ZEISS LSM 880 with Airyscan

Your New Standard for Fast and Gentle Confocal Imaging

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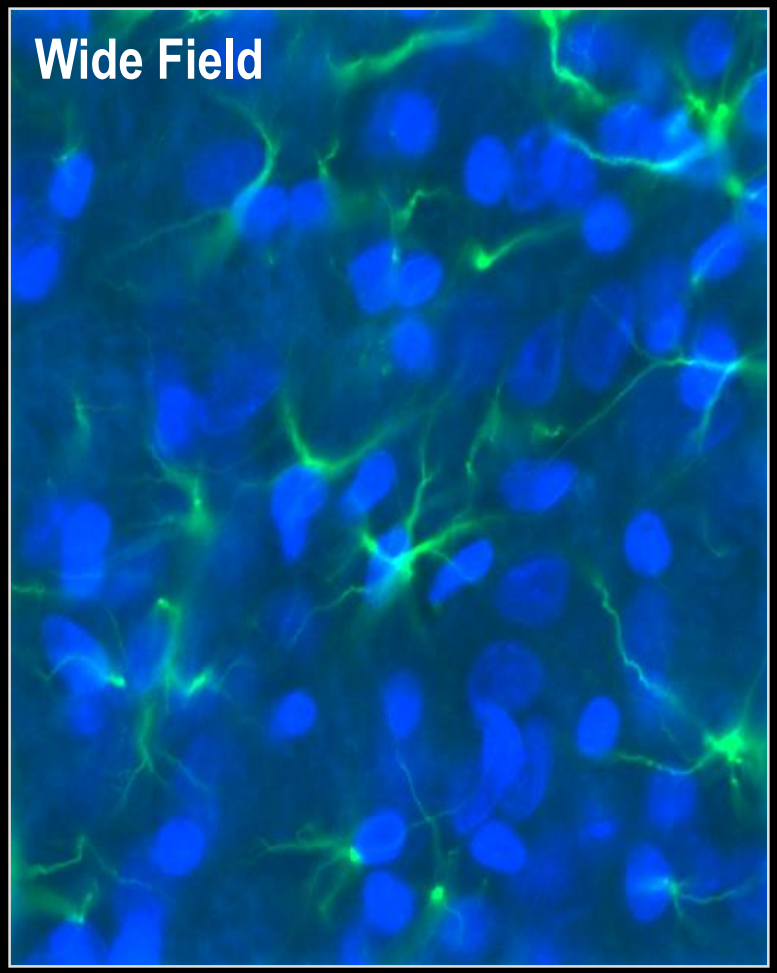
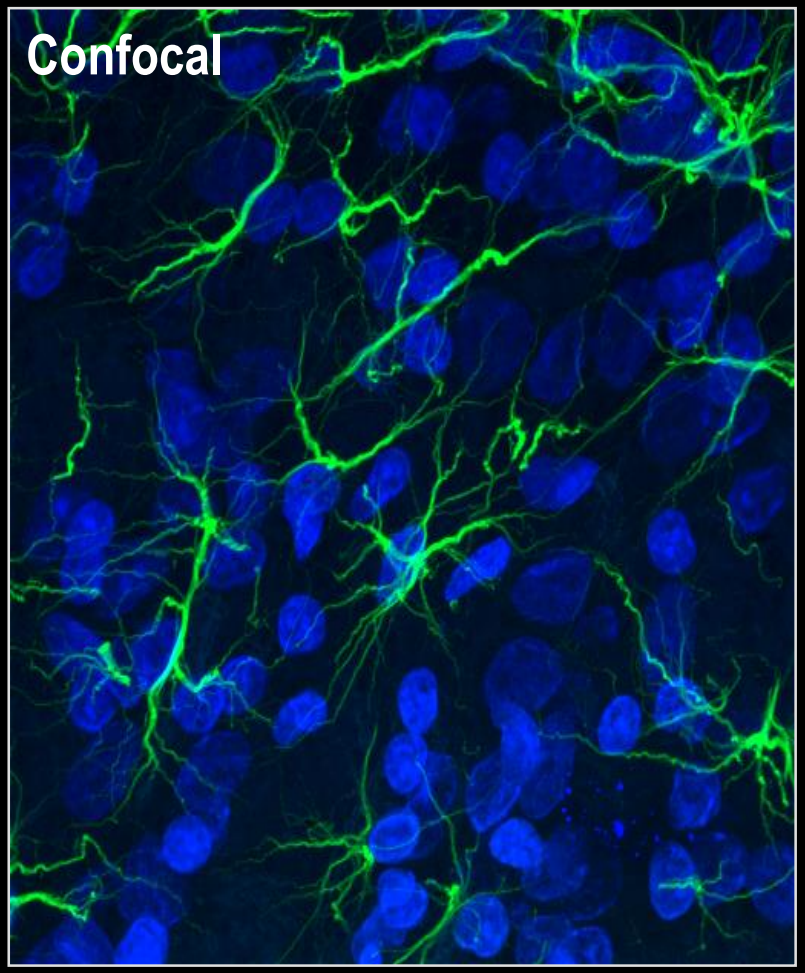
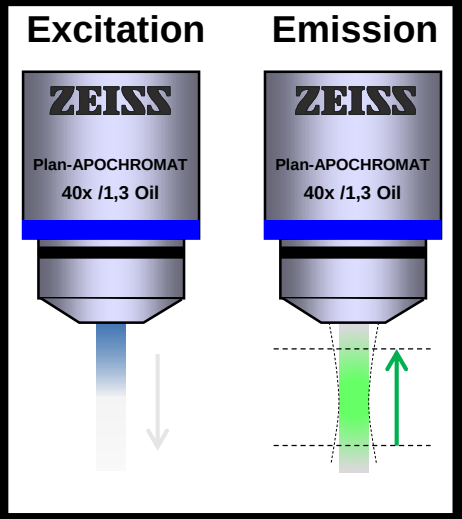
Carl Zeiss RMS

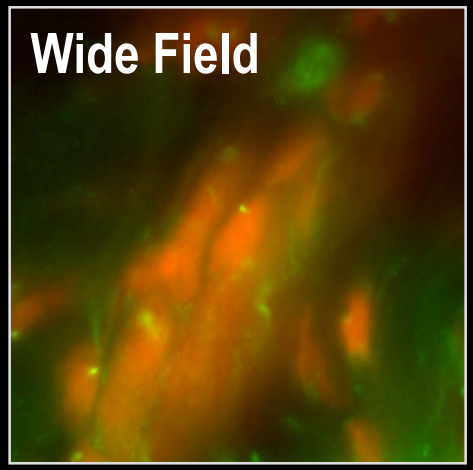
Confocal Principle

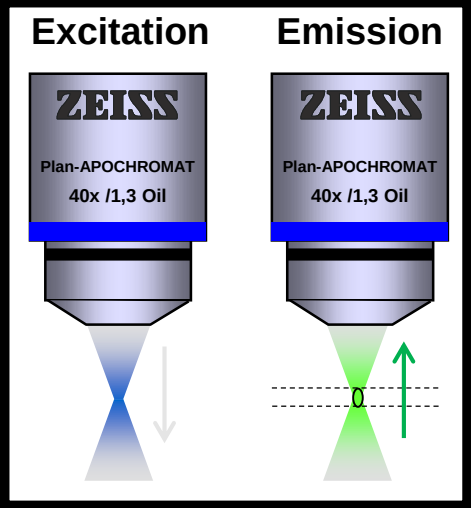


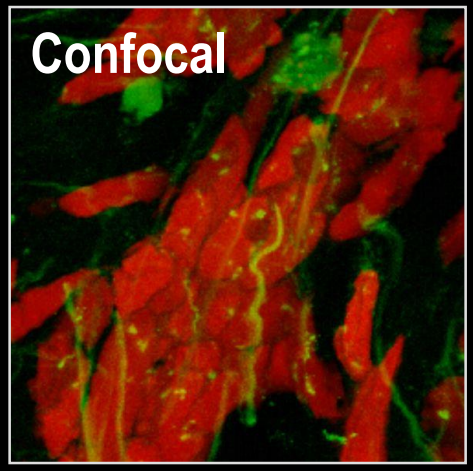


Confocal Principle

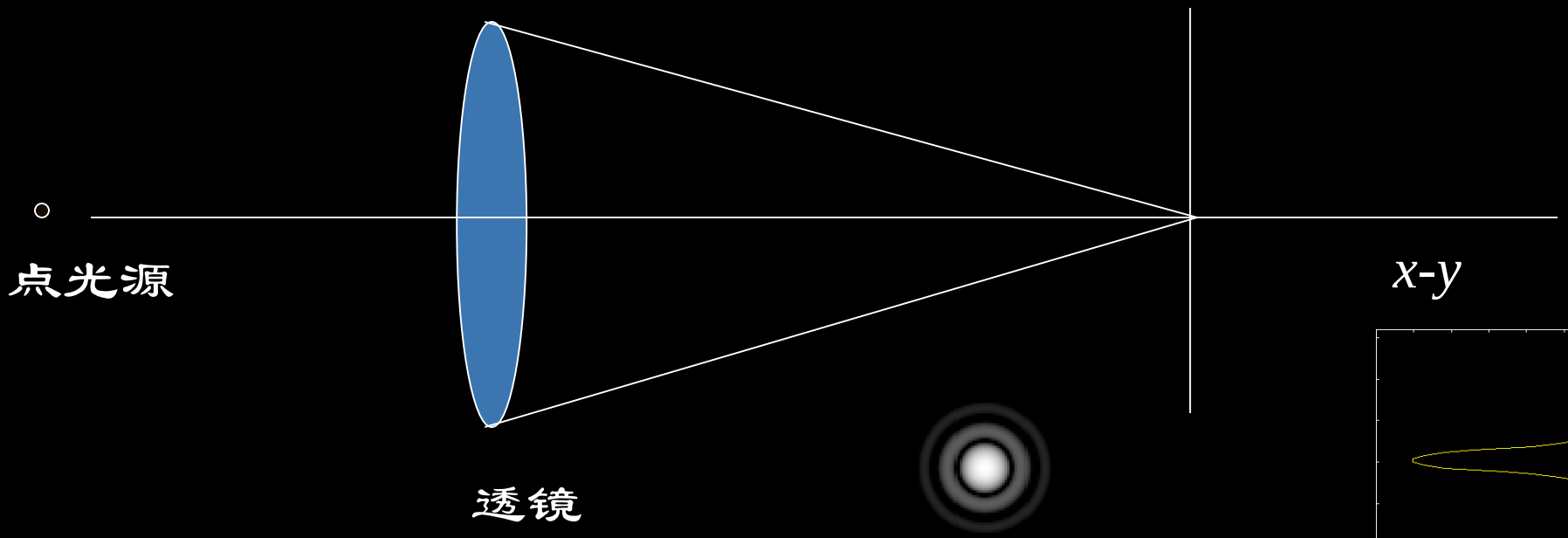




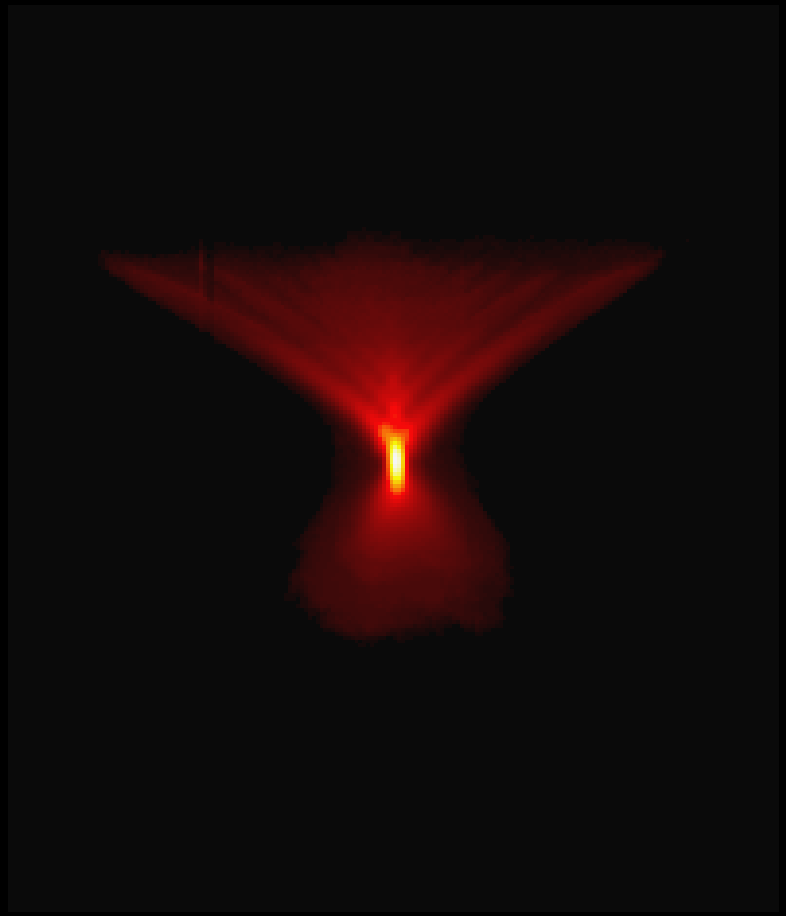


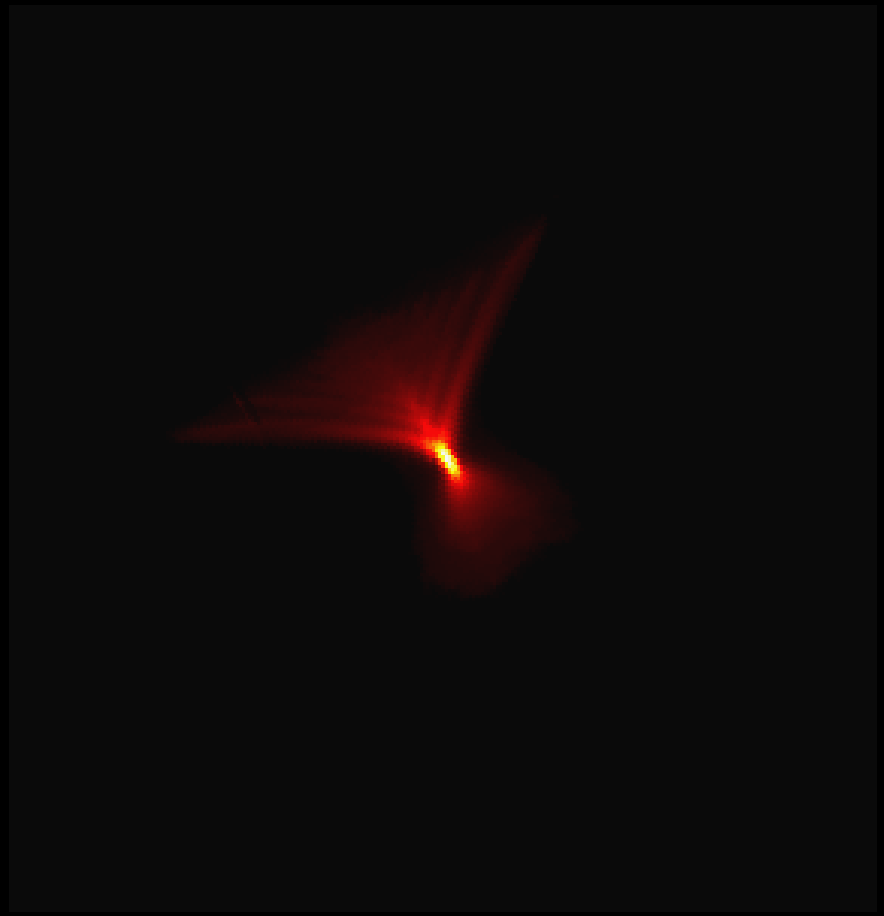


显微镜成像原理

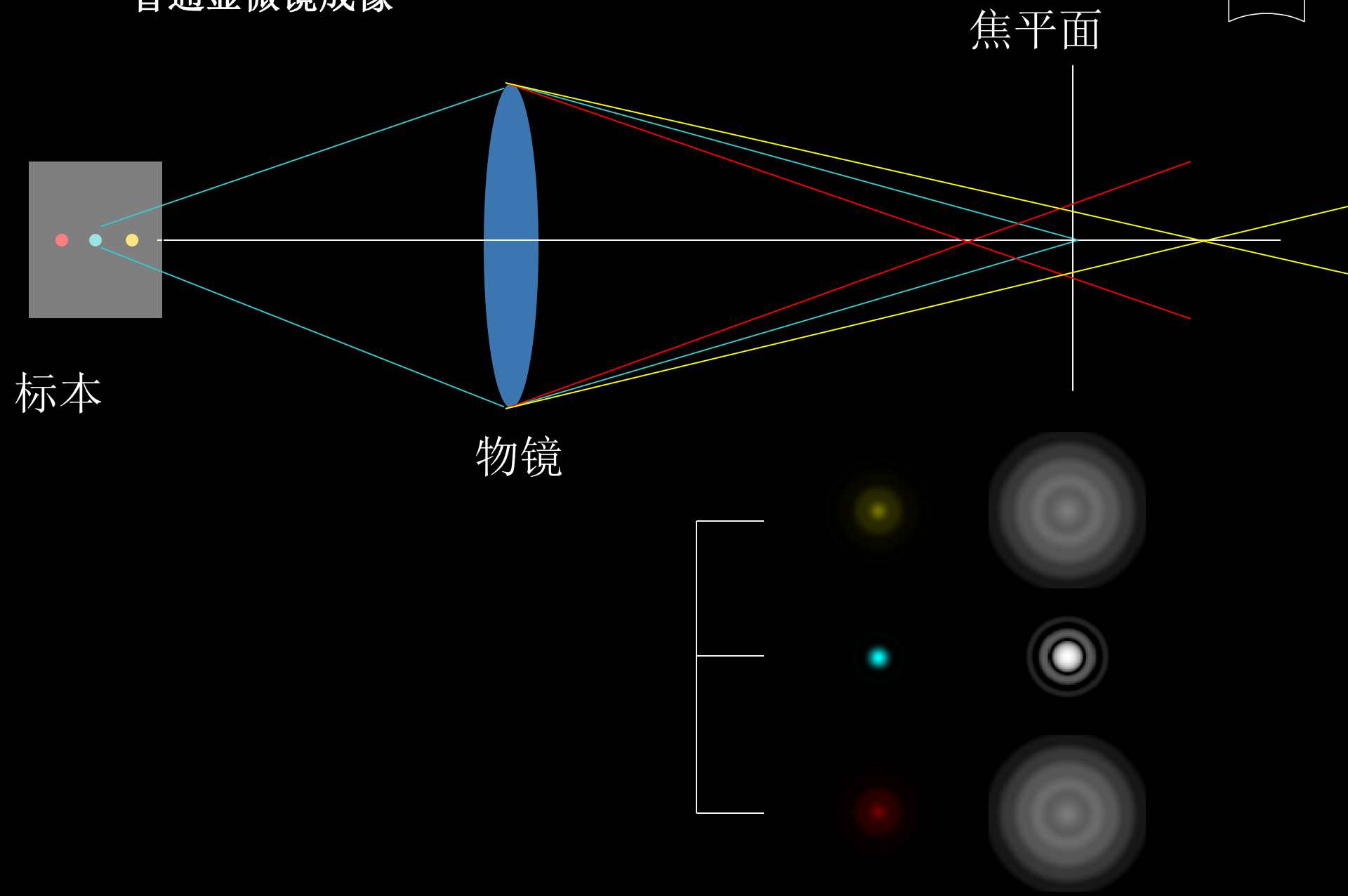


点扩散函数 (PSF)

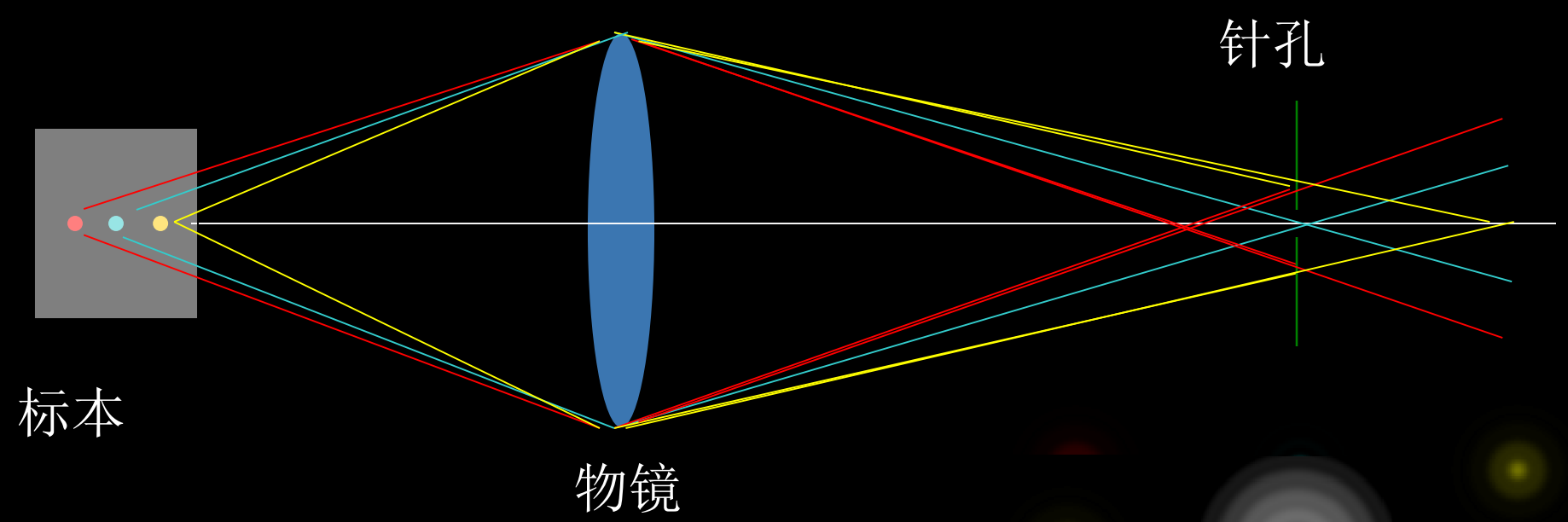


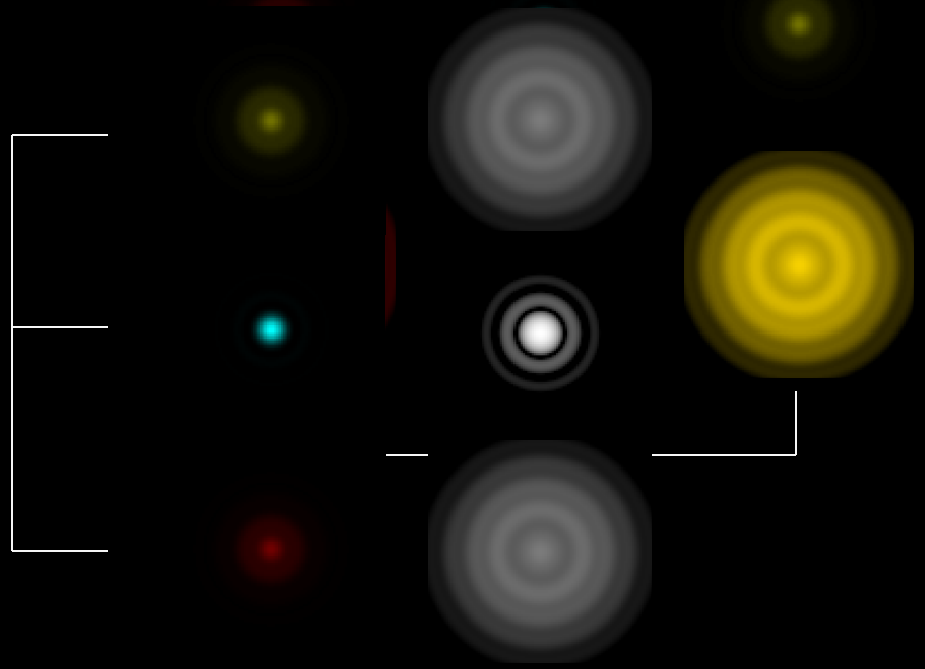


普通显微镜成像



共聚焦显微镜成像





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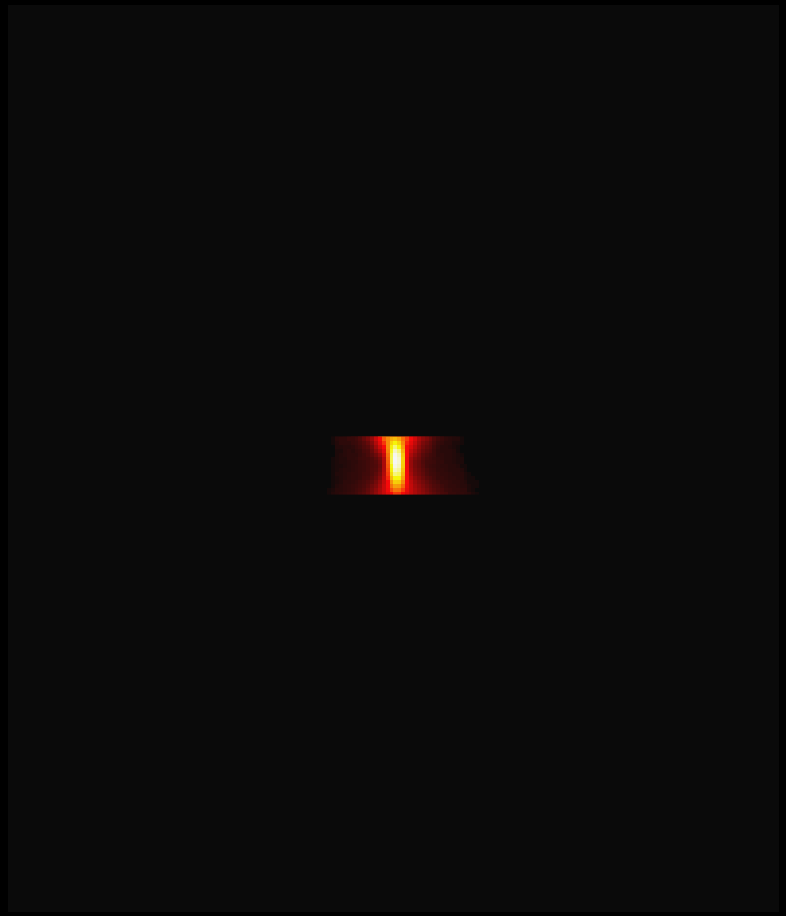
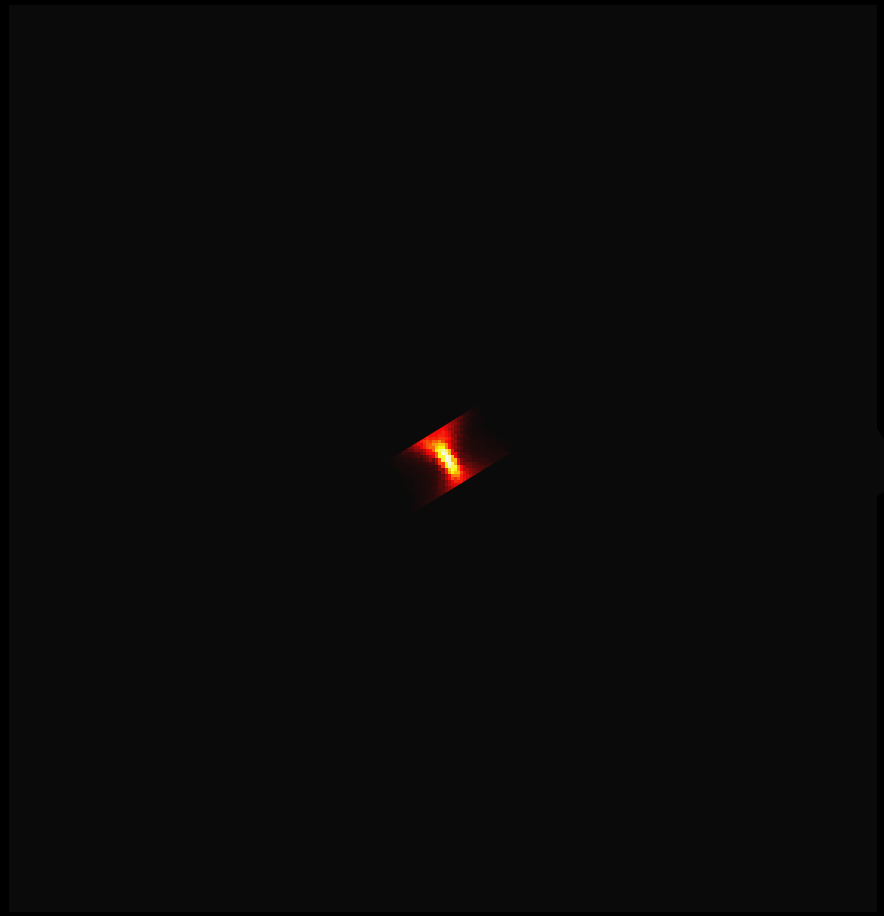
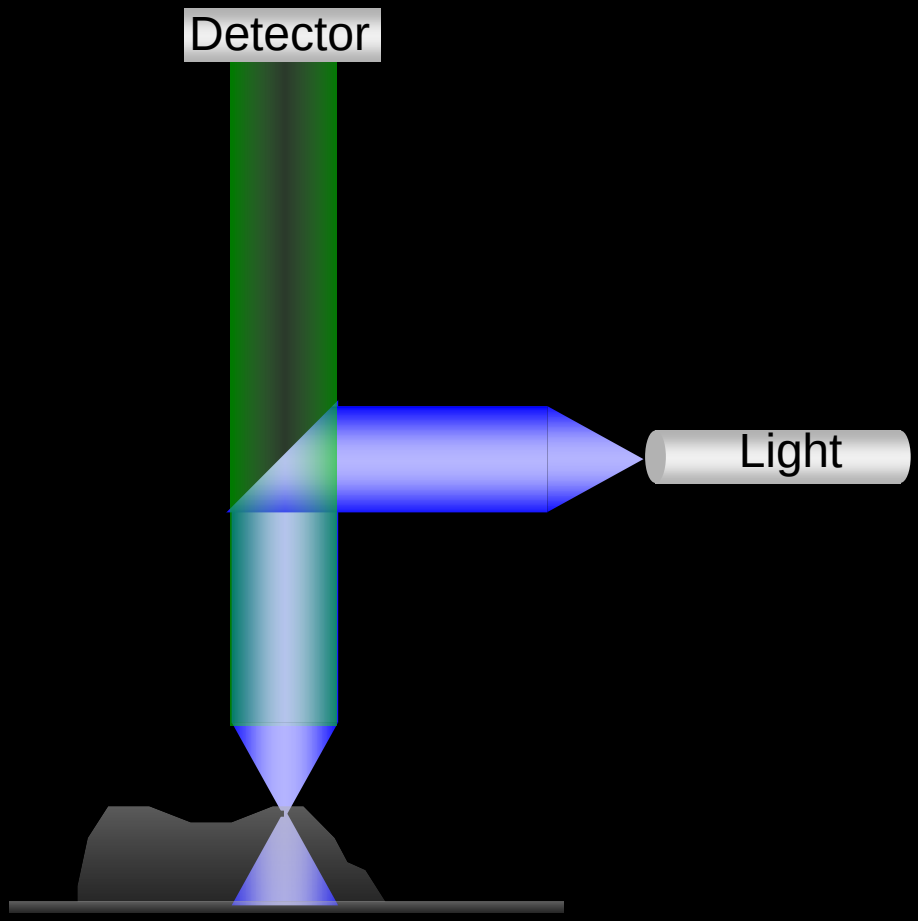
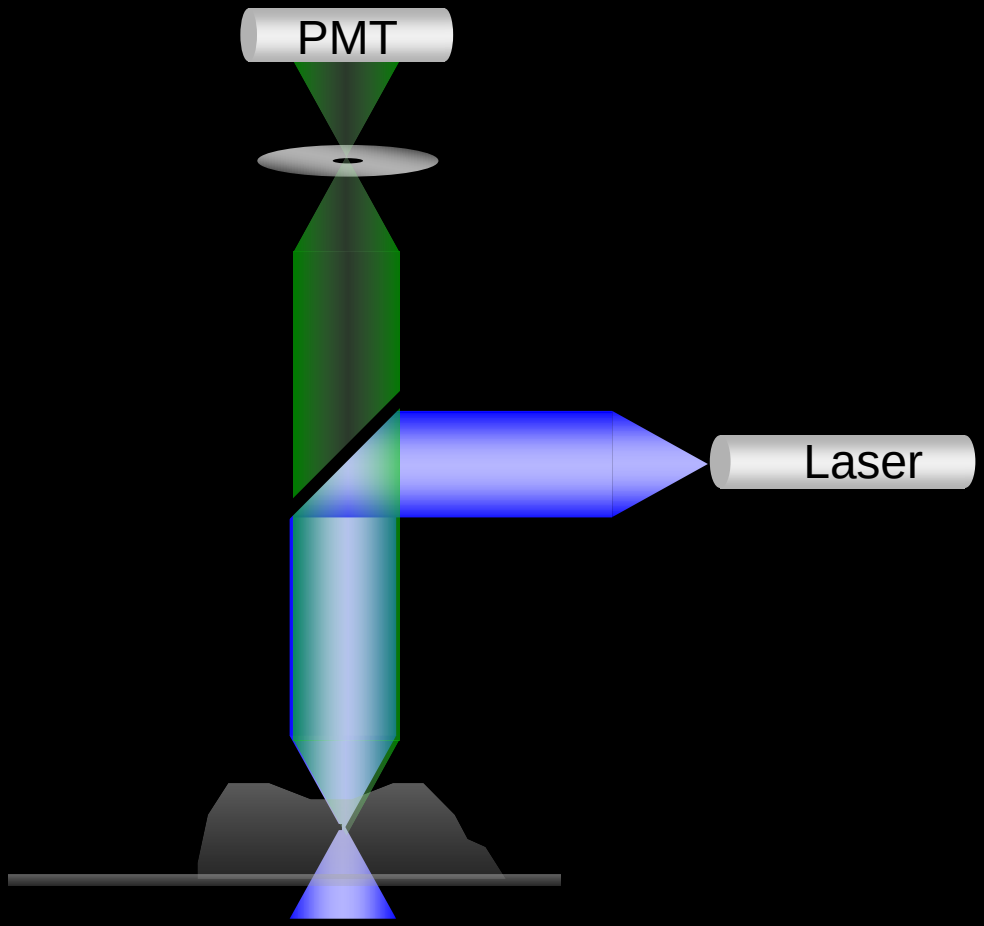




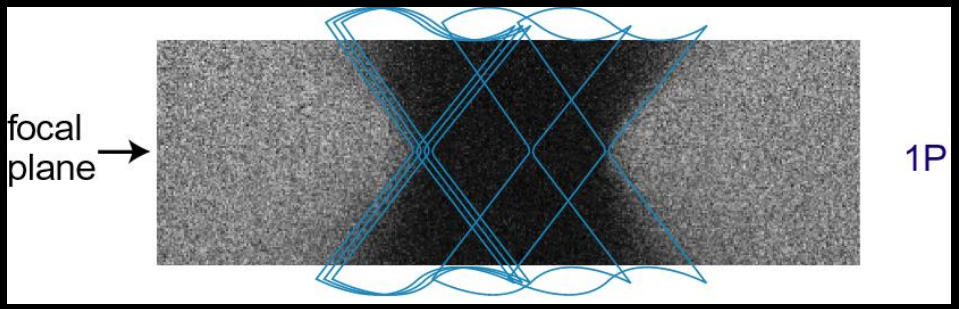
The confocal principle

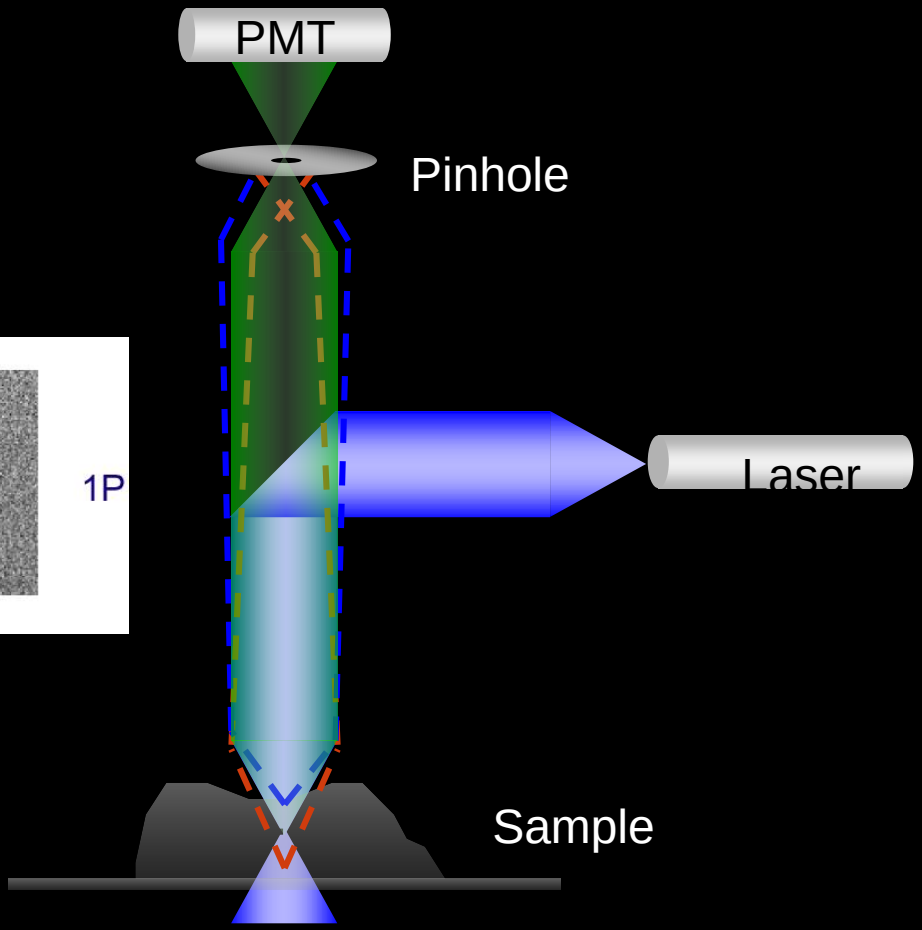


The confocal principle



The confocal principle





Laser Scanning Microscopy

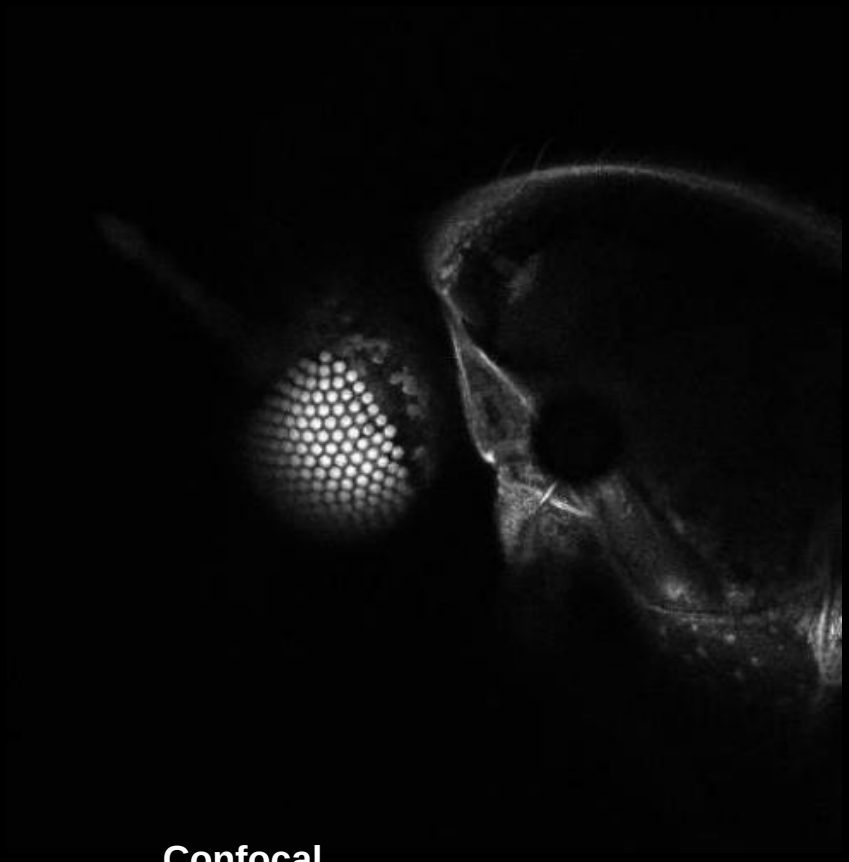
The power of optical sectioning

Wide Field

(out-of-focus light blurs the image)

Laser Scanning Microscopy

The power of optical sectioning



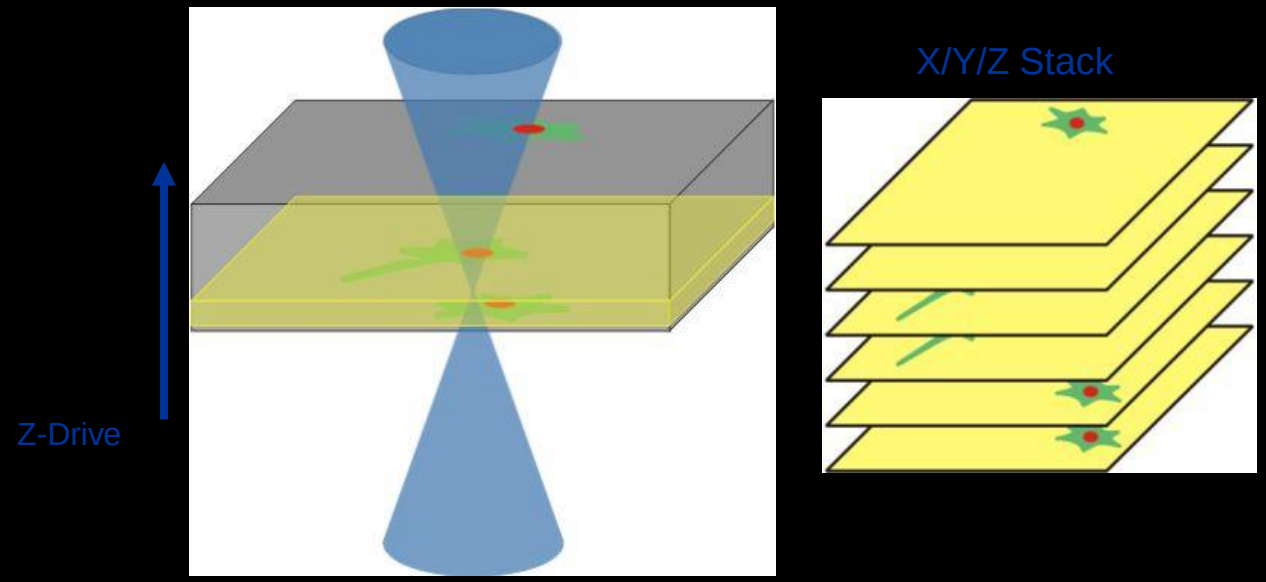
Confocal

(optical sectioning rejects out-of-focus light)

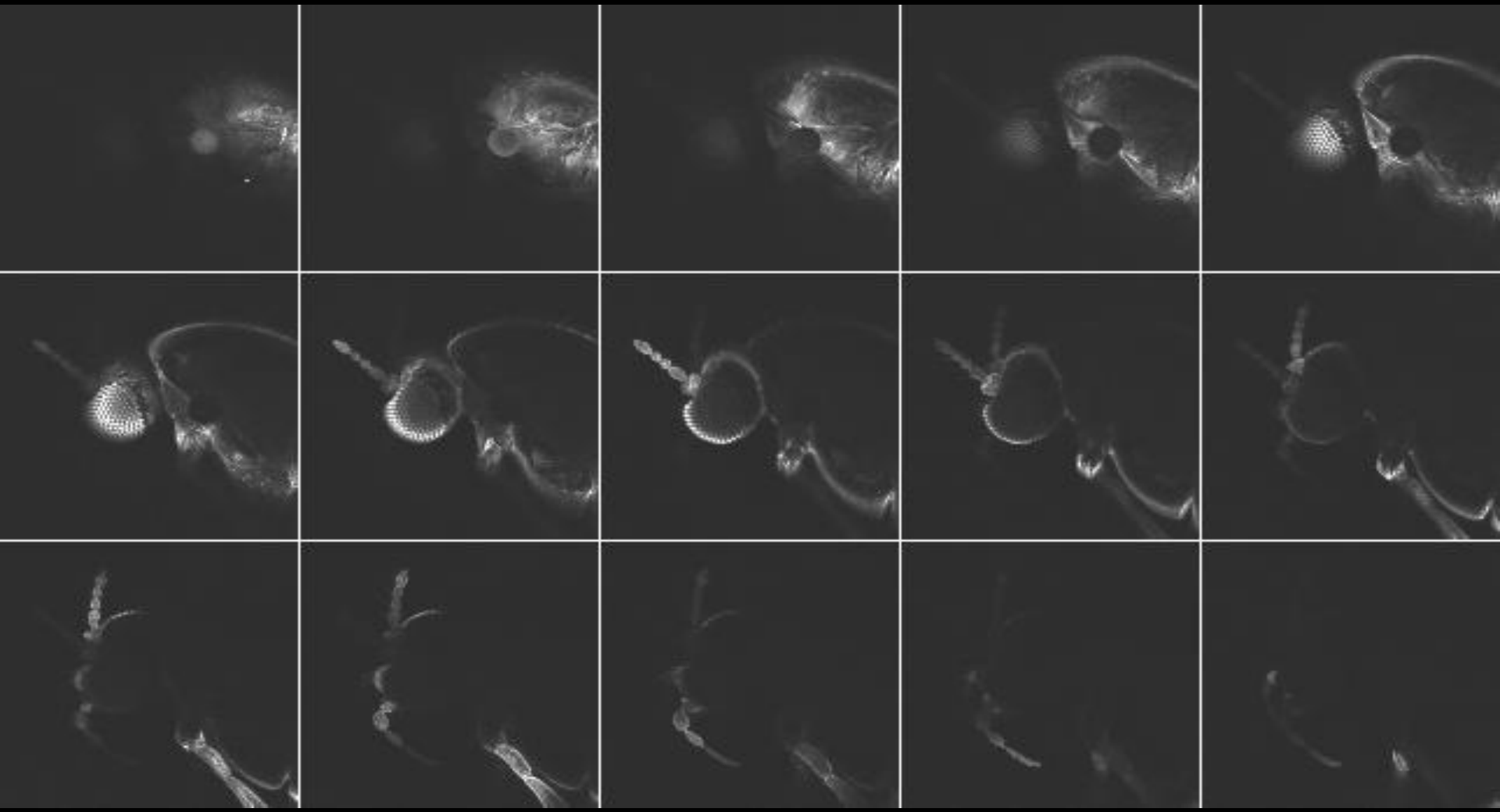
Confocal Microscopy

Acquisition of Optical Sections in Confocal Microscopes

3 D information is acquired by moving the excitation focus not only in XY direction but also in Z direction. The result is a 3 D data stack consisting of number of XY images representing different optical sections from the specimen



另一个重要概念----光切片 **(optical slice)**

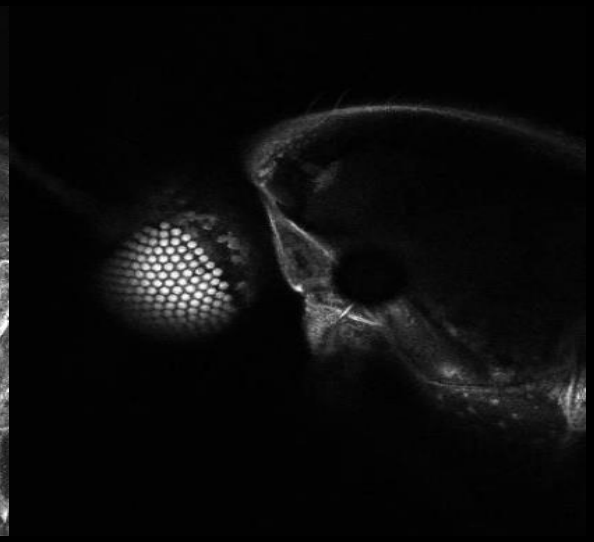


Confocal Microscopy

Acquisition of optical sections

Wide Field
(out-of-focus light blurs the image)

Confocal
(projection of Z-stack)

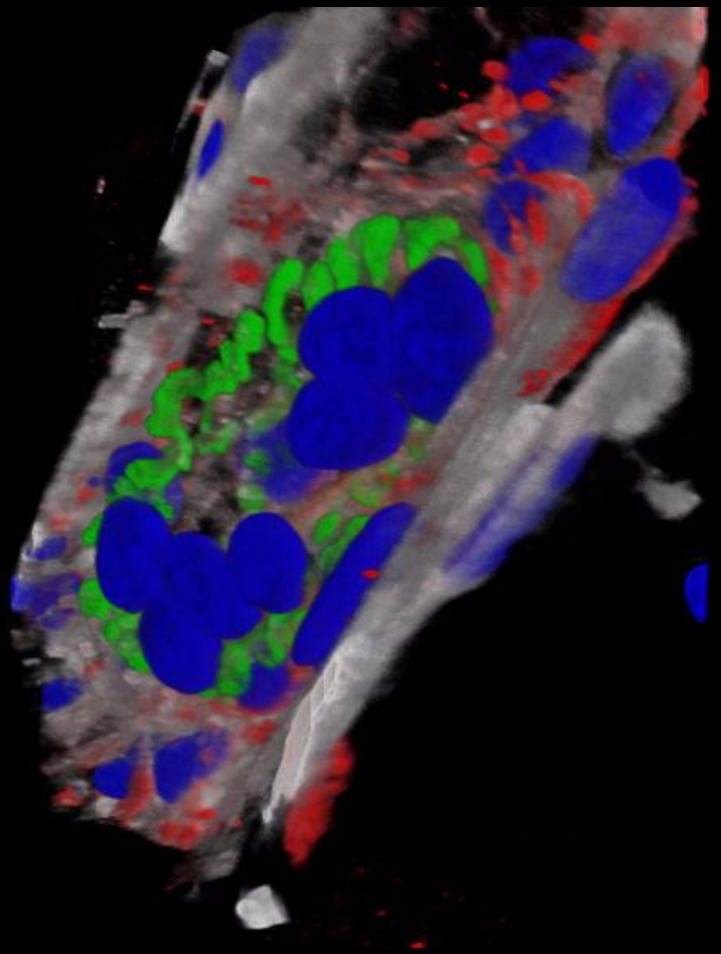


Confocal
(optical sectioning rejects out-of-focus light)

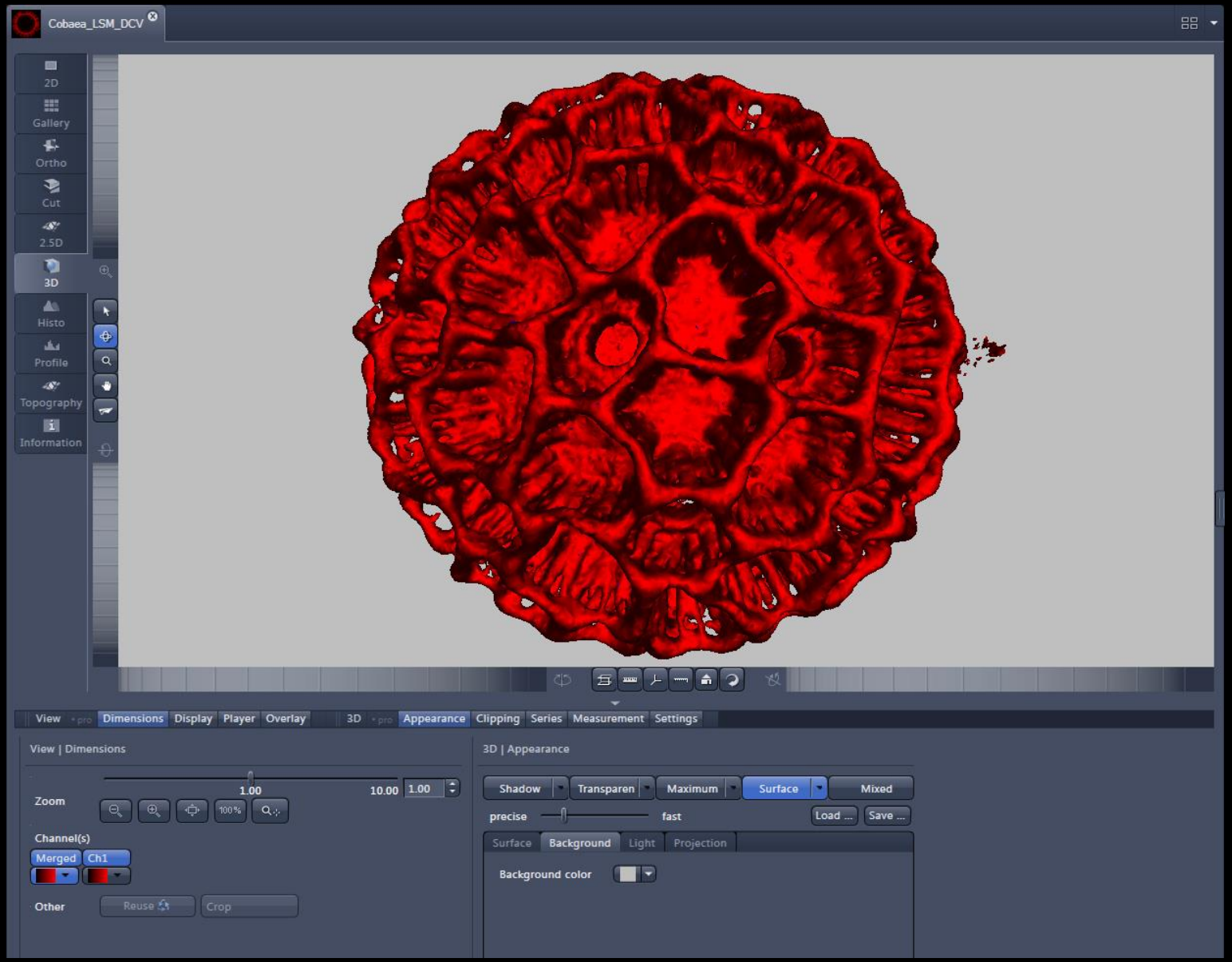
3D Rendering and Animation

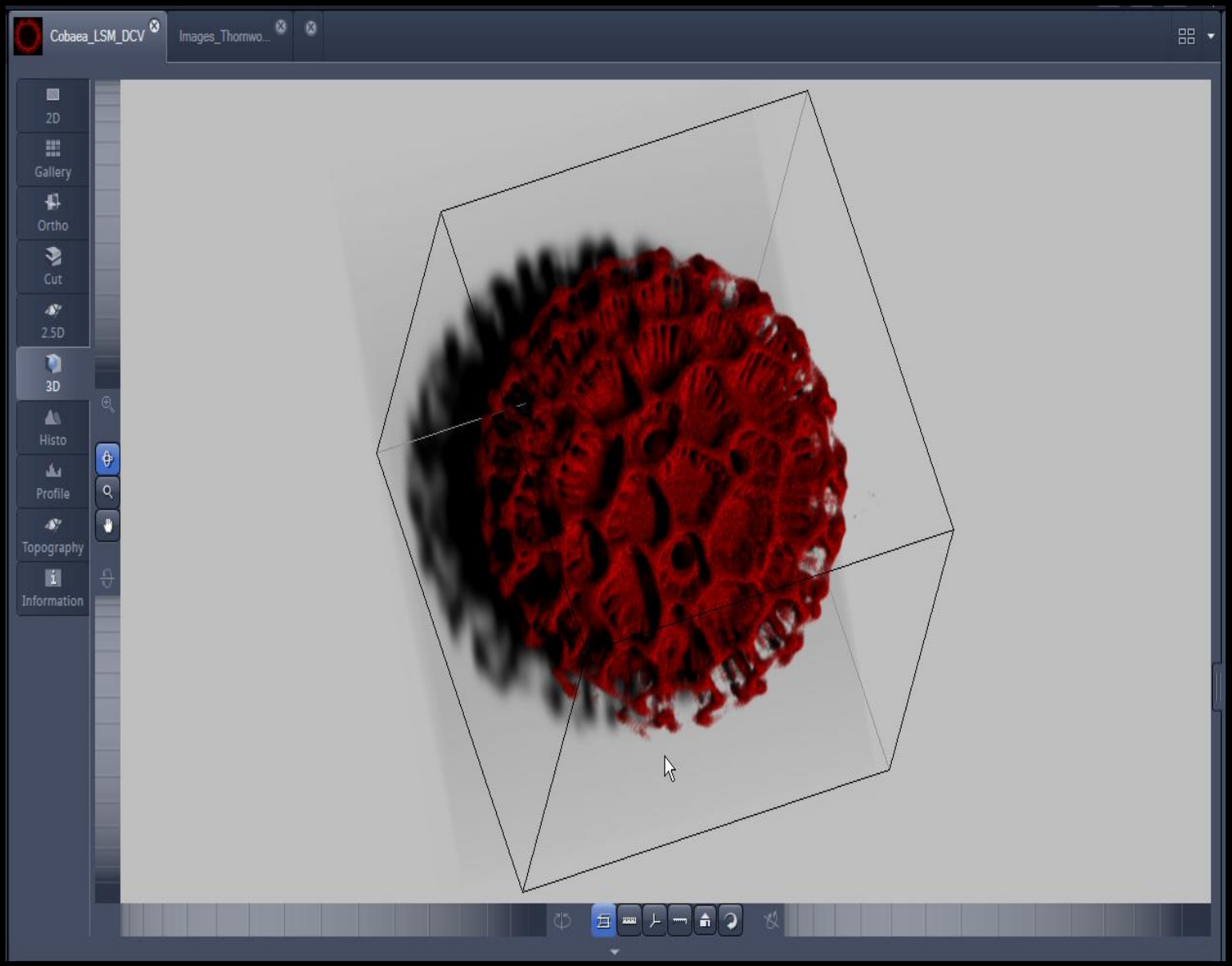
样品：大鼠神经肌肉节点

■ 乙酰胆碱受体
alpha-金环蛇毒素 / Alexa 488
■ 神经膜细胞
S100蛋白/ Alexa 555
■ 细胞核 DAPI
■ CD44 黏附分子/ Atto 647N

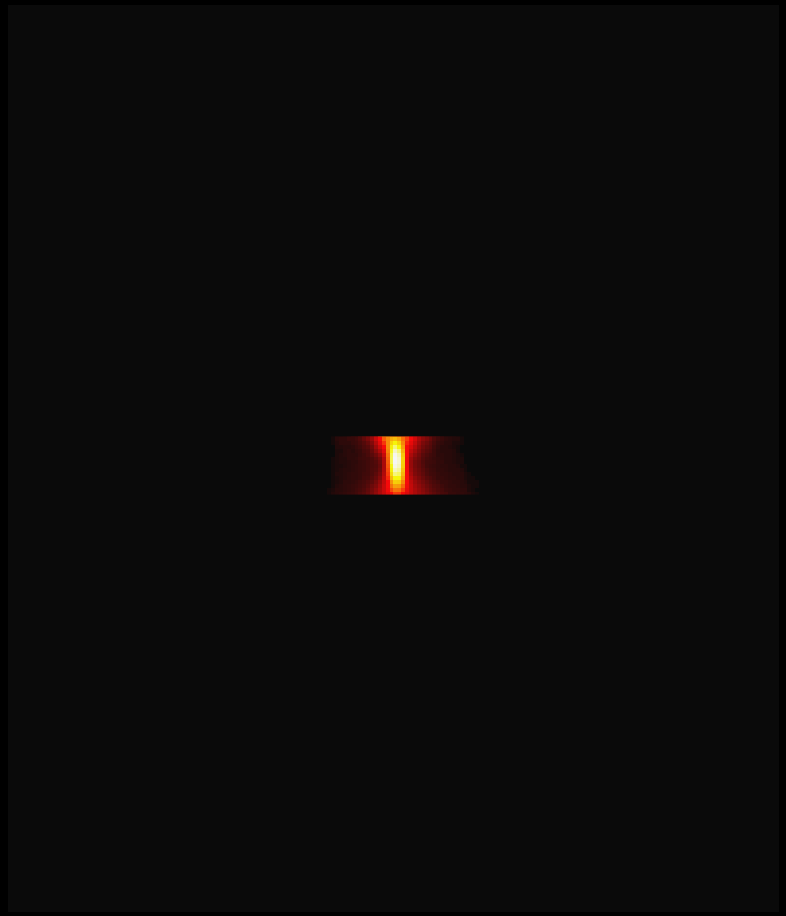


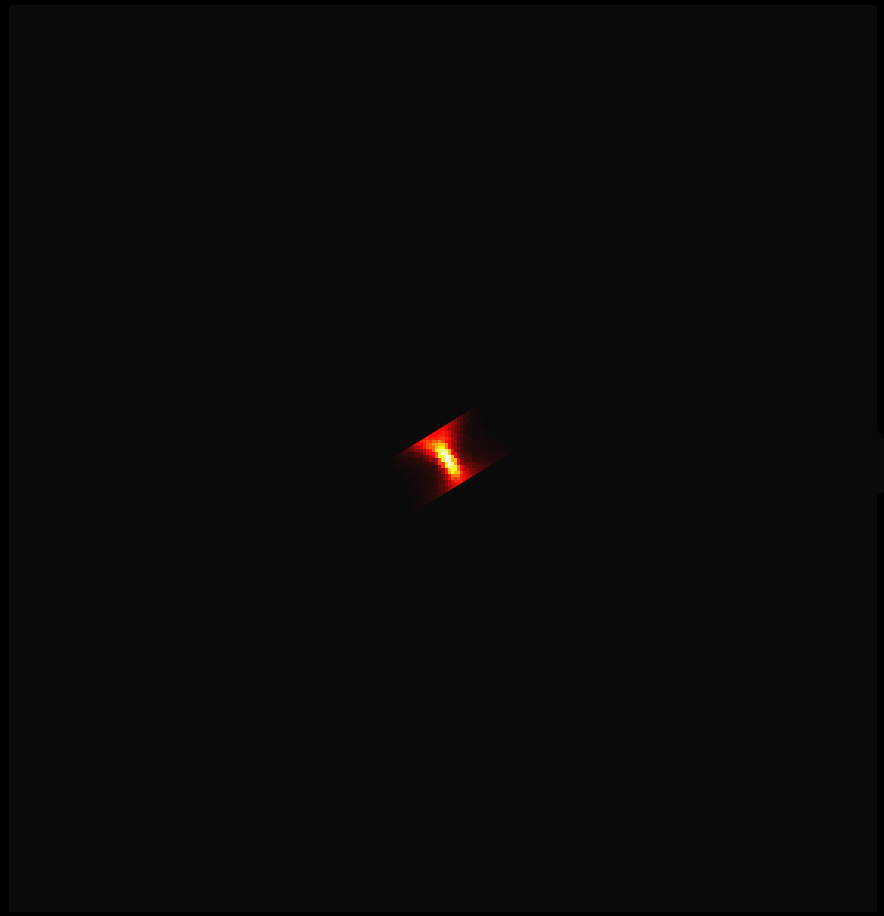
来源:
Dr. Grzegorz Wilczynski
Nencki Institute
Warsaw, Poland


Cobaea_LSM_DCV
2D
Gallery
Ortho
Cut
2.5D
3D
Histo
Profile
Topography
Information
View
Dimensions
Display
Player
Overlay
3D
Appearance
Clipping
Series
Measurement
Settings
View | Dimensions
Zoom
1.00
10.00
1.00
100%
Channel(s)
Merged
Ch1
Other
Reuse
Crop
3D | Appearance
Shadow
Transparen
Maximum
Surface
Mixed
precise
fast
Load ...
Save ...
Surface
Background
Light
Projection
Background color


Cobaea_LSM_DCV
Images_Thornwo...
2D
Gallery
Ortho
Cut
2.5D
3D
Histo
Profile
Topography
Information

点扩散函数 (PSF)



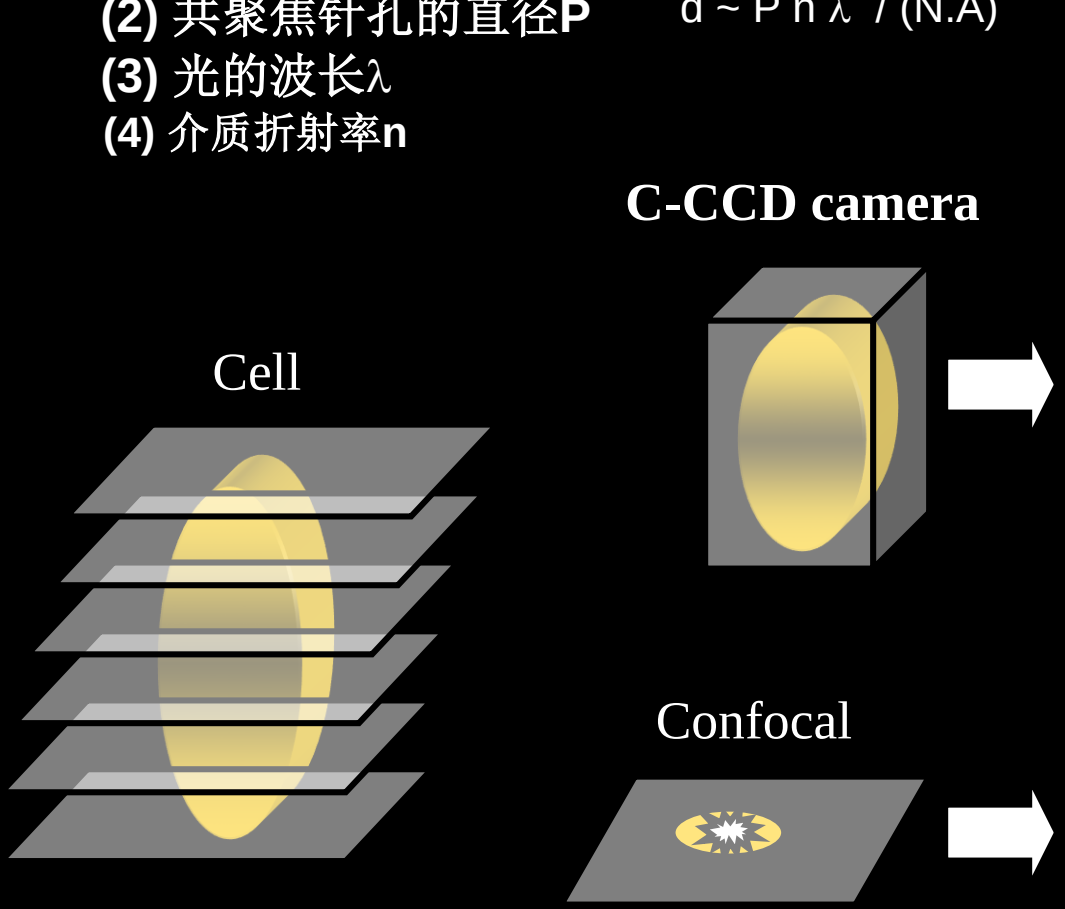


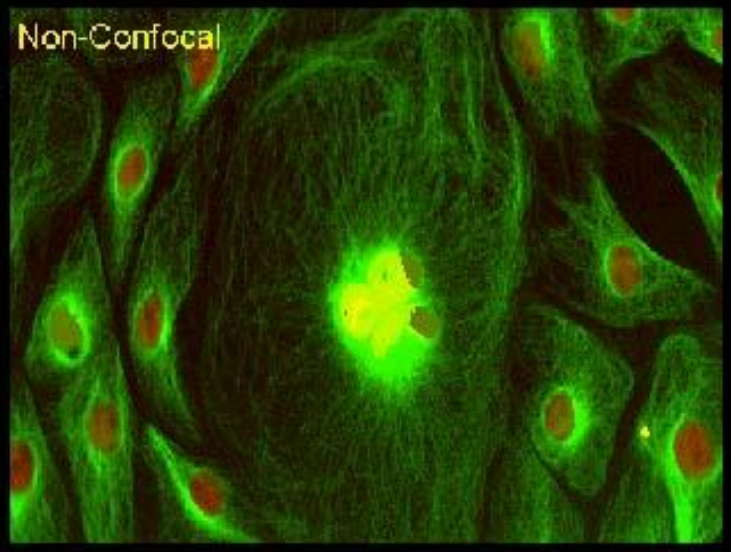
Confocal vs C-CCD: Optical Section

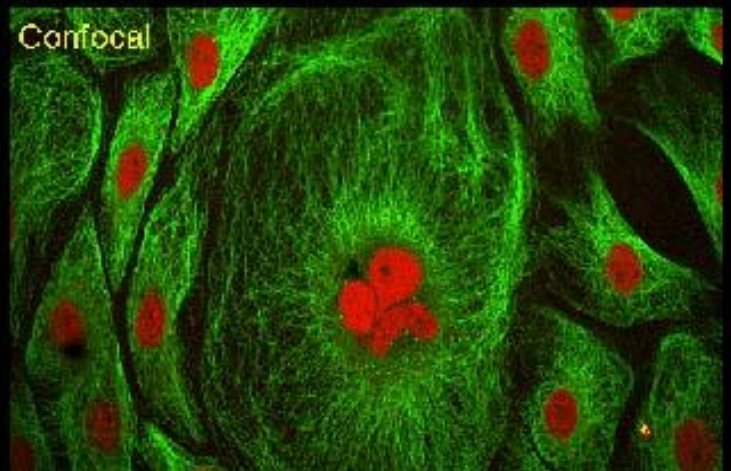
光学切片的厚度 **d** 取决于：

(1) 物镜数值孔径（**NA**）
(2) 共聚焦针孔的直径**P**
(3) 光的波长λ
(4) 介质折射率**n**

$$d \sim P\, n\, \lambda\ /\ (N.A)^2$$





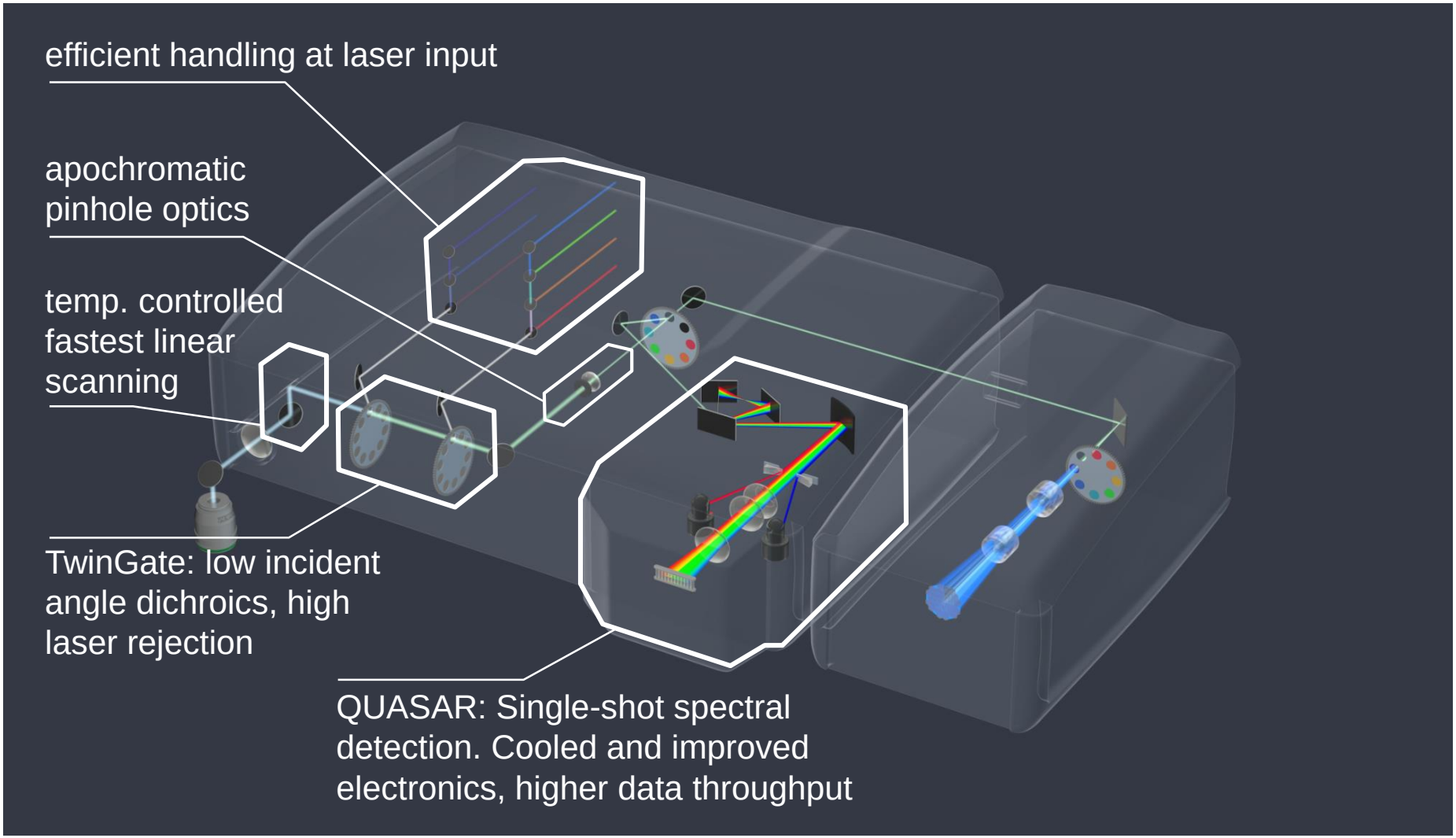


Optimize Sectioning

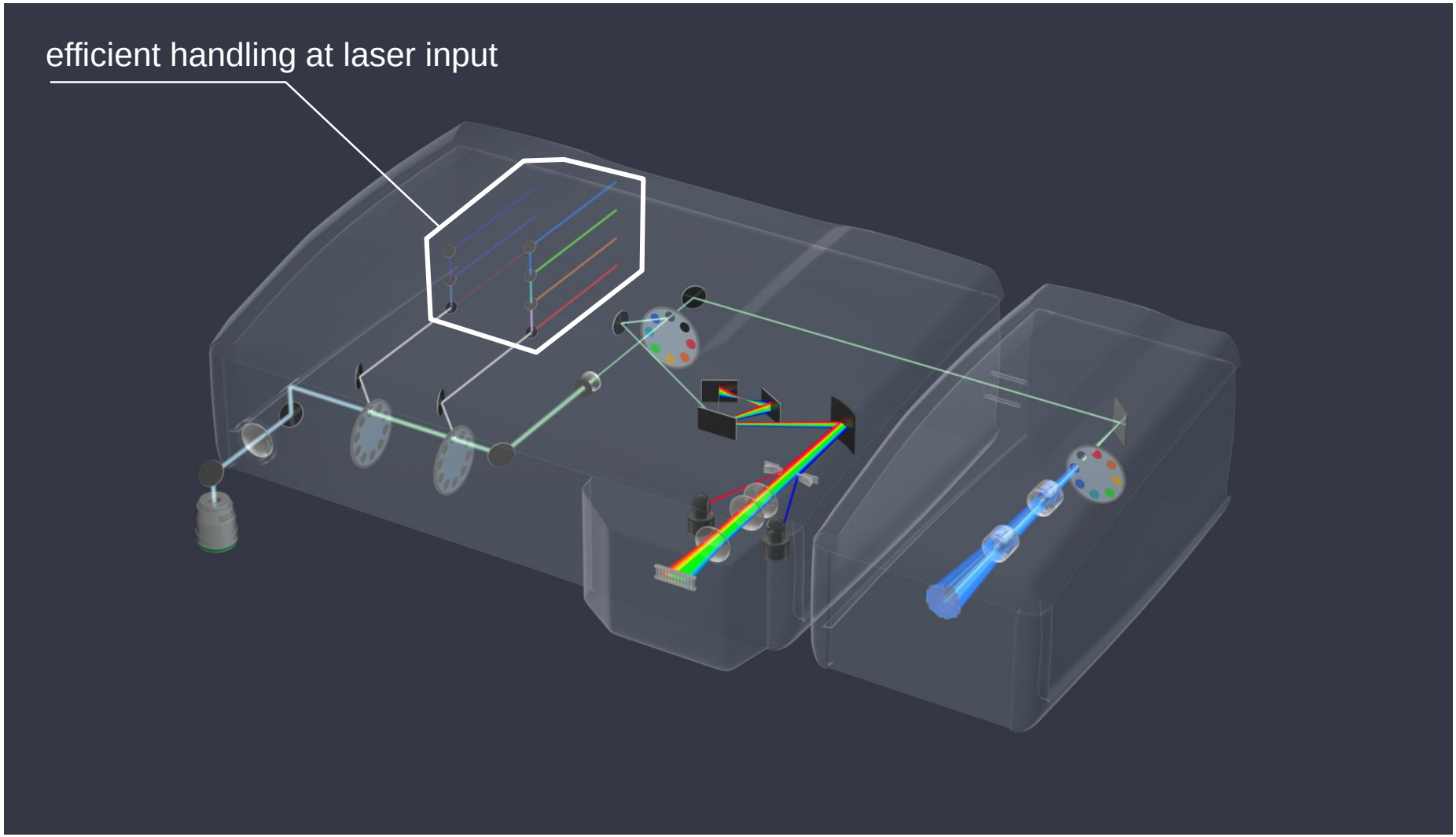
Optimize Sectioning and Step: Optimal Interval is set starting with one Airy unit for all channels

Optimize Sectioning and Step: Match Pinhole to Step resulting in equal optical sections for all channels

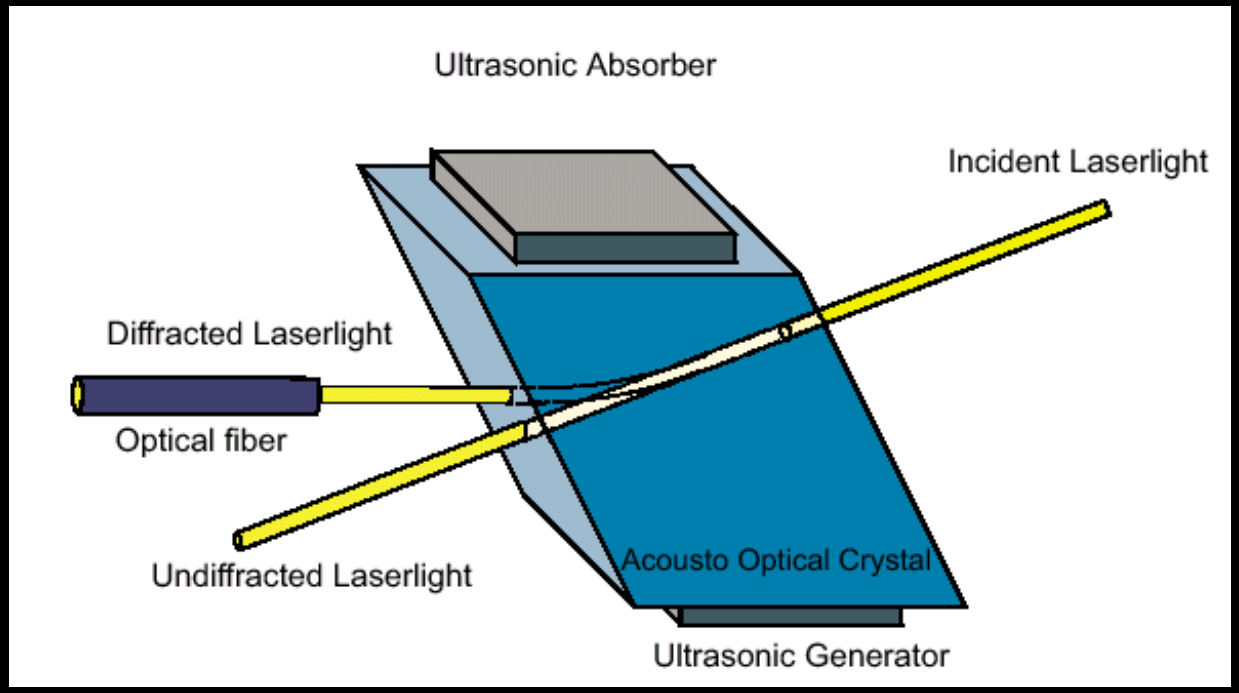
LSM 880 – Scanner

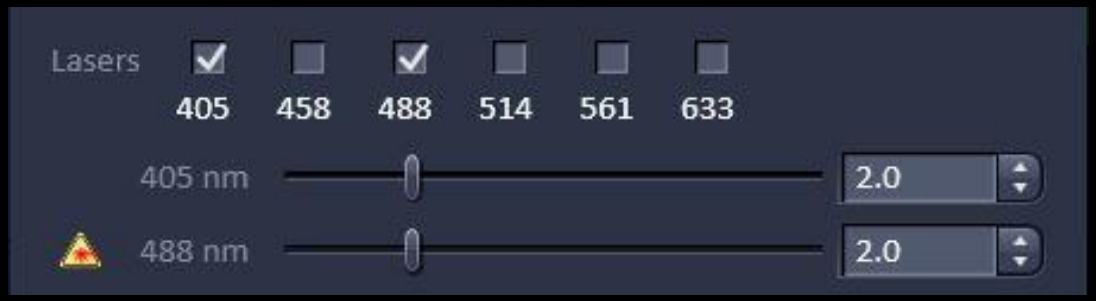


LSM 880 – Scanner

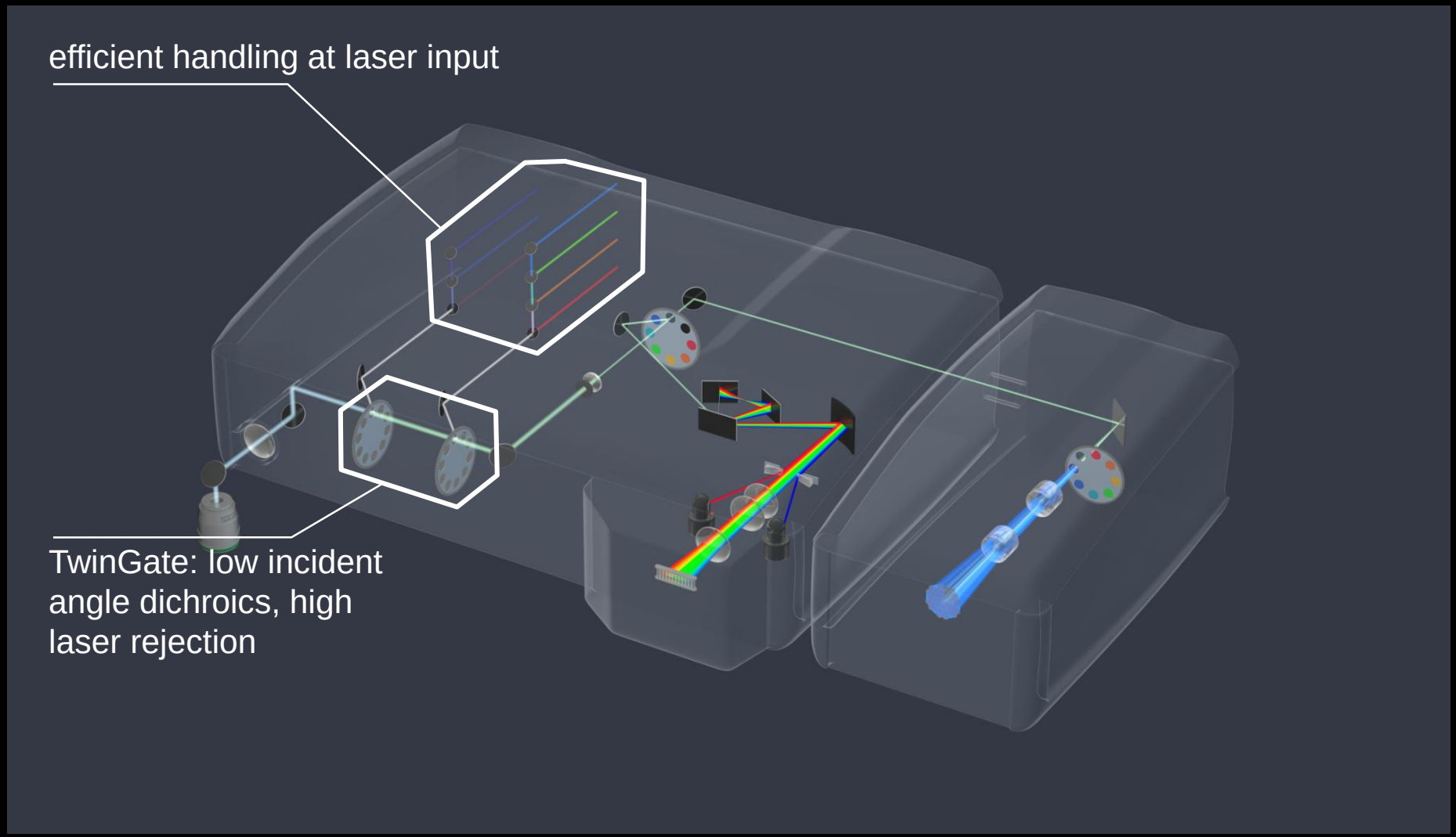


AOTF


Ultrasonic Absorber
Incident Laserlight
Diffracted Laserlight
Optical fiber
Undiffracted Laserlight
Acousto Optical Crystal
Ultrasonic Generator


Lasers
405
458
488
514
561
633
405 nm
2.0
488 nm
2.0

LSM 880 – Scanner



MBS

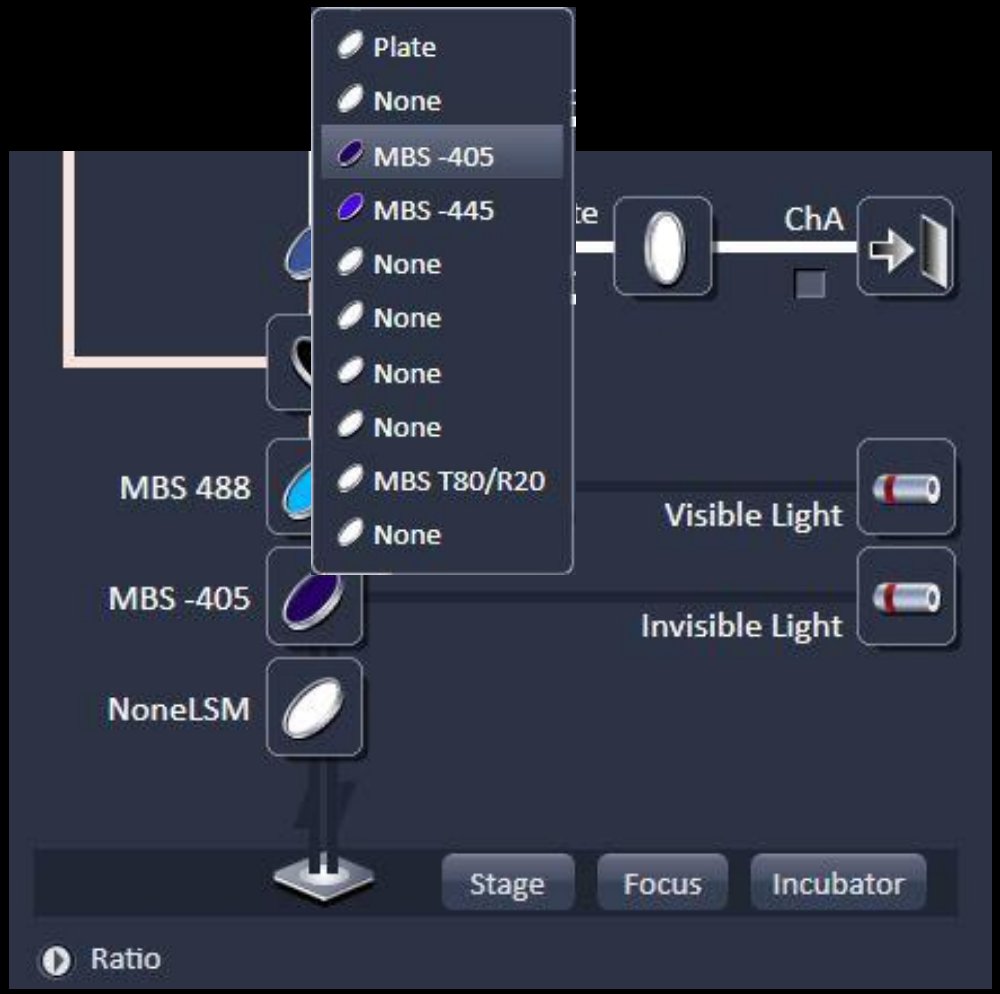
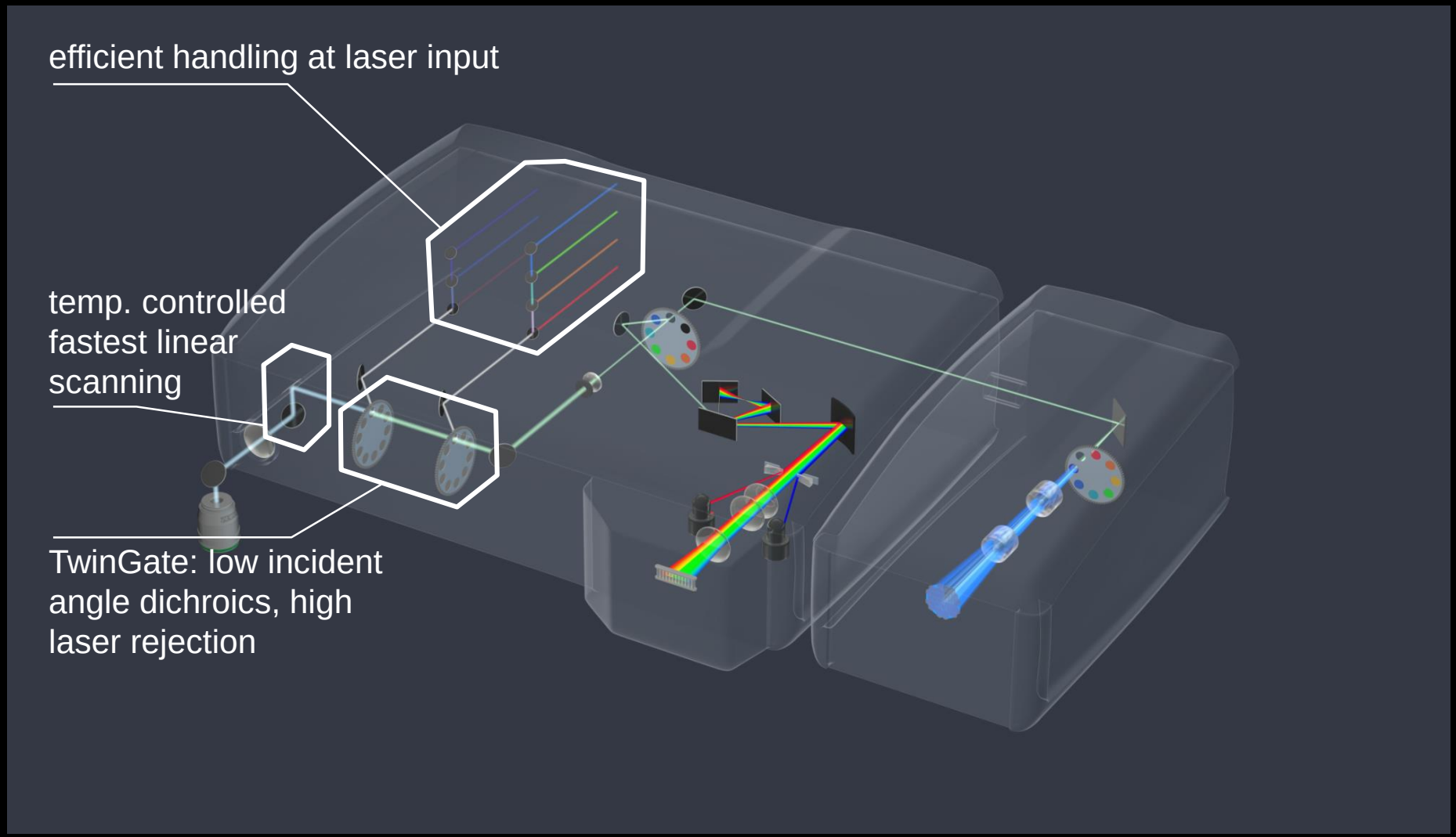
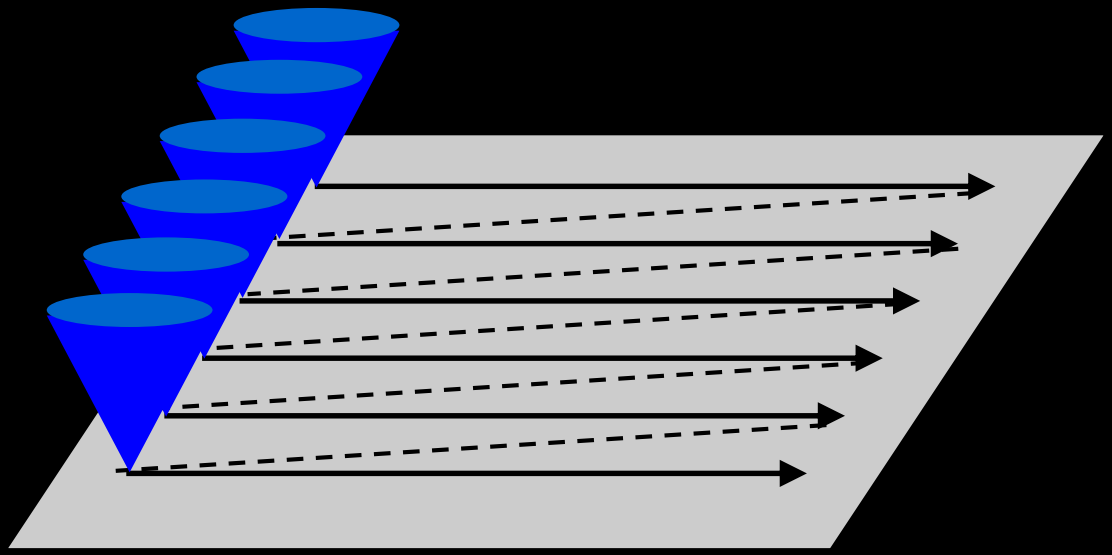


Plate
None
MBS -405
MBS -445
None
None
None
None
MBS T80/R20
None
ChA
MBS 488
MBS -405
NoneLSM
Visible Light
Invisible Light
Stage
Focus
Incubator
Ratio

LSM 880 – Scanner



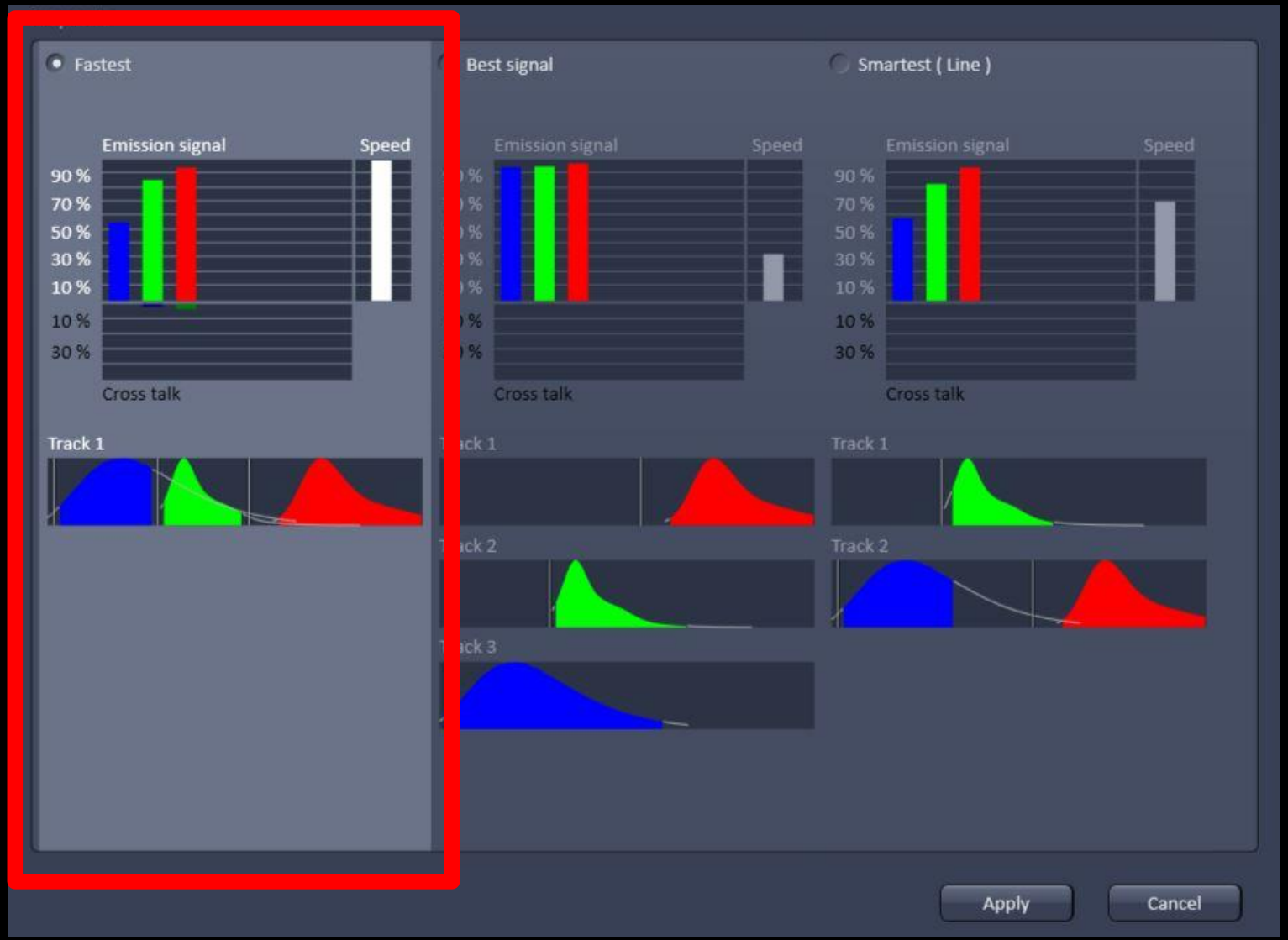
Confocal: Point Scanning



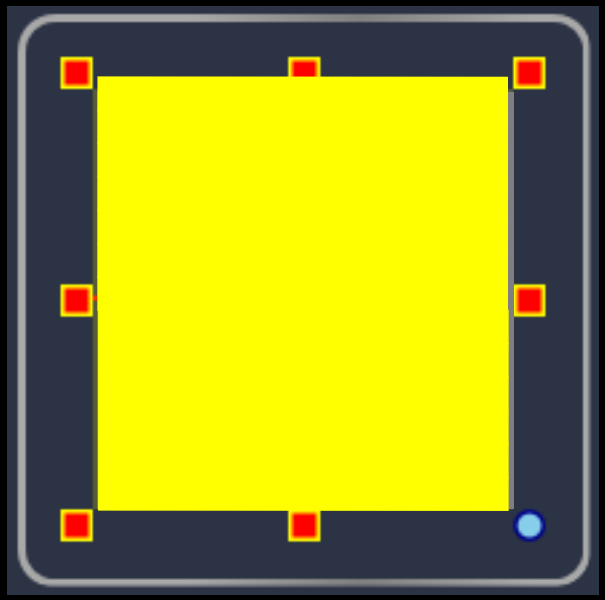
XY scanning

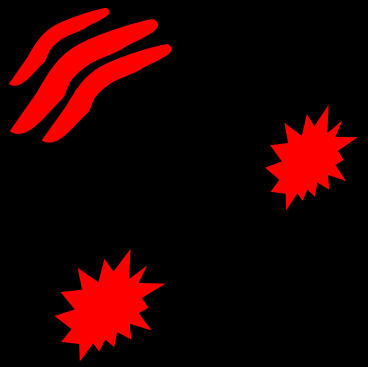
Point scanning confocal systems

Smart Setup


Fastest
Emission signal
Speed
90 %
70 %
50 %
30 %
10 %
10 %
30 %
Cross talk
Track 1
Best signal
Emission signal
Speed
Cross talk
Track 1
Track 2
Track 3
Smartest (Line)
Emission signal
Speed
90 %
70 %
50 %
30 %
10 %
10 %
30 %
Cross talk
Track 1
Track 2
Apply
Cancel

Fastest image acquisition:



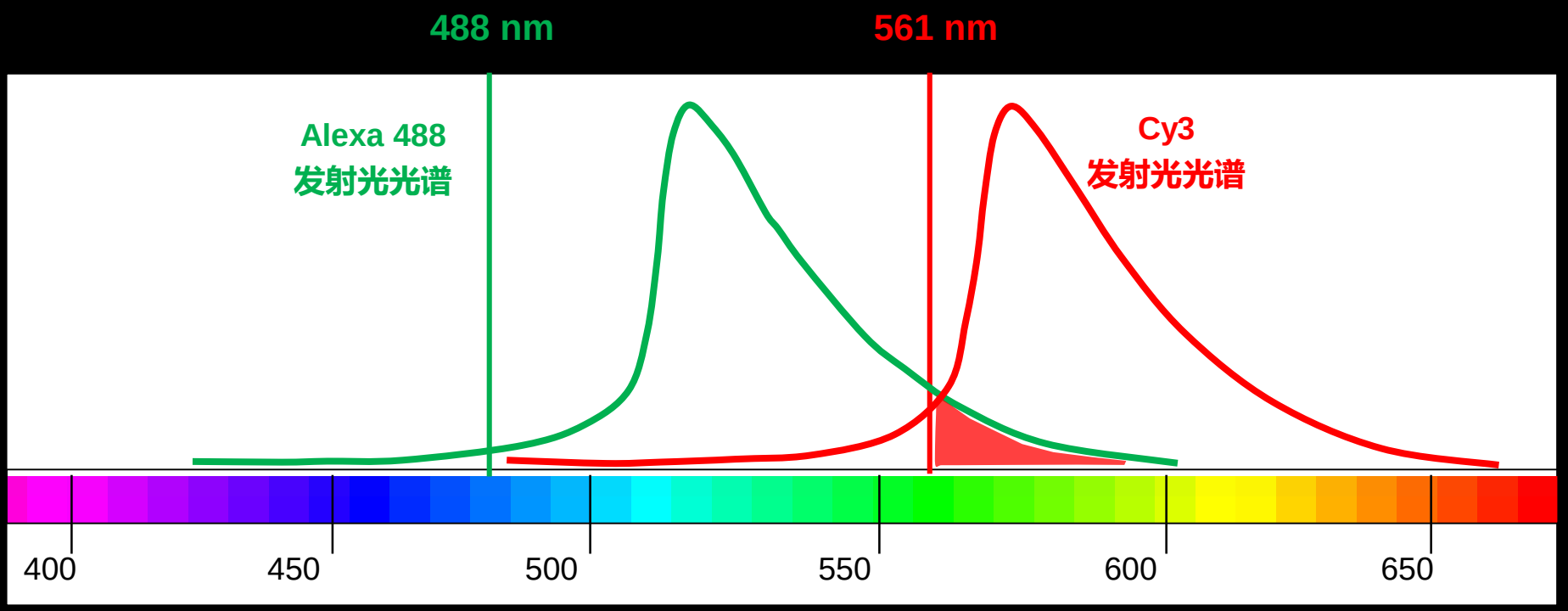


<u>Only one Track acquisition:</u>

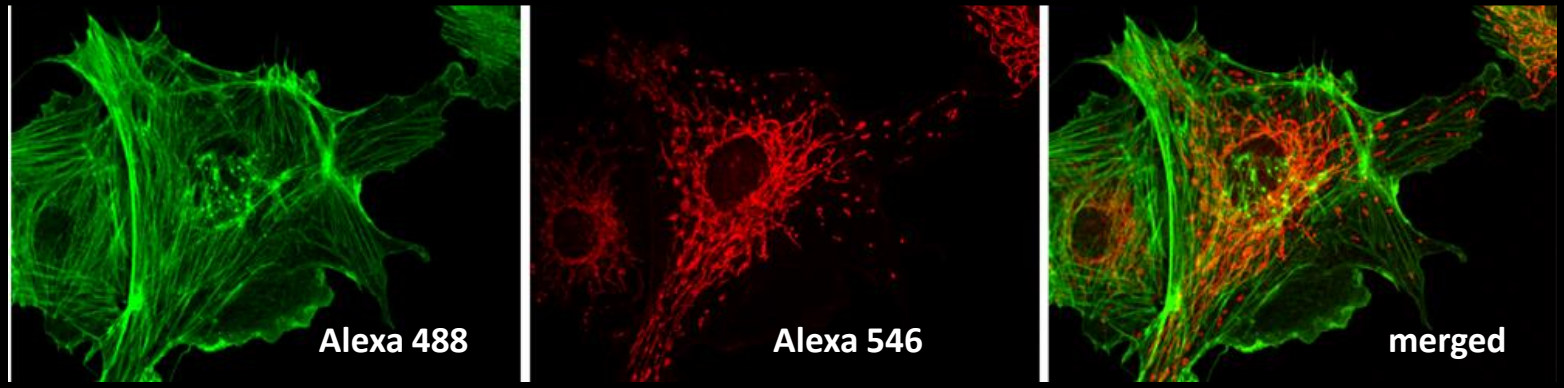
\+ Reduced or no spatial shift in moving samples.
\+ Fastest image acquisition

\- **Crosstalk ! ! !**

Emission Crosstalk
发射光串色

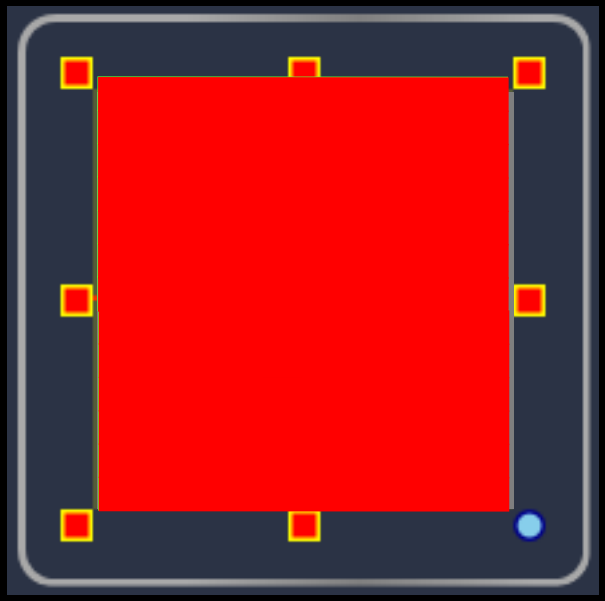


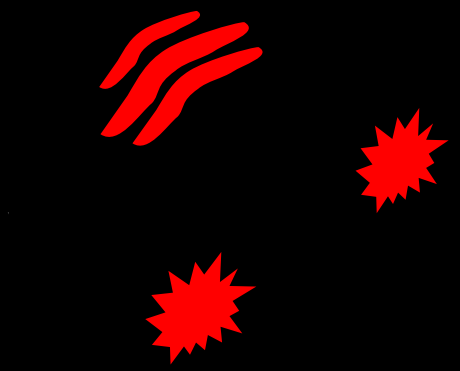
Emission Crosstalk
发射光串色



Sequential image acquisition: Framewise

Up to 4 individual tracks can be setup





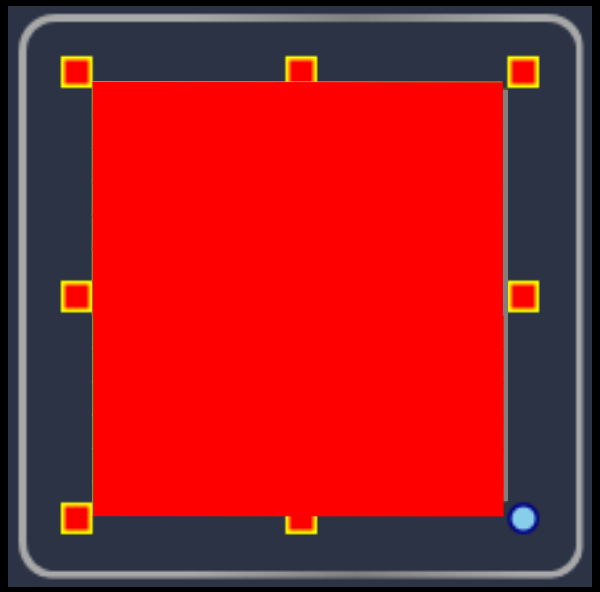
<u>Switch Track every Frame:</u>

\+ Hardware settings can be changed inbetween tracks (e.g. pinhole diameter, beamsplitters)

\- A moving sample can create a spatial shift of the two channels

Sequential image acquisition: Linewise

Up to 4 individual tracks can be setup

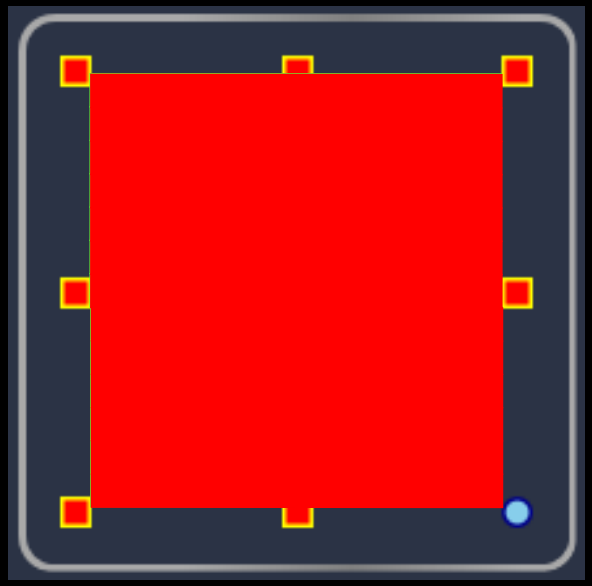


Switch Track every Line:

+ Reduced or no spatial shift in moving samples.
+ Fast image acquisition

- No hardware settings can be changed inbetween tracks (e.g. pinhole diameter, beamsplitters)

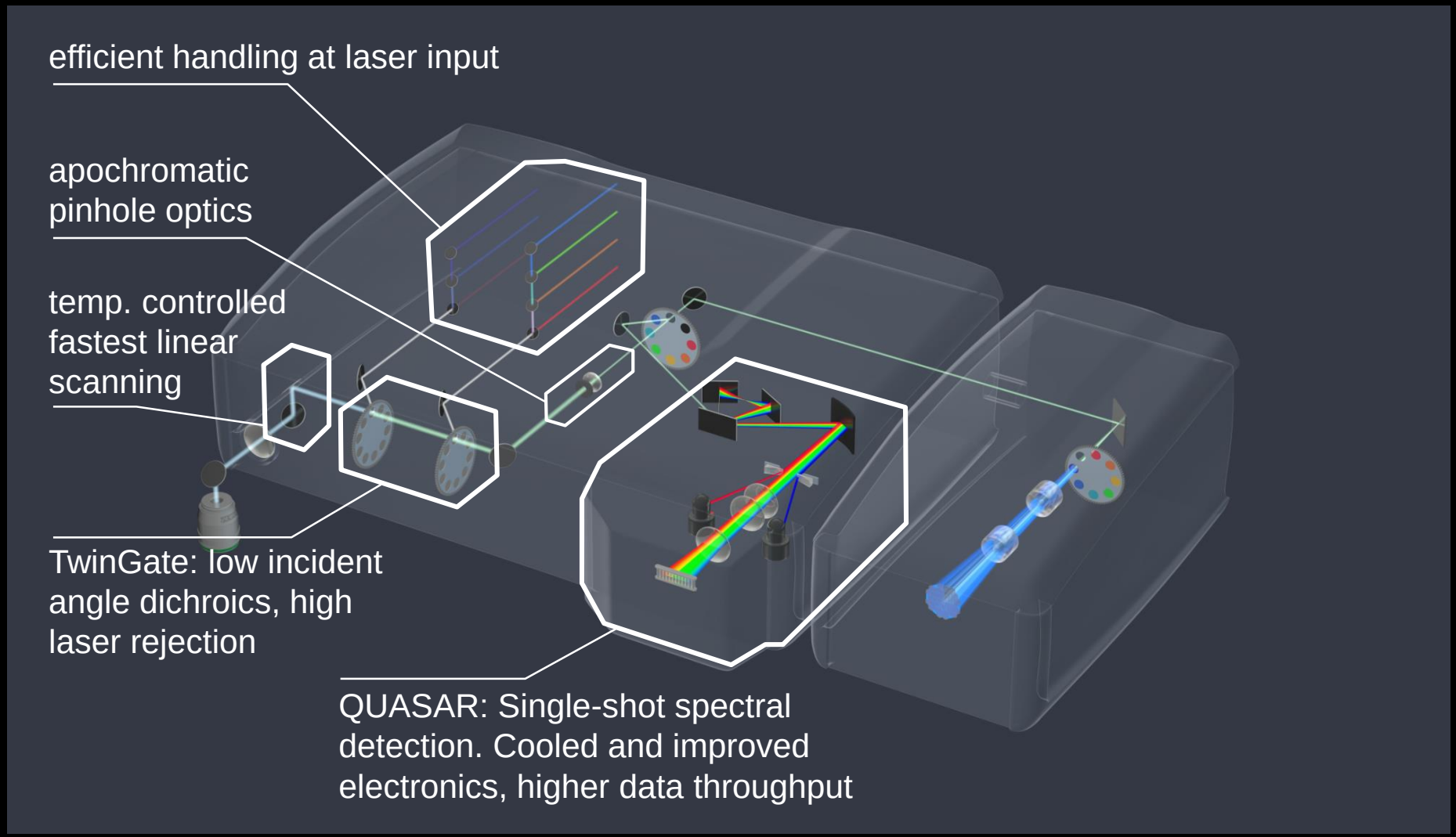
Sequential Image Acquisition: Linewise bidirectional



Fastest switching between tracks - Switch Track every Line bidirectional:

+ Reduced or no spatial shift in moving samples.
+ Fast image acquisition - no fly back time for laser
- No hardware settings be changed inbetween tracks (e.g. pinhole diameter, beamsplitters)
- The channel images of the independent laser movements (from left to right and from right to left) need to be corrected (Corr X and Corr Y) for perfect fitting.

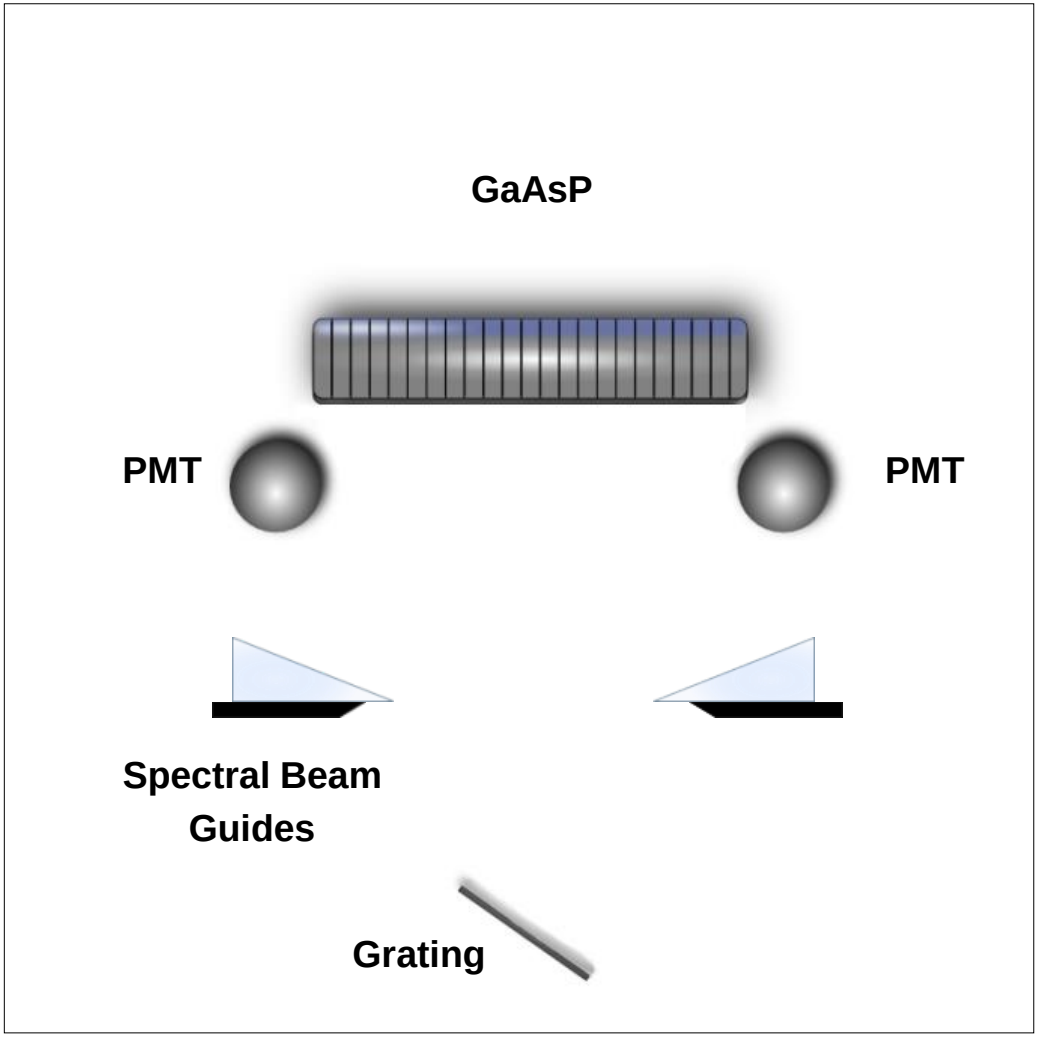
LSM 880 – Scanner



LSM 880

Operation modes of the *QUASAR* detection unit

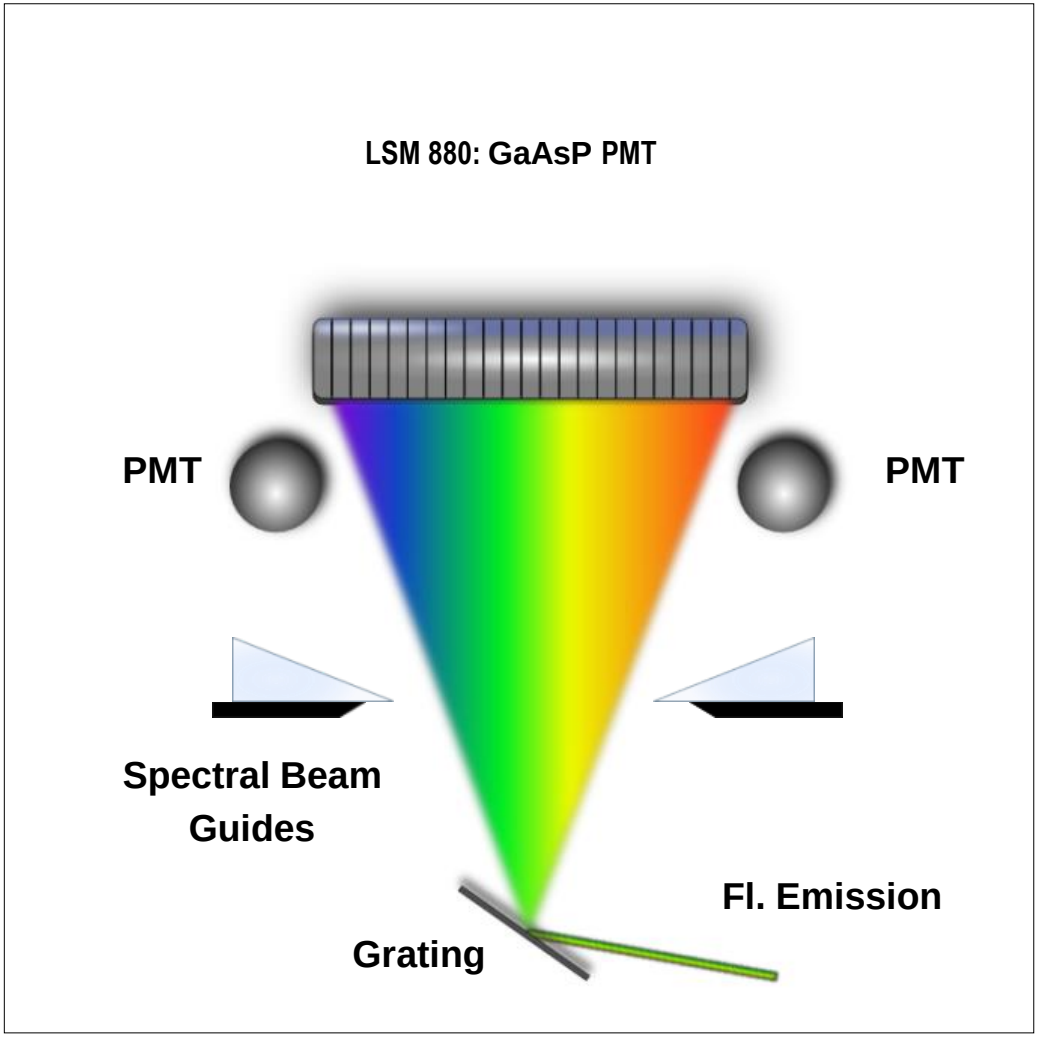
Unmatched flexibility in signal detection!



LSM 880

Operation modes of the *QUASAR* detection unit

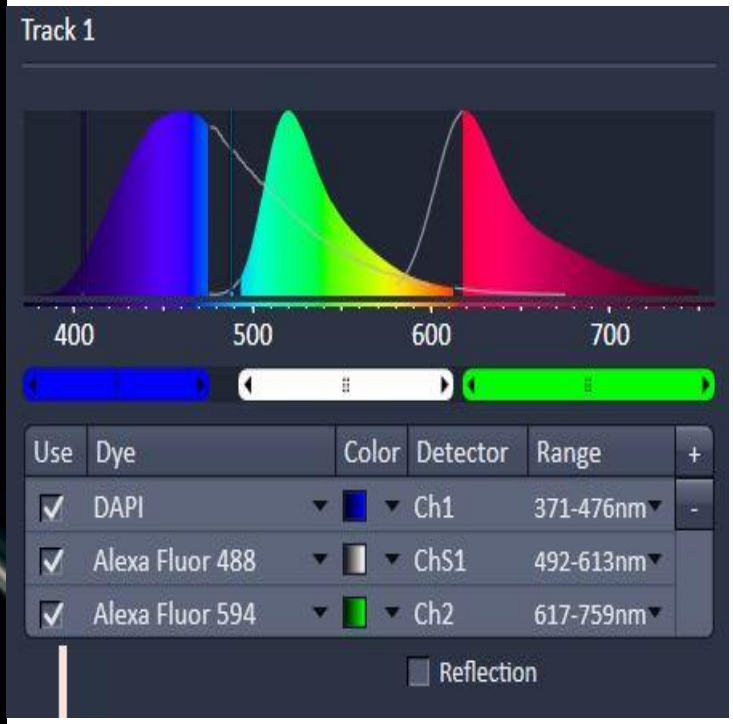
Unmatched flexibility in signal detection!



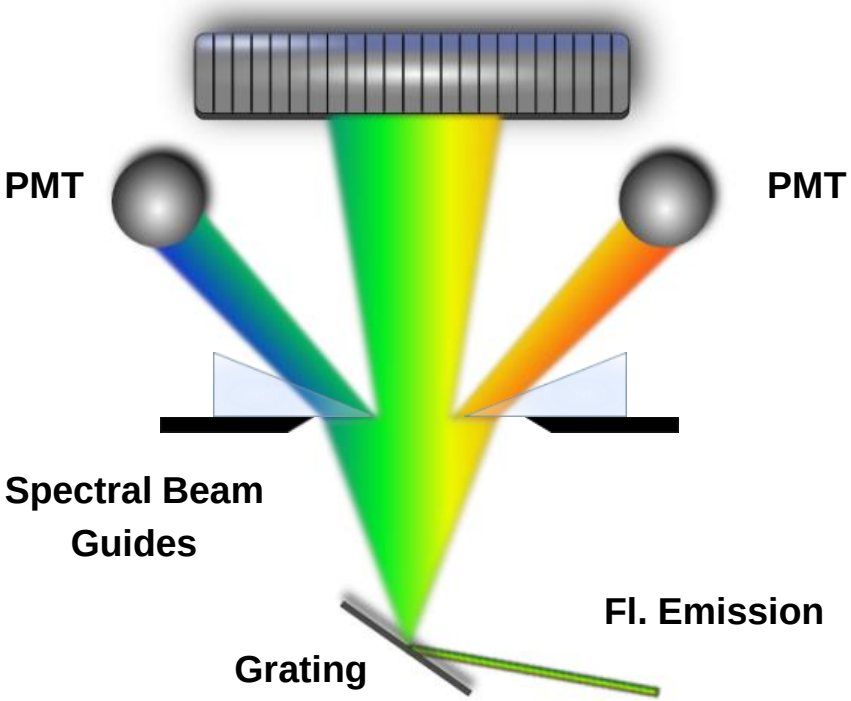
LSM 880

Operation modes of the *QUASAR* detection unit

Unmatched flexibility in signal detection!

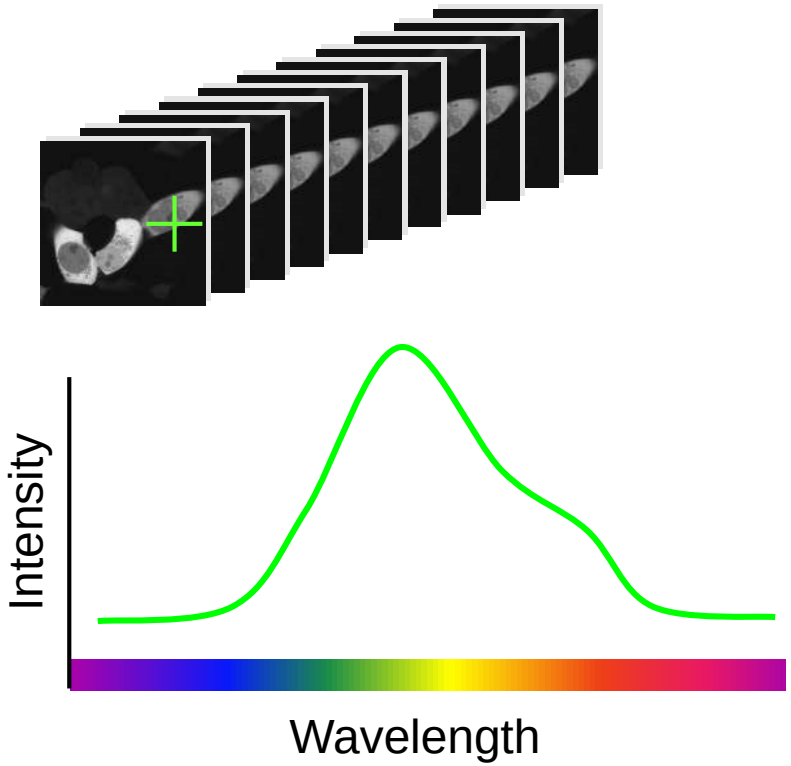


LSM 880: GaAsP PMT



Spectral detection with the LSM 880

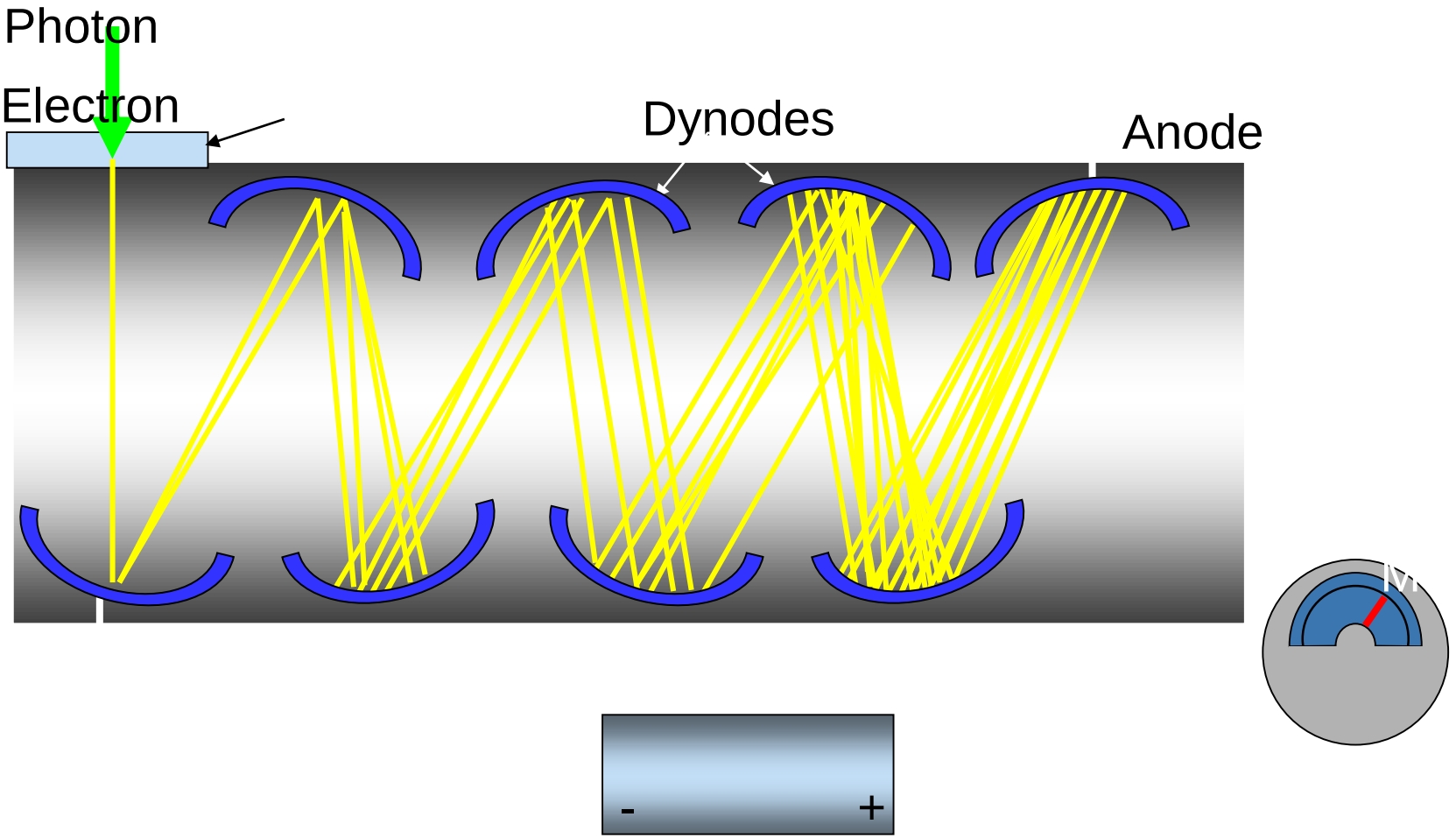
Parallel detection of spectral information of the emission signal!



Measurement of relative intensity provides spectral information of emission signal for every pixel!

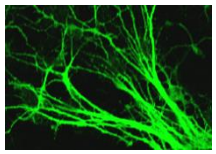
Confocal Principle: Emission Pathway

PMT Detector



omultiplier Tube / PMT Detectors

How does a PMT work?



Photon

p

Photoelectric effect:
Photocathode

Dynodes

Anode

Electron

e^-

Output
Meter

-

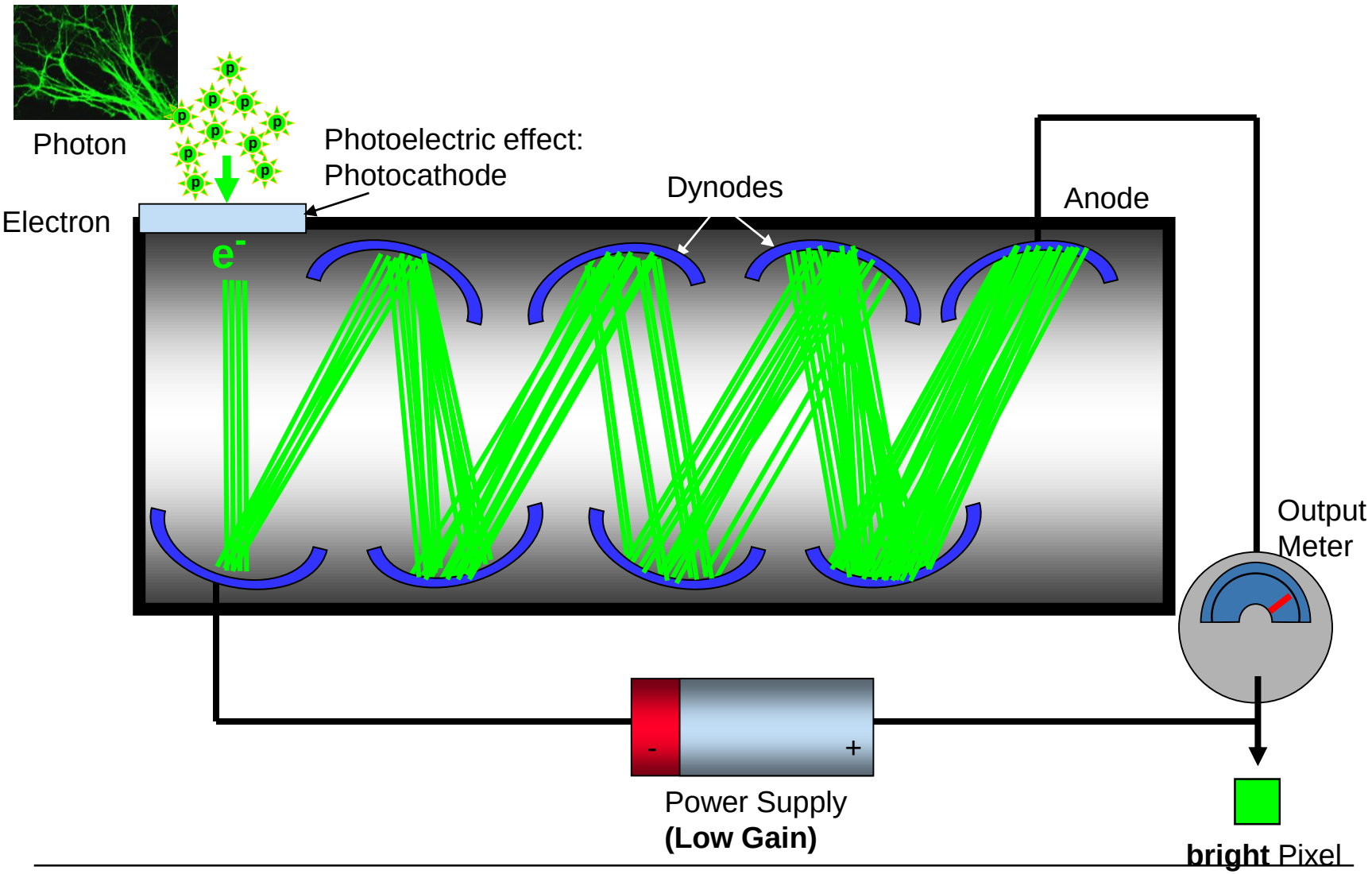
+

Power Supply
(Gain = Volts)

+ - bright Pixel

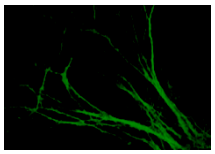
Photomultiplier Tube / PMT Detectors

Assuming a bright sample



Photomultiplier Tube / PMT Detectors

Assuming a dark sample



Photon

Photoelectric effect:
Photocathode

Electron

Dynodes

Anode

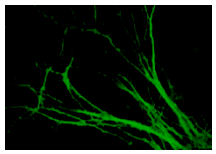
Output
Meter

Power Supply
(Low Gain)

dark Pixel

Photomultiplier Tube / PMT Detectors

Increase a dark sample´s signal with more Gain



Photons

Photoelectric effect:
Photocathode

Electrons

Dynodes

Anode

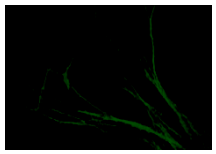
Output
Meter

Power Supply
(Higher Gain)

bright Pixel

Assume a really dimm sample -

Extreme Gain values result in Noise



Photons

Red lines indicate electrons that were generated but have not come from the photons within the sample

Photoelectric effect:
Photocathode

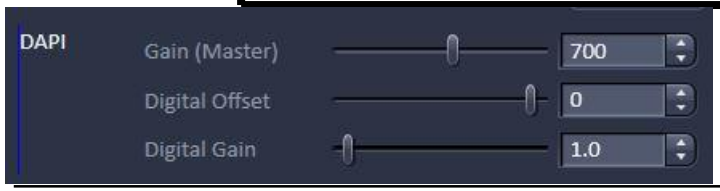
Dynodes

Anode

Electrons

e^-

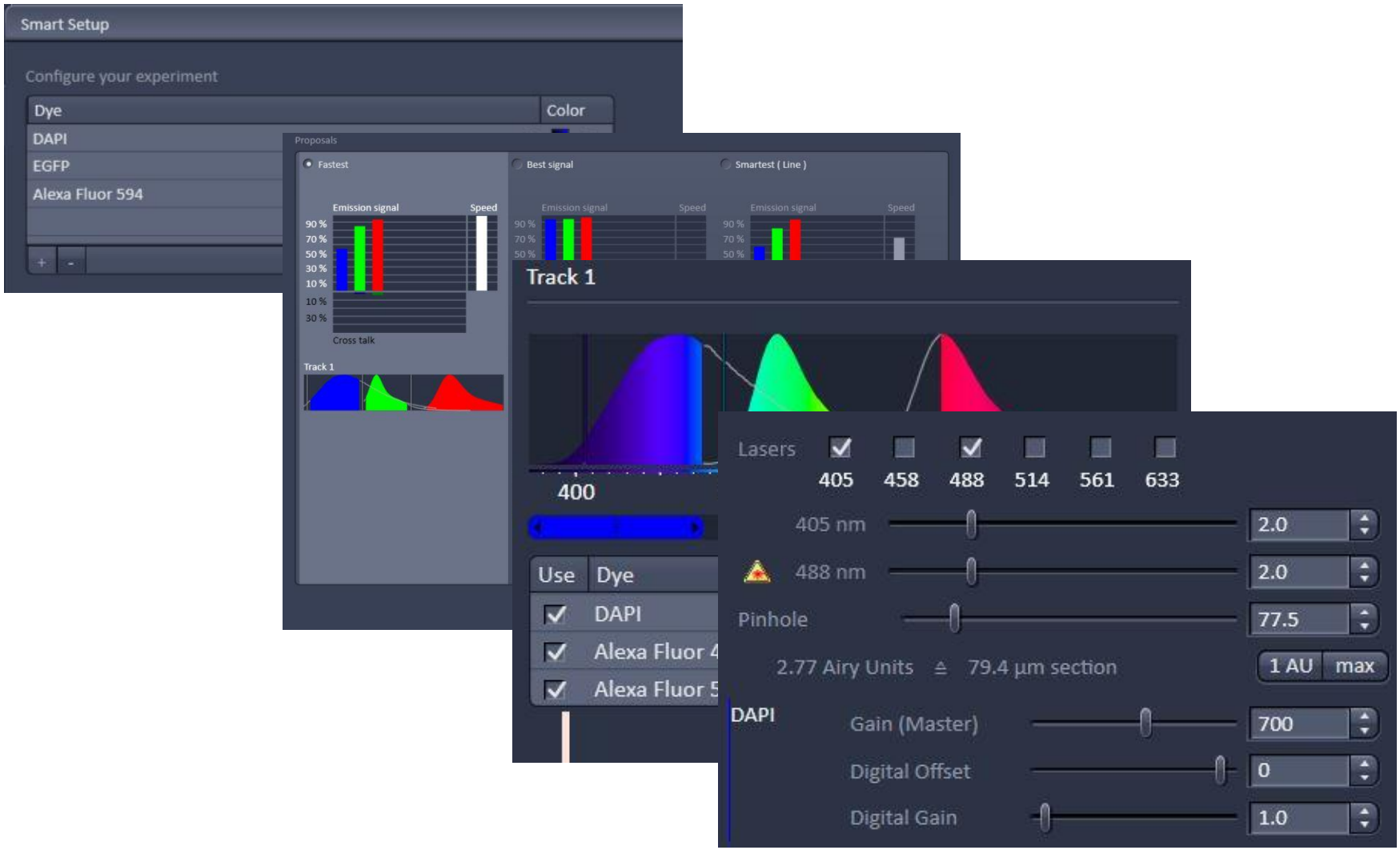
Output Meter



Power Supply
(Highest Gain)

bright Pixel,
but Noise

Summary

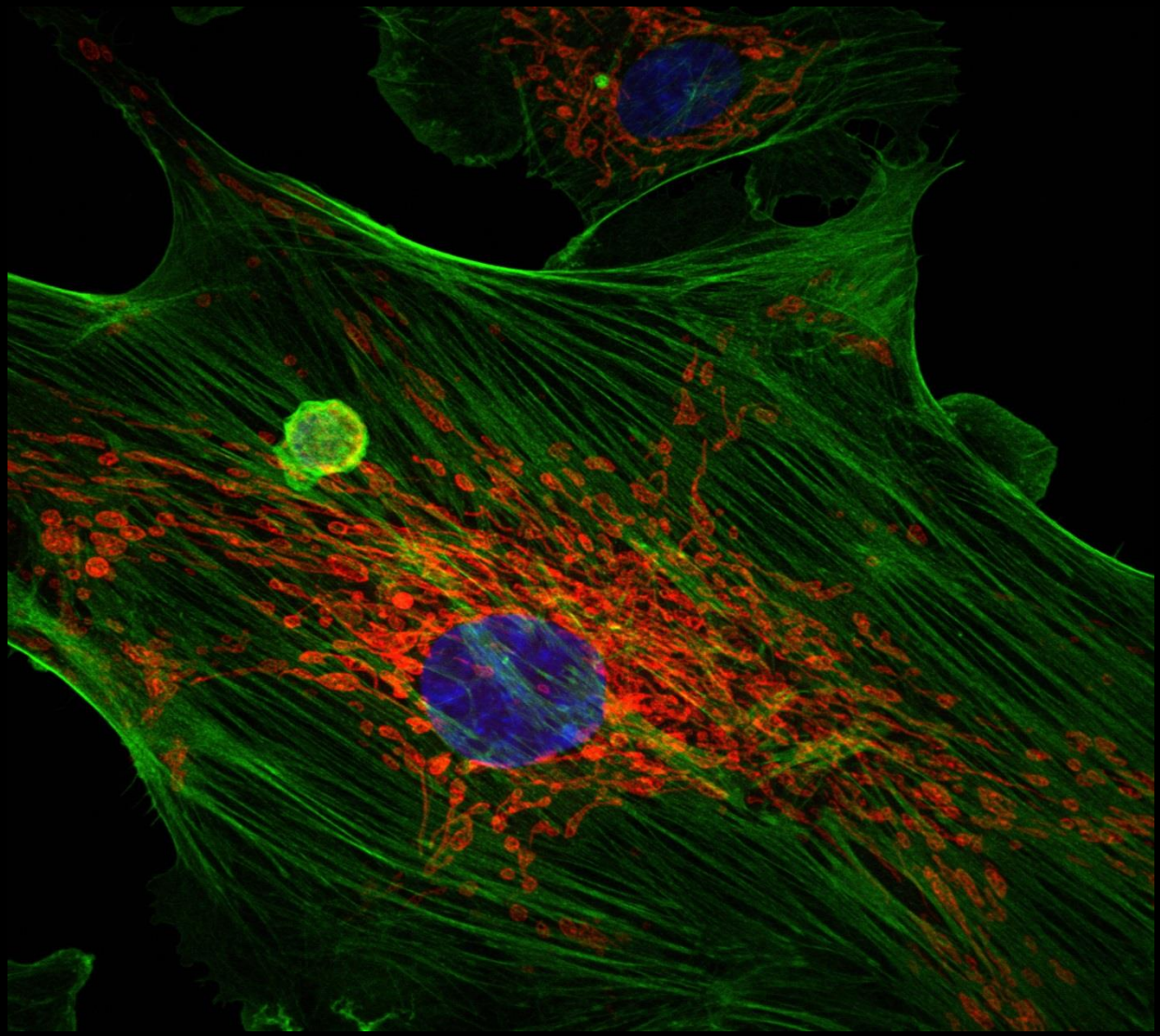

Smart Setup
Configure your experiment
Dye
Color
DAPI
EGFP
Alexa Fluor 594
Proposals
Fastest
Best signal
Smartest (Line)
Emission signal
Speed
Cross talk
Track 1
400
Use
Dye
DAPI
Alexa Fluor 4
Alexa Fluor 5
Lasers
405
458
488
514
561
633
405 nm
2.0
488 nm
2.0
Pinhole
77.5
2.77 Airy Units ≙ 79.4 µm section
1 AU
max
DAPI
Gain (Master)
700
Digital Offset
0
Digital Gain
1.0

LSM 880 multichannel imaging

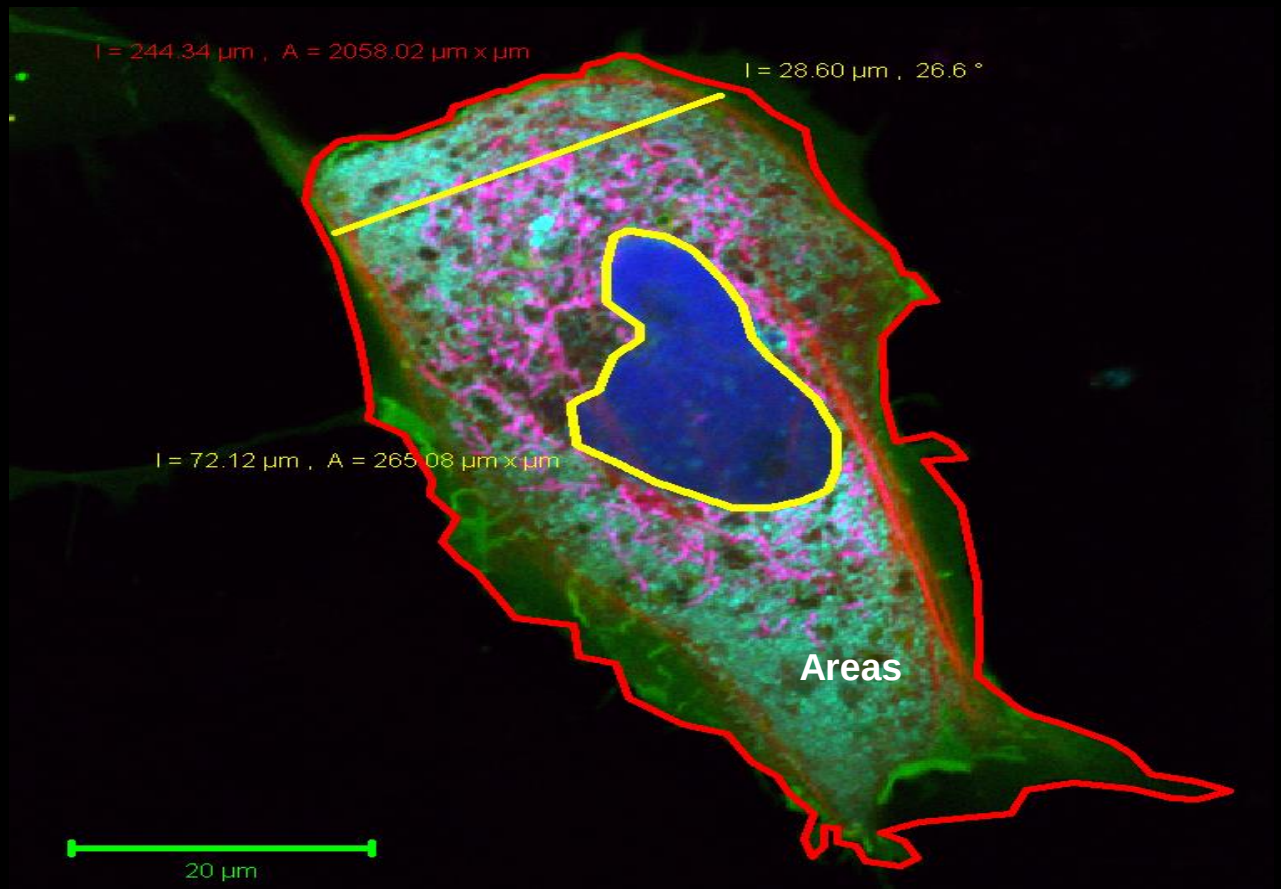
- ***No limitation multiple colors***
- ***Up to 3 channel simultaneously (3GaAsP)***
- ***Photo counting mode for weak FL signal***
- ***High image contrast***

Today’s Applications

Multifluorescence Imaging



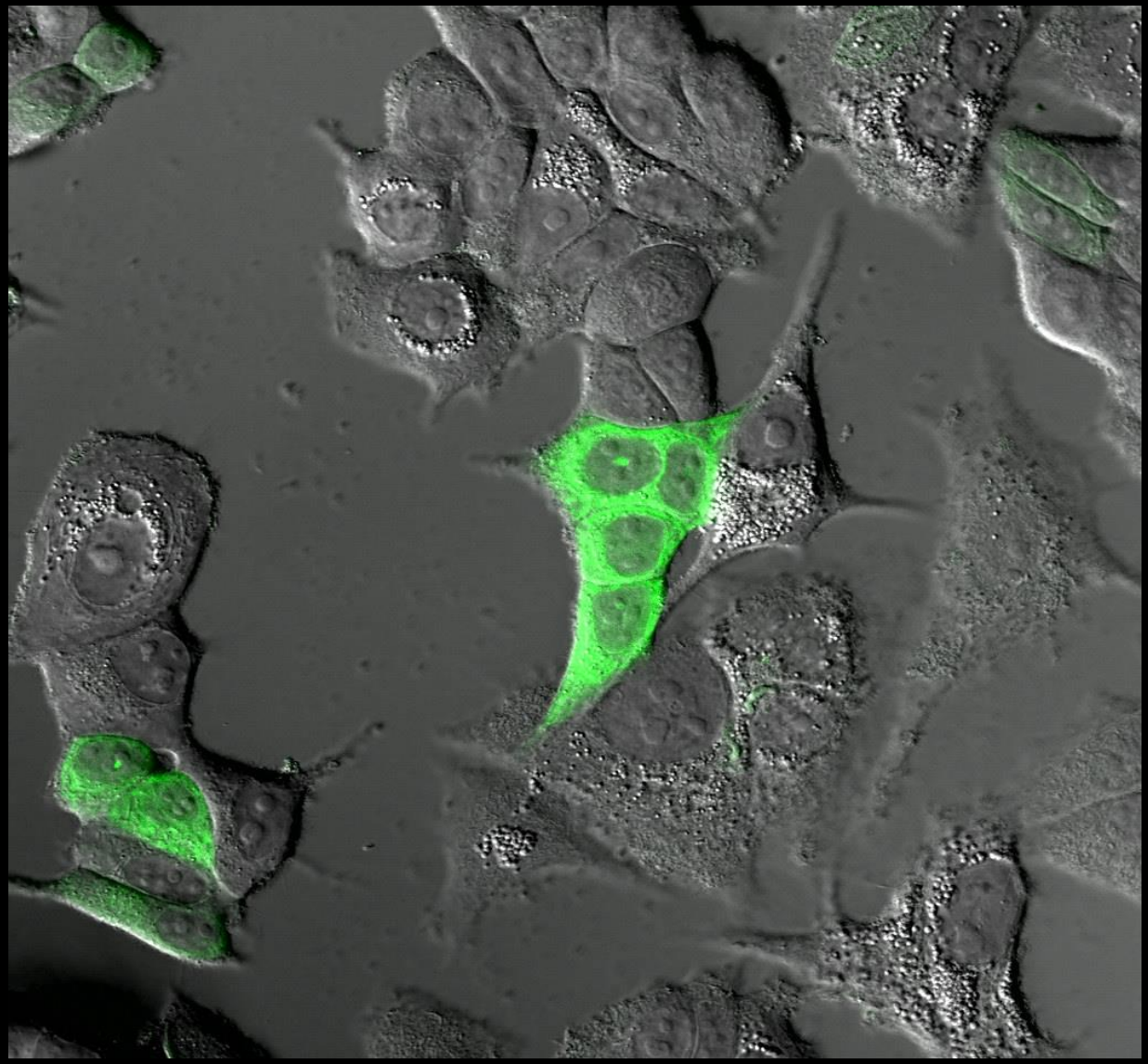
Quantifying image information



	Name	Scene	Area[µm²]	Channel_1_AF...	Channel_2_AF.
	A	B	C	D	E
1	Rectangle	1	10,478.00	36.88	39.2
2	Spline Contour	1	1,132.00	33.69	45.3
3	Line	1			

Today's Applications

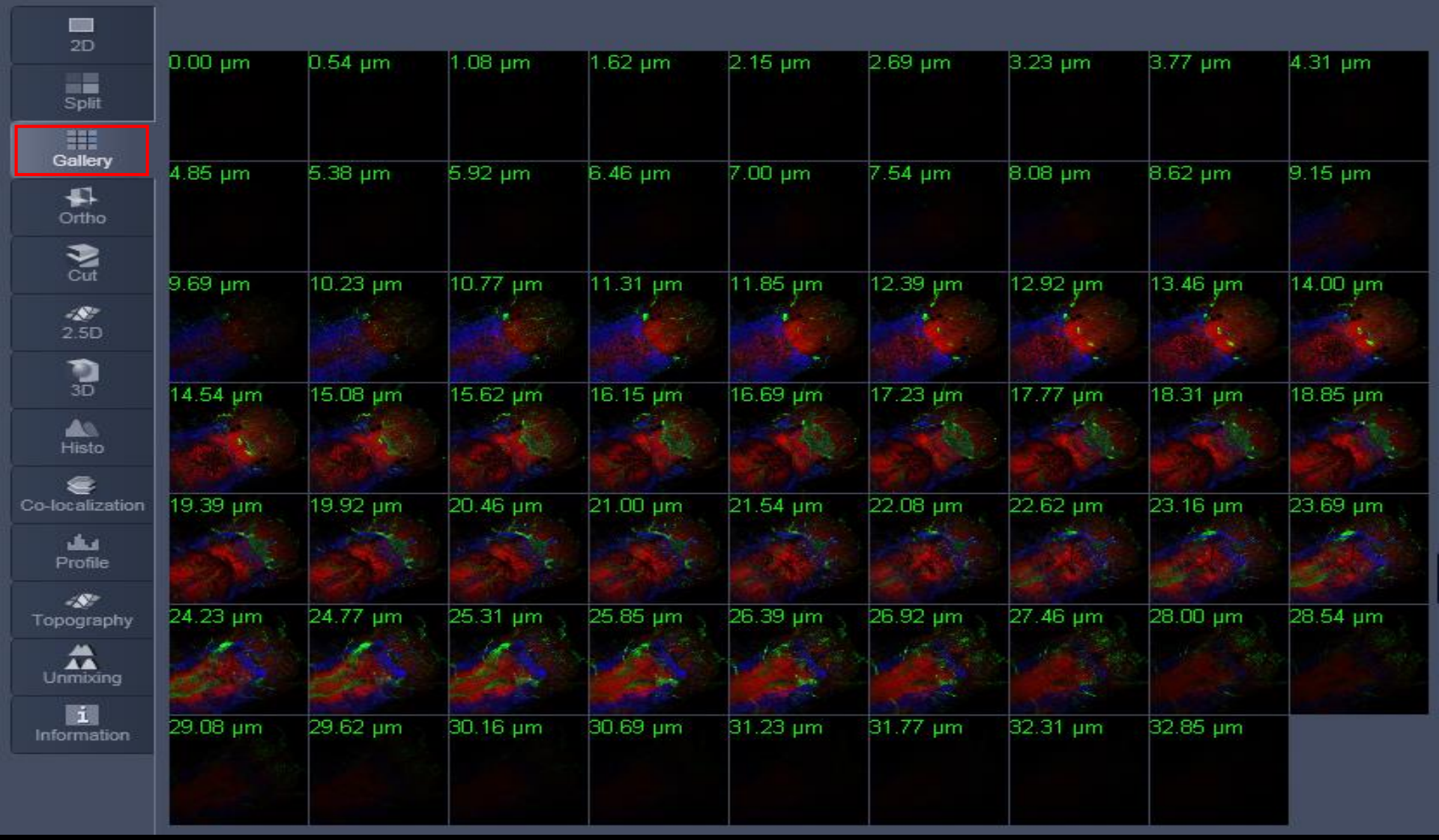
Multifluorescence Imaging



LSM 880
3D Z stack imaging

- ***Z drive down to 10nm/step***
- ***Extremely fast Z driver for fast Z stack imaging***

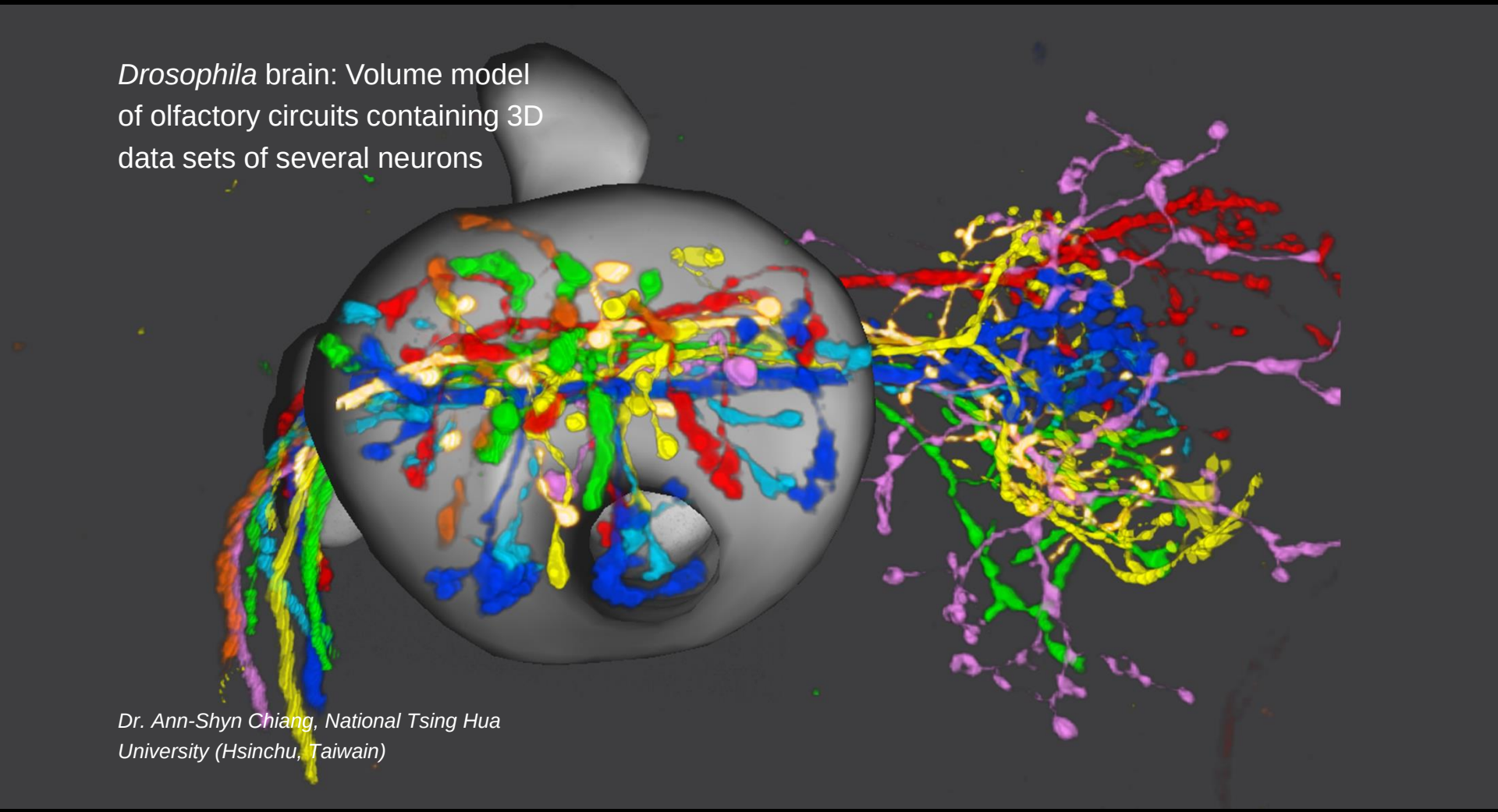
LSM 880 Z stack image
3D reconstruction display



Today’s Applications

Visualization of 3D structures in neurosciences

Drosophila brain: Volume model of olfactory circuits containing 3D data sets of several neurons



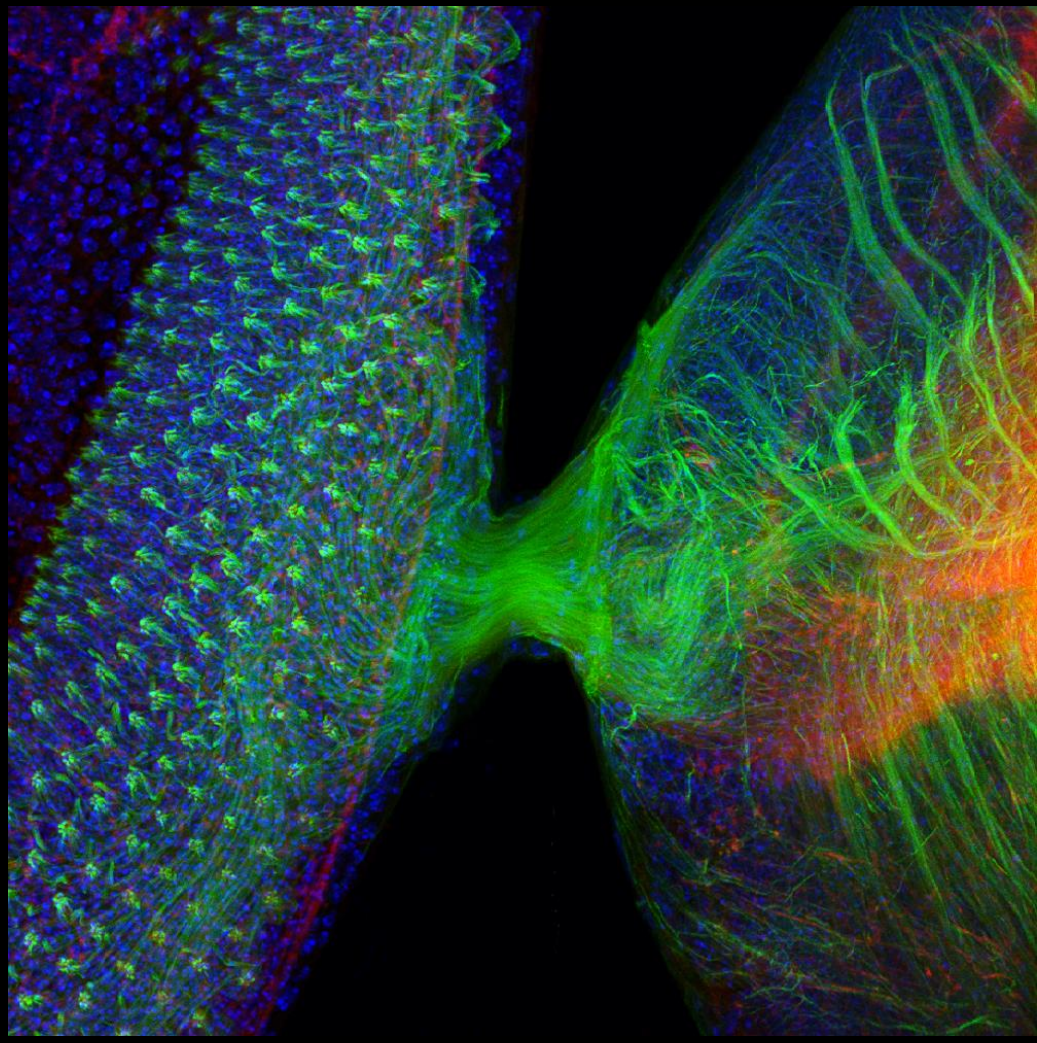
Dr. Ann-Shyn Chiang, National Tsing Hua University (Hsinchu, Taiwain)

Today’s Applications

Visualization of 3D structures in neurosciences

样品: 果蝇眼睛和大脑感受神经元GFP
肌动蛋白Red
细胞核Blue

图像: Z-stack最大强度投影

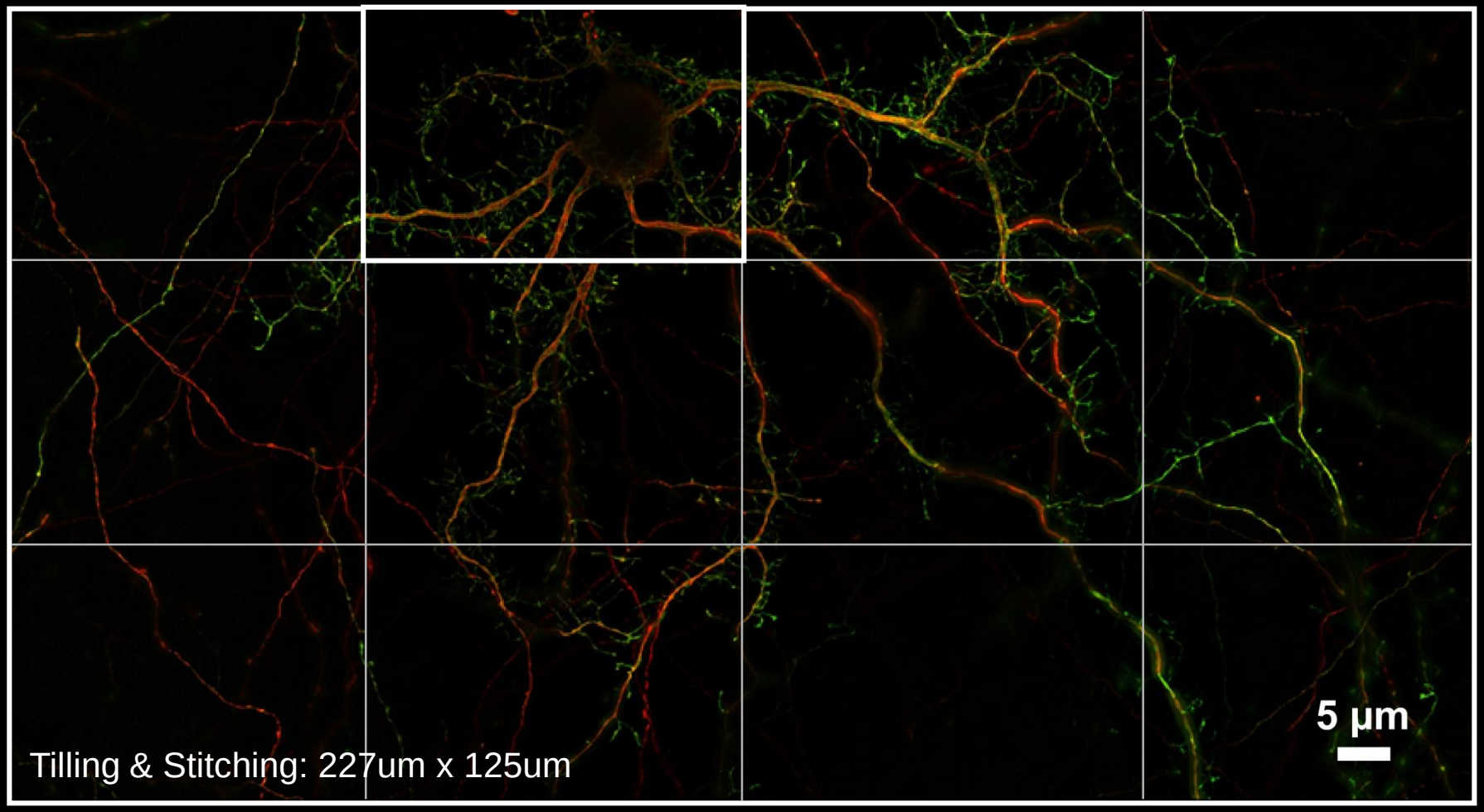


来源:
Anand Tiwari, Banaras Hindu University, Varanasi (India)

LSM 880
Tile Scan Imaging

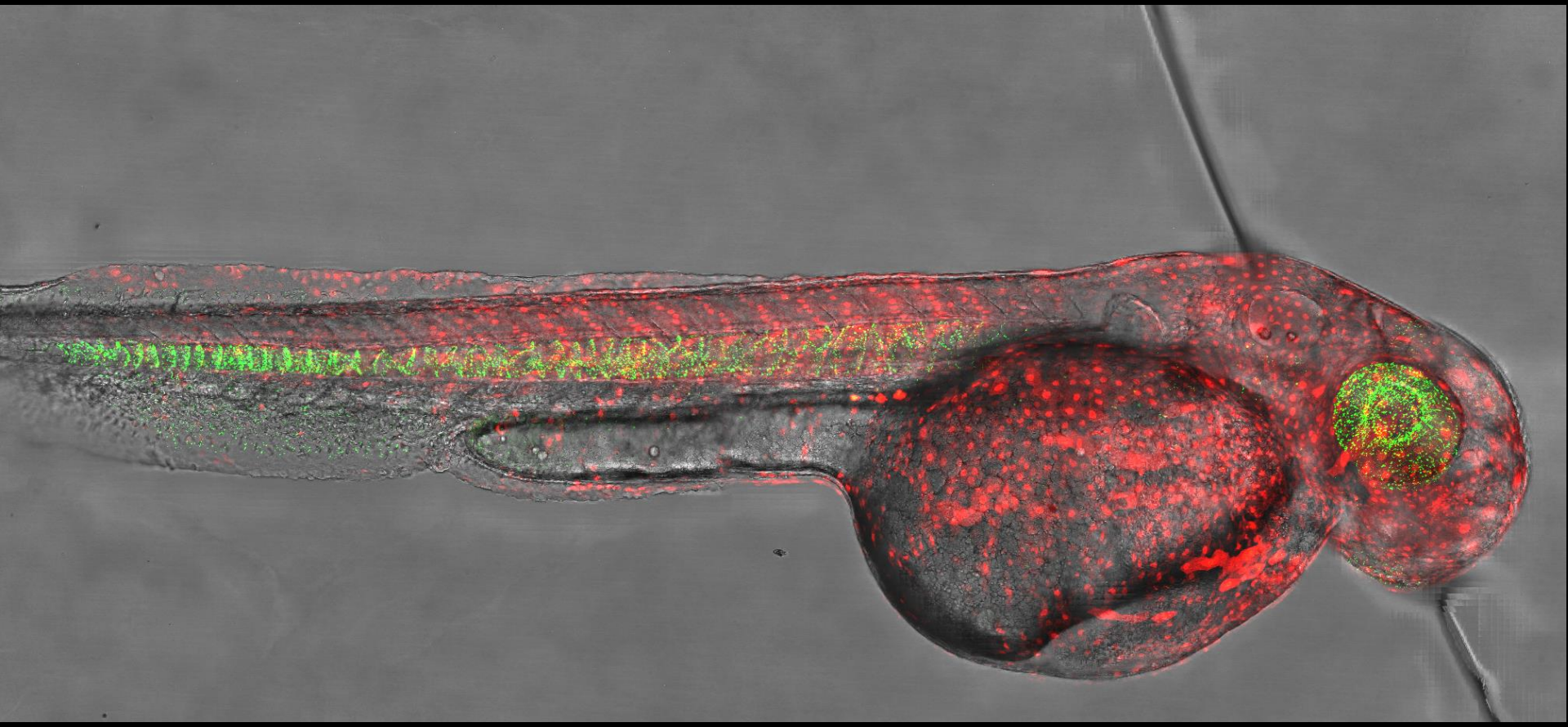
- Large FOV

Today's Applications



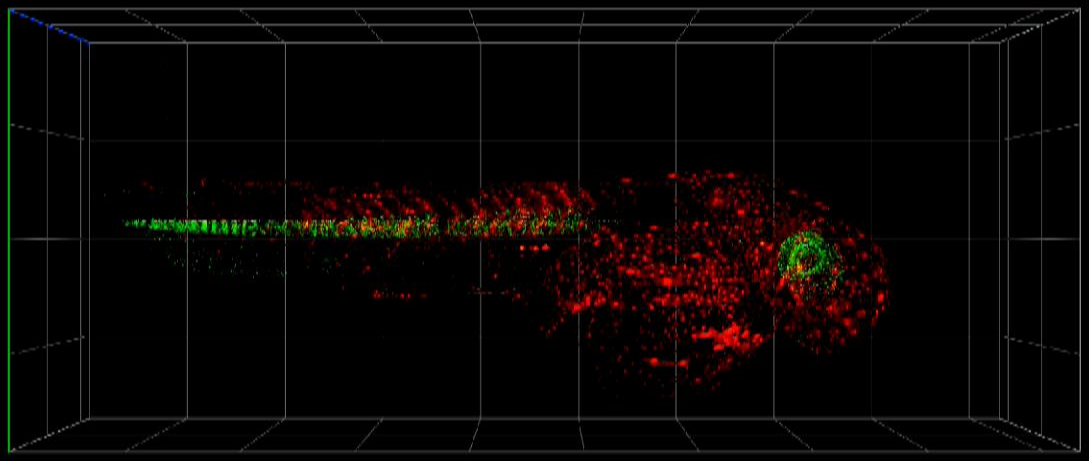
Today’s Applications

Today's Applications



X-Y-Z-Tiles

Today’s Applications



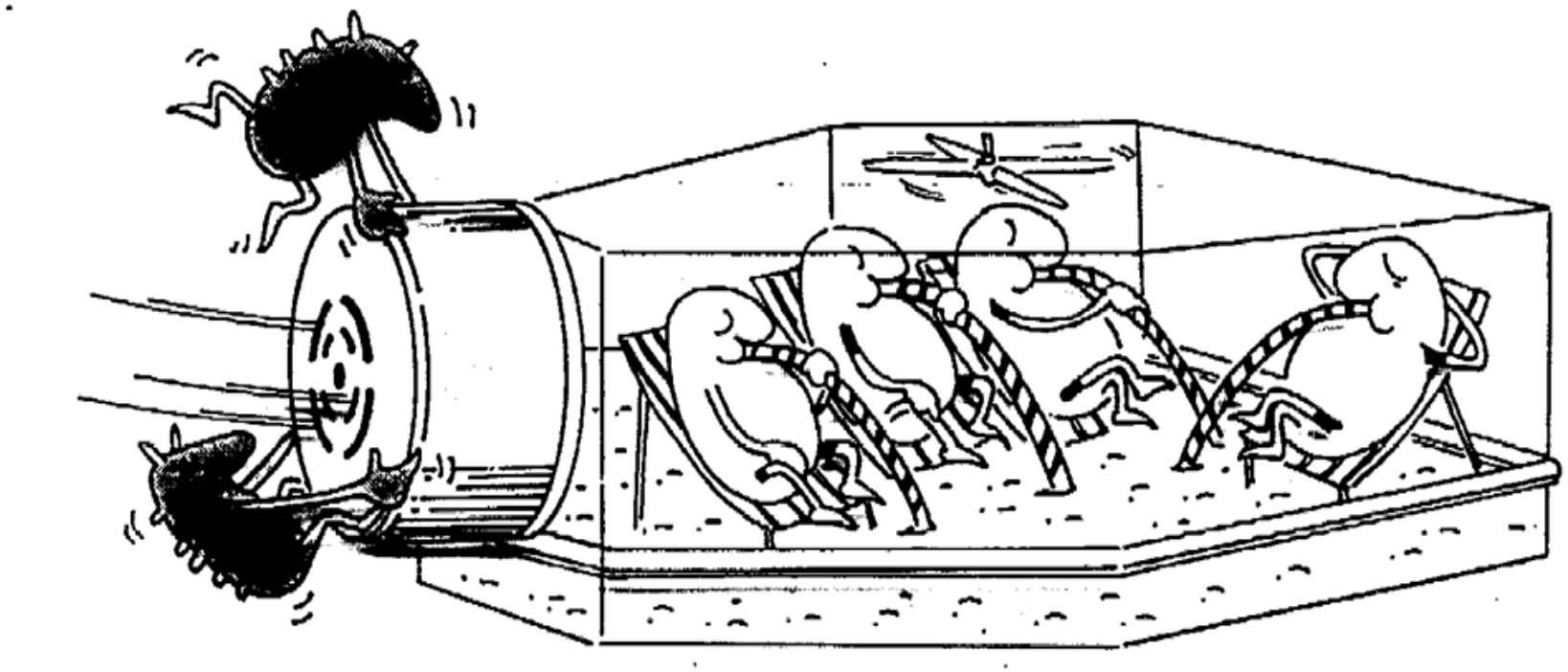
LSM 880
Live Cell Imaging

- ***Linear scanning system improve image quality***
- ***13fps @ 512 x 512***
- ***Gentle Imaging with minimal laser power***

LSM 880 – Long Term Living Cell Imaging

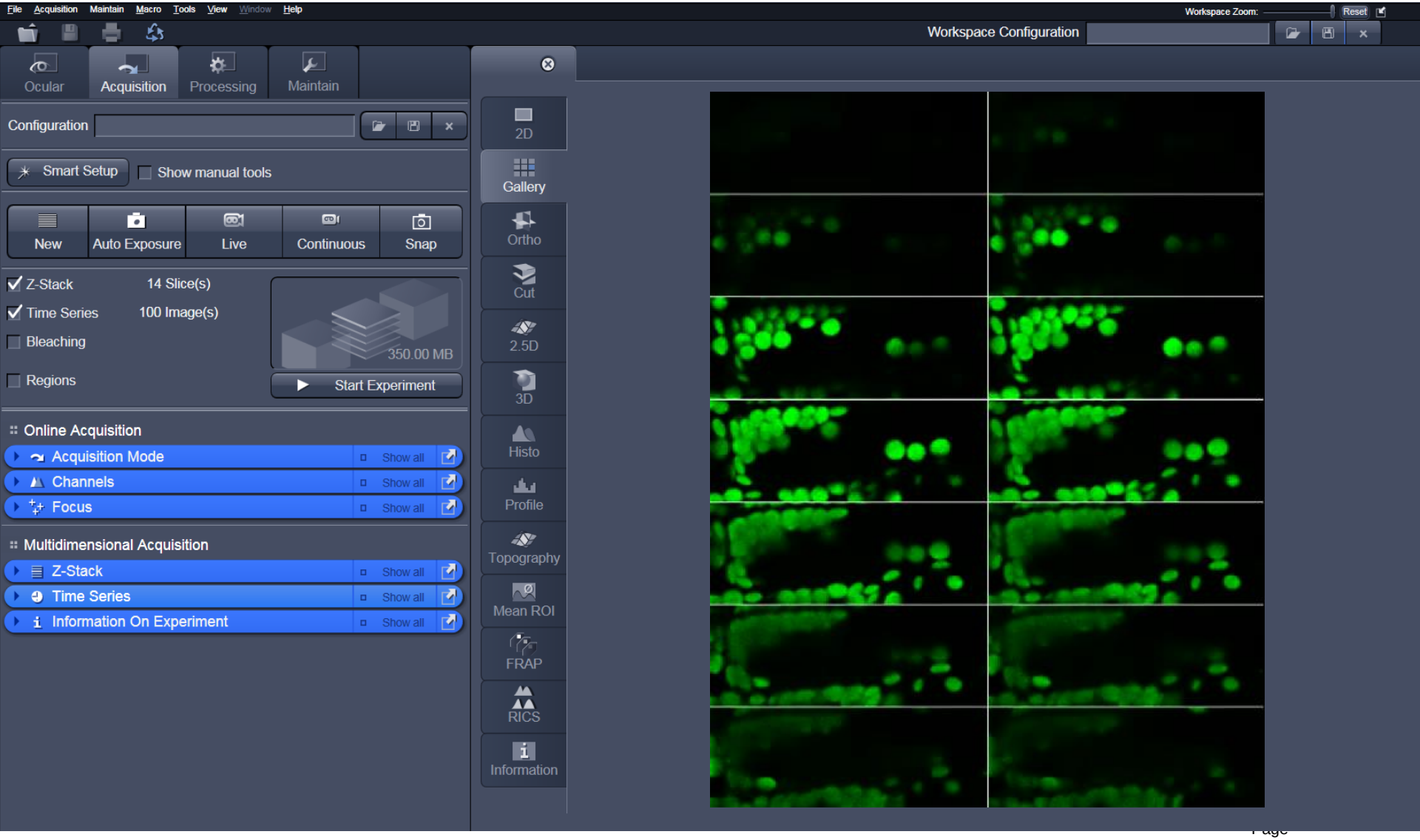
Our Goal

Copy in-vivo conditions „to make your cells happy“!



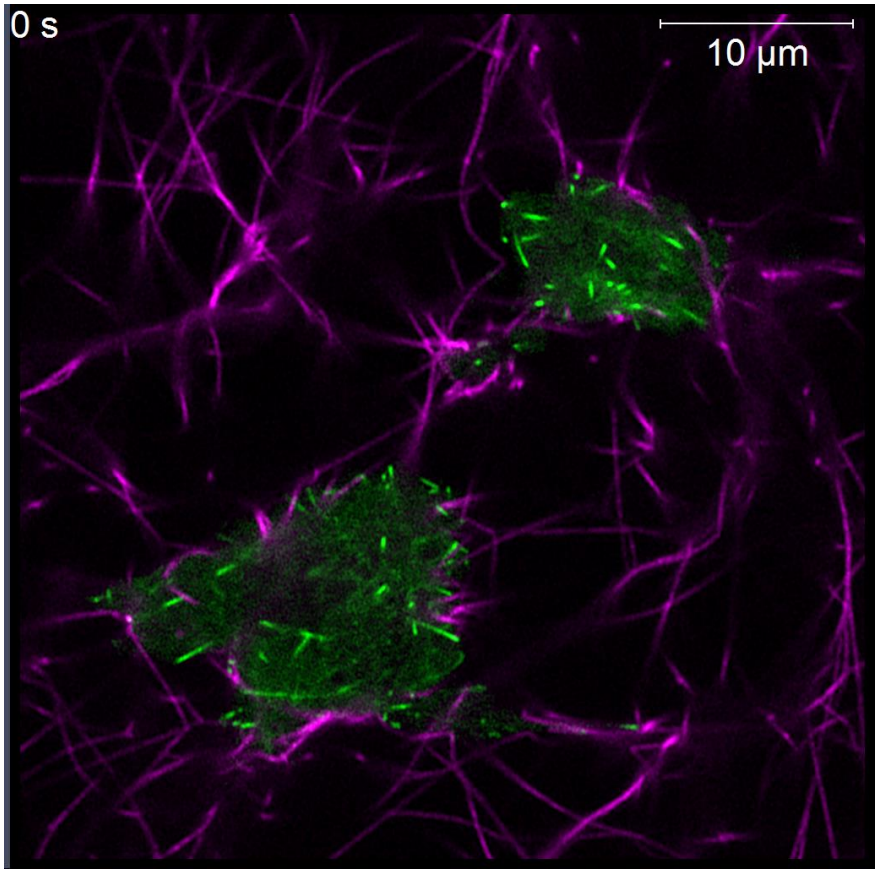
LSM 800 Time Series

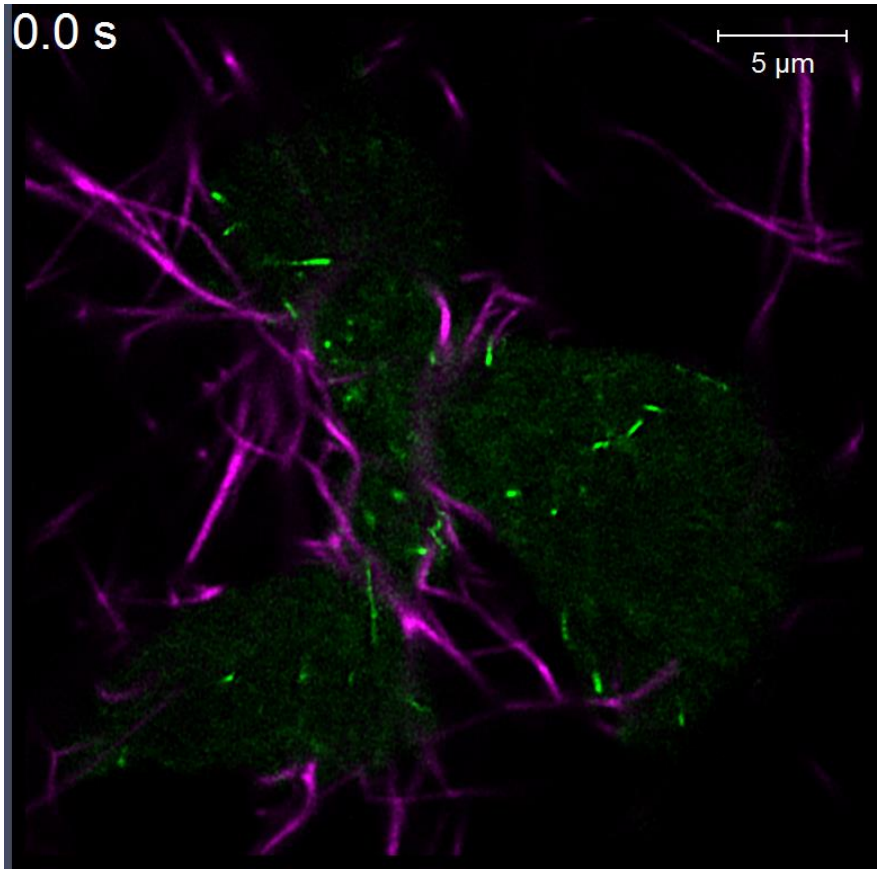
ZEN 2: Multi-dimensional imaging made easy



LSM 880 Time Series

High contrast image with high speed





Cells (EB3, green) in a 3D collagen fiber matrix (magenta)

LSM 800 System Sensitivity

Gentle Live Cell Imaging

Fox lung cells are observed for 24 hours while dividing.

一体化整合的活细胞工作站

CO2的湿度、温度控制

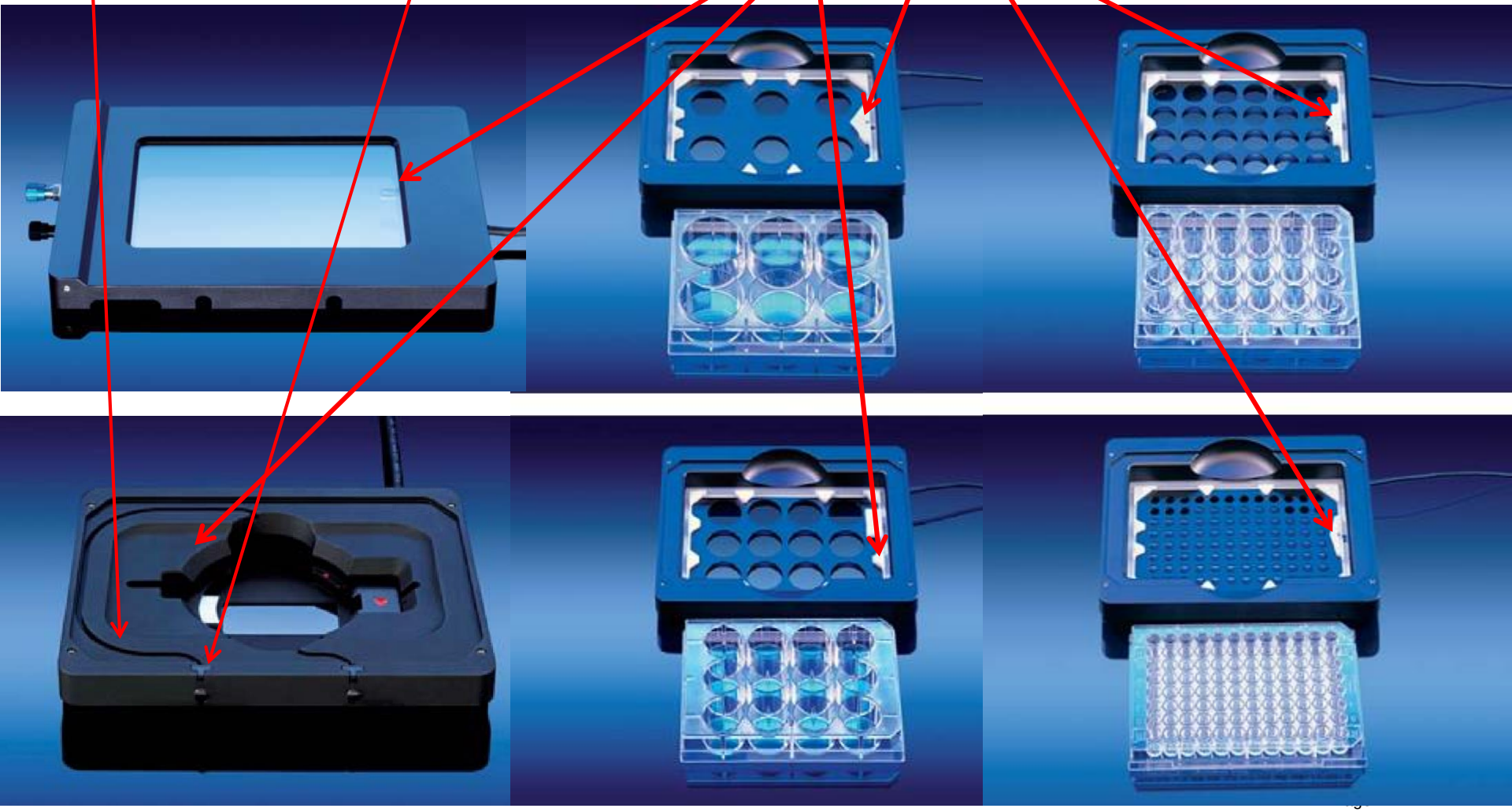
内层培养装置

活细胞培养装置

一体化整合的活细胞工作站

- 四通道温度精确控制，范围室温至 60° C，精度：0.01° C
- CO_2浓度设定范围：0-8%，精度：0.01

加药预热槽　　管道夹口　　TemperatureSensor



LSM 800 – Long Term Living Cell Imaging

Incubation System

TempModule S1

Basic module for controlling the temperature of 4 independent heating channels

- *Supplies additional control modules with power and control signals*
- *The TempModule S1 is controlled by AxioVision (from version 4.6 upwards) or by the Axio Observer.Z1 TFT touch screen display*
- *The control characteristic can be freely selected for each channel. Eight parameter sets are available*
- *An additional channel for the external Control Sensor T S1 allows the temperature to be measured directly in the culture vessel in preliminary experiments (calibration)*
- *Communication takes place via CAN (microscope) or USB (PC)*
- *Internal resolution: 0.01°C*
- *Setpoint value range for connected heating components: ambient temperature to 60.0°C (recommended: ambient temperature to 45°C)*

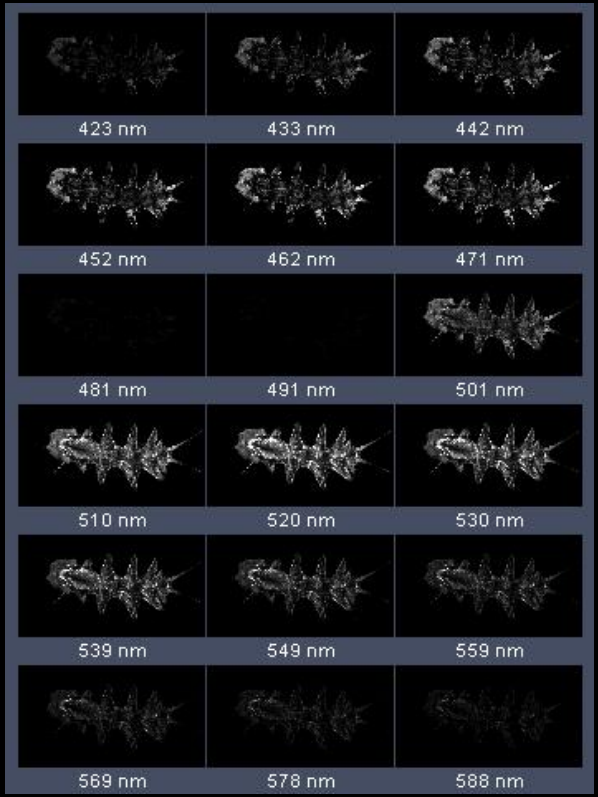
CO_2 Module S1

Control module for CO_2 regulation in incubators or under CO_2-Covers

- *CO_2 control, together with a carbonate buffer system, allows a stable pH value in the cell culture medium over a long period of time*
- *A built-in CO_2 sensor continually measures the current CO_2 concentration*
- *Fluctuations in concentration are eliminated as a result of the continuous addition of very small amounts of CO_2*
- *For low gas flows in small incubators and CO_2-Covers or for medium gas flows with Incubator S TIRF S1 or Laser Safety Incubator Refl/Transm Light S1*
- *Internal resolution: 0.01%*
- *Setpoint value range: 0.0-8.0%*

Today's Applications

Elimination of cross-talk problem with spectral imaging



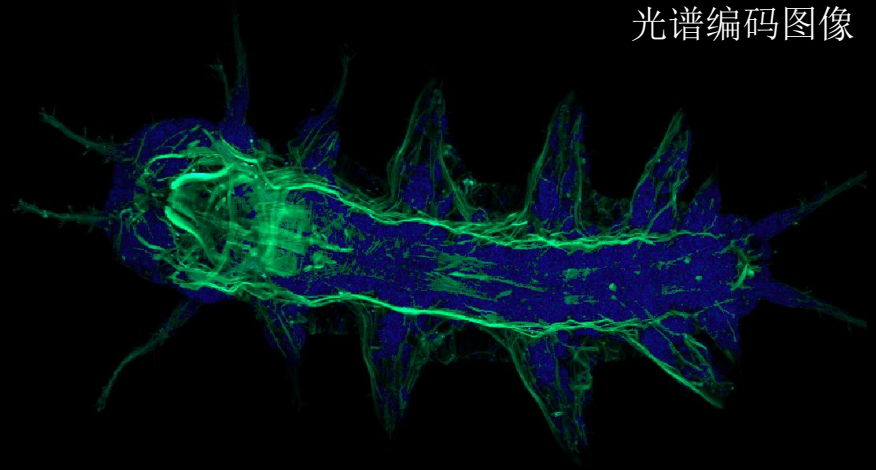
刚毛海蚯蚓 (环节蠕虫

细胞核：DAPI

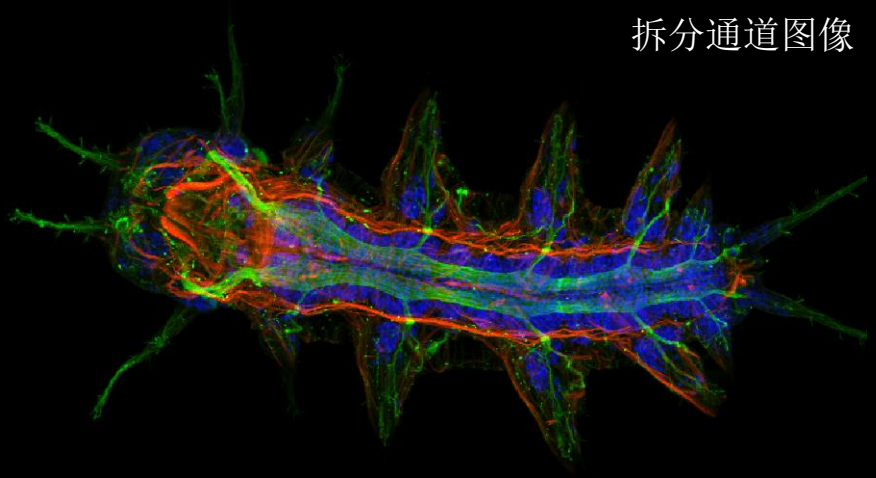
肌肉：Alexa 488

神经系统：Cy2

光谱编码图像



拆分通道图像



Today's Applications

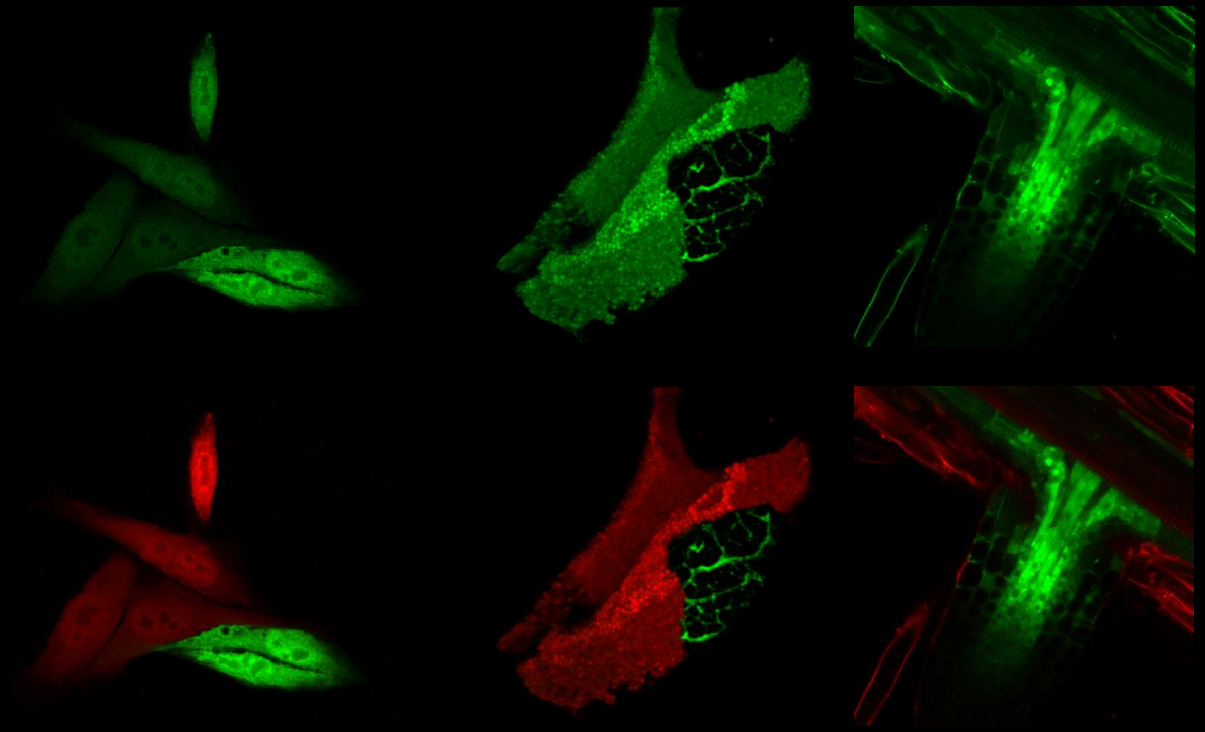
Elimination of cross-talk problem with spectral imaging

Separation of dyes with overlapping spectra

Separation of fluorescent labels from autofluorescence

without linear unmixing

result of linear unmixing

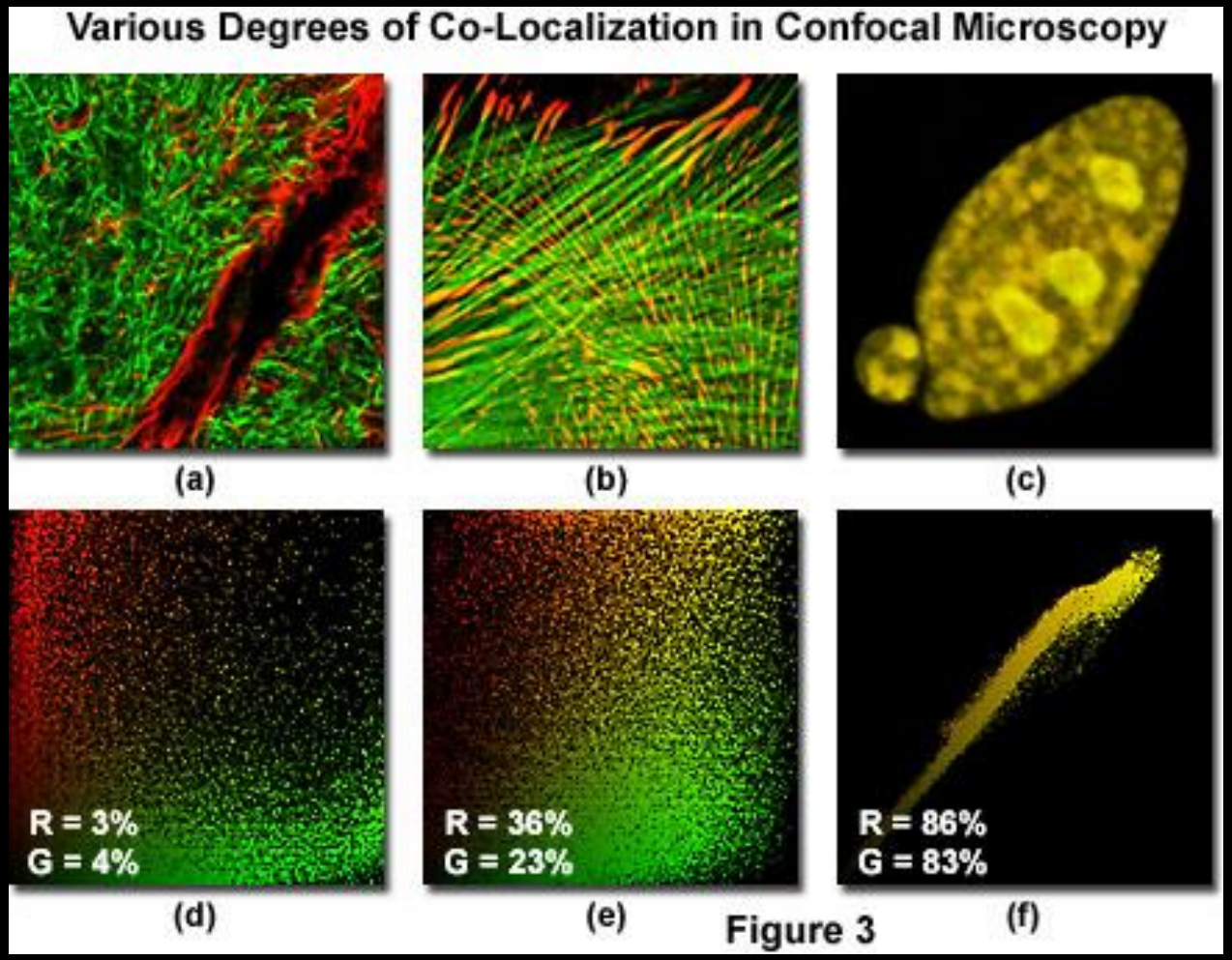


Cultured Cells (GFP, YFP)

Zebrafish Embryo (GFP)

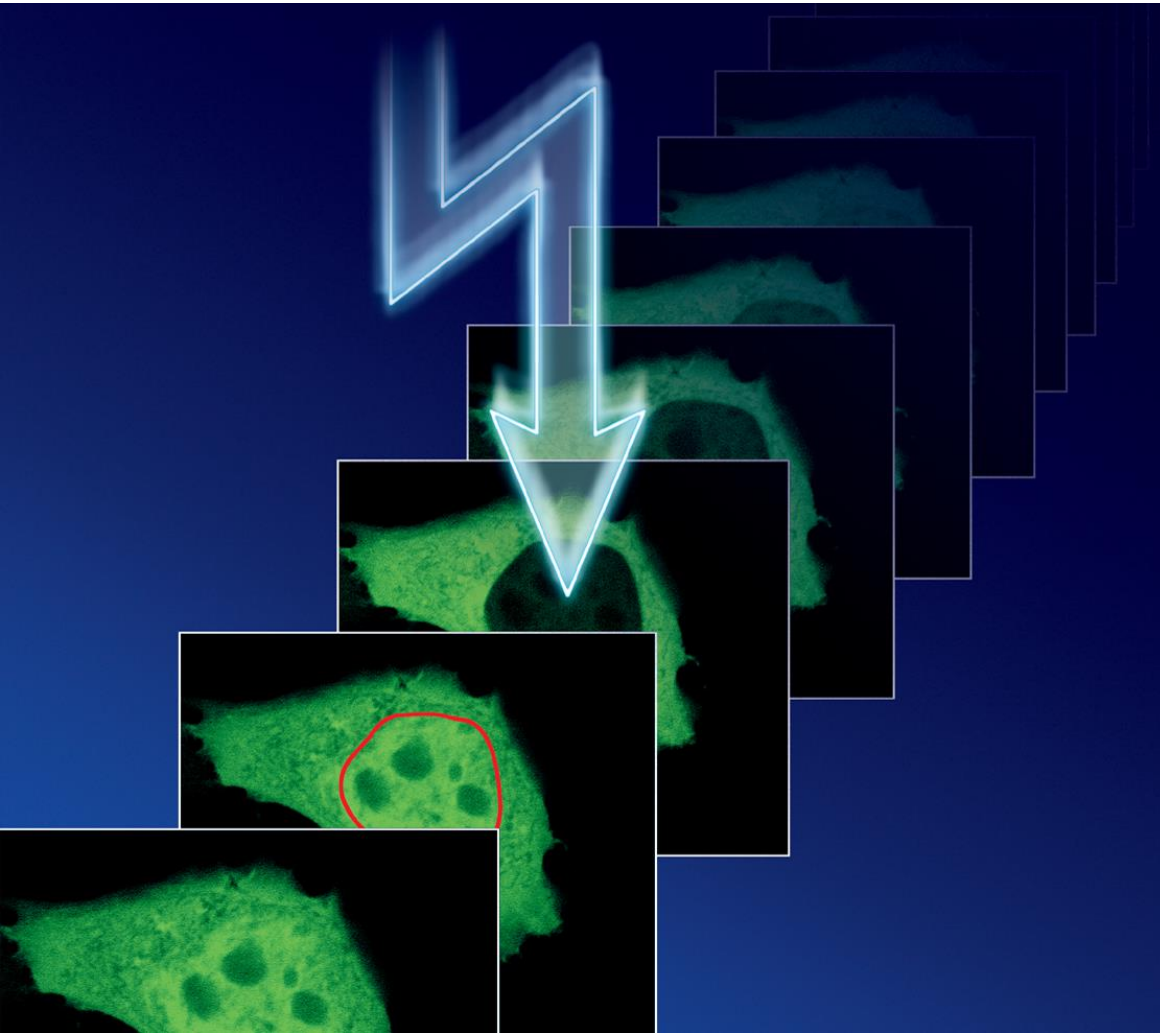
Arabidopsis (GFP)

Co-localization



$$R_r = \frac{\sum_i (S1_i - S1_{aver}) \cdot (S2_i - S2_{aver})}{\sqrt{\sum_i (S1_i - S1_{aver})^2 \cdot \sum_i (S2_i - S2_{aver})^2}}$$

Measurements of kinetics after precise photomanipulation



Photomanipulation

- Photobleaching (e.g. FRAP)
- Photoactivation
- Photoconversion
- Uncaging
- Laser Ablation

WHAT IS FRAP?

什么是FRAP?

Fluorescence *R*ecovery *A*fter *P*hotobleaching

荧光漂白后恢复

For Quantifying Molecular Mobility

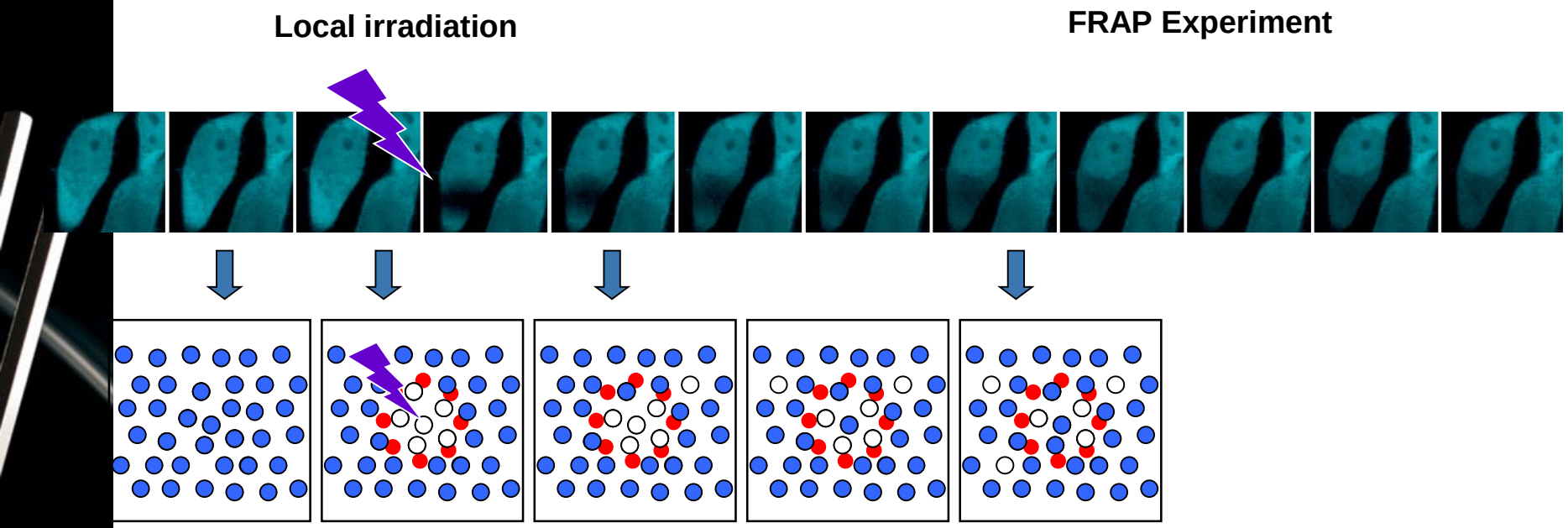
用于量化分子流动性

What is FRAP?

什么是FRAP?

***F*luorescence *R*ecovery *A*fter *P*hotobleaching**

荧光漂白后恢复

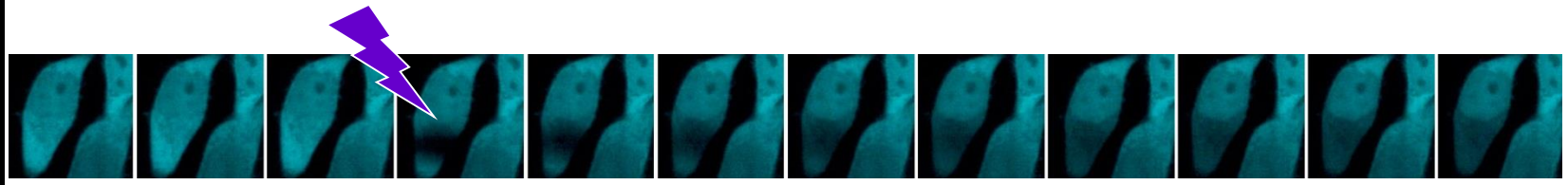


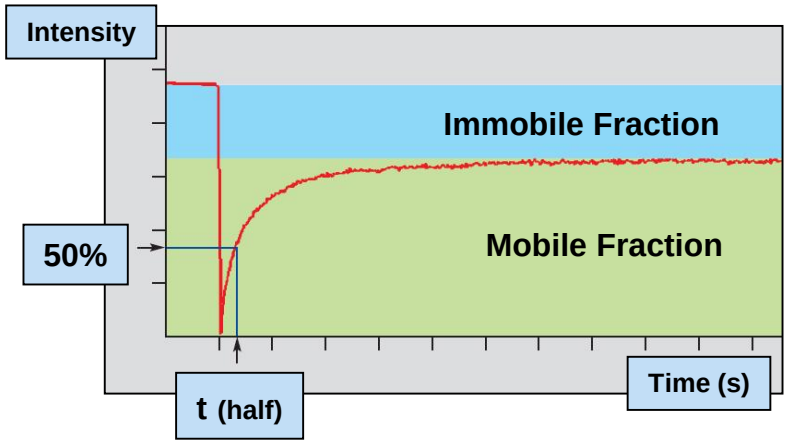
FRAP: For Quantifying Molecular Mobility

FRAP 用于量化分子流动性

Local irradiation

FRAP Experiment

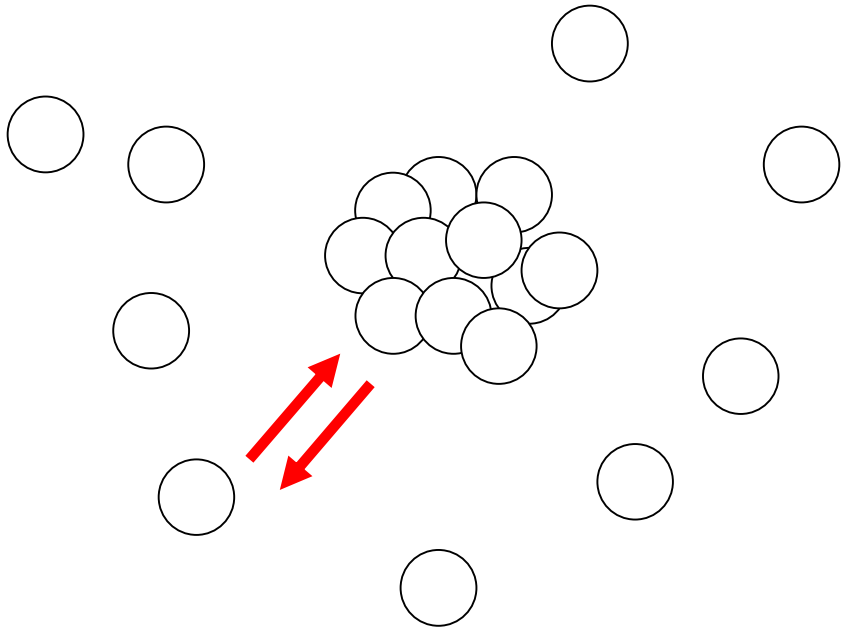




Fluorescence intensity measured within photobleached region

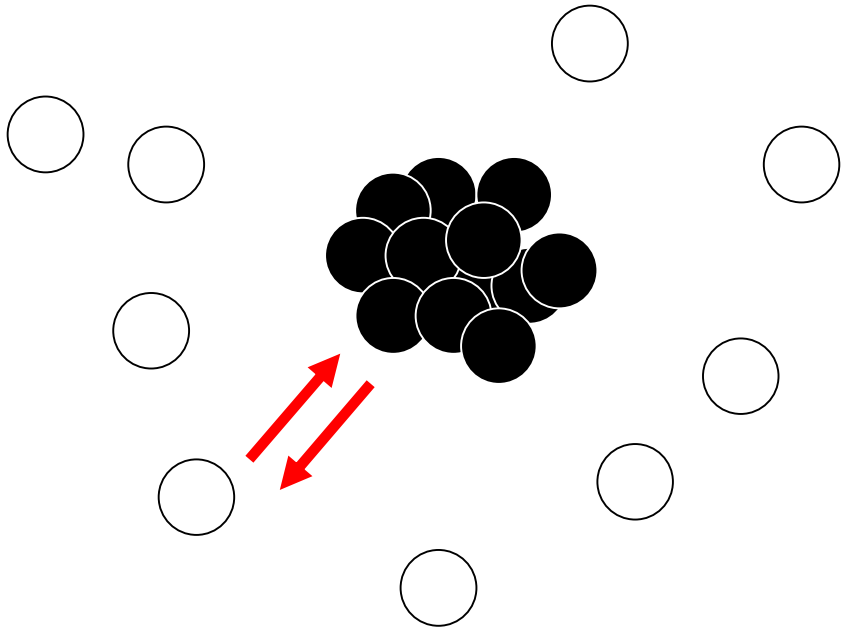
Model „protein cluster“

Analysis of the continuous molecule exchange



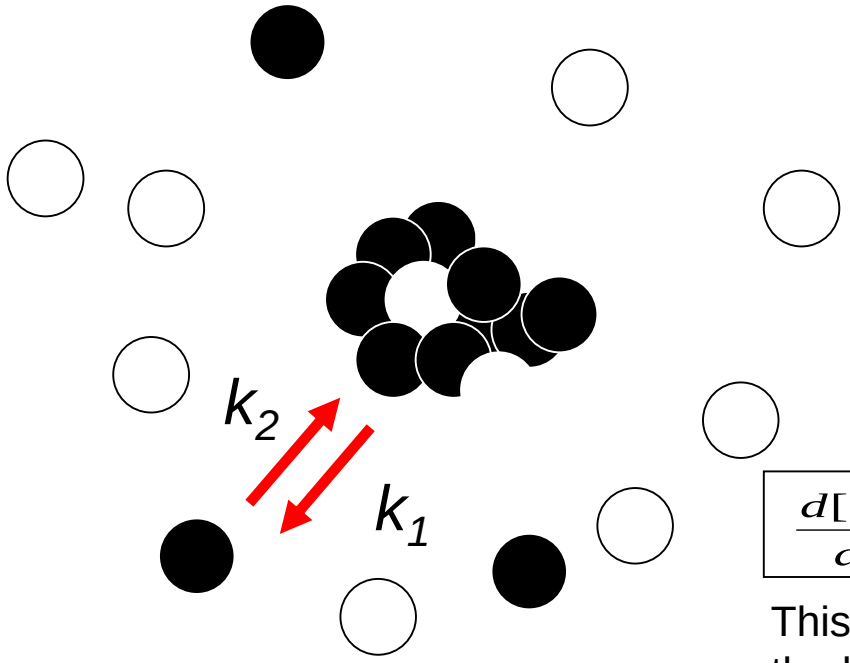
Model „protein cluster“

Bleach cluster



Model „protein cluster“

Find k1 and k2 and check for feasibility

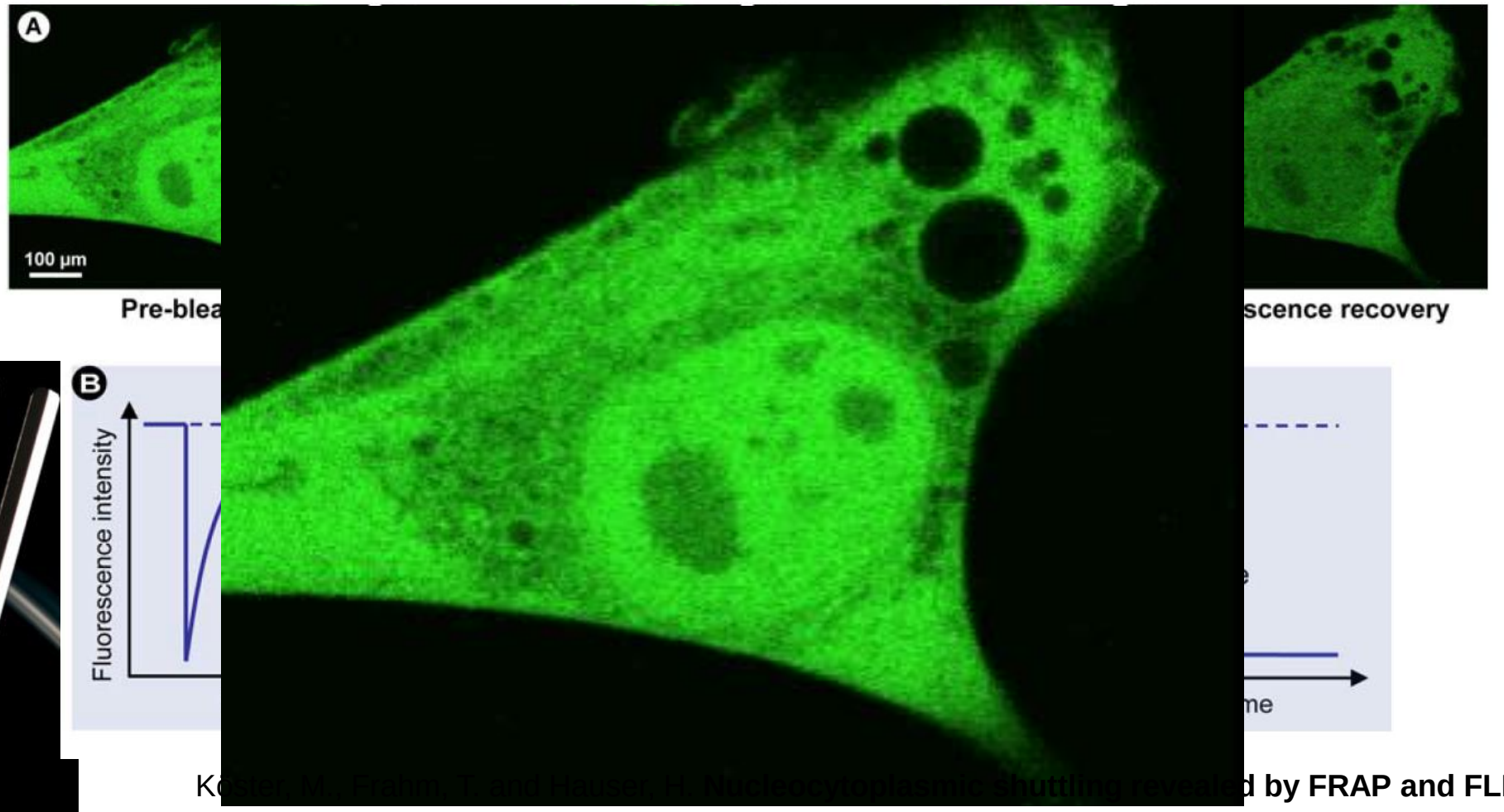


$$\frac{d[F_1]}{dt} = -k_1[F_1] + k_2[F_2]$$

This equation can be the key for one of the unknown terms

Modelling explains individual curve features via specific protein interactions (models) and related equation terms

Using FRAP and mathematical modeling to determine the in vivo kinetics of nuclear proteins



Köster, M., Frahm, T. and Hauser, H. Nucleocytoplasmic shuttling revealed by FRAP and FLIP technologies. ***Current Opinion in Biotechnology*** *16*: 28-34 (2005).

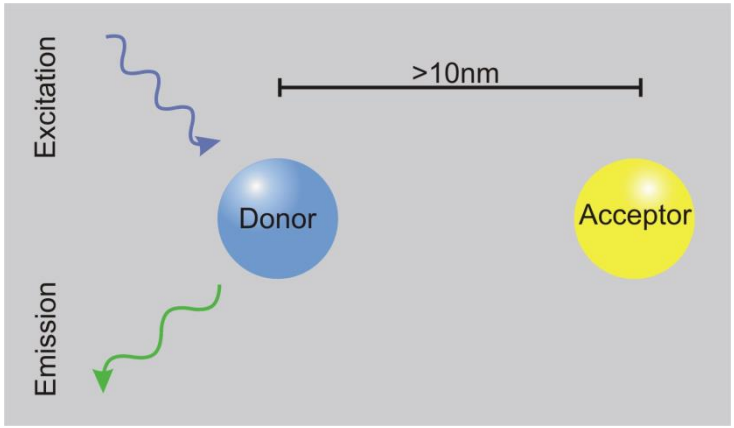
FRET Microscopy with LSM Systems
Fluorescence resonance energy transfer

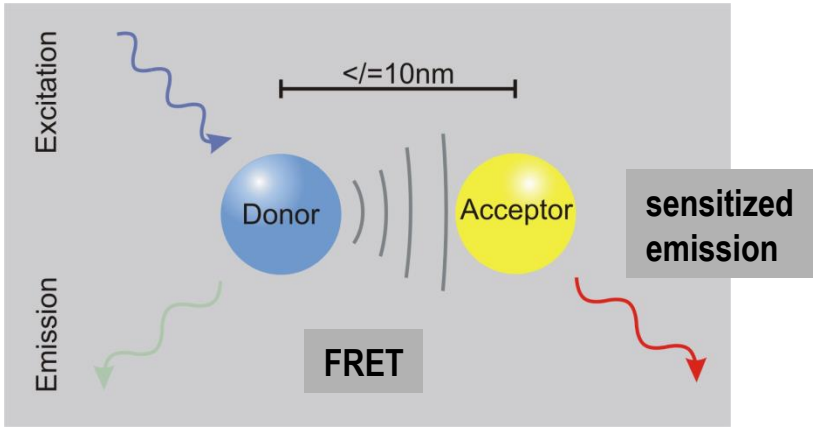
Employing FRET as a molecular ruler in microscopy

FRET is a non-radiative transfer of an excited state from one fluorophore (donor) to another (acceptor).

FRET occurs if donor and acceptor are in close proximity (1-10 nm).

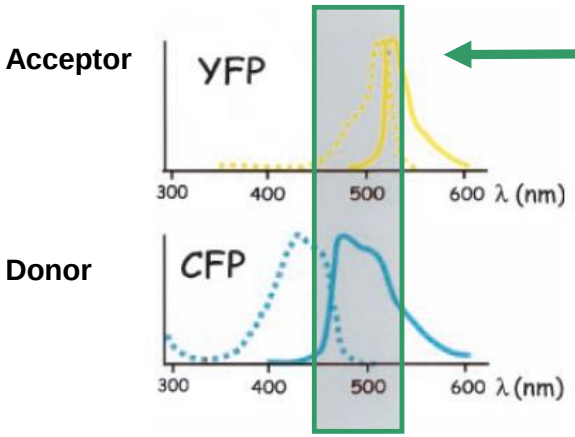
FRET permits microscopic proximity assays at the molecular level!





Prerequesites for FRET:

A suitable FRET Pair



The Acceptor´s excitation spectrum must overlap with the Donor´s emmission spectrum

Important!

FRET is an electron-based energy transfer.

(Don´t think that -in this example- CFP produces cyan light and then excites YFP, so YFP then fluoresces… that´s wrong!)

Possible FRET Pairs:

Fluorescent Protein Pair	Donor Excitation Maximum (nm)	Acceptor Emission Maximum (nm)	Donor Quantum Yield	Acceptor Extinction Coefficient	Förster Distance (nm)	Brightness Ratio
EBFP2-mEGFP	383	507	0.56	57,500	4.8	1:2
ECFP-EYFP	440	527	0.40	83,400	4.9	1:4
Cerulean-Venus	440	528	0.62	92,200	5.4	1:2
MiCy-mKO	472	559	0.90	51,600	5.3	1:2
TFP1-mVenus	492	528	0.85	92,200	5.1	1:1
CyPet-YPet	477	530	0.51	104,000	5.1	1:4.5
EGFP-mCherry	507	510	0.60	72,000	5.1	2.5:1
Venus-mCherry	528	610	0.57	72,000	5.7	3:1
Venus-tdTomato	528	581	0.57	138,000	5.9	1:2
Venus-mPlum	528	649	0.57	41,000	5.2	13:1

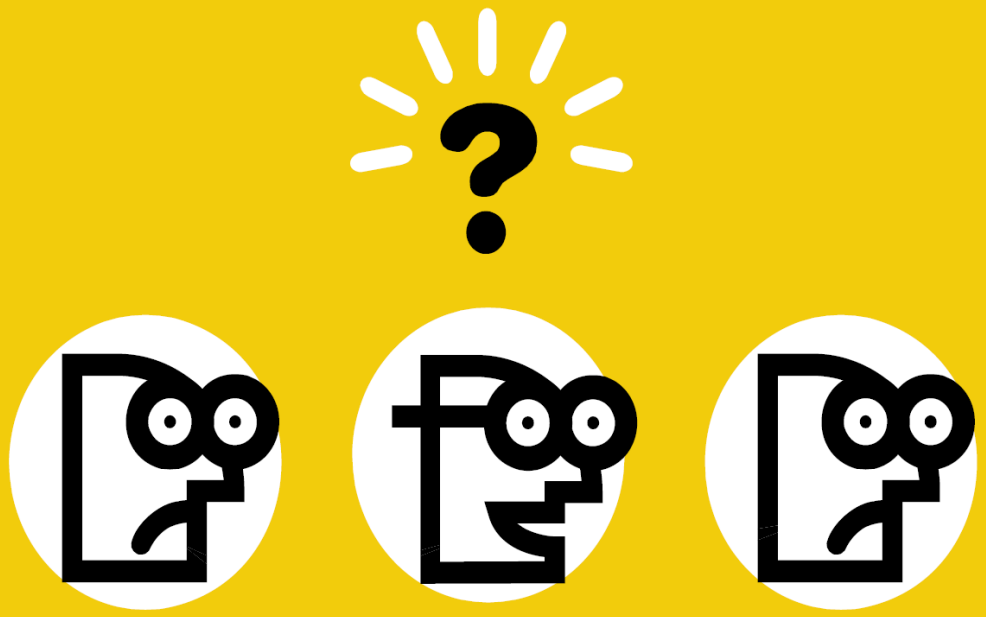
FRET Microscopy with LSM Systems

FRET Detection Methods

The detection methods have different properties and are suited to different samples

Detection of changes: Acceptor photobleaching Donor photobleaching	**=> fixed samples**
Information self-contained: Ratio imaging Sensitized emission	**=> in vivo**

Fluorescence Lifetime Imaging



It's QUESTION TIME!!

We make it visible.