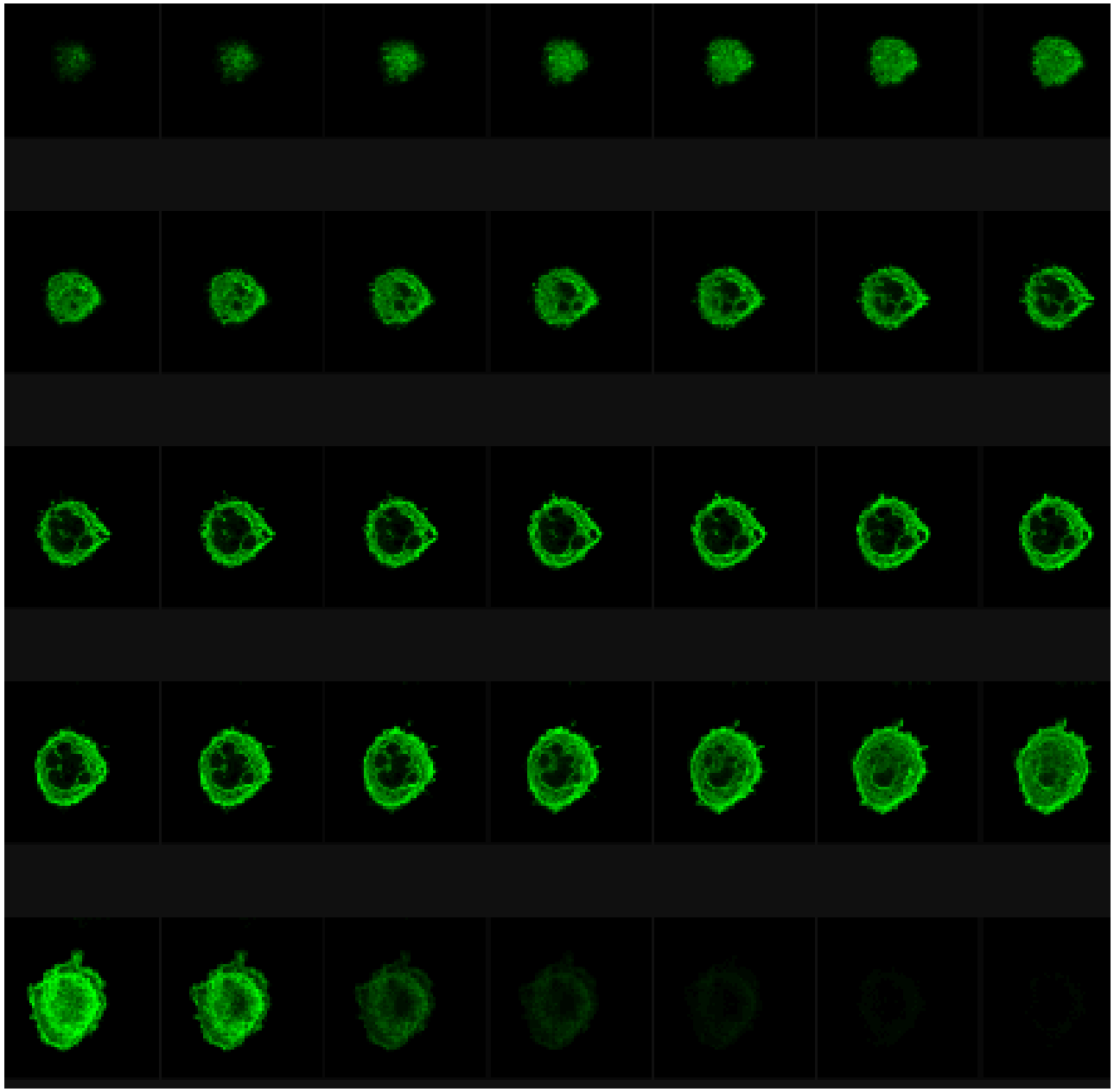
Confocal and Widefield Fluorescence Microscopy



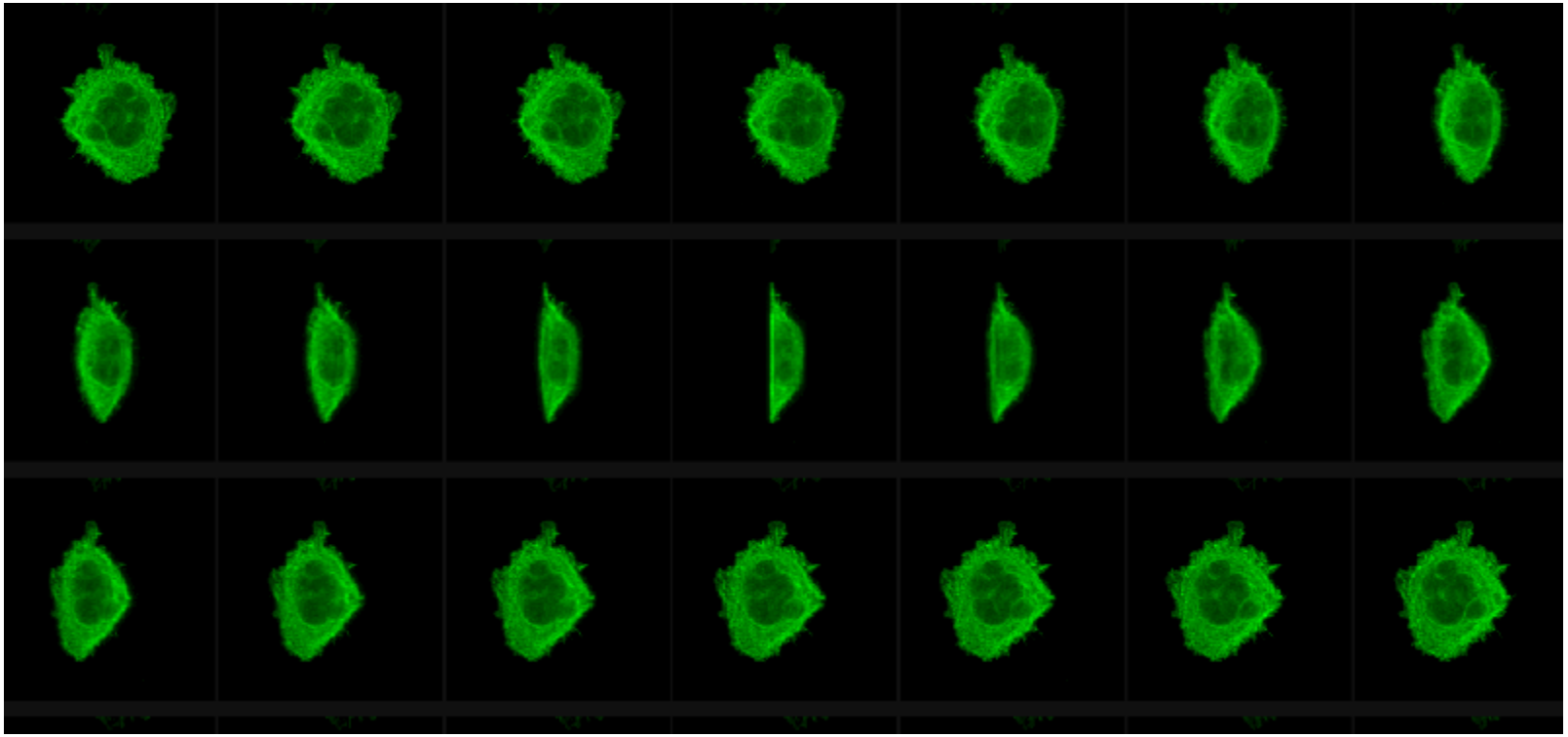
Widefield

Confocal

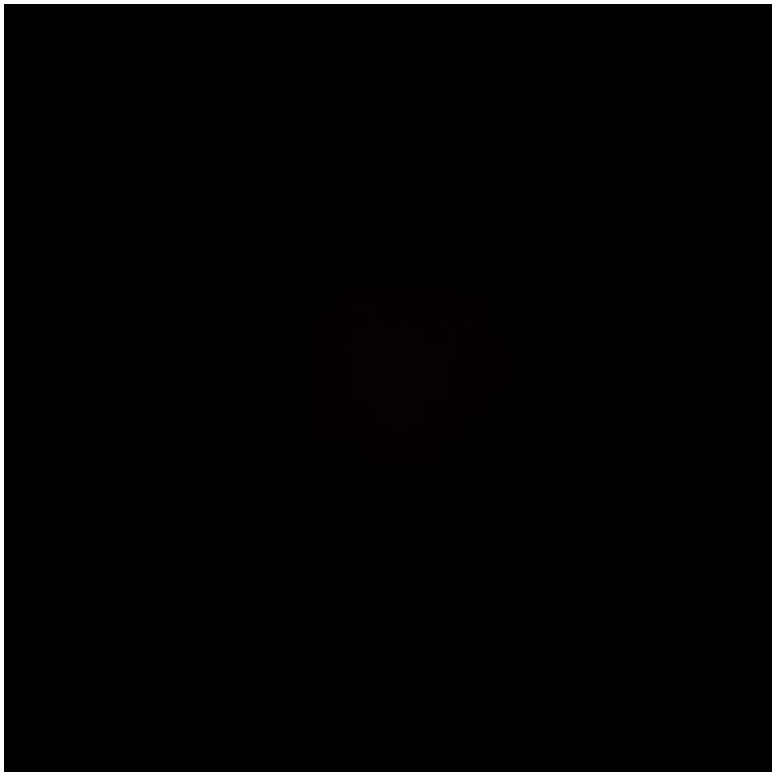
human medulla **rabbit muscle fibers** **sunflower pollen grain**

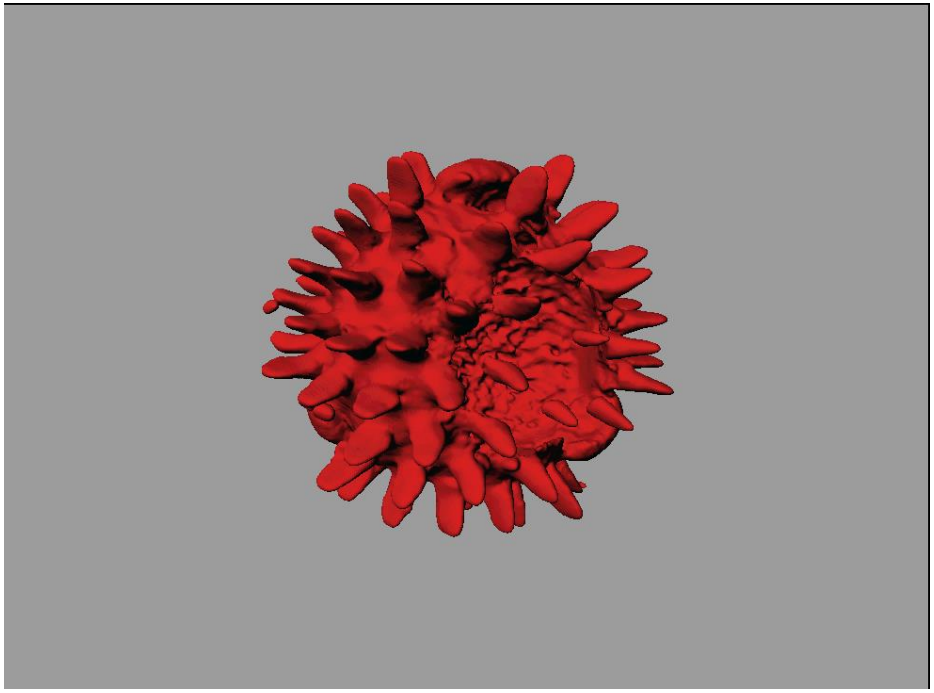


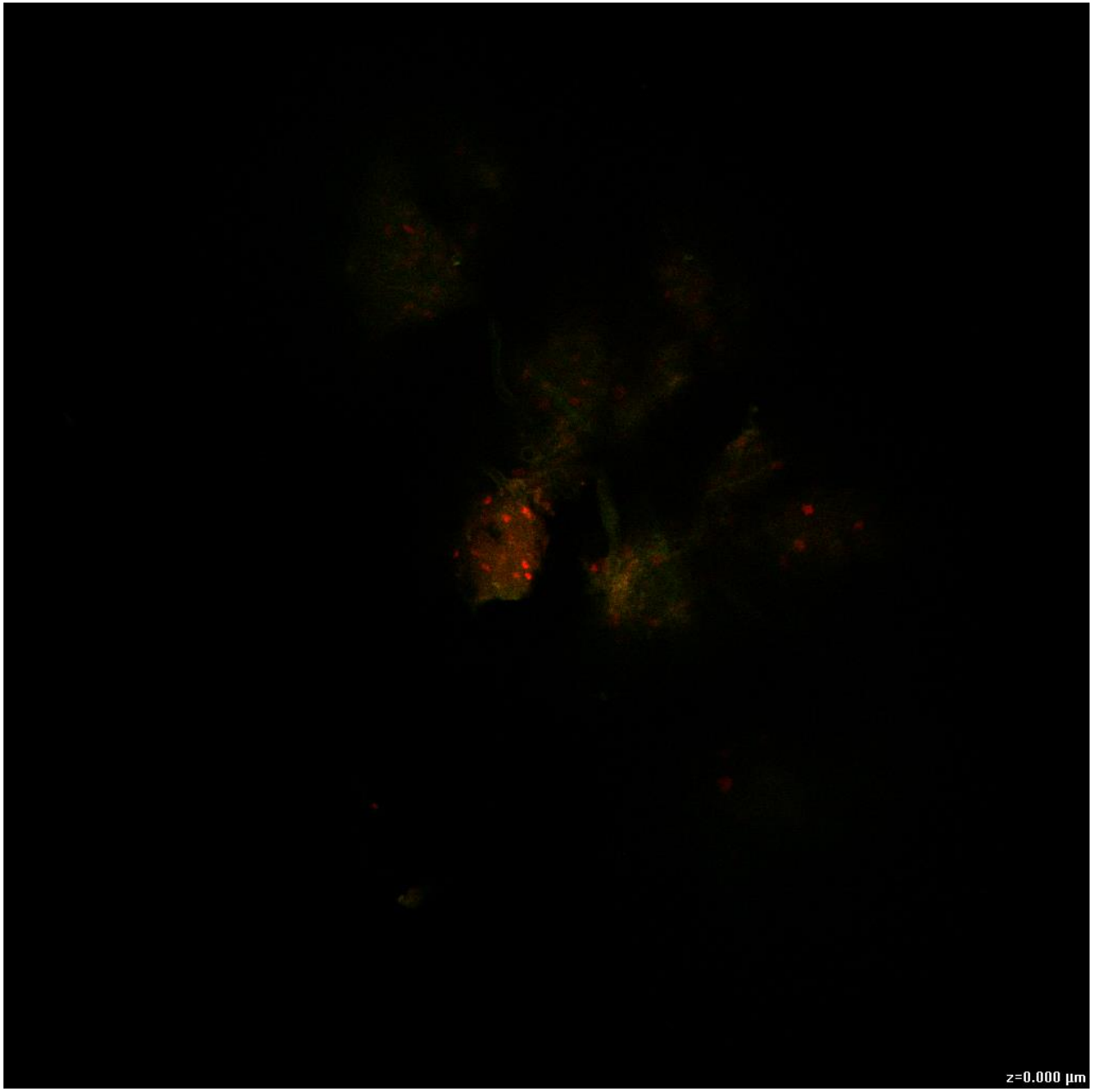
A z-series scan of a cell, from top to bottom.

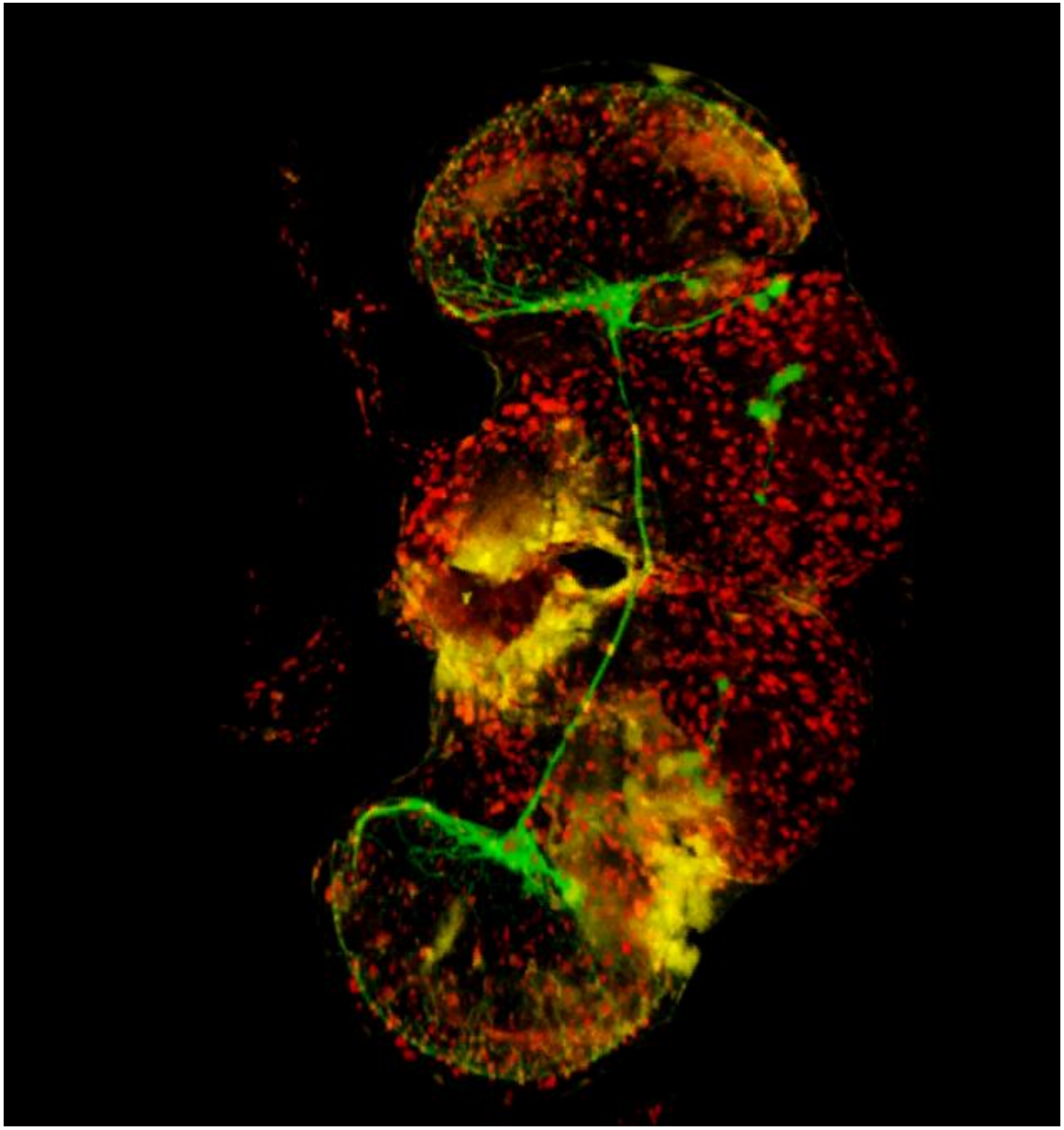


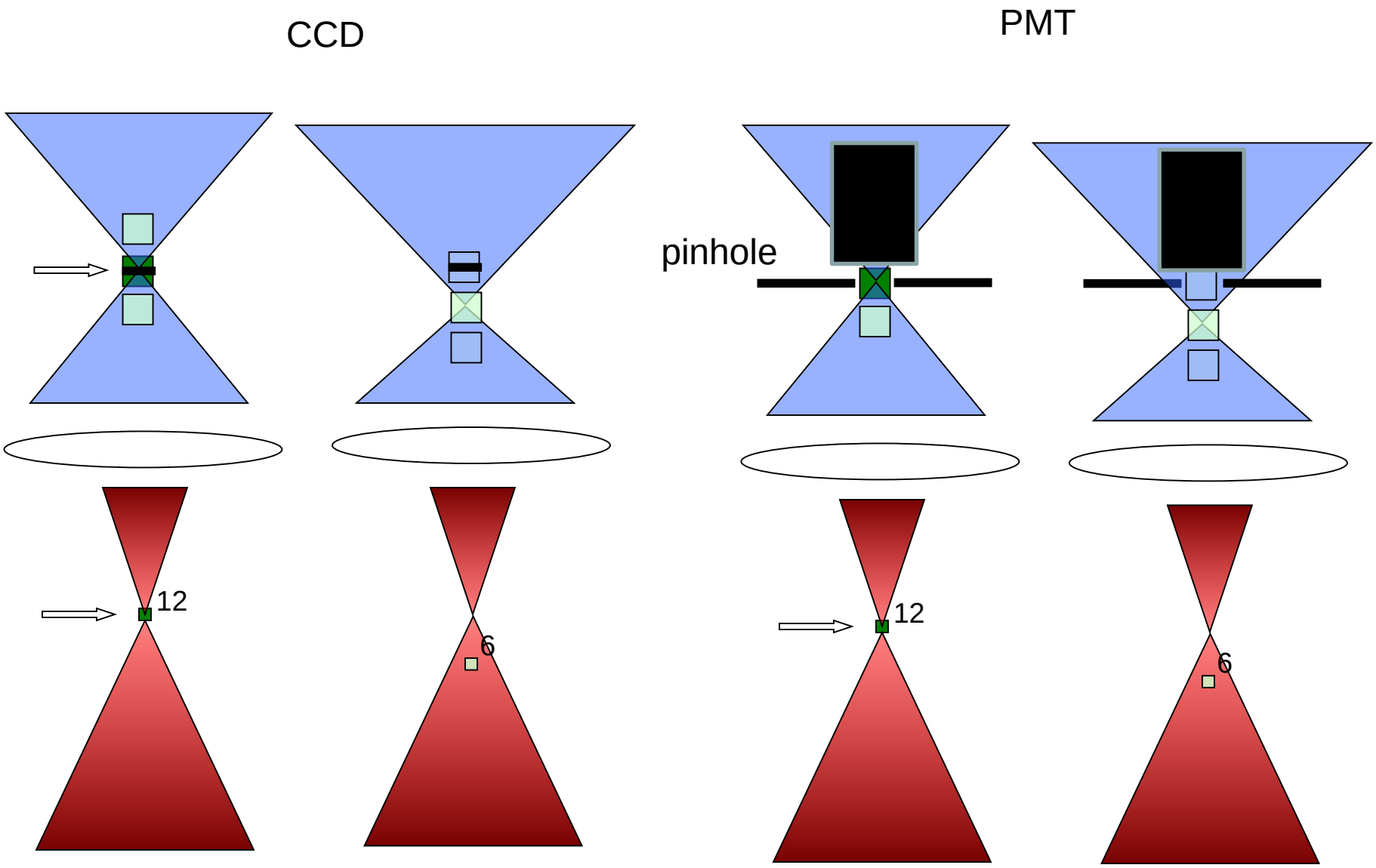
Reconstruct the 3D structure






z=0.000 µm




CCD
PMT
pinhole
12
6
12
6

The concept is first described by Marvin Minsky in 1950s.

Marvin Lee Minsky
(1927-Present) (1927 – 2016)

Toshiba Professor of Media Arts and Sciences
Professor of E.E. and C.S., M.I.T

Dec. 19, 1961 M. MINSKY 3,013,467
MICROSCOPY APPARATUS
Filed Nov. 7, 1957

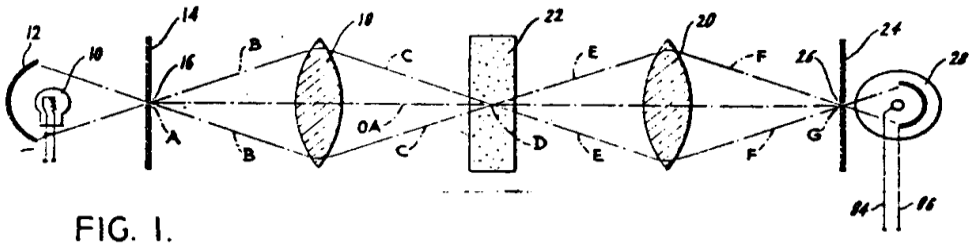


FIG. 1.

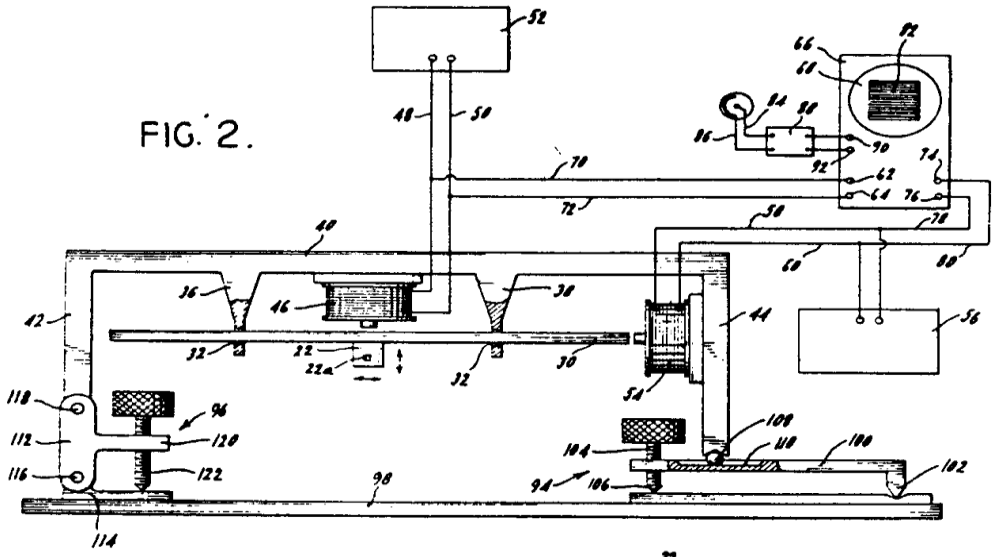


FIG. 2.

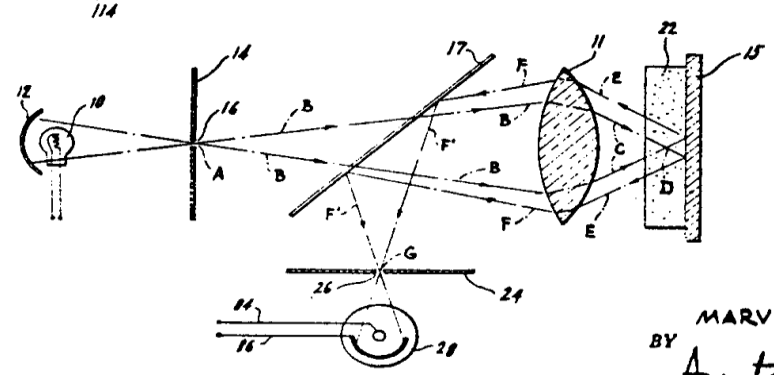
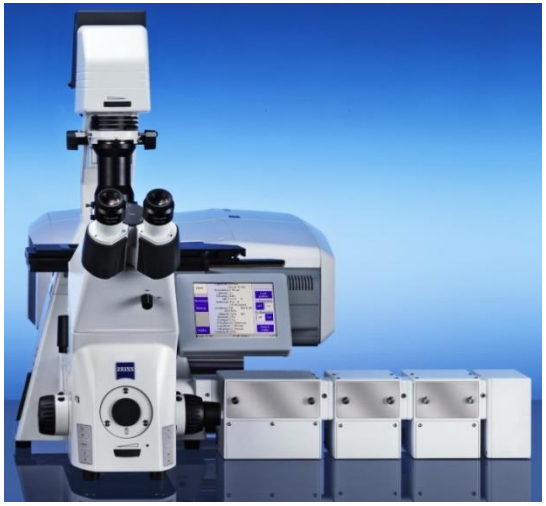


FIG. 3.

INVENTOR.
MARVIN MINSKY
BY Ameter & Levy



Zeiss LSM 810

Leica TCS SP8

Nikon A1R

Olympus FV1200

Confocal is Good

Superb lateral and axial resolution

What do we give up to achieve the resolution?

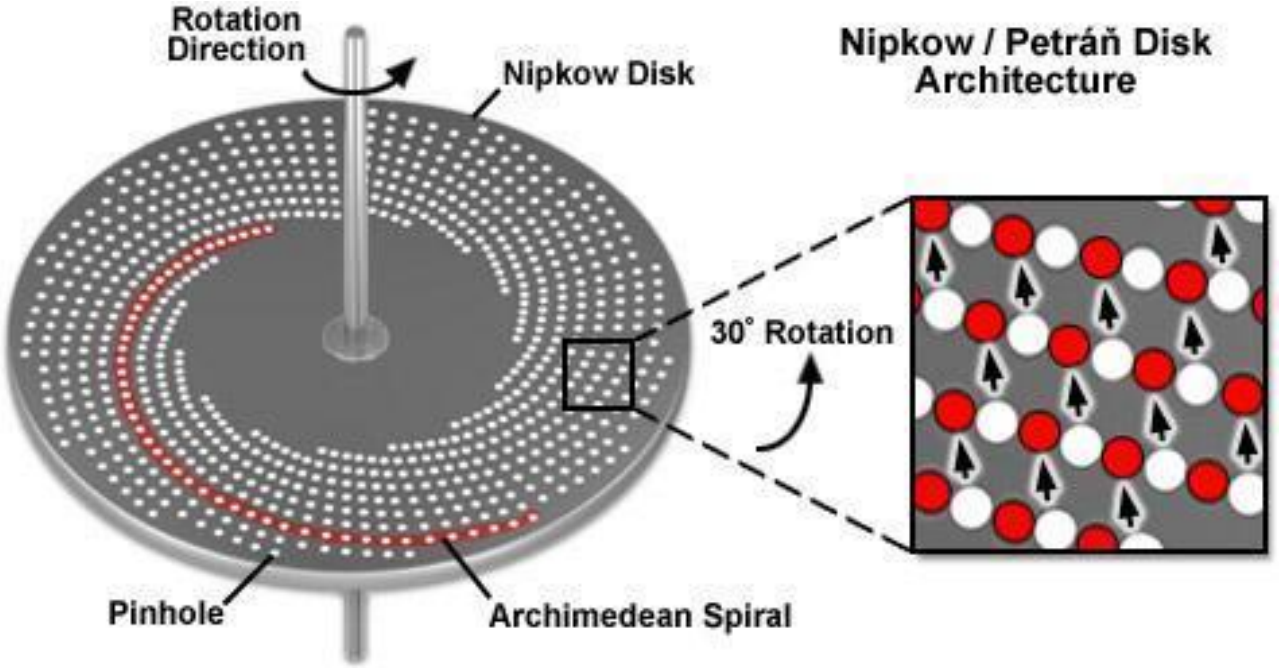
Typical temporal resolution : several seconds per frame

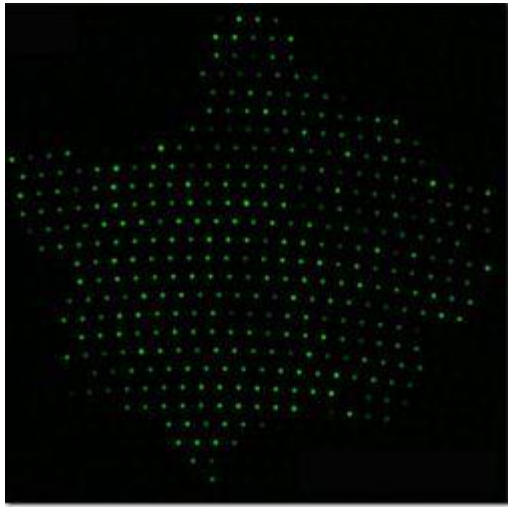
We sacrifice temporal resolution for spatial resolution

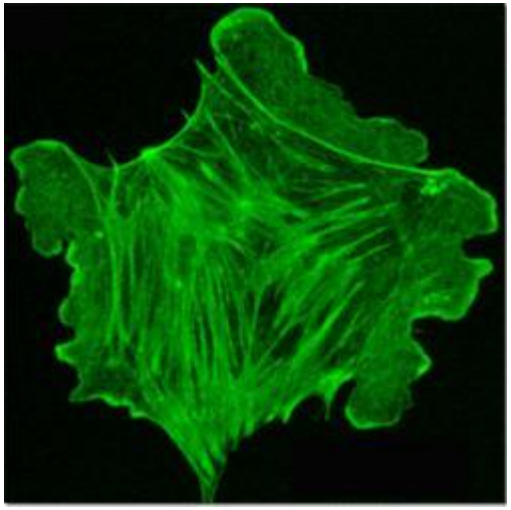
Maybe we can scan more points at one time

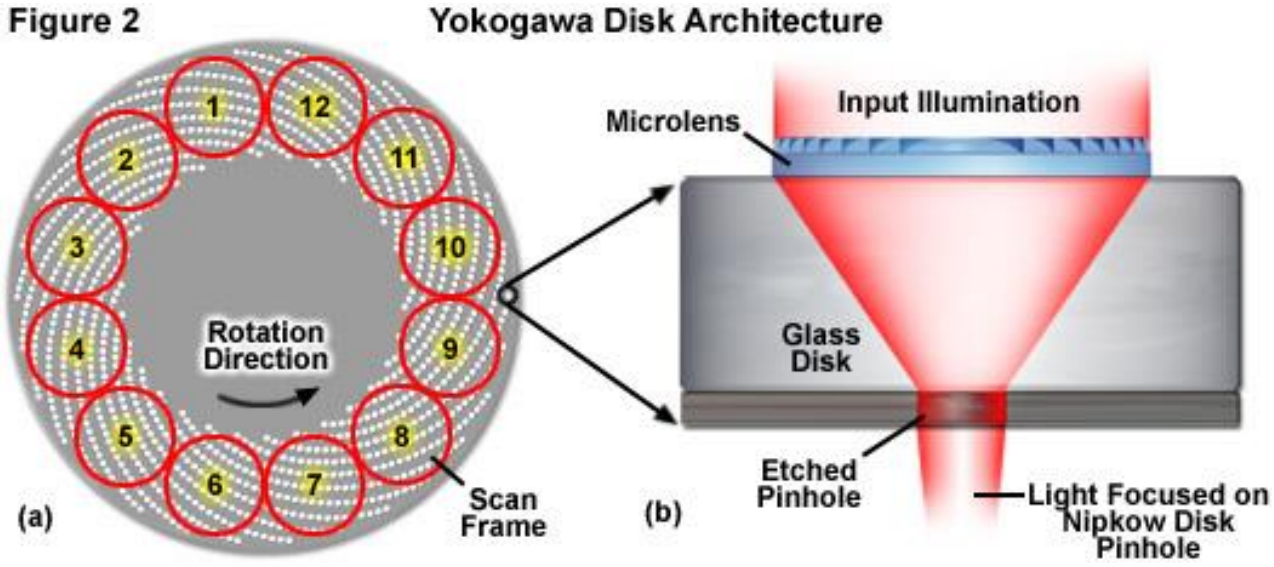
If they are far apart from each other

How far? ~ 10 times the resolution limit


Rotation Direction
Nipkow Disk
Nipkow / Petráň Disk Architecture
30˚ Rotation
Pinhole
Archimedean Spiral

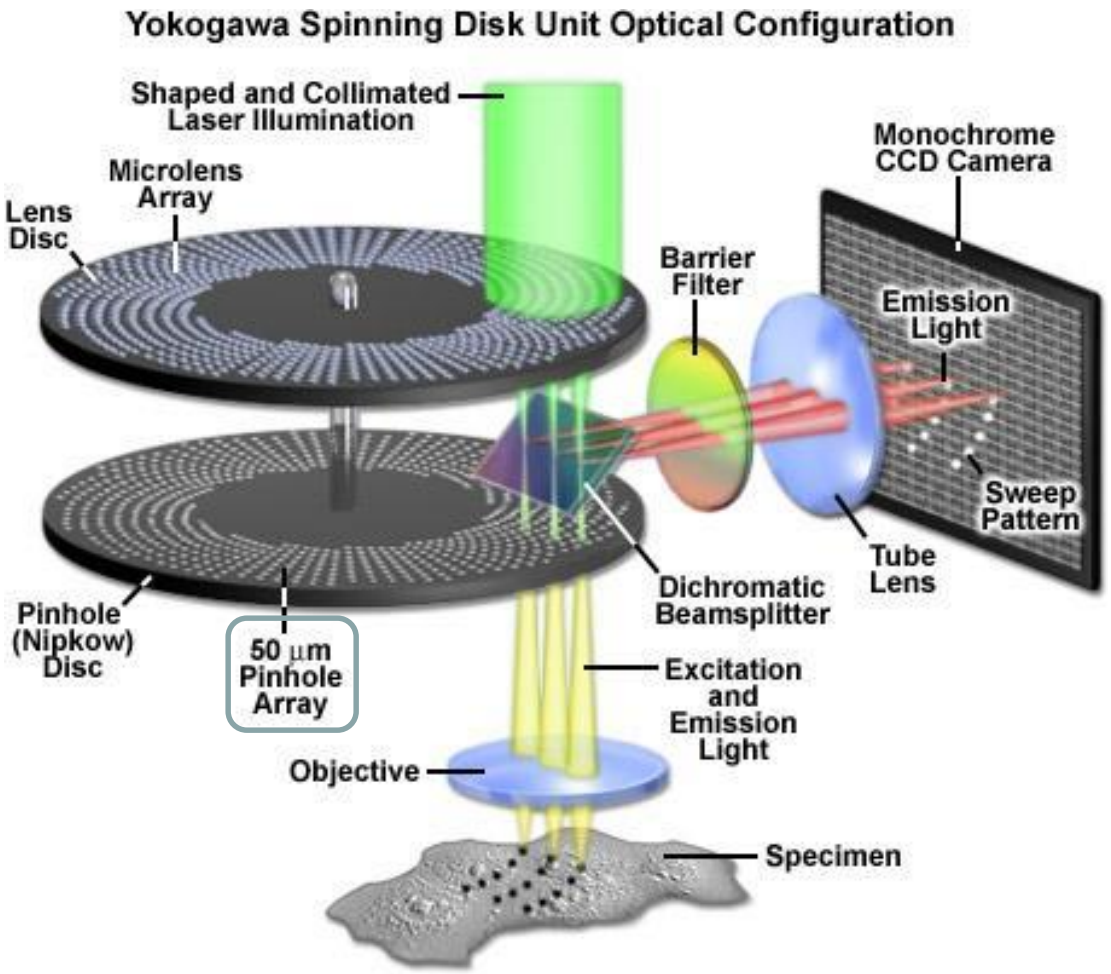




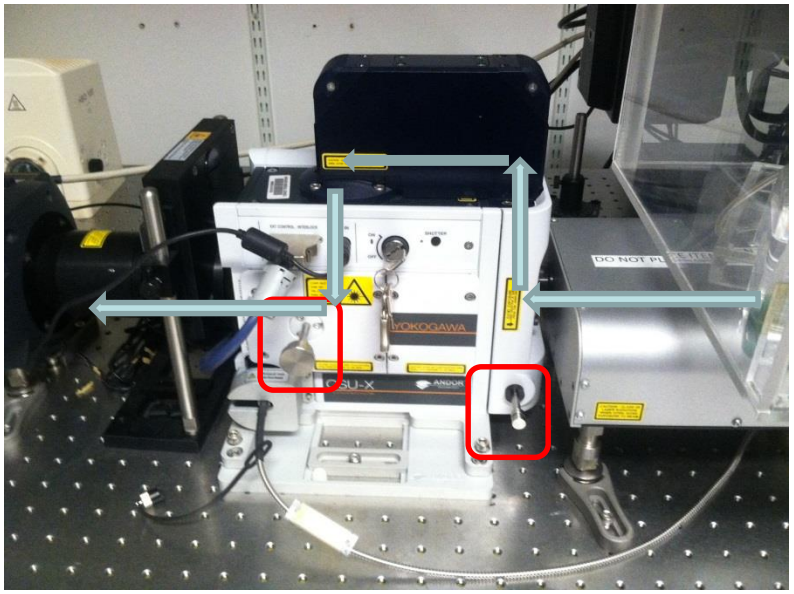


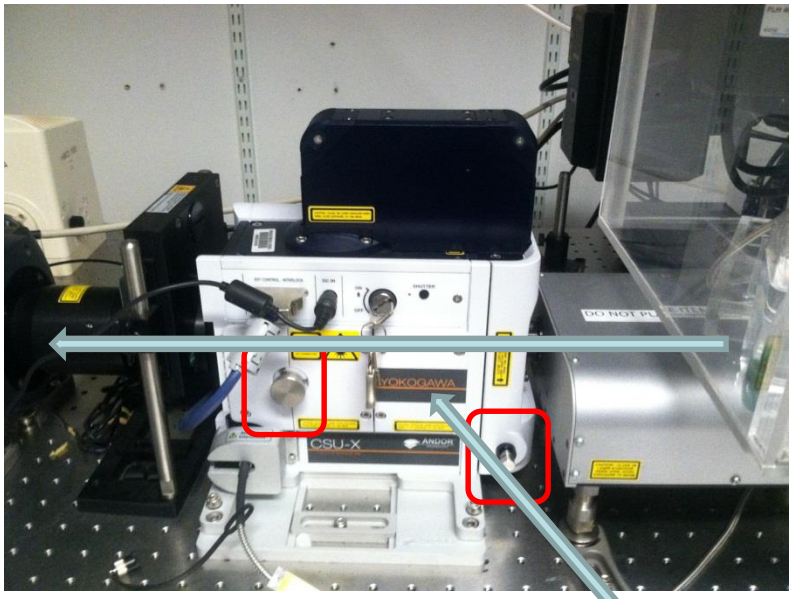
Improve light throughput from 1% to ~70%

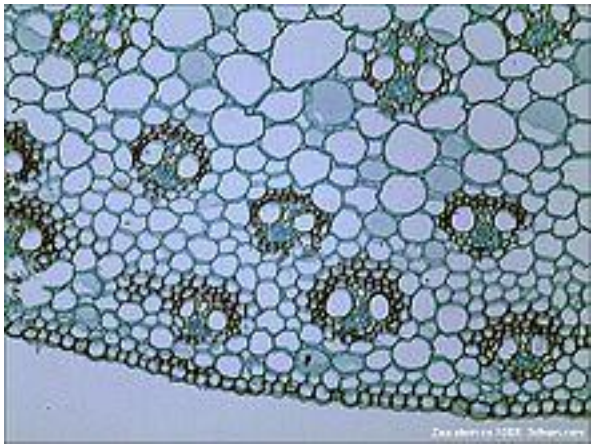
Yokogawa system

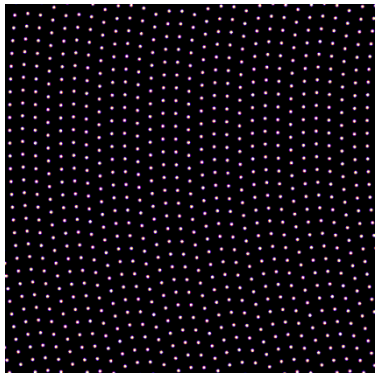


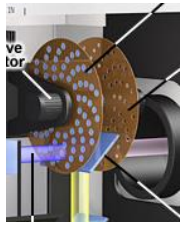
SDC pinhole demo

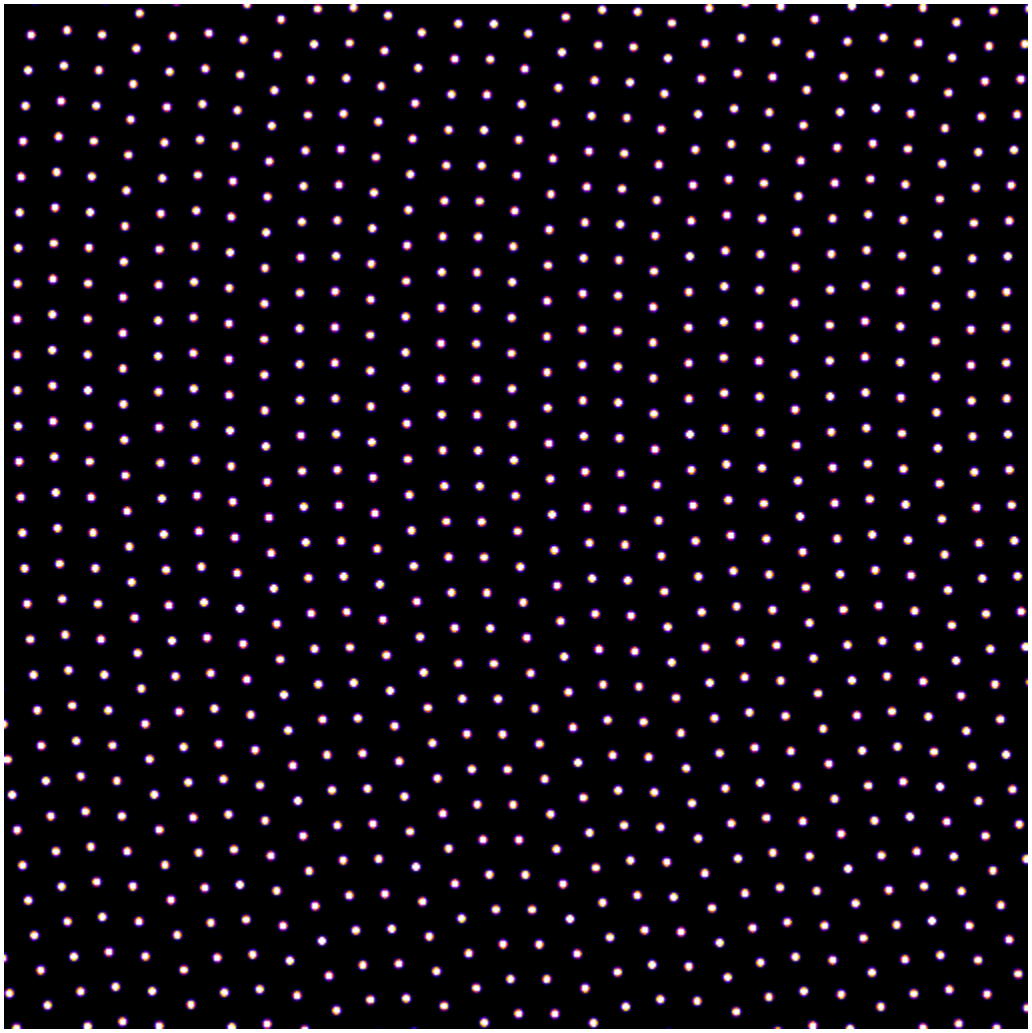

YOKOGAWA
CSU-X


YOKOGAWA
CSU-X





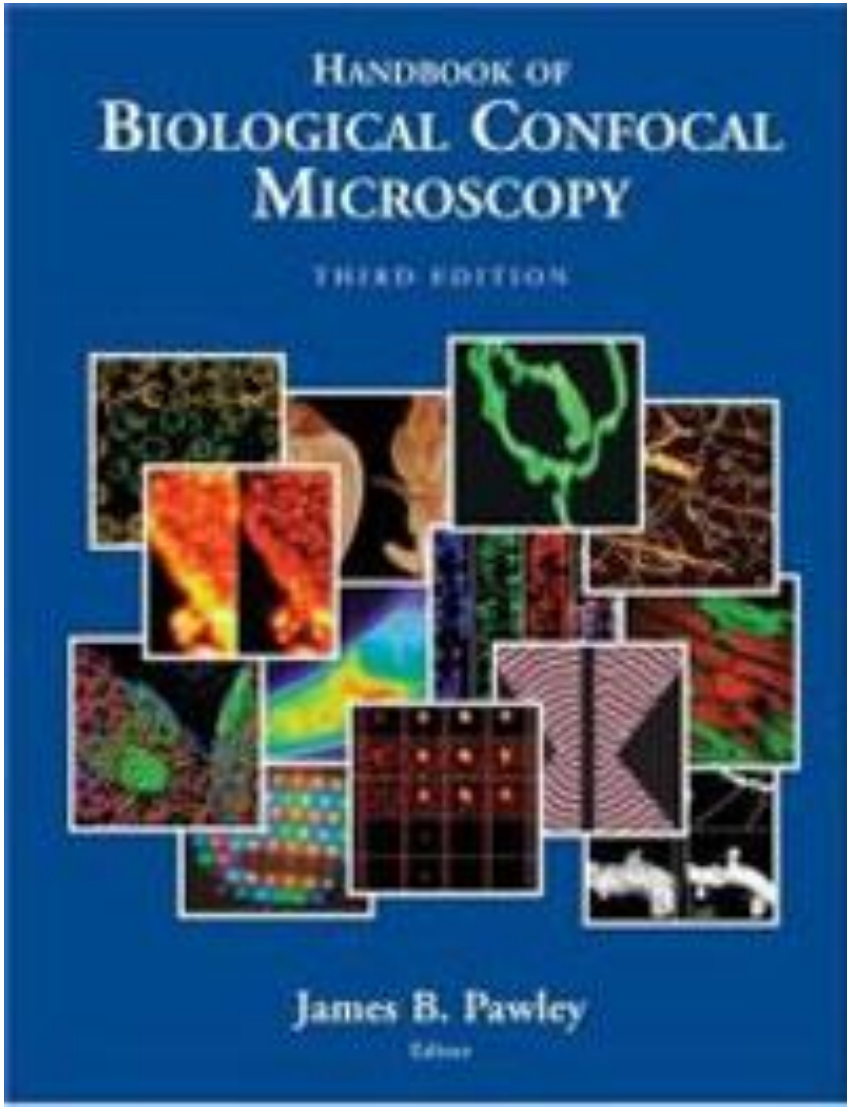




Pinhole projection on CCD camera

How many pinhole in the FOV?

About 850 pinhole inside
the Field Of View


HANDBOOK OF
BIOLOGICAL CONFOCAL
MICROSCOPY
THIRD EDITION
James B. Pawley
Editor