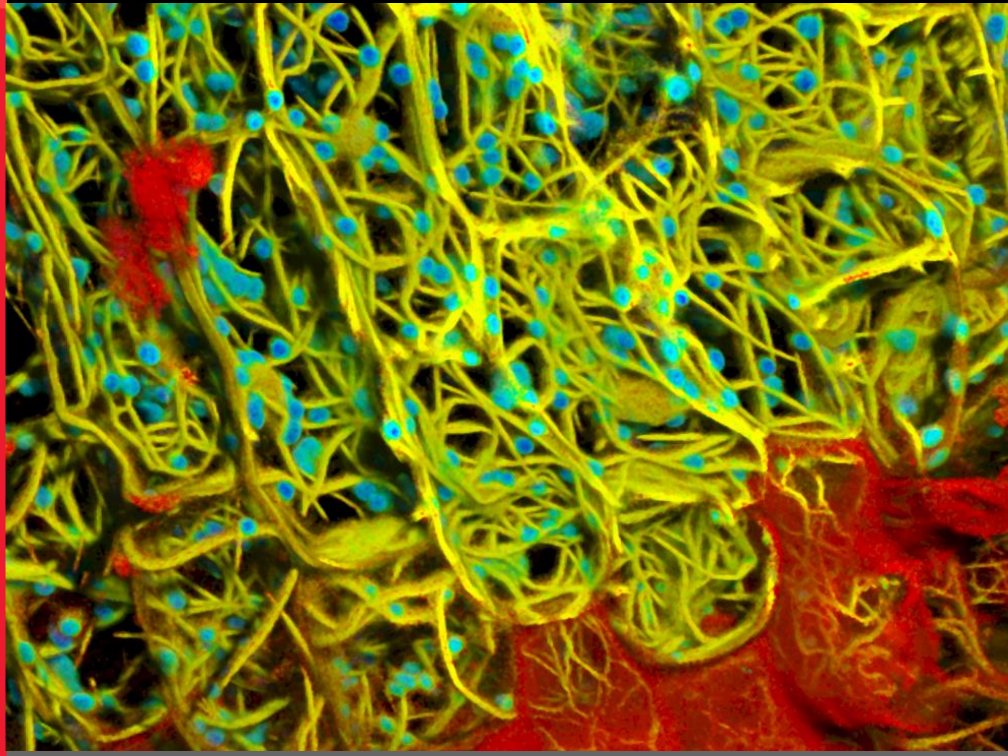
STELLARIS 8

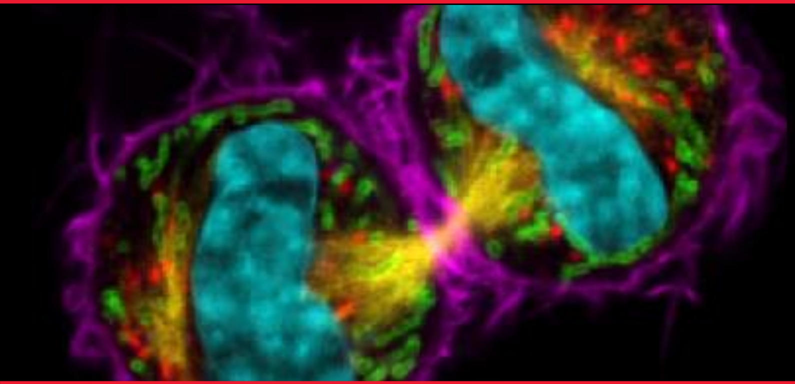
#CONFOCALREIMAGINED

2020.08.19



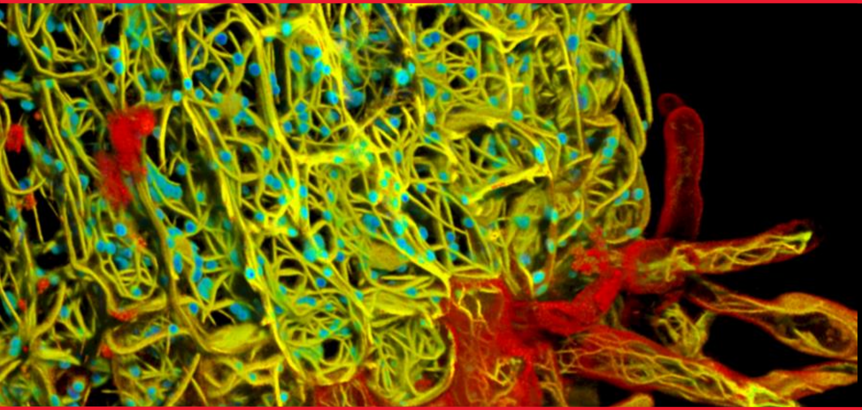
劉思嫺
美嘉儀器股份有限公司
www.major.com.tw

STELLARIS Is Built On These Key Attributes



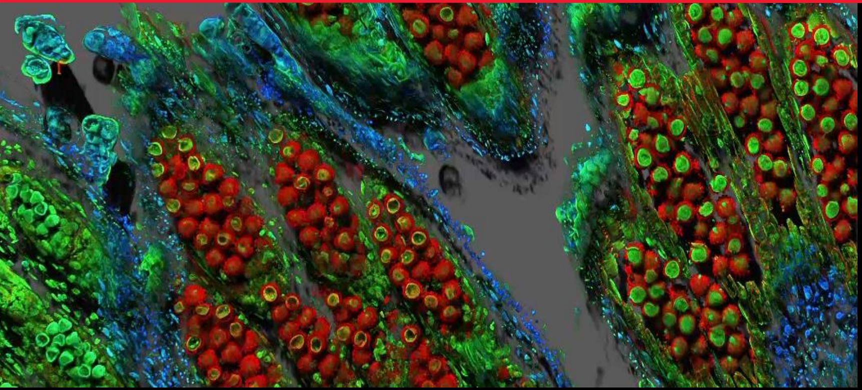
POWER

SEE MORE



POTENTIAL

DISCOVER MORE



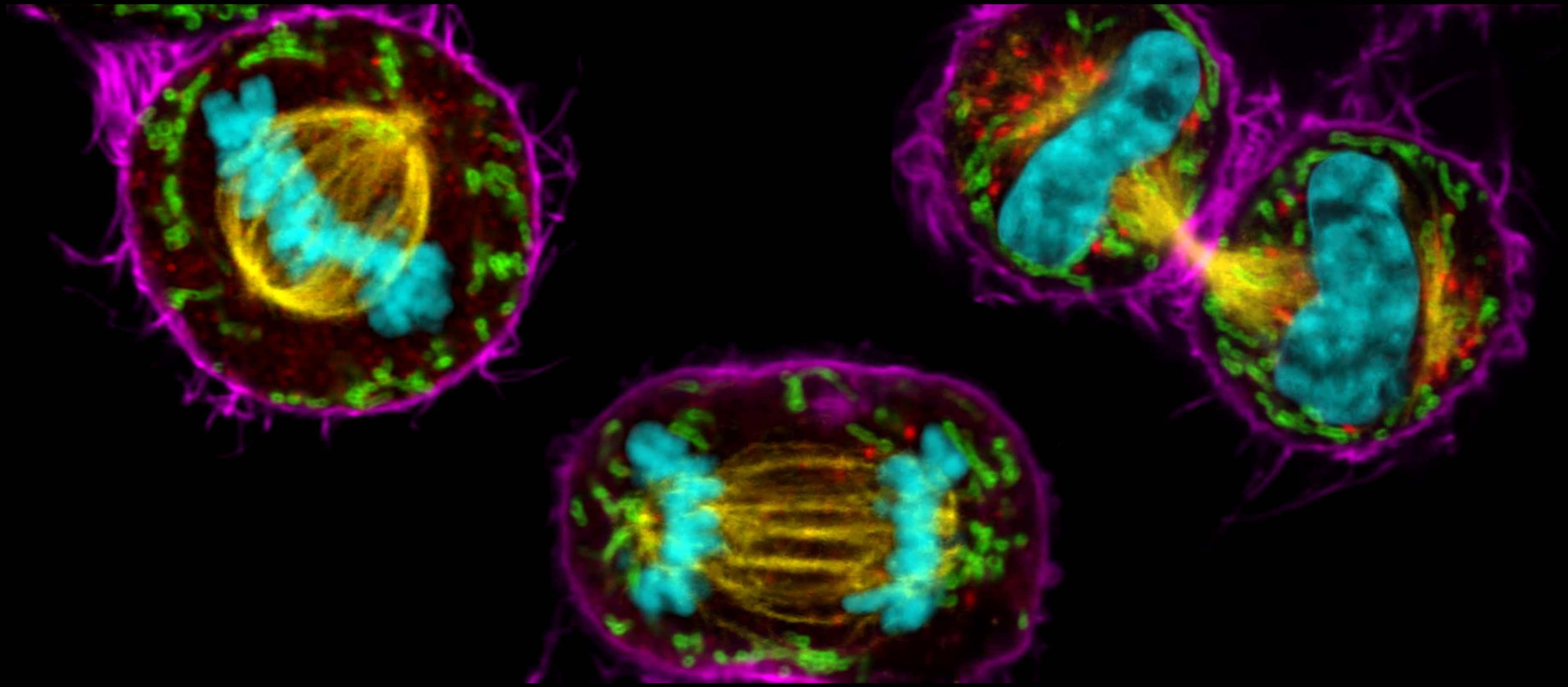
PRODUCTIVITY

DO MORE

#CONFOCALREIMAGINED

Leica

POWER
SEE MORE



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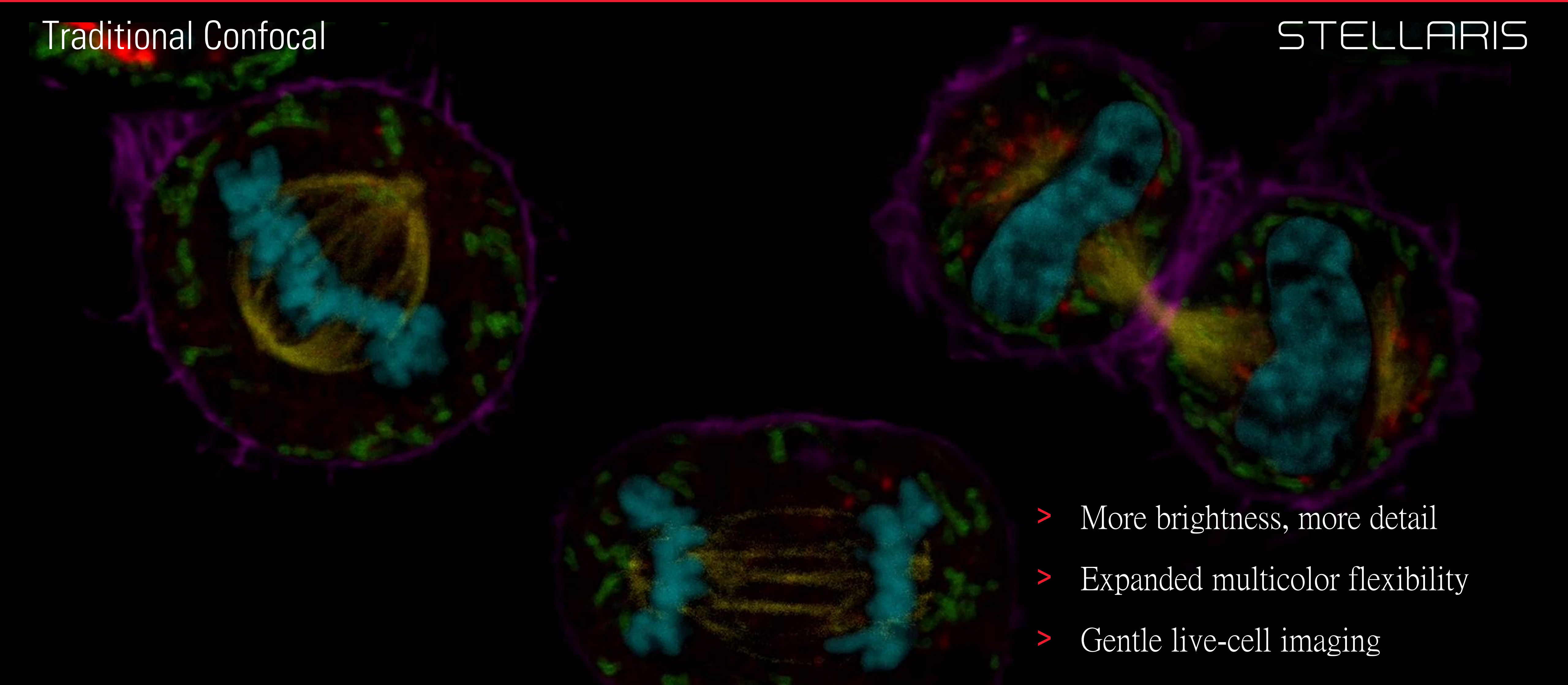
STELLARIS Gives You More Brightness, More Detail



- More brightness, more detail
- Expanded multicolor flexibility
- Gentle live-cell imaging

COS7 mitotic cells. Chromatin (cyan, mCherry), mitotic spindle (yellow, EGFP), Golgi (red, Atto647N), mitochondria (green, AF532), actin filaments (magenta, SiR700)
Sample courtesy of Jana Döhner, Urs Ziegler, University of Zürich; cells expressing mCherry were a kind gift of Daniel Gehrlich. SiR was a kind gift of Spirochrome.

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The First Key Innovation: The New Power HyD Detector Family

Power HyD™ S
The new standard for STELLARIS

- All-round detector, high performance throughout the spectrum
- Leica Si-based technology, hybrid analog/photon counting modes

Power HyD™ X
The new eXcellence in functional imaging

- Specialized for fast FALCON and τ-STED in the visible range up to far-red range
- Leica GaAsP-hybrid technology

Power HyD™ R
The new choice for near infraRed detection

- Specialized for fast FALCON and τ-STED in the Near-Infrared range
- Leica GaAsP-hybrid technology

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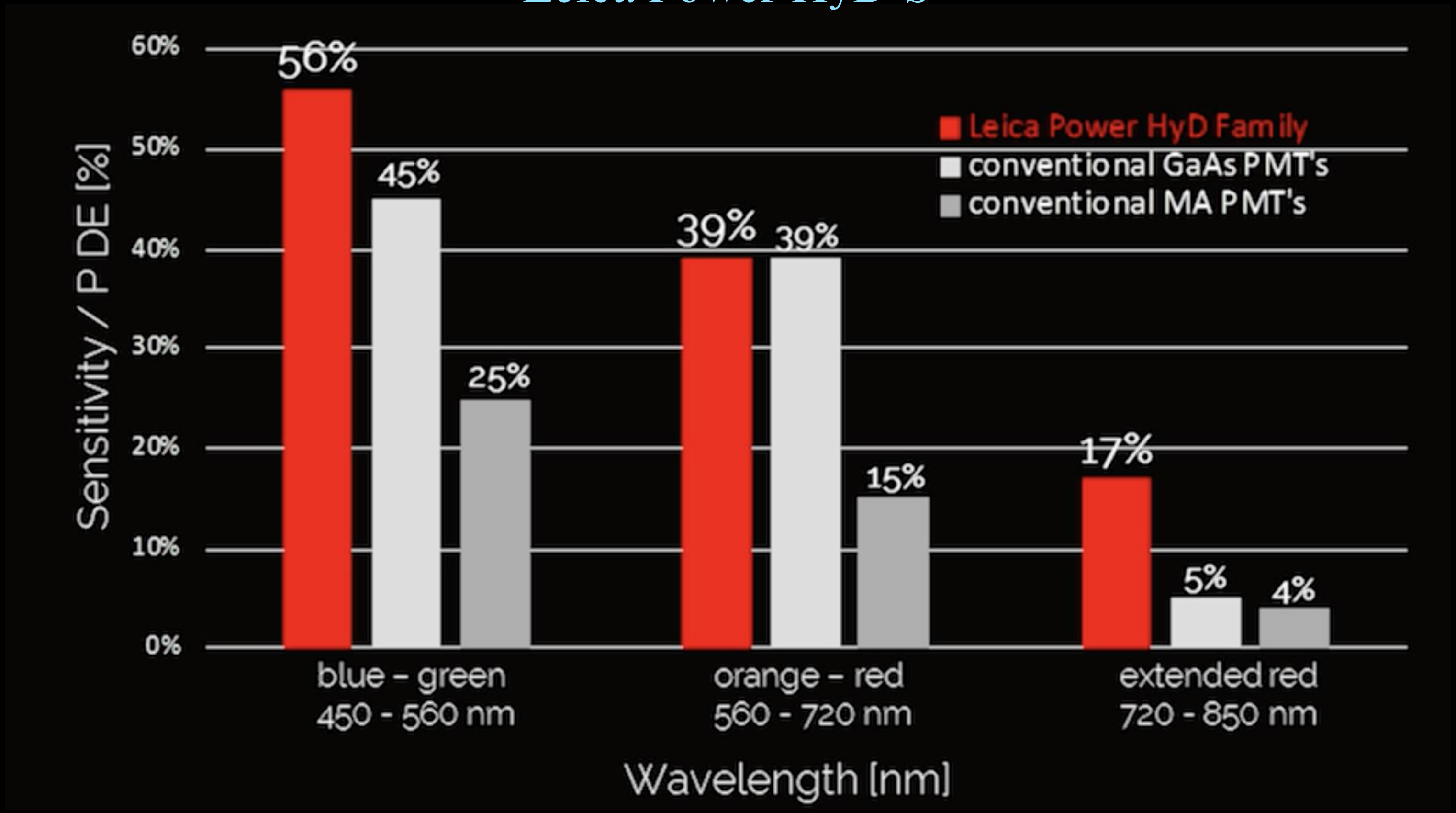
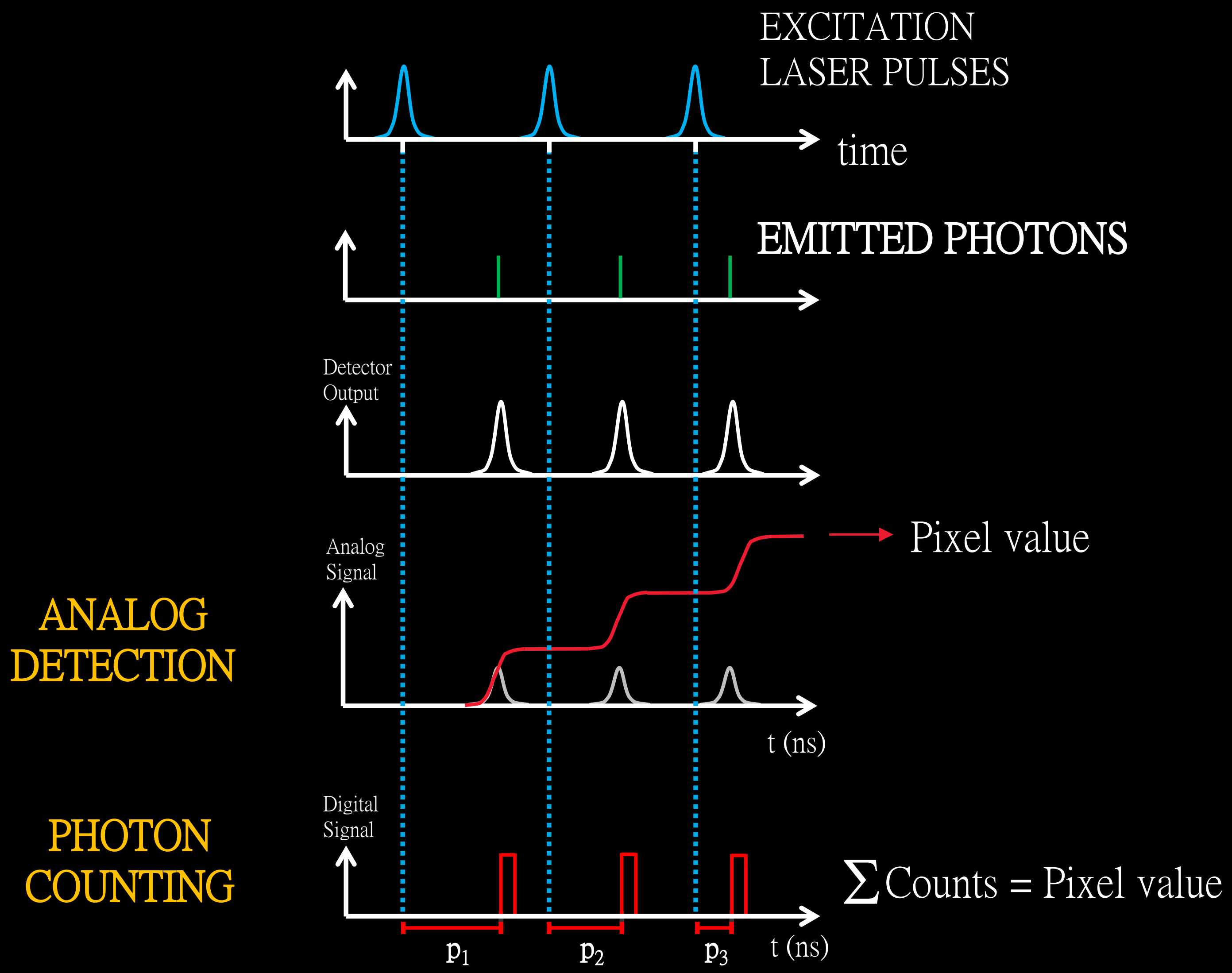
Enhanced Spectral Freedom: STELLARIS 8

Leica Power HyD S



The Power HyD Family Covers The Needs Of Applications Throughout The Spectrum

Power HyD S: The New Standard For STELLARIS



ANALOG DETECTION

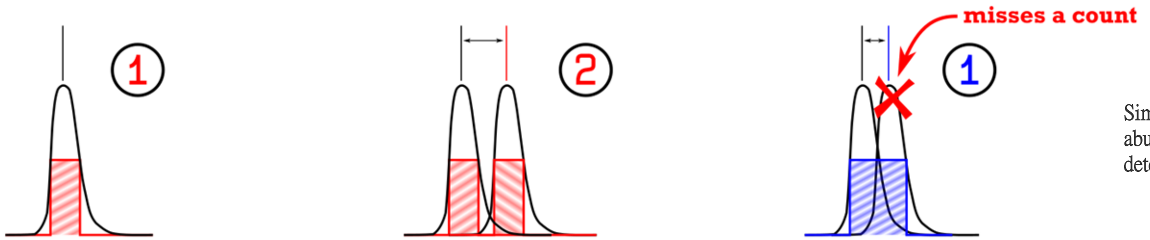
- High dynamic range
- Traditional mode for confocal

PHOTON COUNTING

- Sensitivity to faintest signals
- Quantitative applications
- Fluorescence Lifetime-based applications FALCON, TauSense)

Power Counting: The New Photon Counting In STELLARIS

Traditional Photon Counting



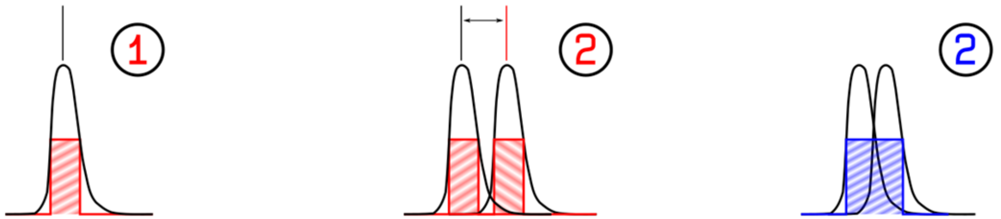
Sparse occurrence

Frequent occurrence at distance above pulse pair resolution

Frequent occurrence at distance below pulse pair resolution

Simple thresholding of detector signals misses abundant photons, resulting in a dead time for detection and a saturation of counts.

STELLARIS Power Counting



Sparse occurrence

Frequent occurrence at distance above pulse pair resolution

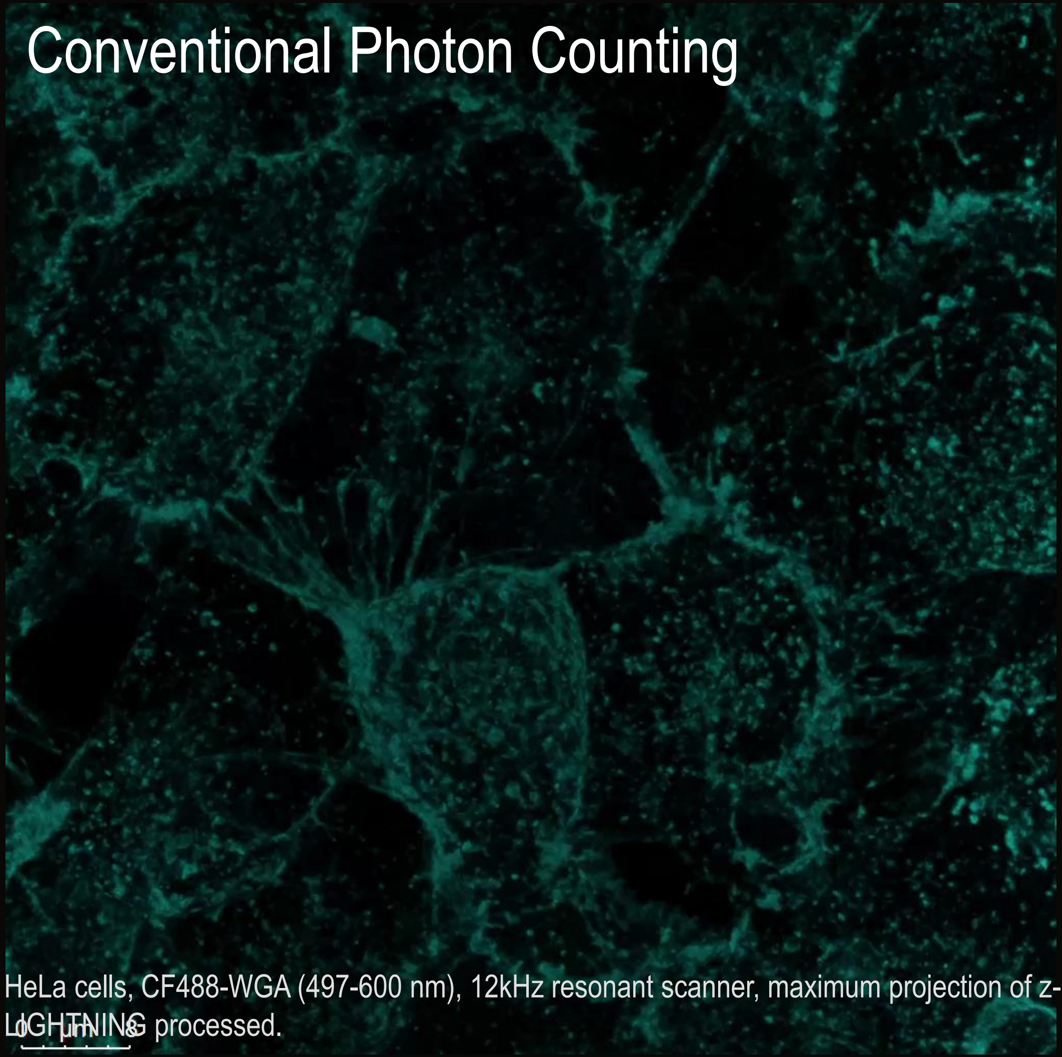
Frequent occurrence at distance below pulse pair resolution

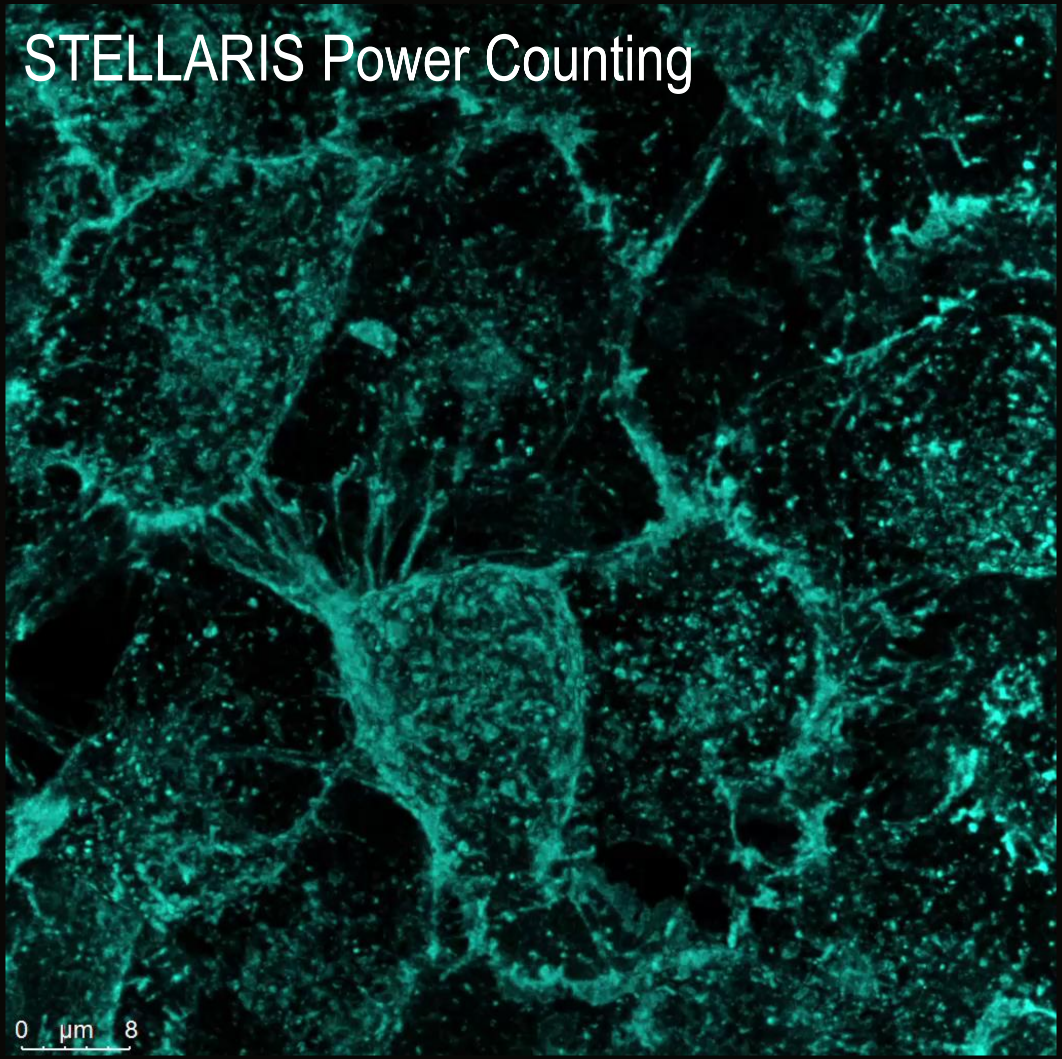
Recognition of two overlapping photons is enabled by taking the pulse width into account

Low signal intensities:
Power counting and traditional counting results match

High signal intensities:
Power counting significantly enhances detection sensitivity and dynamic range

What Is The Benefit Of Power Counting?





HeLa cells, CF488-WGA (497-600 nm), 12kHz resonant scanner, maximum projection of z-stack; LIGHTNING processed.

Power Counting Extends Signifiantly Dynamic Range And Linearity

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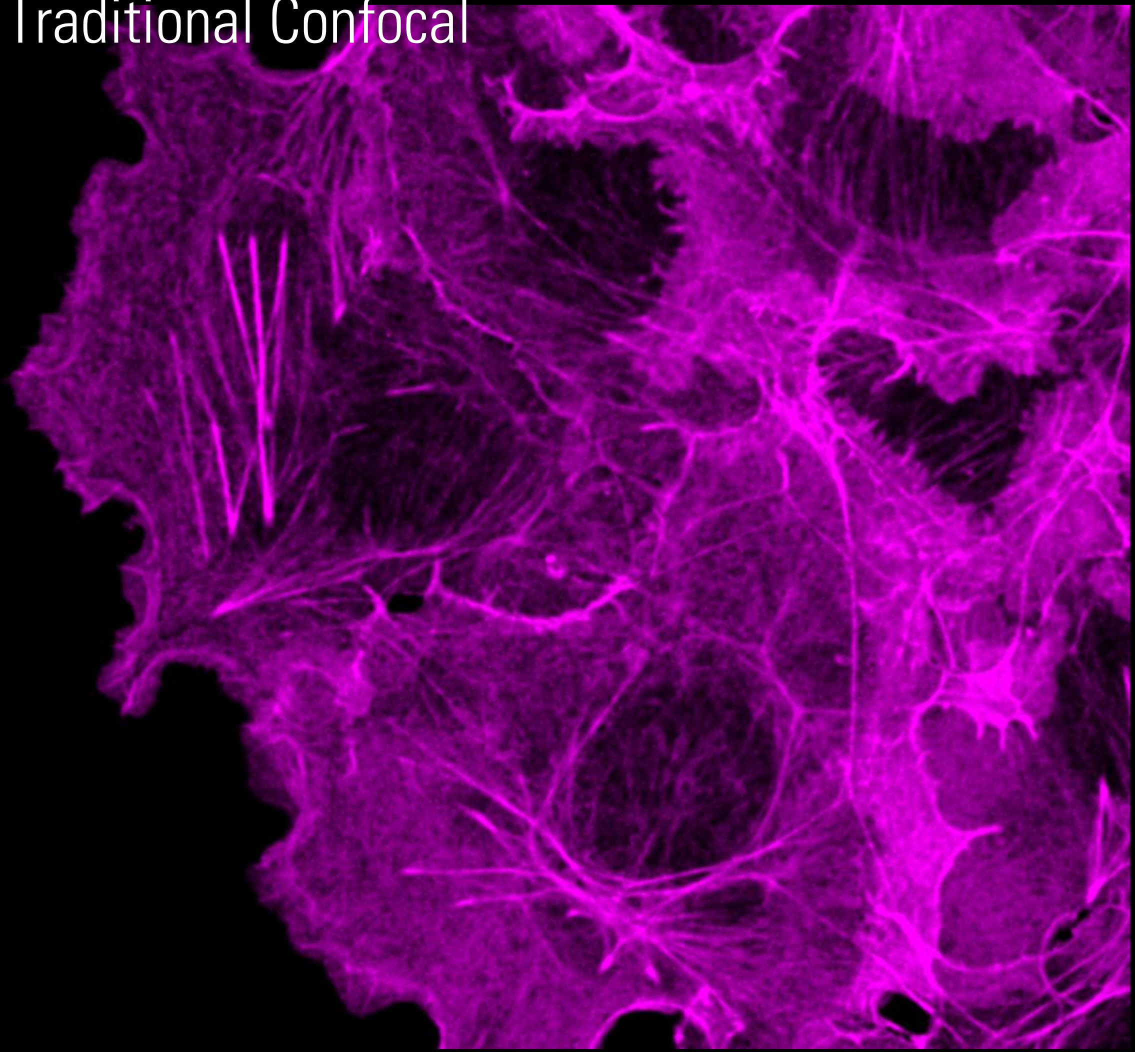
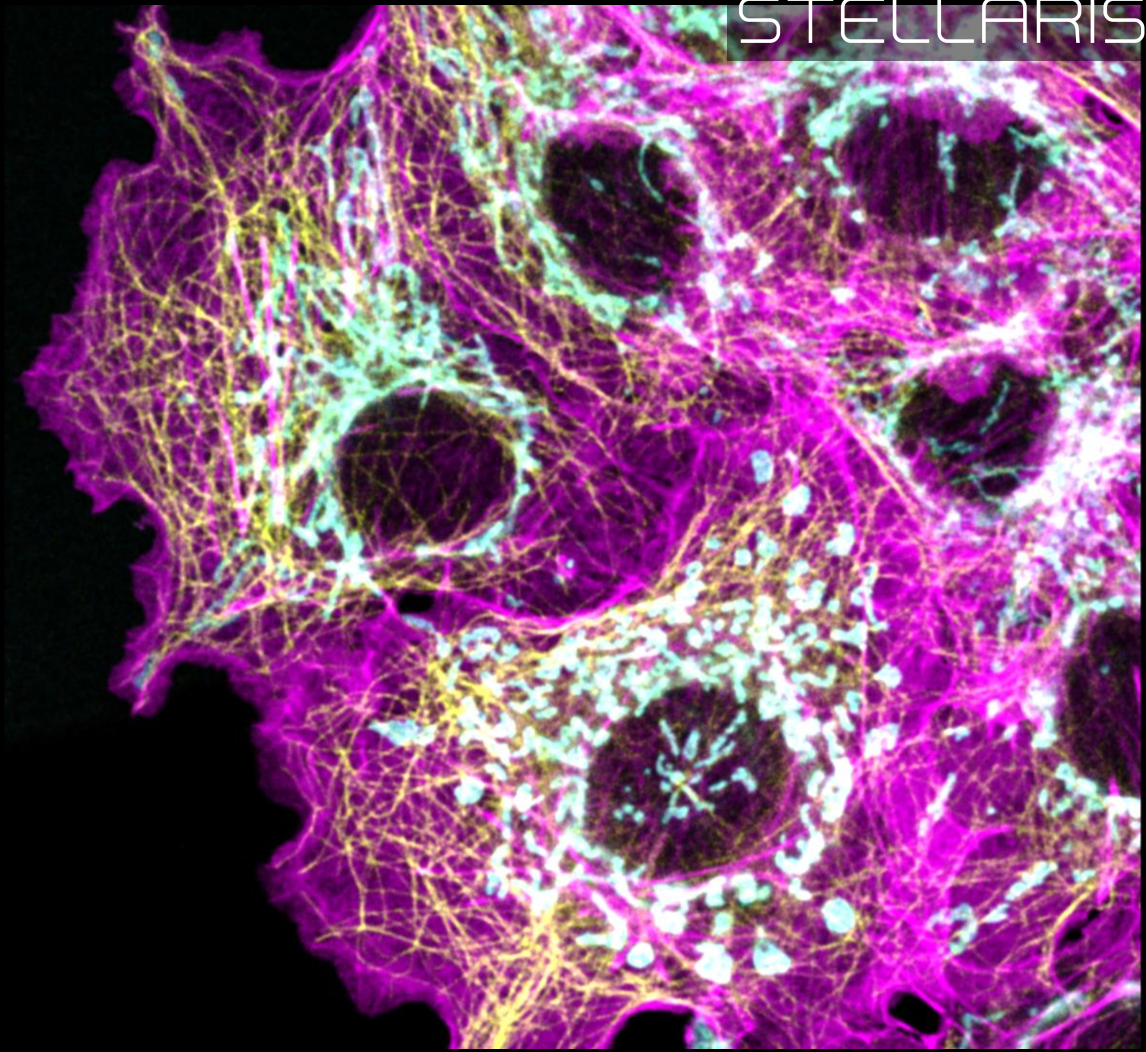
Leica

STELLARIS Gives You Expanded Multicolor Flexibility





COS7 cells. Actin (magenta, SiR-Actin 657-740 nm), Mitochondria (cyan, AF750 760-790 nm), Microtubules (yellow, AF790 810-850 nm)
Sample Courtesy: Jana Döhner, Urs Ziegler, University of Zurich

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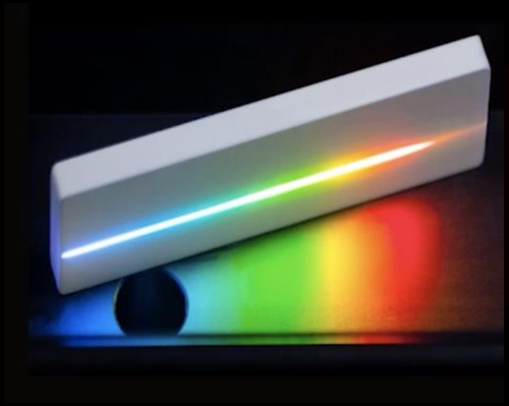
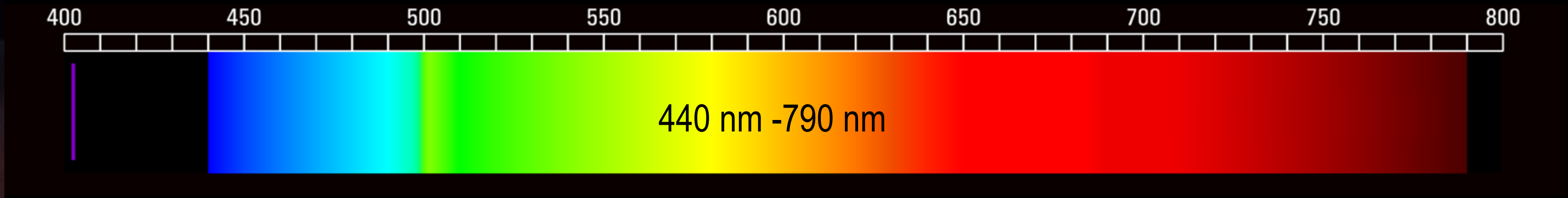
The Second Key Innovation: The Next Generation White Light Lasers

Traditional Confocal

STELLARIS





- Complete spectral freedom with excitation perfectly matched to the fluorochrome
- Less complexity, more flexibility: a single laser to do the work of many. Up to 8 single excitation lines can be used simultaneously

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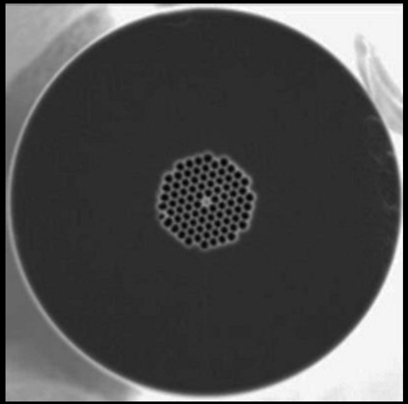
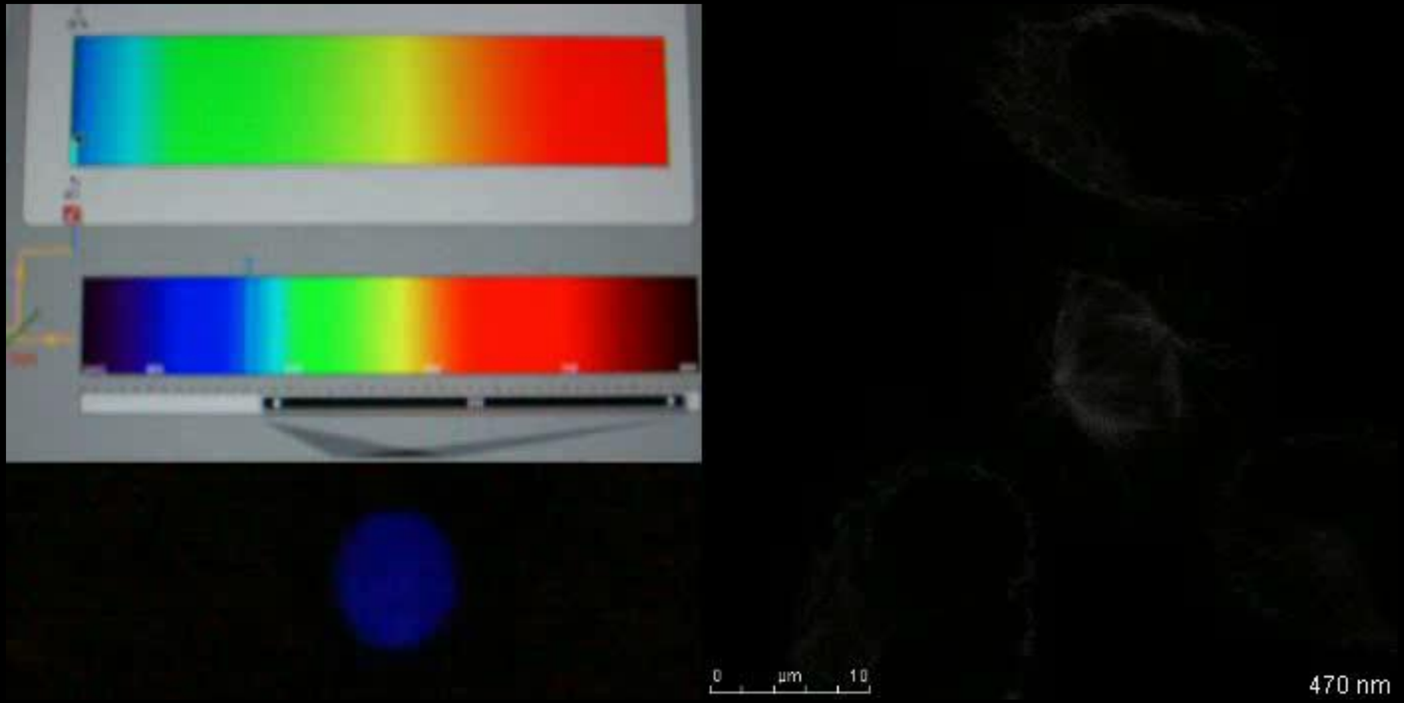
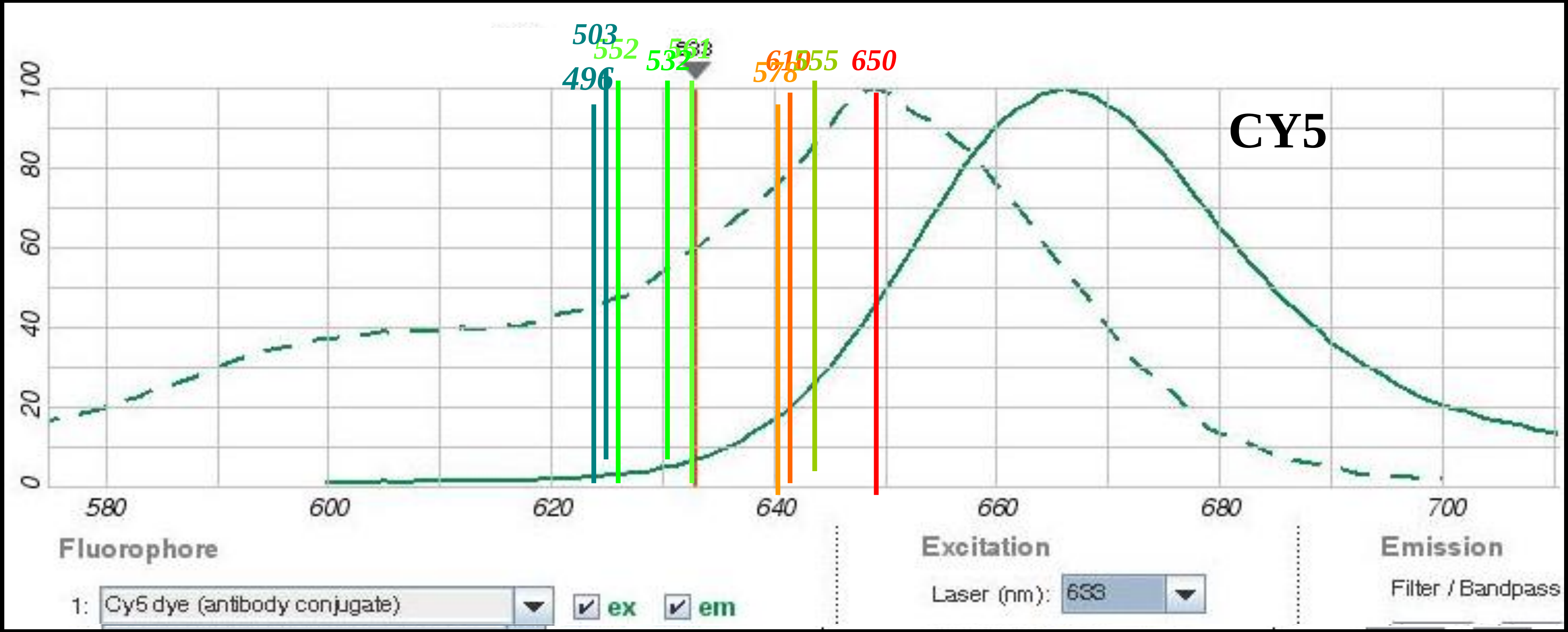
White Light Laser

- 440-790 nm tunable

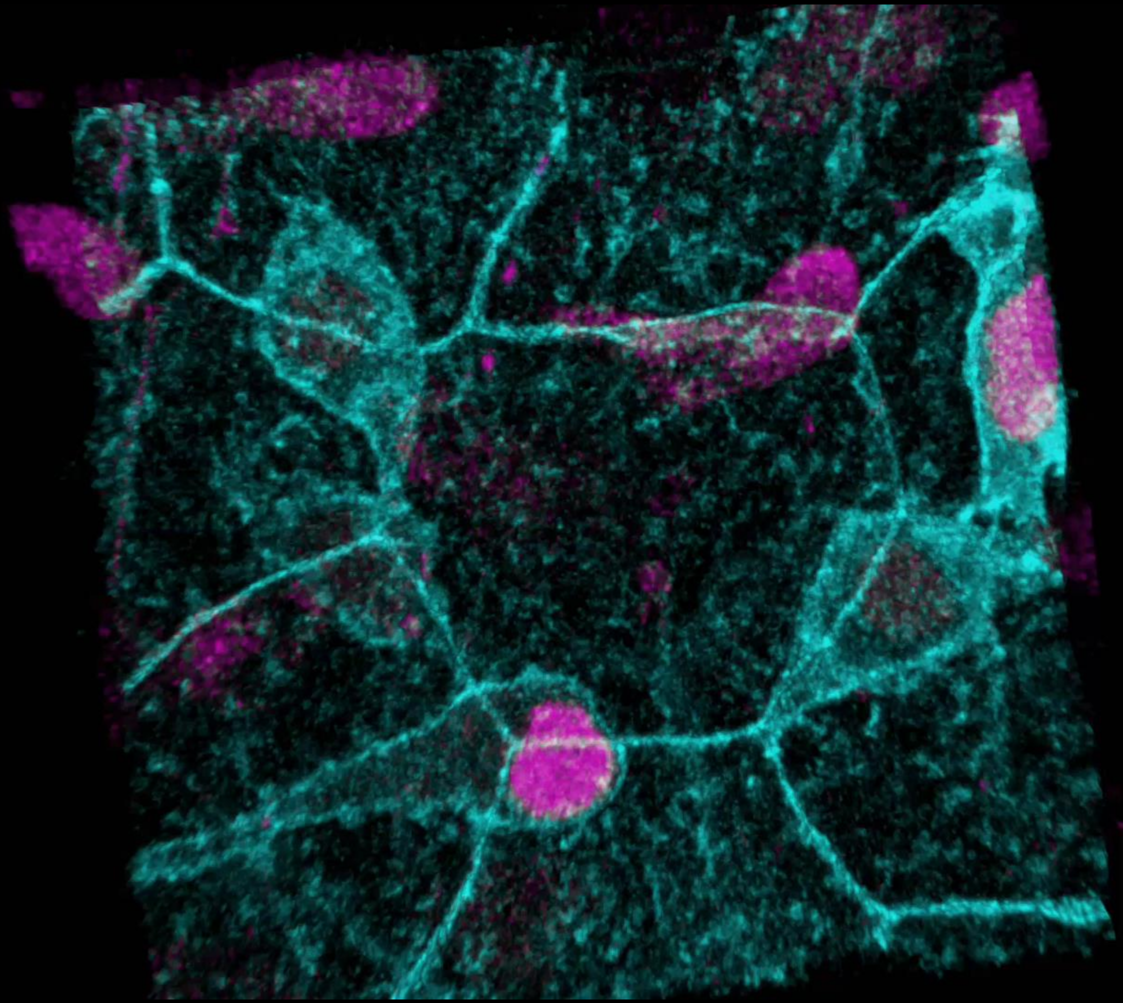






CY5
503
552
532
496
578
650
100
80
60
40
20
0
580
600
620
640
660
680
700
Fluorophore
1: Cy5 dye (antibody conjugate)
ex
em
Excitation
Laser (nm): 633
Emission
Filter / Bandpass

STELLARIS Gives You Gentle Live Cell Imaging



Zebrafish posterior lateral line primordium migration. Cell membrane (cyan), Nuclei (magenta)
Sample Courtesy: Jonas Hartmann, Gilmour Group, EMBL Heidelberg.

- Perform imaging for longer periods, since both excitation as well as detection are optimally tuned
- Preserve sample integrity through efficient signal acquisition at the lowest required levels of illumination
- Possible thanks to redesigned optics for optimized transmission

#CONFOCALREIMAGINED

The Red Extended Benefits Of Our Next Generation WLLs

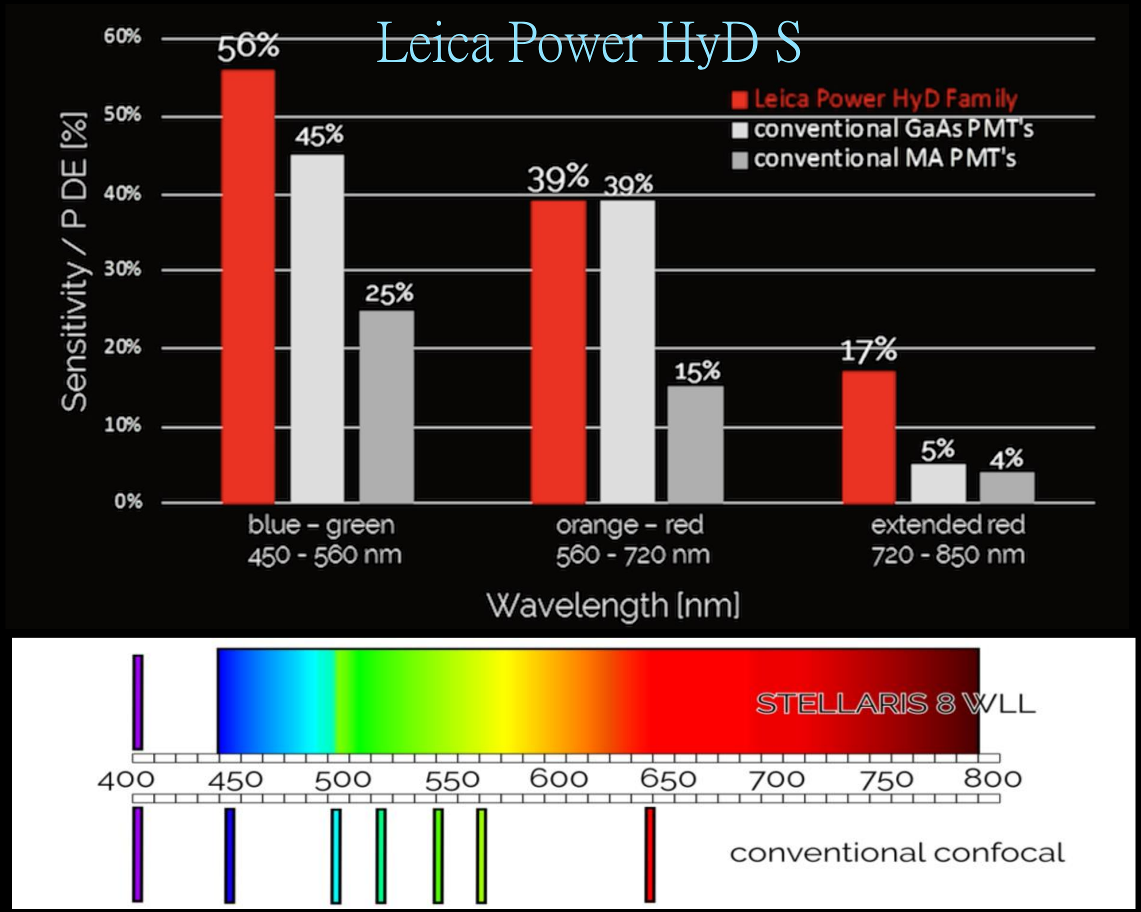
- Excite each fluorophore optimally at its excitation peak
- Enhance multiplexing capabilities by adding up to 3 more fluorophores in the NIR range

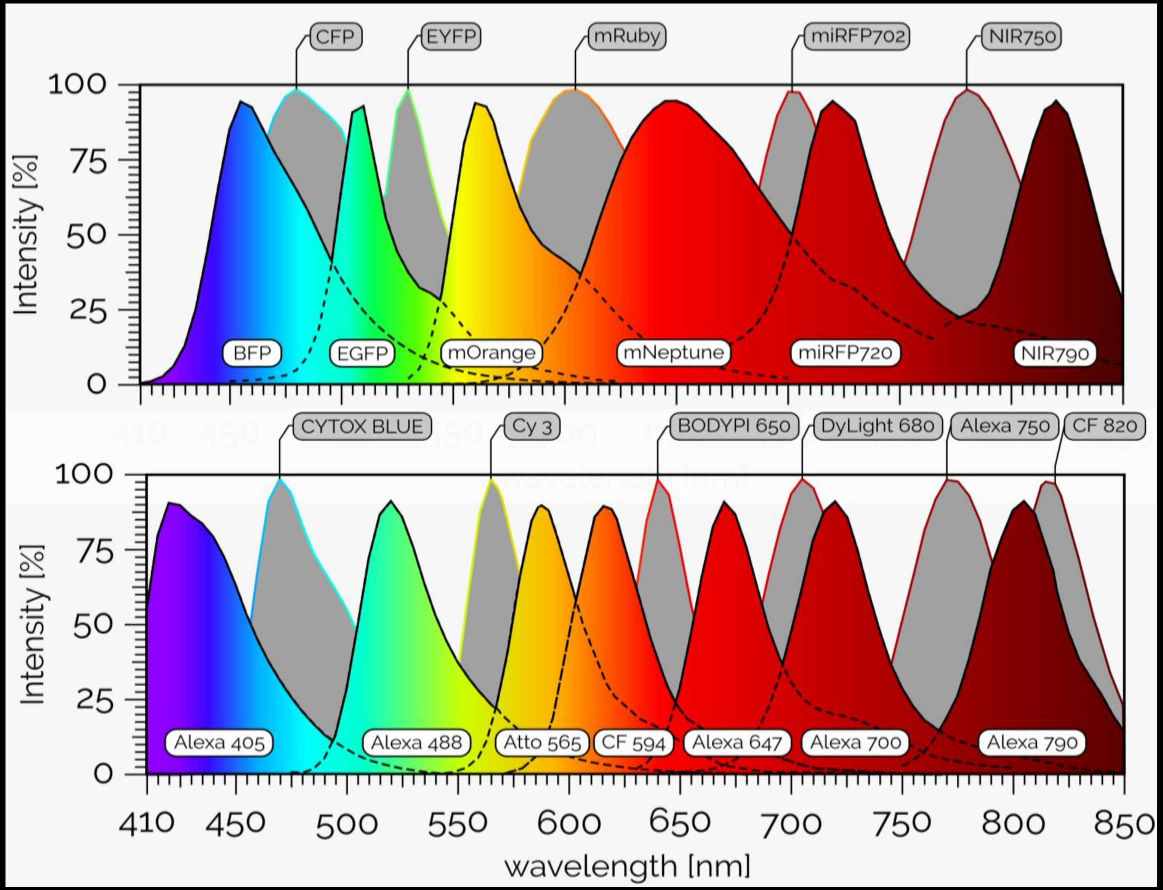
Some 685 nm excitable dyes:

ATTO 740
CF680
CellBrite NIR680
CF750
BioTracker NIR750
Alexa 700
CellBrite NIR700

ATTO 700
CellBrite NIR750
Alexa 750
CF700
MitoView720
CellBrite NIR770
Alexa 680
ATTO 680
ATTO 725

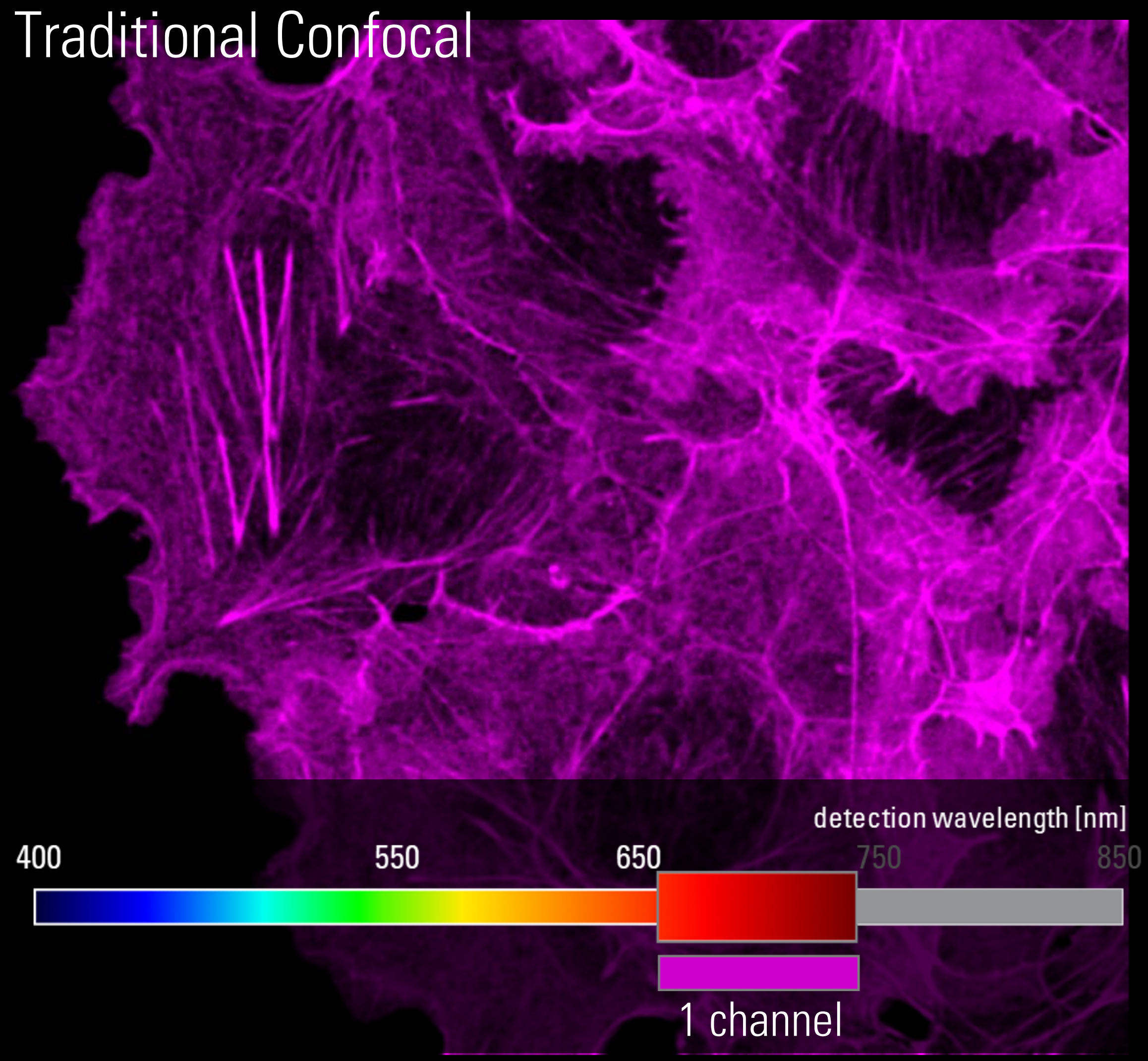
Enhanced Spectral Freedom: STELLARIS 8

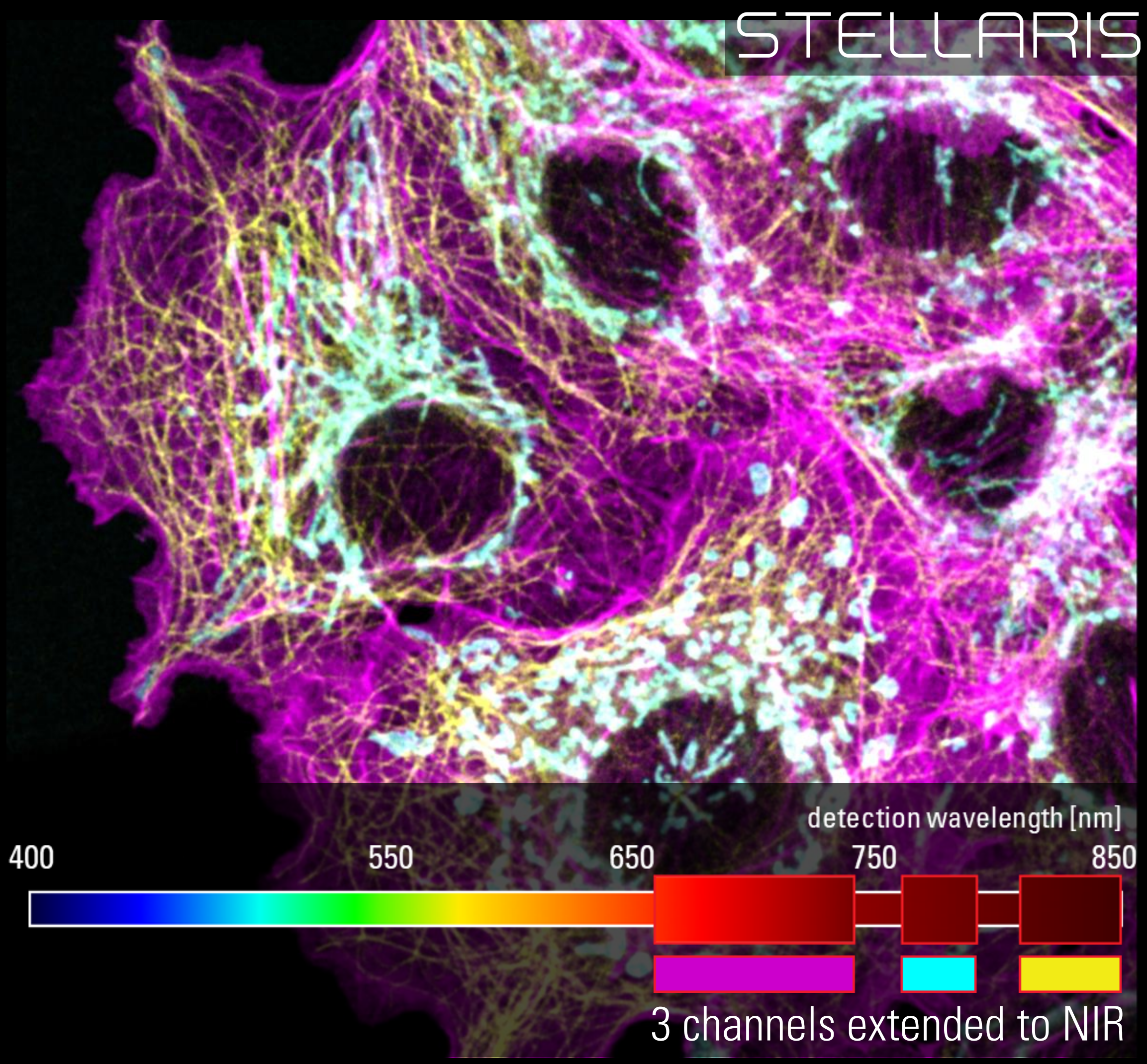




The Power HyD Family Covers The Needs Of Applications Throughout The Spectrum

STELLARIS Gives You Expanded Multicolor Flexibility





COS7 cells. Actin (magenta, SiR-Actin 657-740 nm), Mitochondria (cyan, AF750 760-790 nm), Microtubules (yellow, AF790 810-850 nm)
Sample Courtesy: Jana Döhner, Urs Ziegler, University of Zurich

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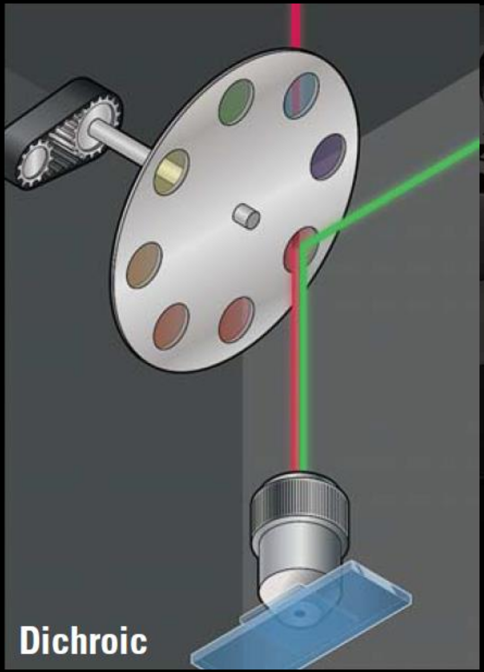
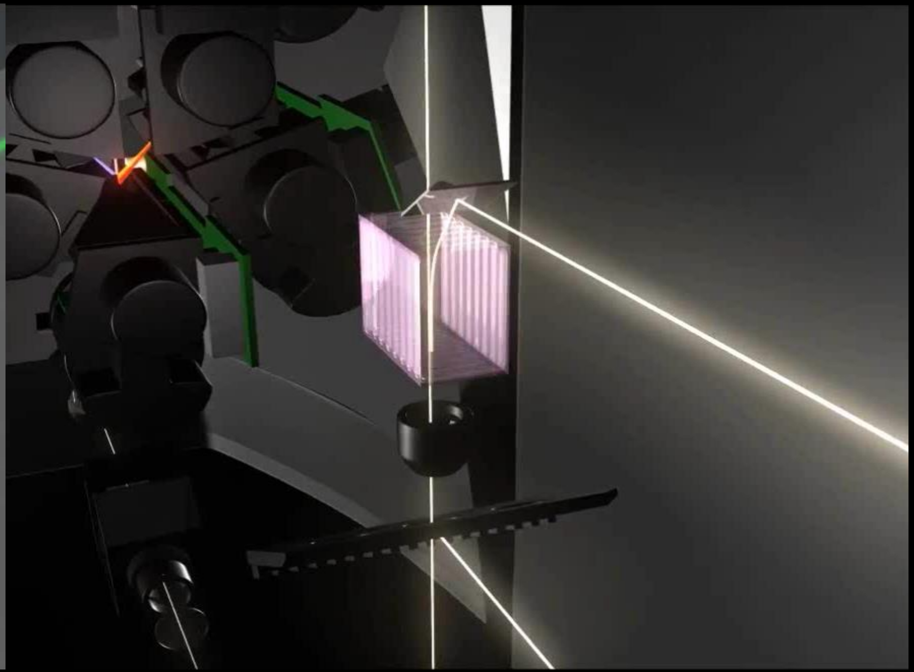
What Is Behind The White Light Laser Technology?

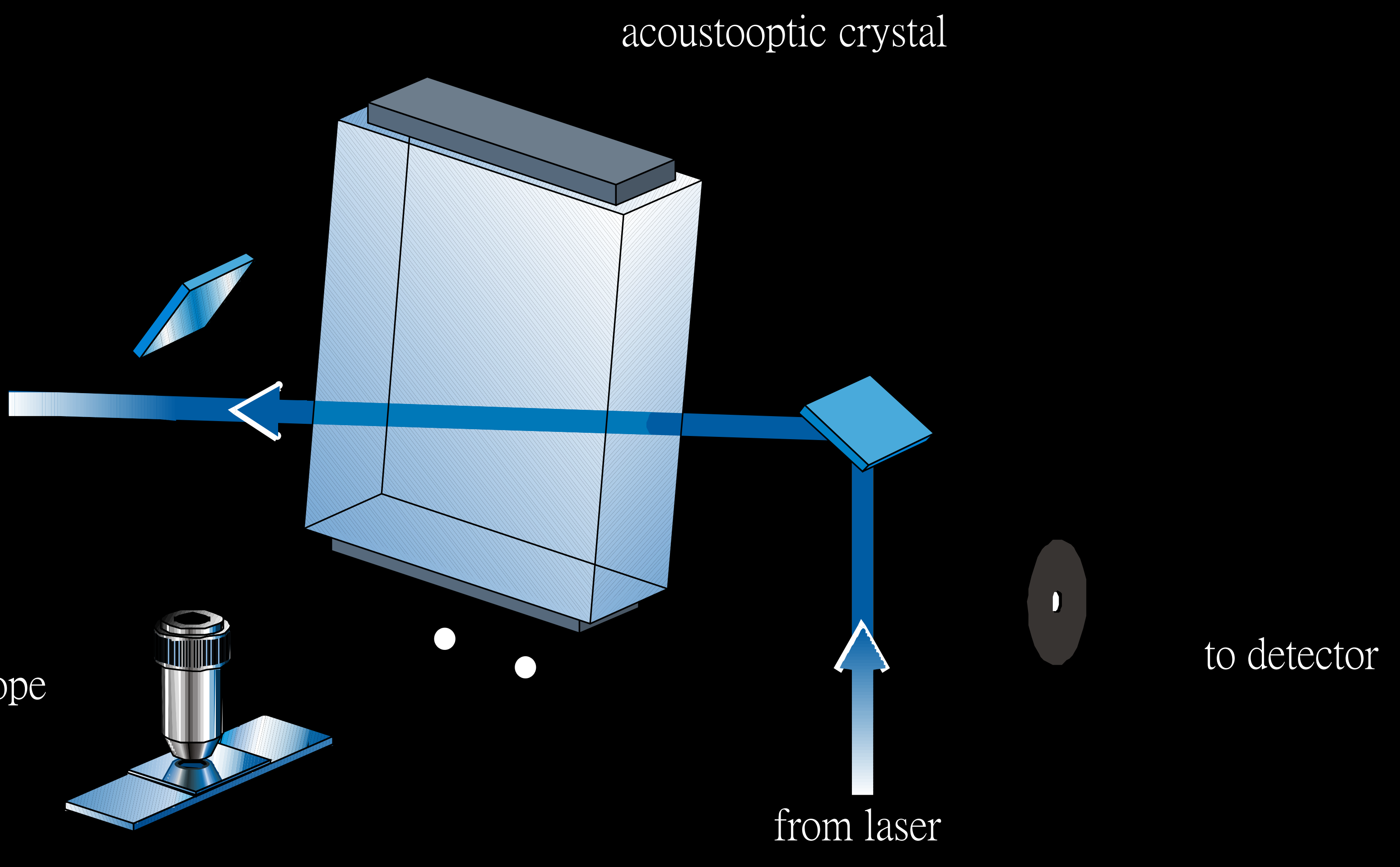
Leica AOBS

- Tunability is achieved with the Acousto Optical Beamsplitter (AOBS)
- Replaces dichroic/multi-dichroic mirrors/wheel-sliders needed for beam splitting
- Microsecond switching time for line sequential acquisition
- Free choice of wavelength with nm precision across the spectrum

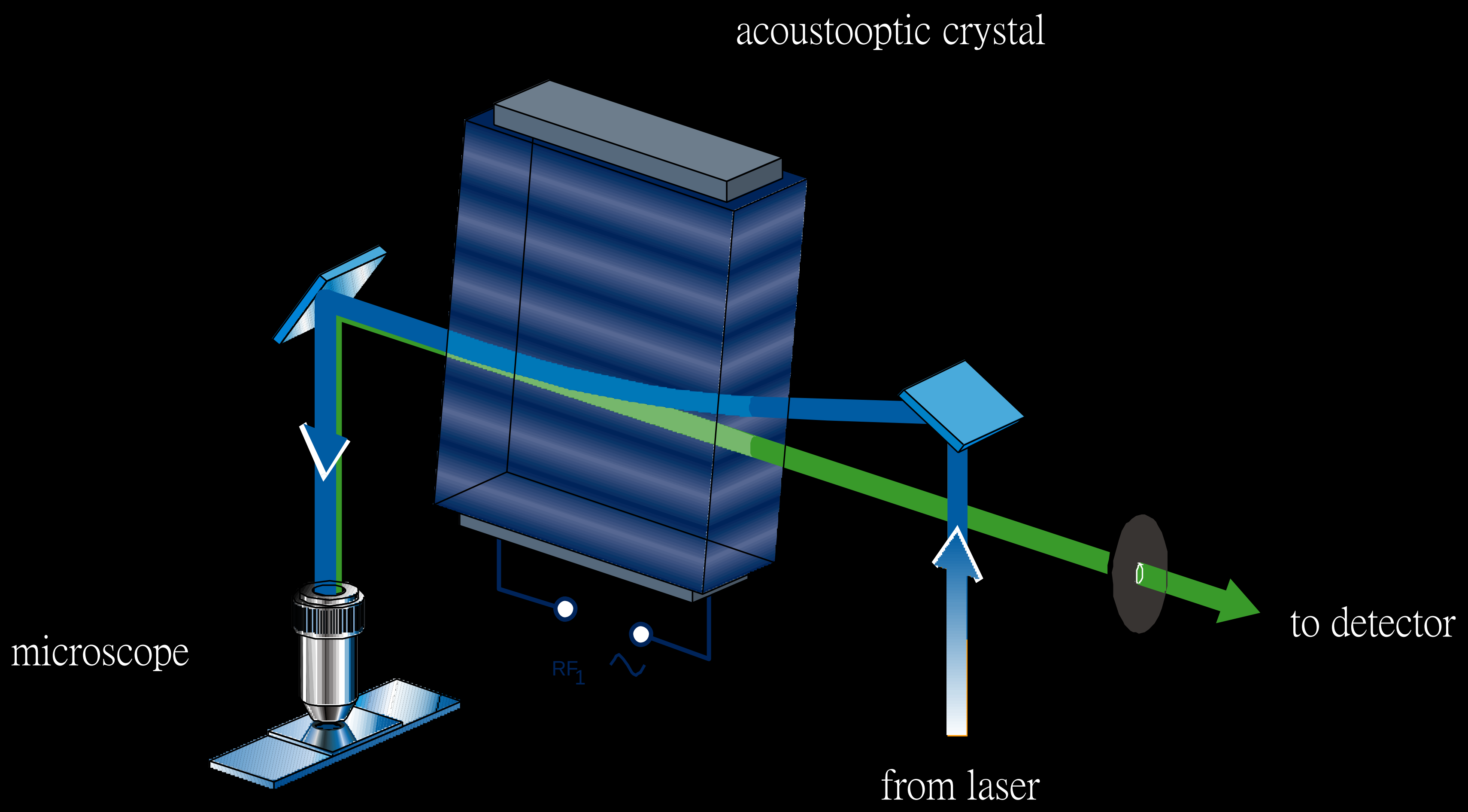




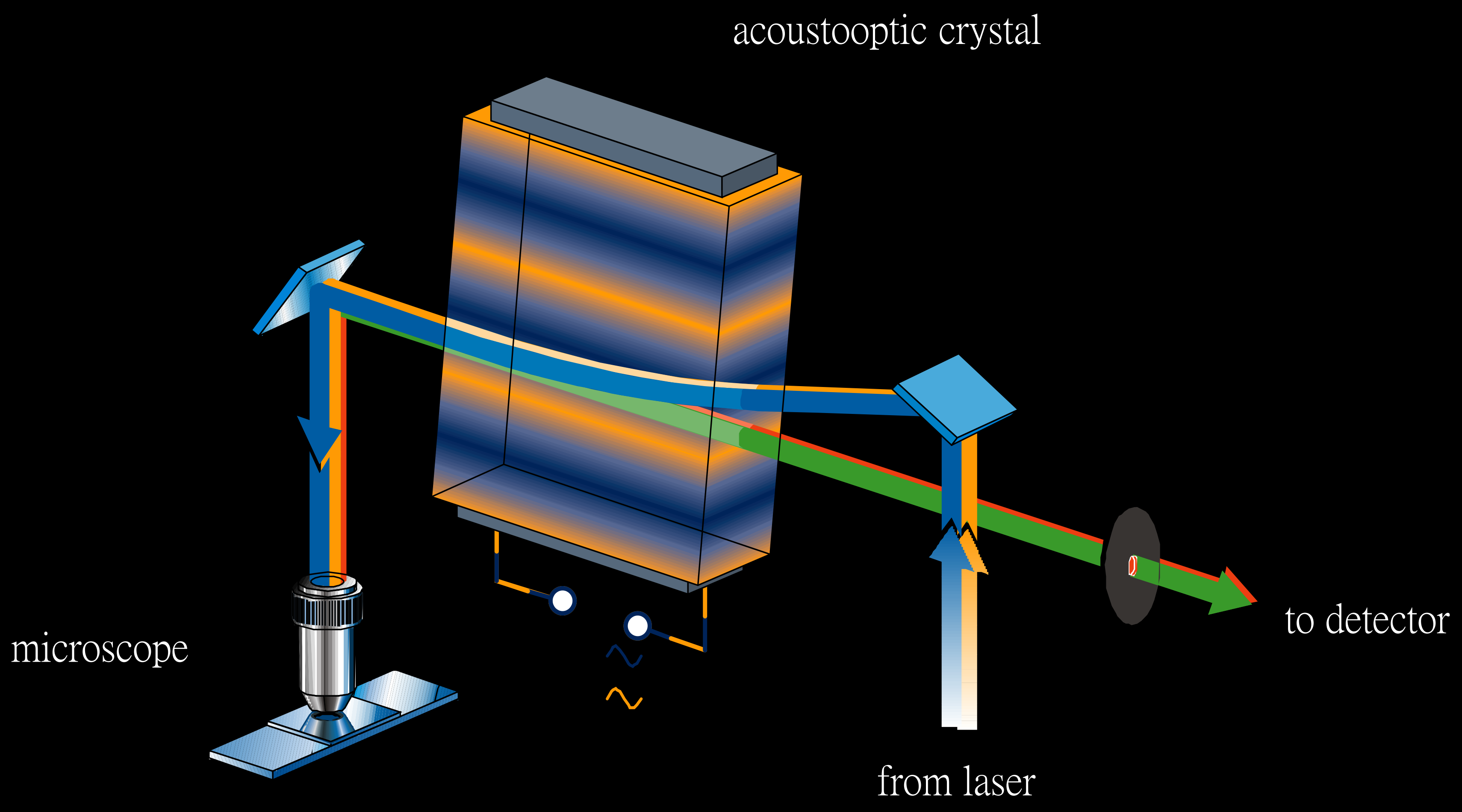
Acousto Optical Beam Splitter (AOBS)



Acousto Optical Beam Splitter (AOBS)



Acousto Optical Beam Splitter (AOBS)



The Leica AOBS Maximizes Signal Collection

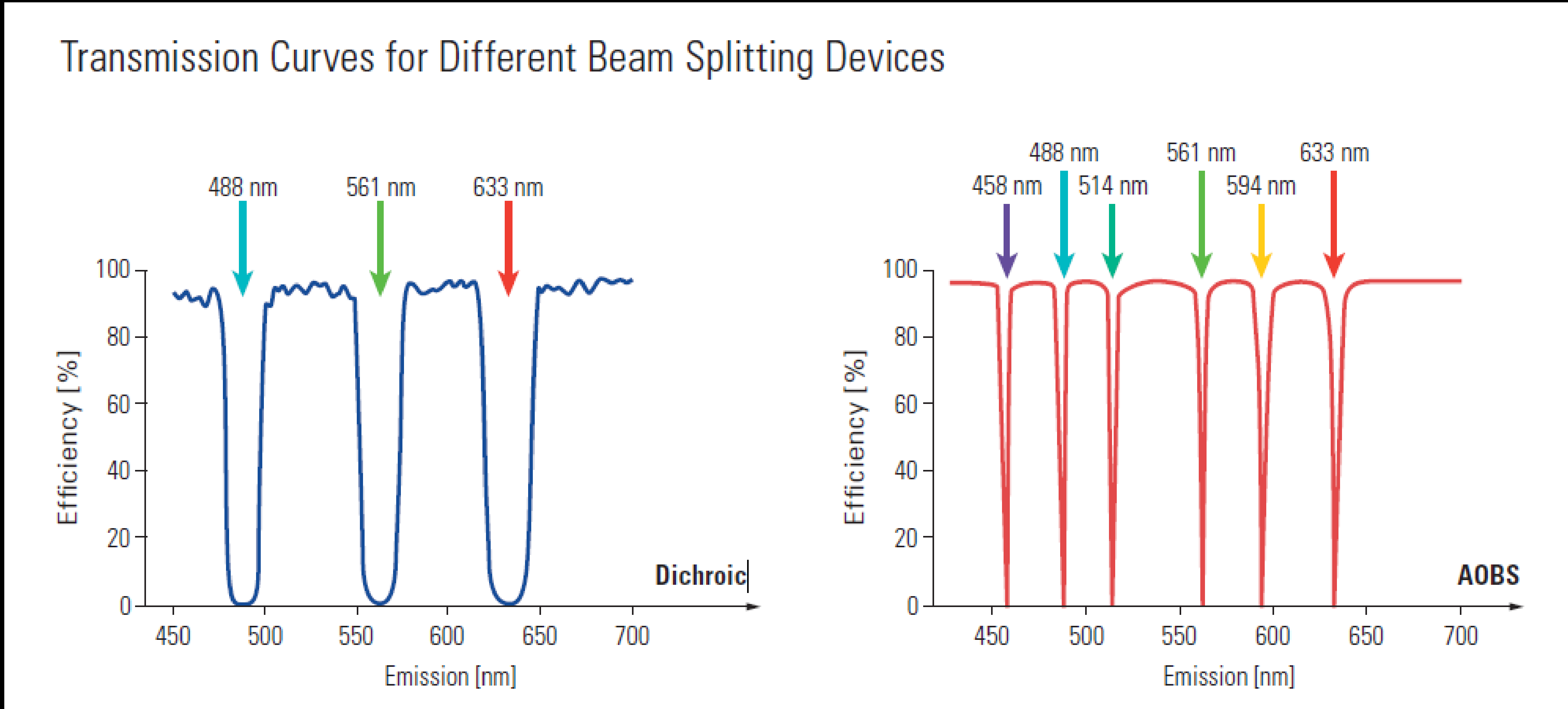
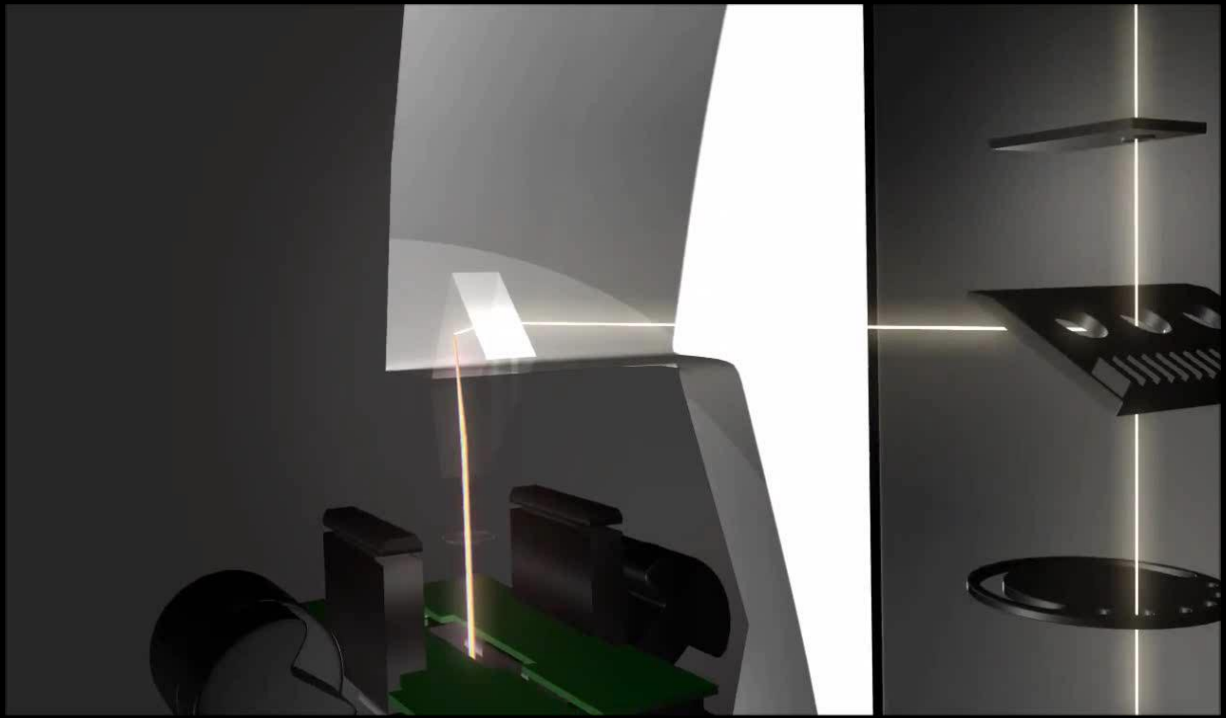


Fig. 1a: Dichroic. Non-flexible reflections on bands.

Fig. 1b: AOBS. All wavelength are fully flexible, adjusting to your experiment.

- Collect more light, thanks to the steep edges and narrow width of the of reflection bands
- Reduce overall light dose, thanks to the flexibility on the excitation side

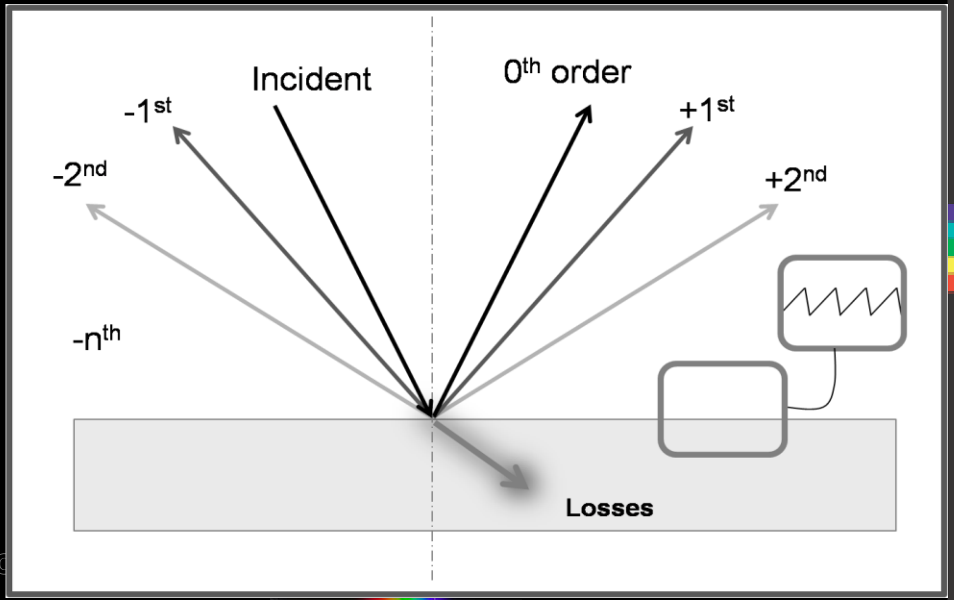
STELLARIS Is Fit For Purpose

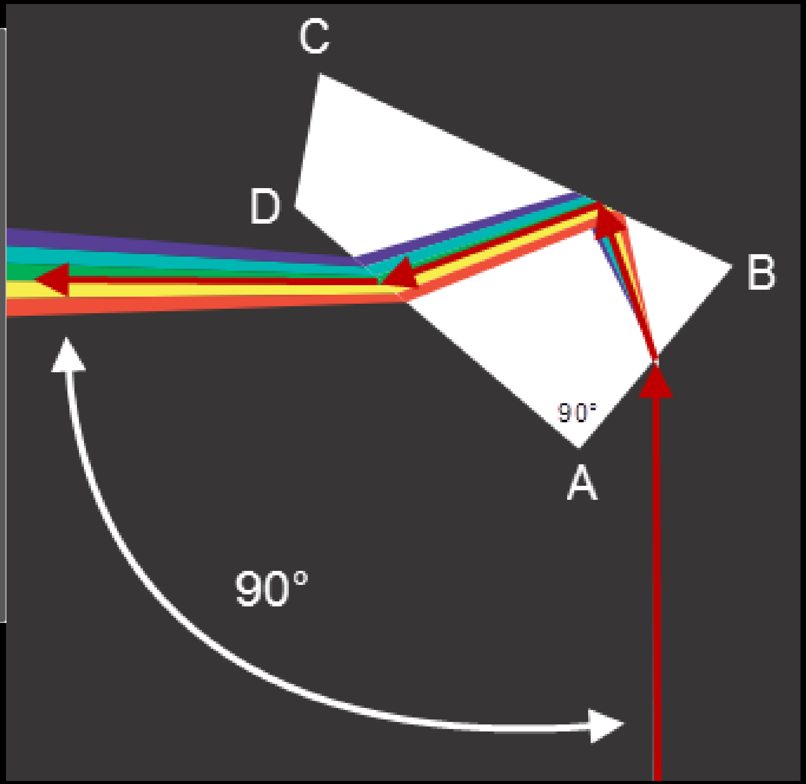


Prism

Photon-preserving dispersion

Dispersive Element: Grating vs Prism





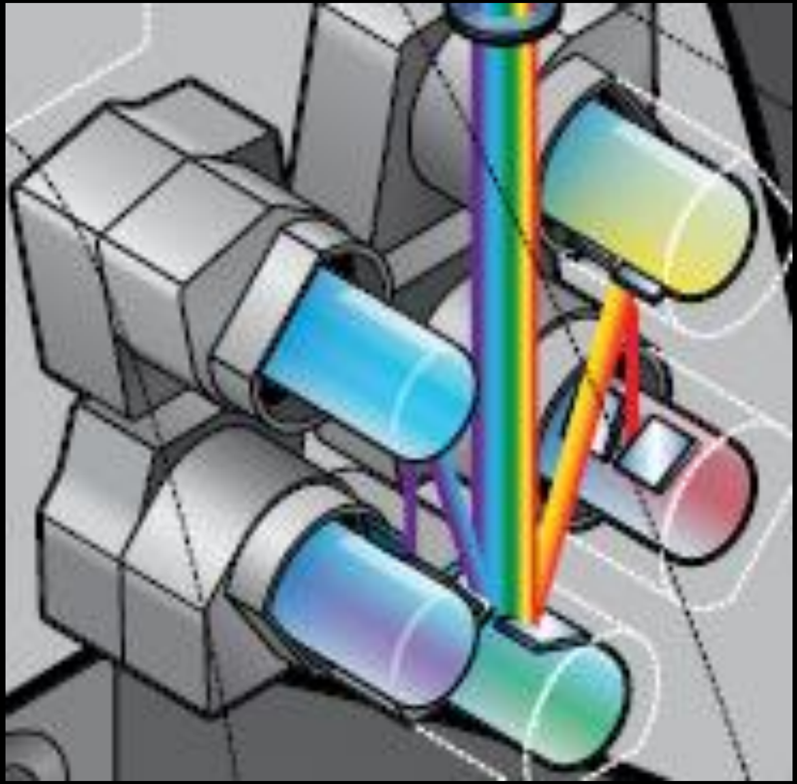
Grating

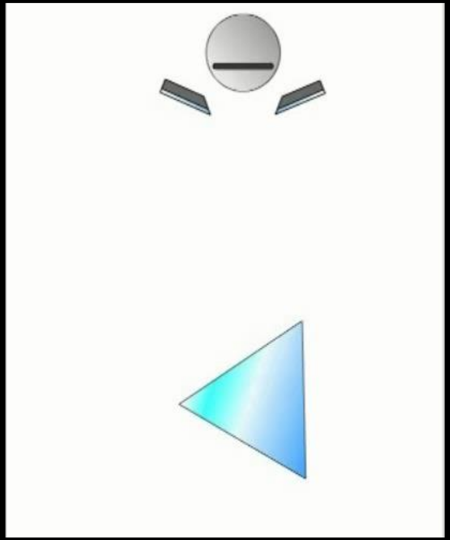
- Light is distributed to many orders, only one is used
- Light is lost by scattering
- Only S-polarized light is used efficiently
- Grating only optimized for one wavelength ("blaze")

Prism

- No additional orders
- Prisms lose only a few percent at the surfaces, this is reduced by anti-reflective coatings

STELLARIS Is Fit For Purpose

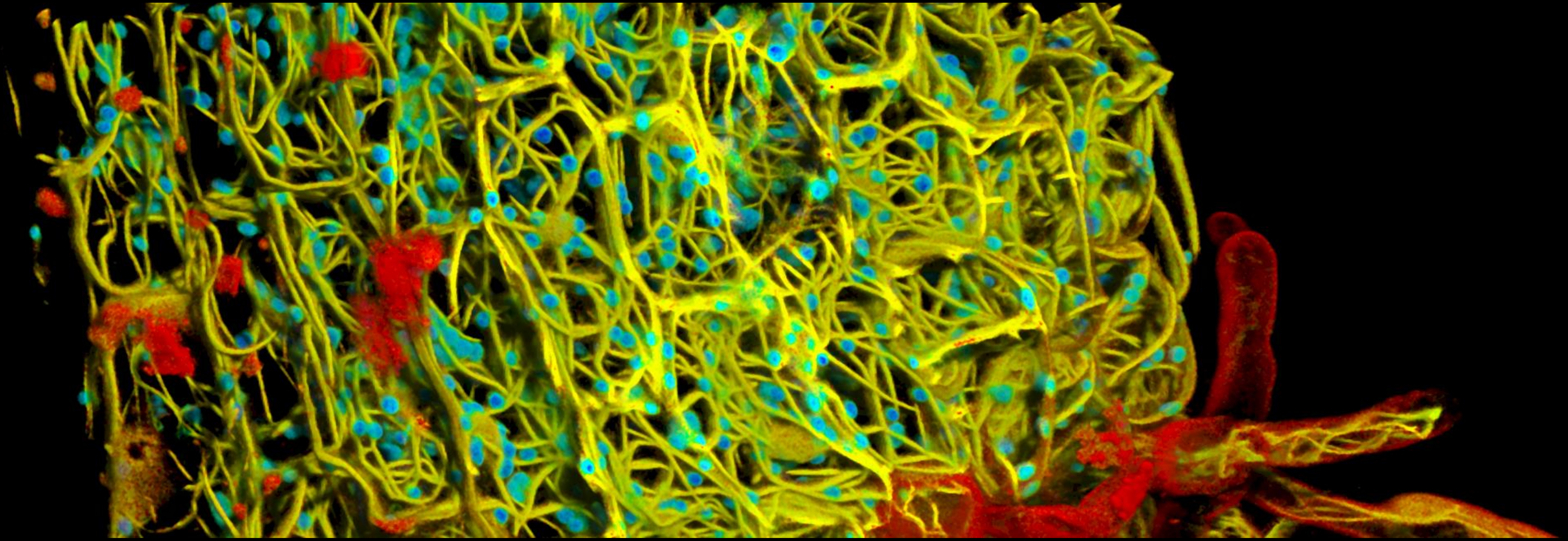




Spectral Detection

POTENTIAL

DISCOVER MORE



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Leica

STELLARIS Potential Delivers The Following Benefits

- Explore new dimensions of information
- Improve image quality
- Multiplex beyond the spectral options

Root-hypocotyl-junction of *Arabidopsis thaliana*. Chloroplasts (endogenous fluorescence); actin (Life-Act Venus: Era et al. Plant Cell Physiol., 2009); membranes (Propidium iodide). Sample courtesy: Melanie Krebs, COS, University of Heidelberg.

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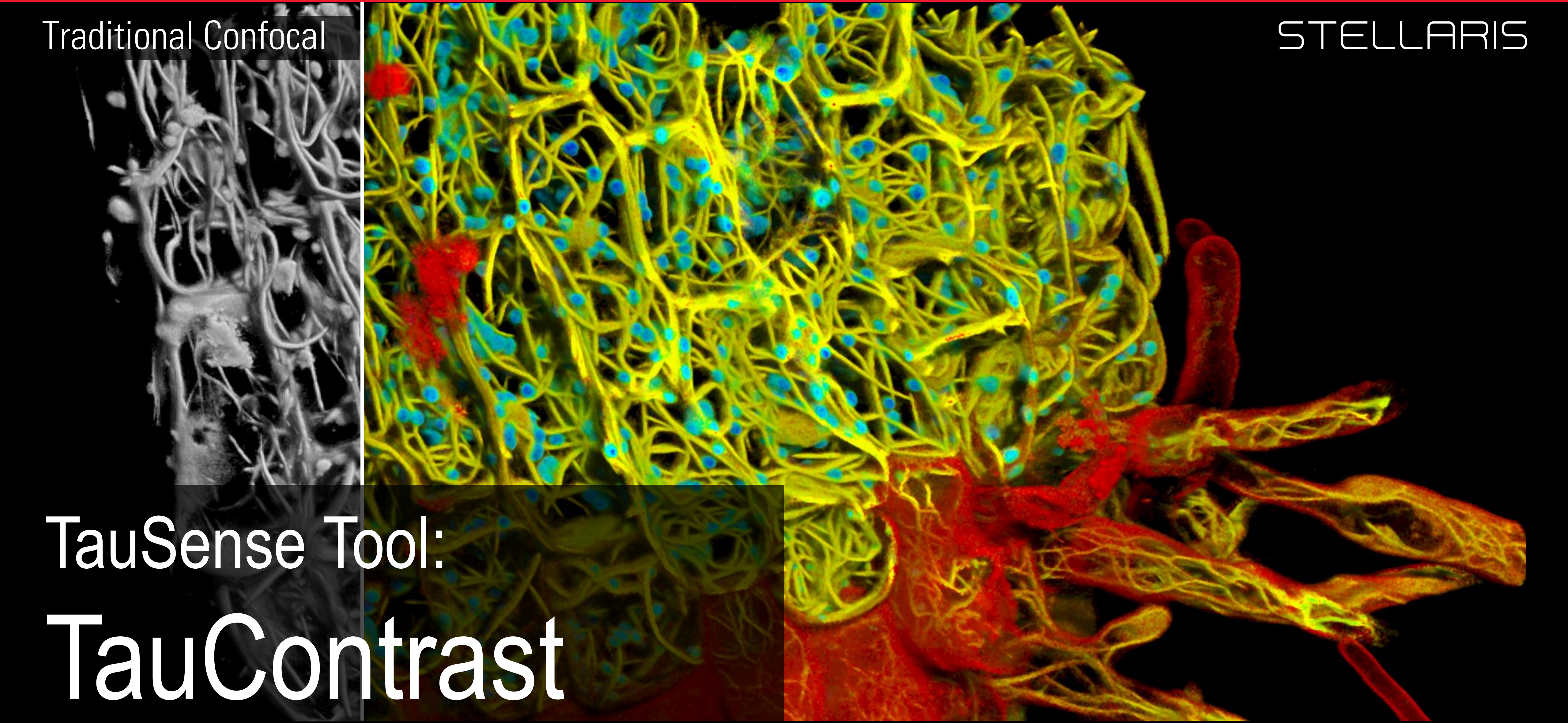
TauSense™

> Set of tools based on fluorescence lifetime

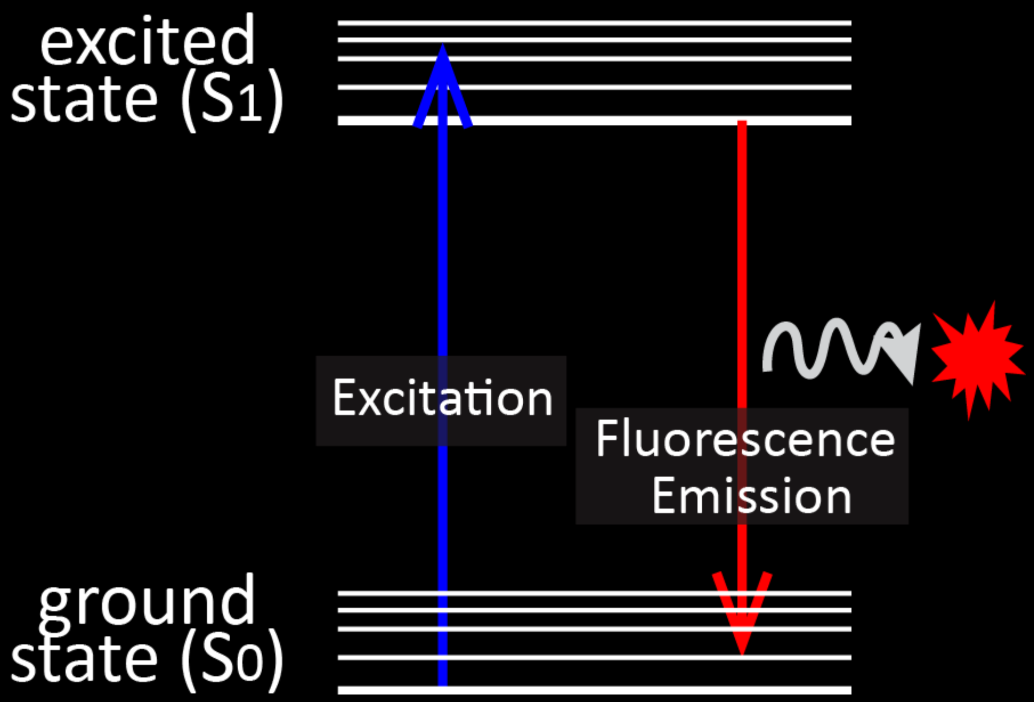
Explore A New Dimension Of Information



TauSense Tool:

TauContrast

The Technology Behind TauContrast

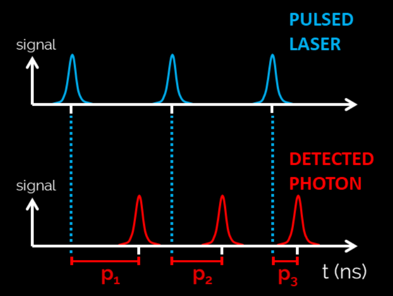


> Fluorescence Intensity ($N_{photons}$)

> Fluorescence Lifetime (ns)

The Technology Behind TauContrast

- > Fluorescence Intensity ($N_{photons}$)
- > Photon Arrival Time (ns)



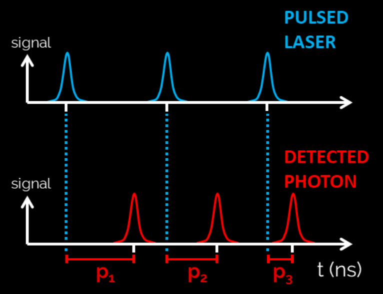
1) FPGA
2) Pixel-by-pixel
3) On the fly

- > Fluorescence Intensity ($N_{photons}$)
- > Average Photon Arrival Times (AAT, ns)

The Technology Behind TauContrast

> Fluorescence Intensity ($N_{photons}$)

> Photon Arrival Time (ns)



1) FPGA
2) Pixel-by-pixel
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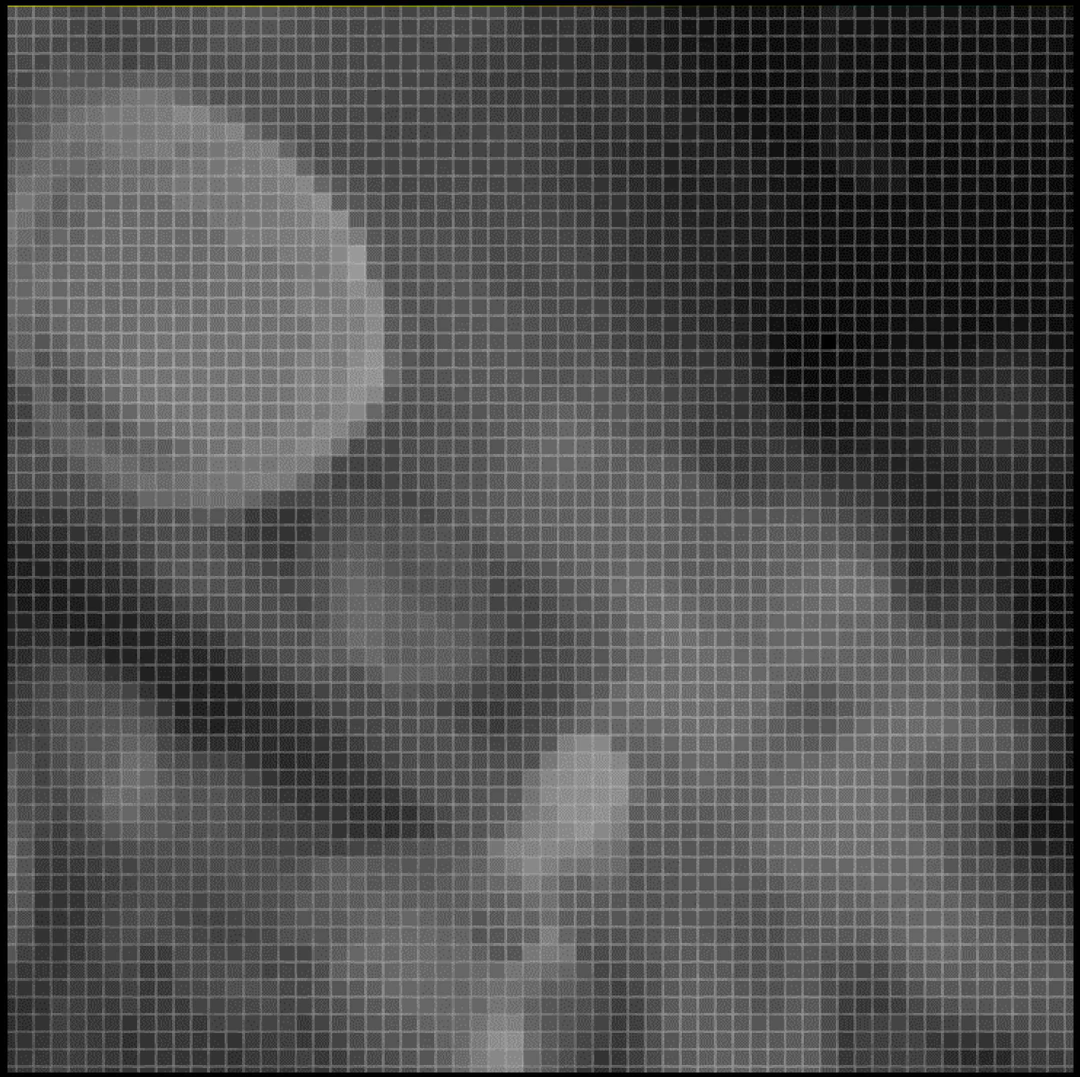
> Fluorescence Intensity ($N_{photons}$)

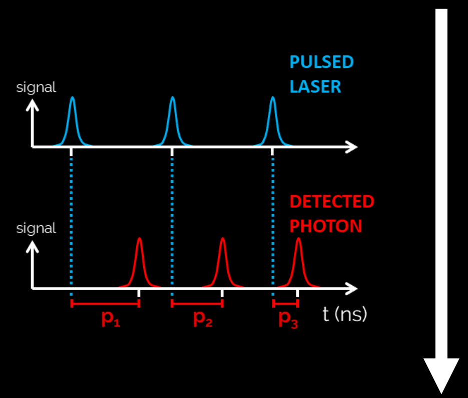
> Average Photon Arrival Times (AAT, ns)



The Technology Behind TauContrast

> Fluorescence Intensity ($N_{photons}$)

> Photon Arrival Time (ns)



1) FPGA
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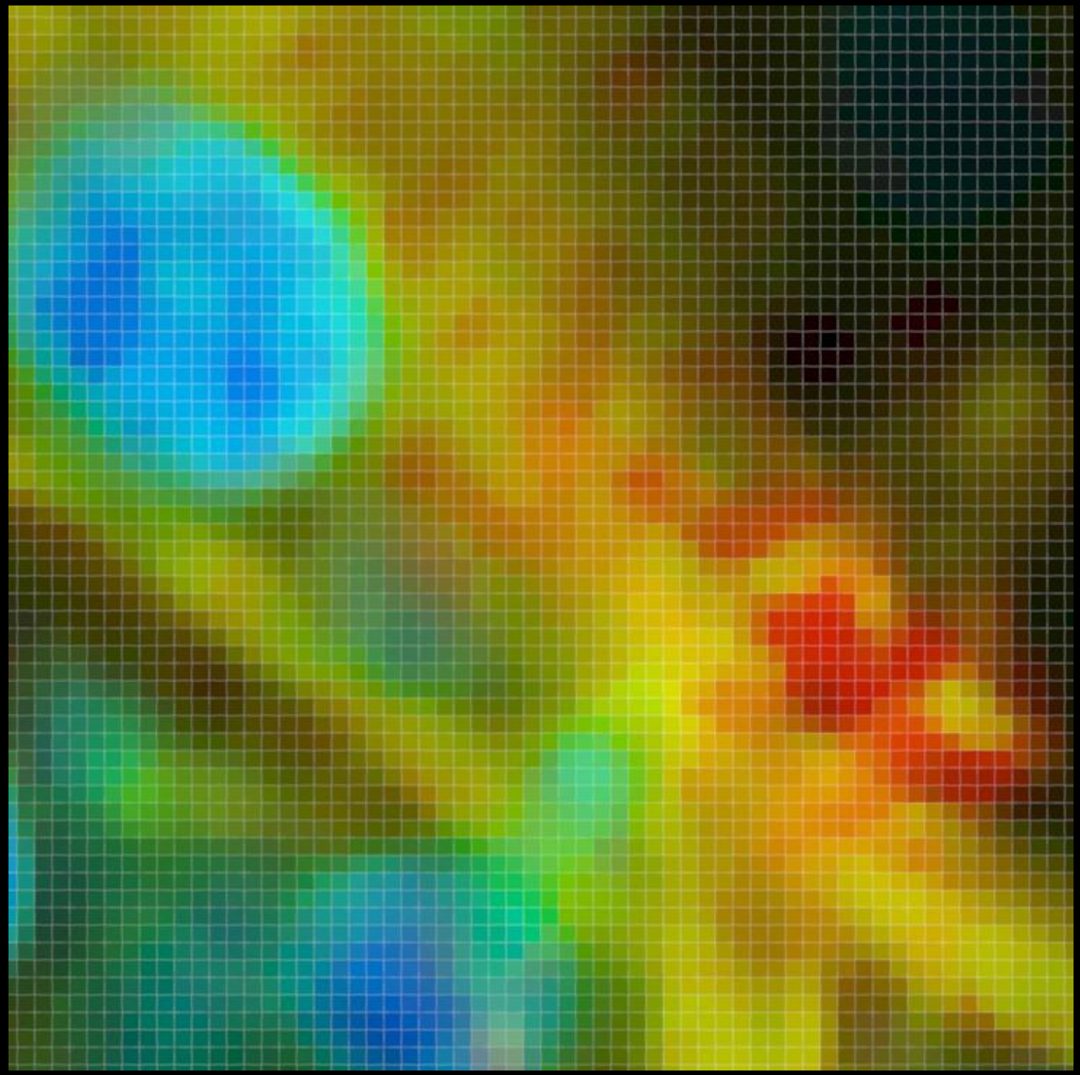
> Fluorescence Intensity ($N_{photons}$)

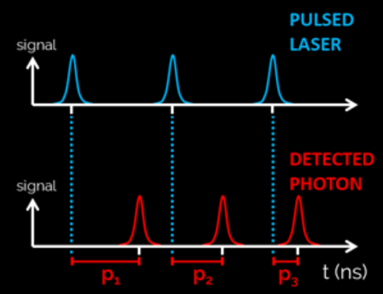
> Average Photon Arrival Times (AAT, ns)



The Technology Behind TauContrast

> Fluorescence Intensity ($N_{photons}$)

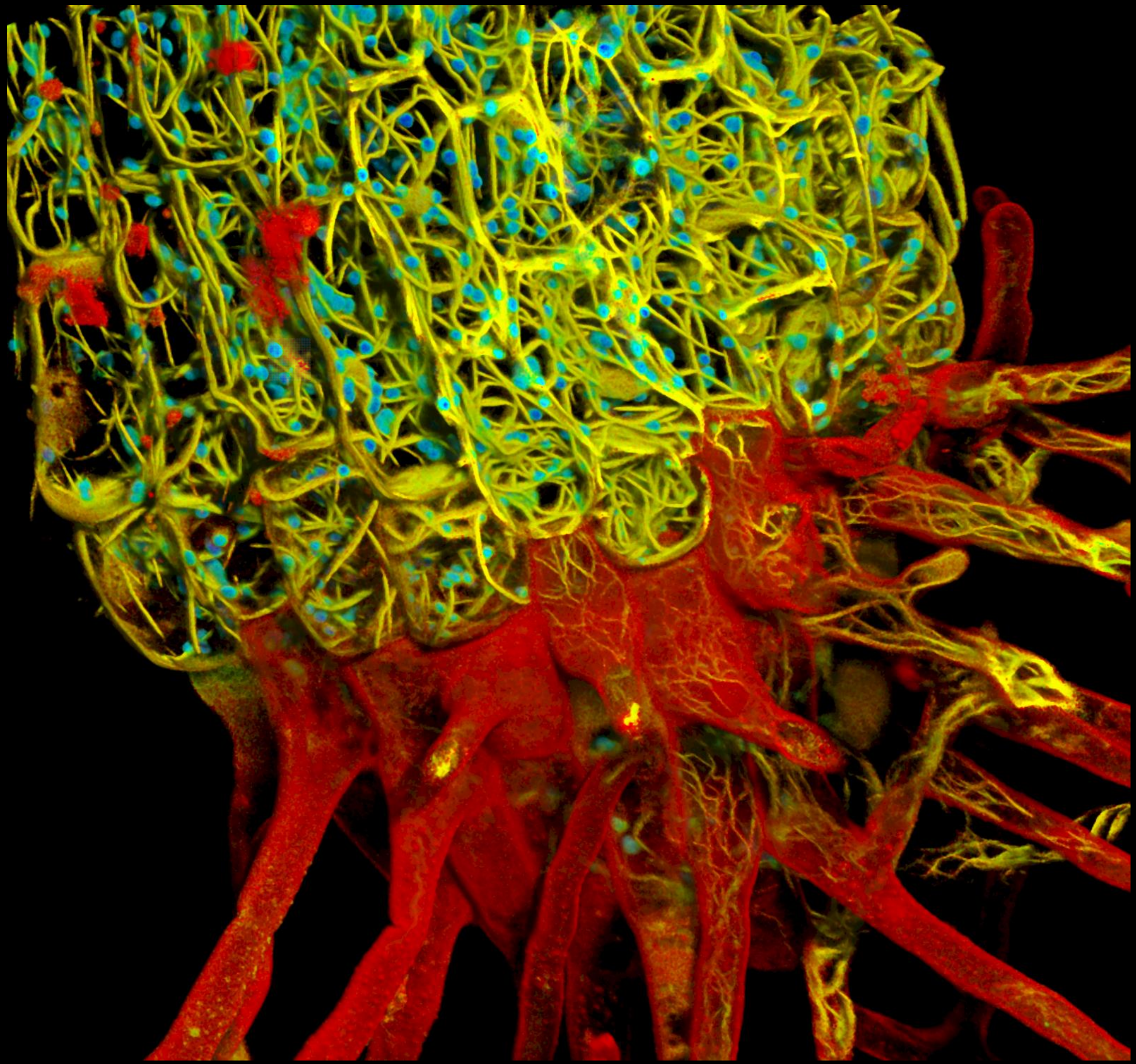
> Photon Arrival Time (ns)



1) FPGA
2) Pixel-by-pixel
3) On the fly

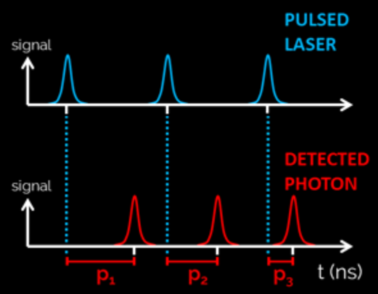
> Fluorescence Intensity ($N_{photons}$)

> **Average** Photon Arrival Times (AAT, ns)



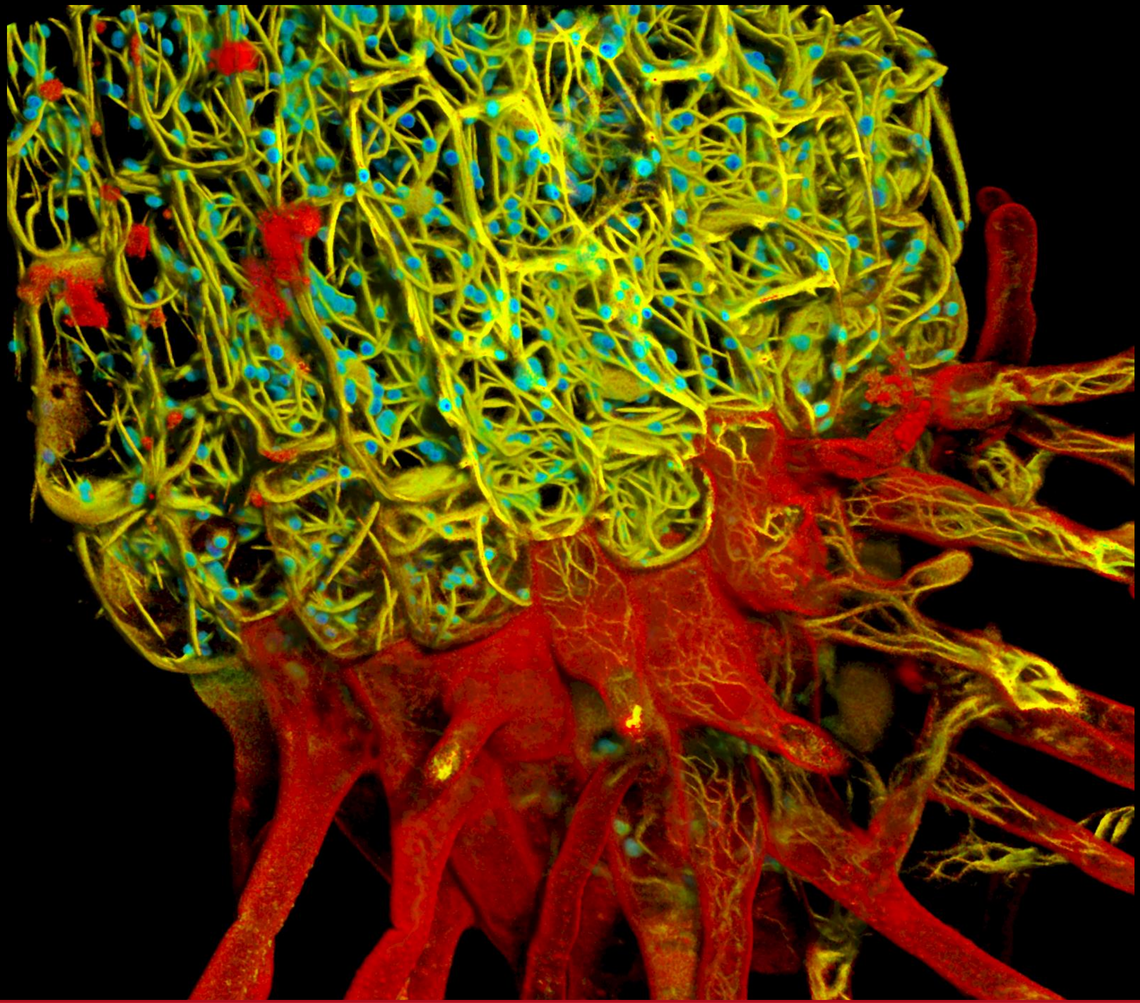
The Technology Behind TauContrast

- > Fluorescence Intensity ($N_{photons}$)
- > Photon Arrival Time (ns)



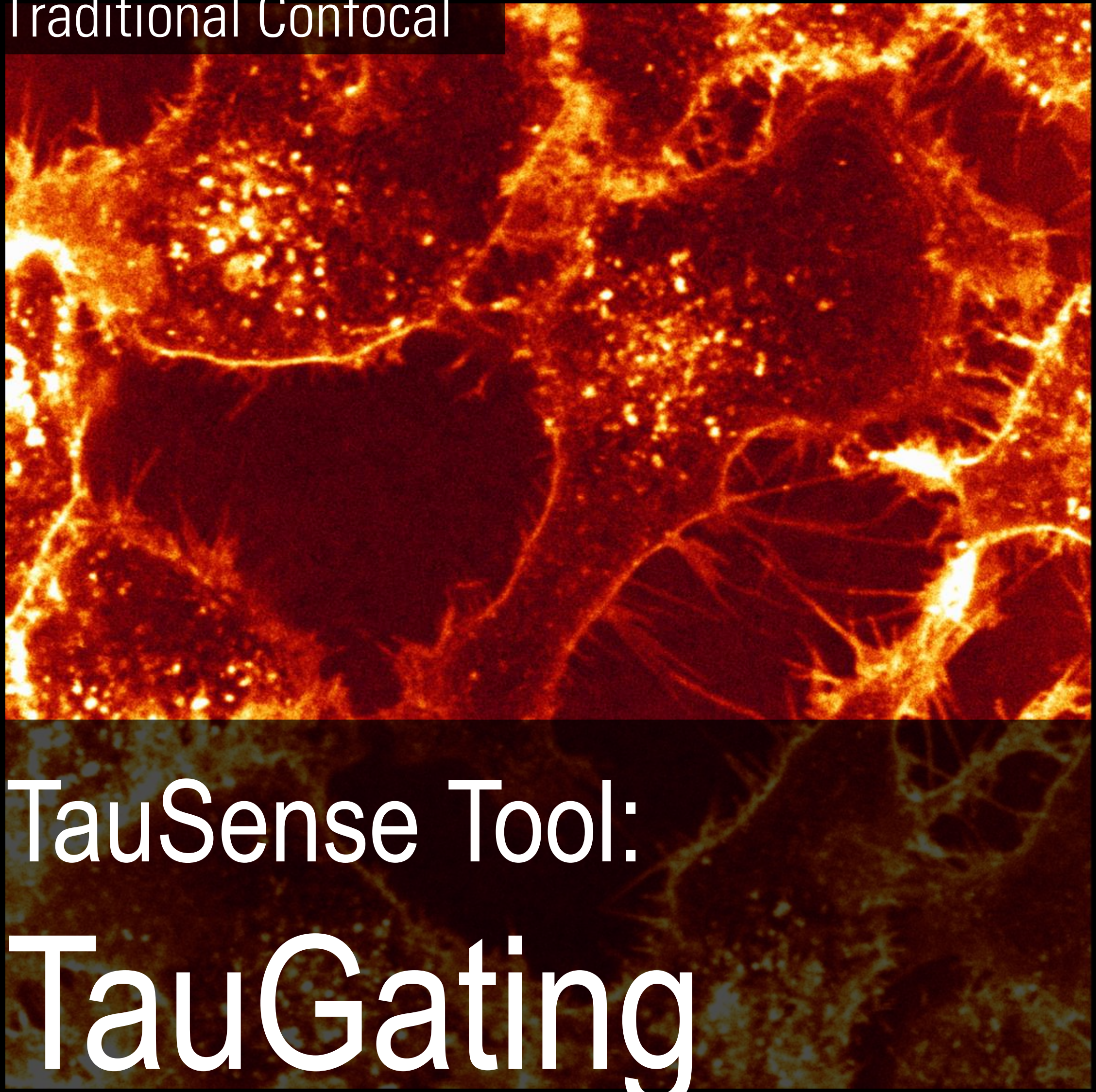
1) FPGA
2) Pixel-by-pixel
3) On the fly

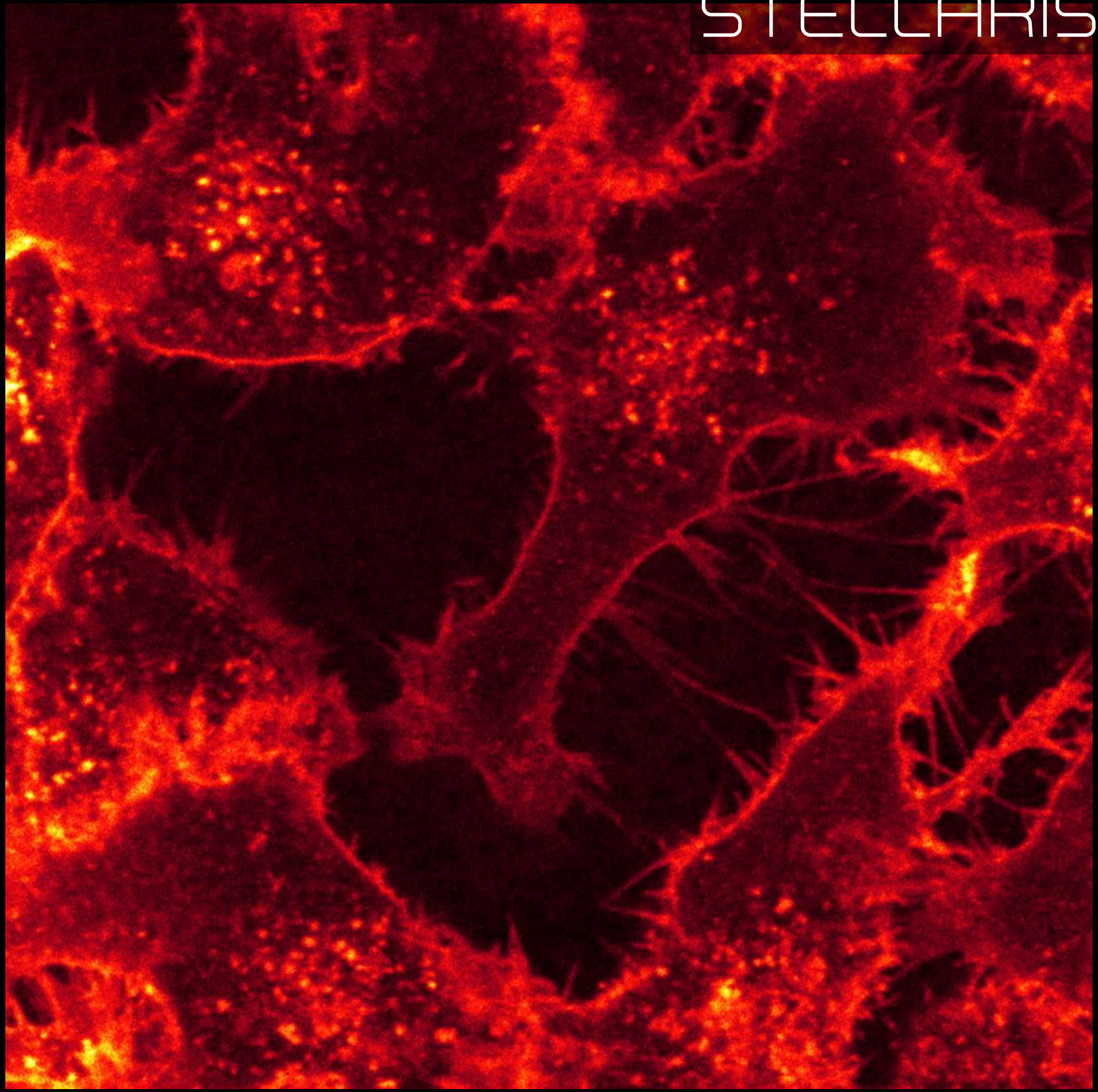
- > Fluorescence Intensity ($N_{photons}$)
- > **Average** Photon Arrival Times (AAT, ns)



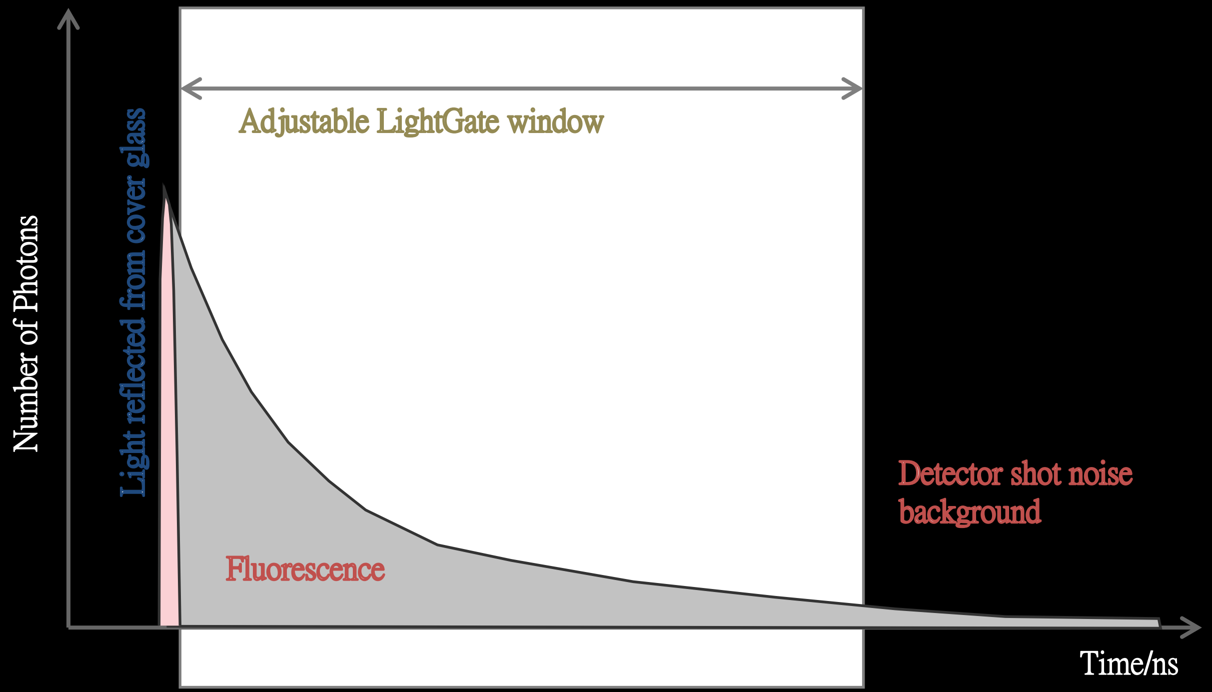
TauContrast Gives Instant, Pixel-by-Pixel AAT, With Every Image (Live/Acquired)

Improve Image Quality

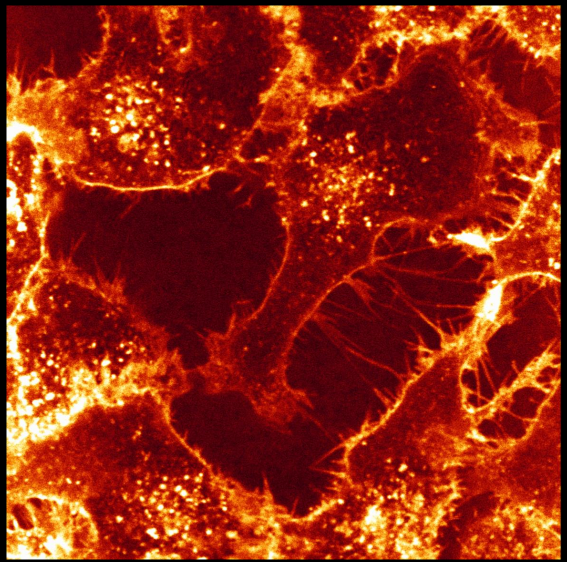




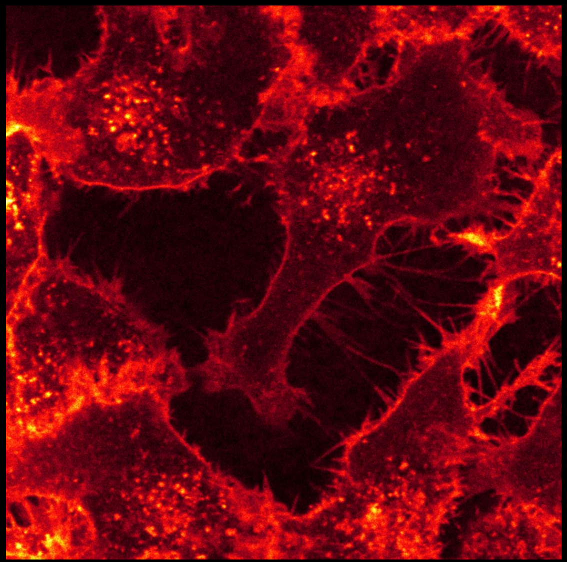
The Technology Behind TauGating



> Digital Gate Channels (Intensity, $N_{photons}$)



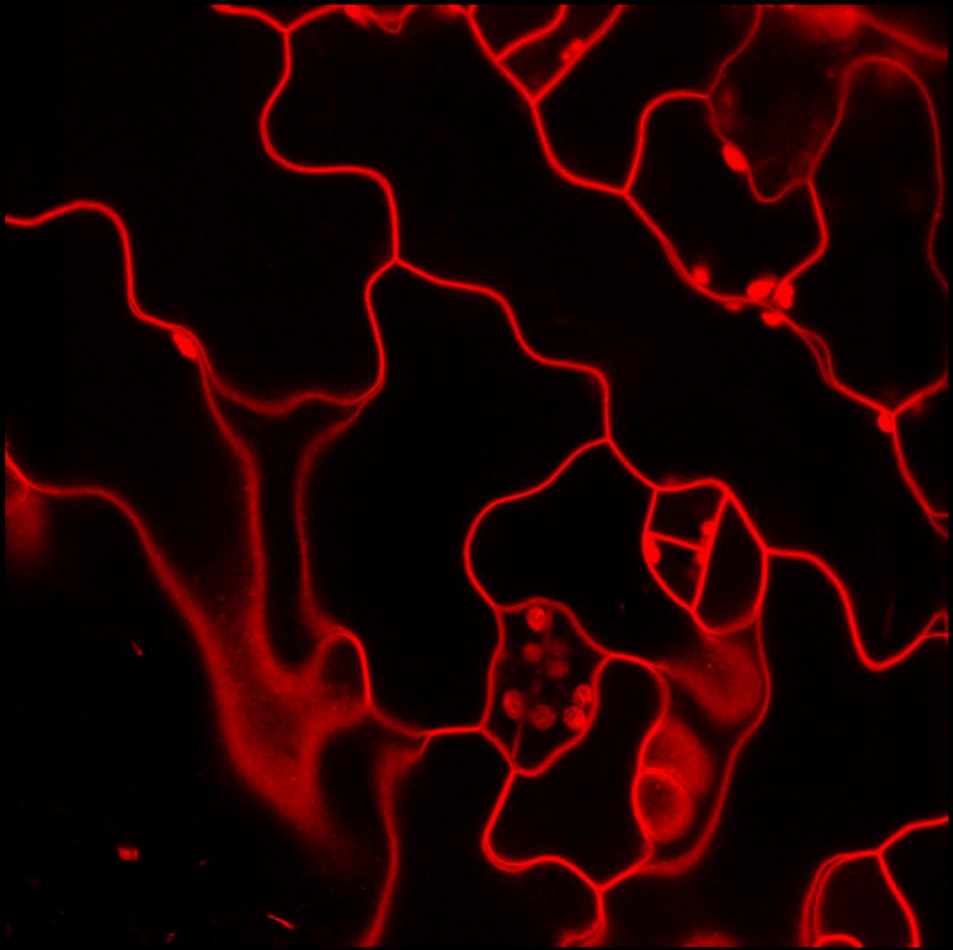
Gate Channel 2



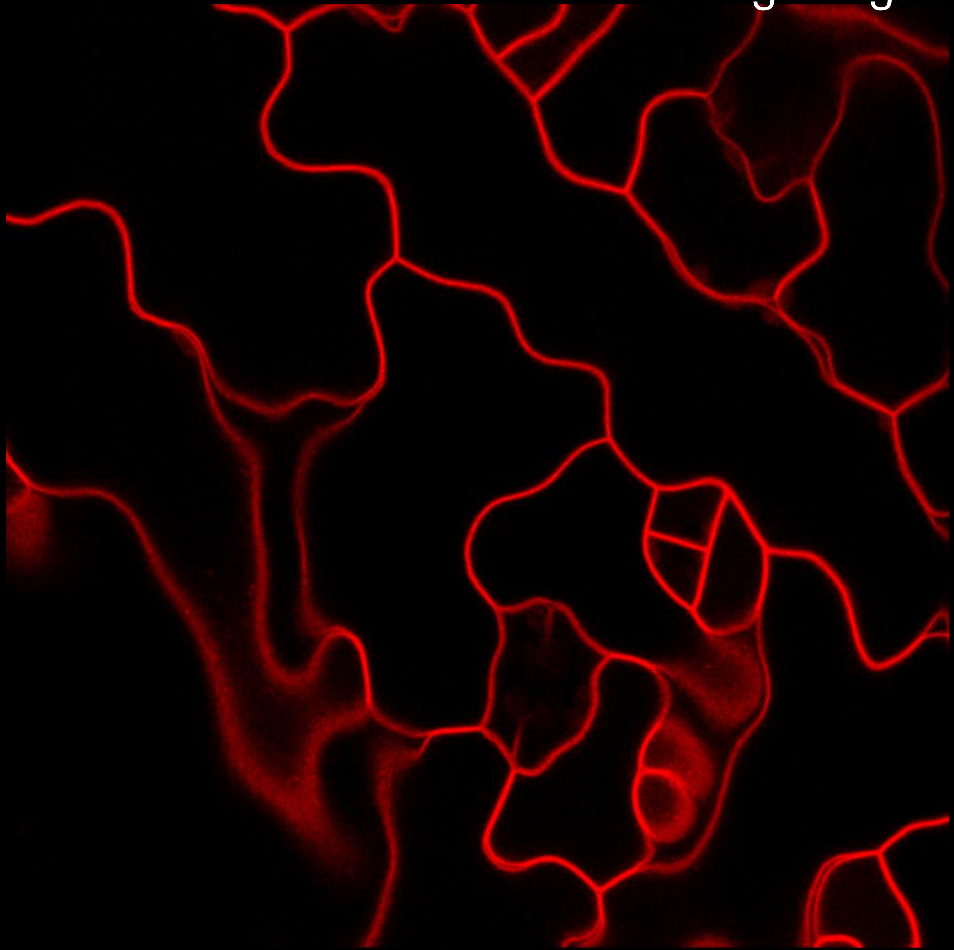
Gate Channel 1

TauGating Gives Digital Gate Channels With Every Image (Live/Acquired)

Traditional Confocal

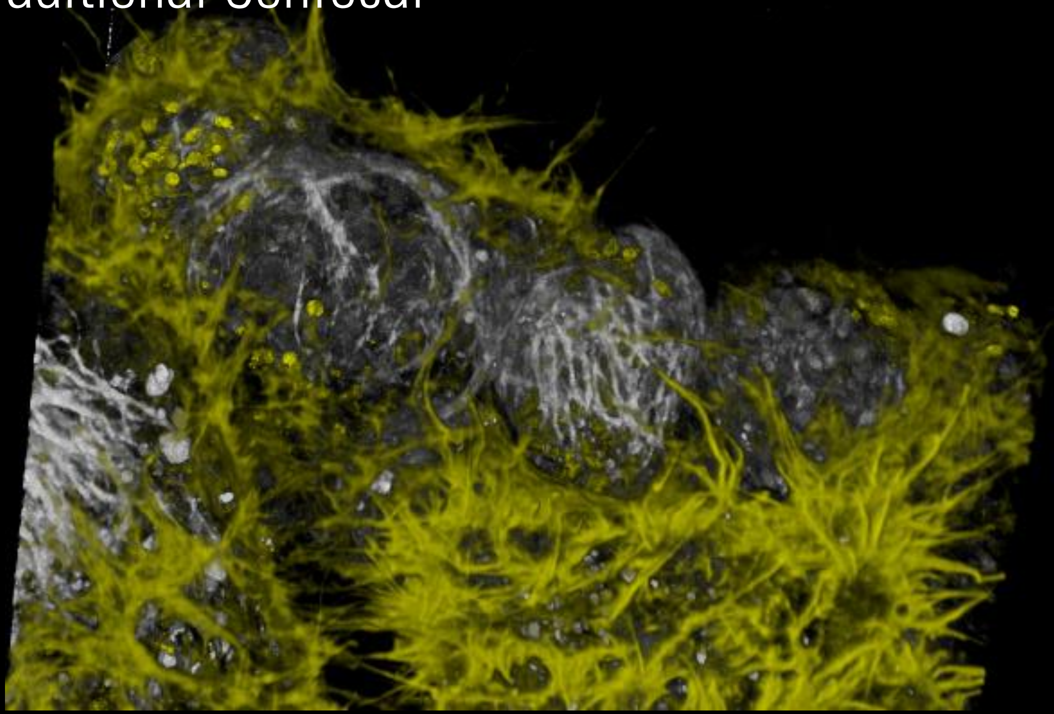


Tau gating

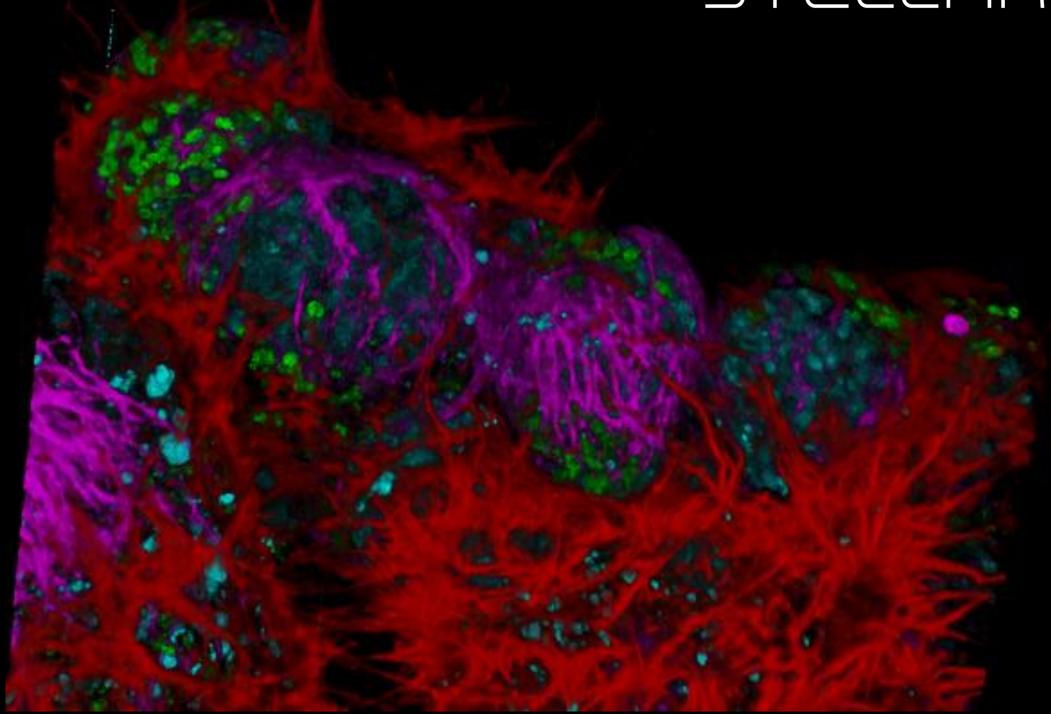


Multiplex Beyond The Spectral Options

Traditional Confocal



STELLARIS

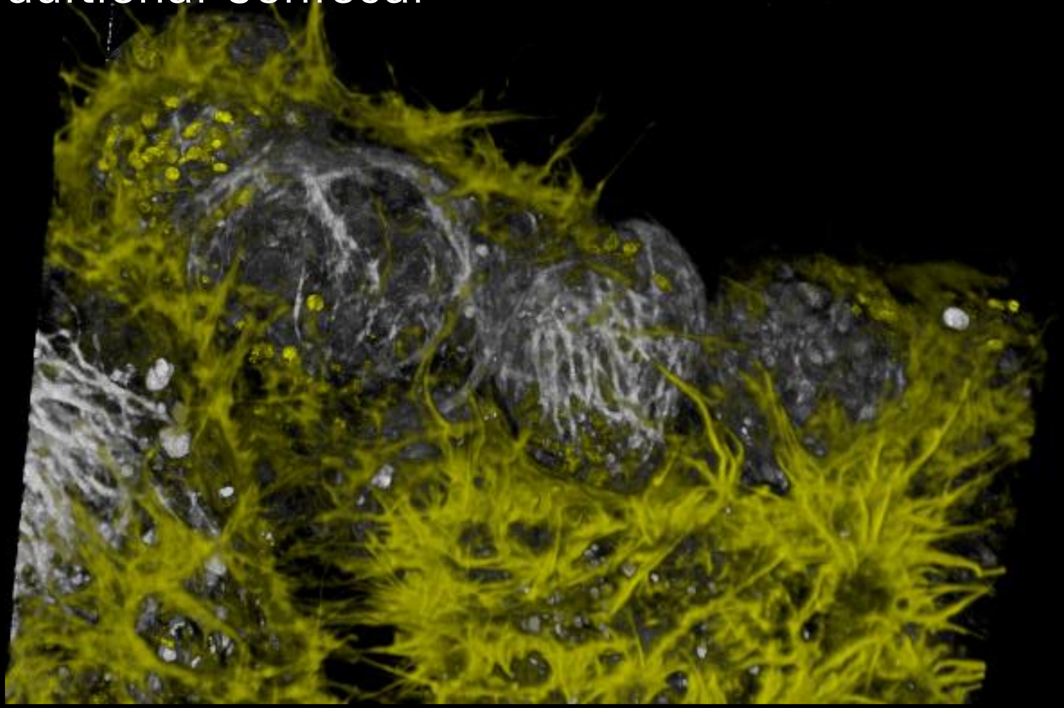


TauSense Tool:

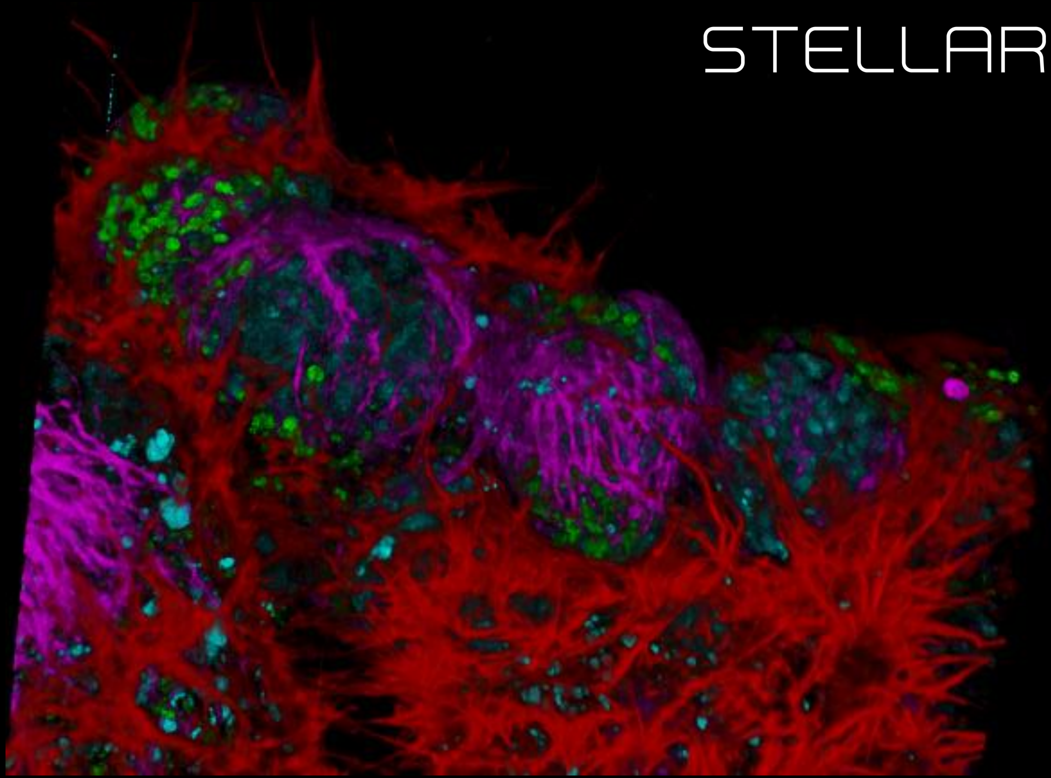
TauSeparation

Multiplex Beyond The Spectral Options

Traditional Confocal



STELLARIS



detection wavelength [nm]

400 550 650 750 850

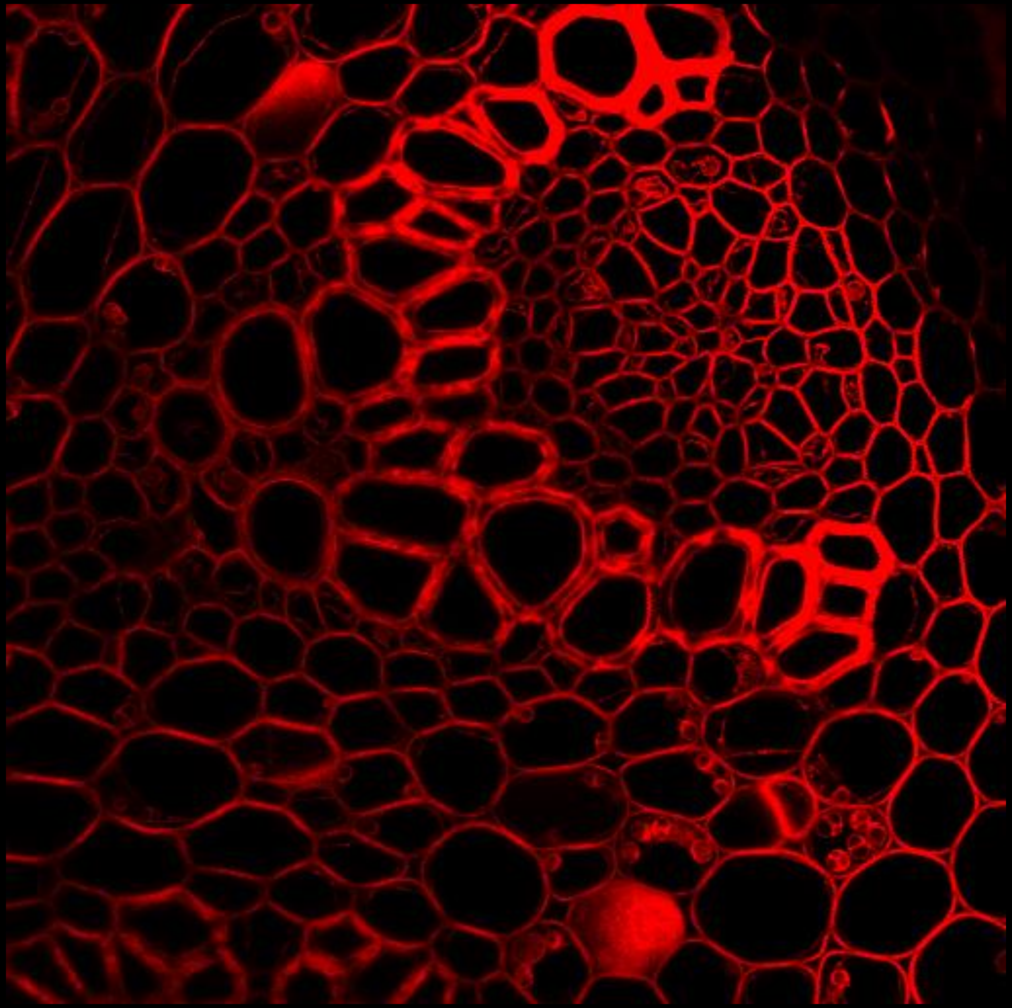
> Separate even fully overlapping fluorophores using lifetime-based information

2 detectors, 2 intensity channels

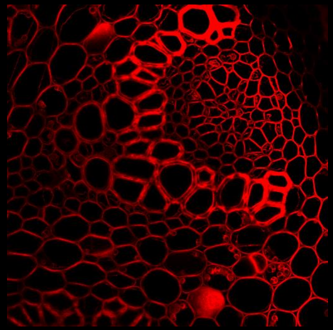
NE-115 cells. LifeAct-mNeonGreen (left: yellow, right: red), MitoTracker Green (left: yellow, right: green), NUC Red (left: gray, right: blue), and SiR-tubulin (left: gray, right: magenta).
Courtesy: Max Heydasch, University of Bern and Spirochrome

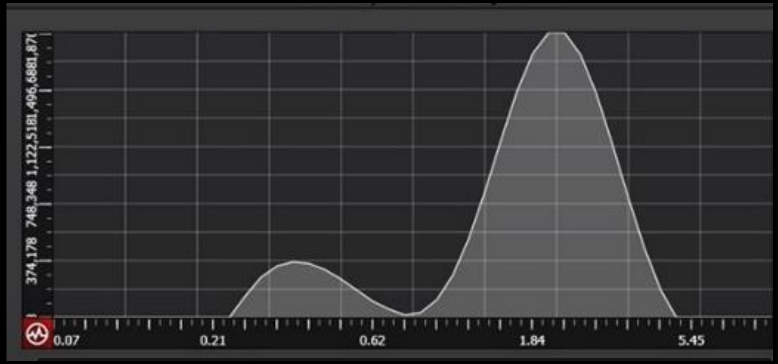
Traditional Confocal -- Intensity

Detector 1
Ex : 631nm
Em : 655-700nm

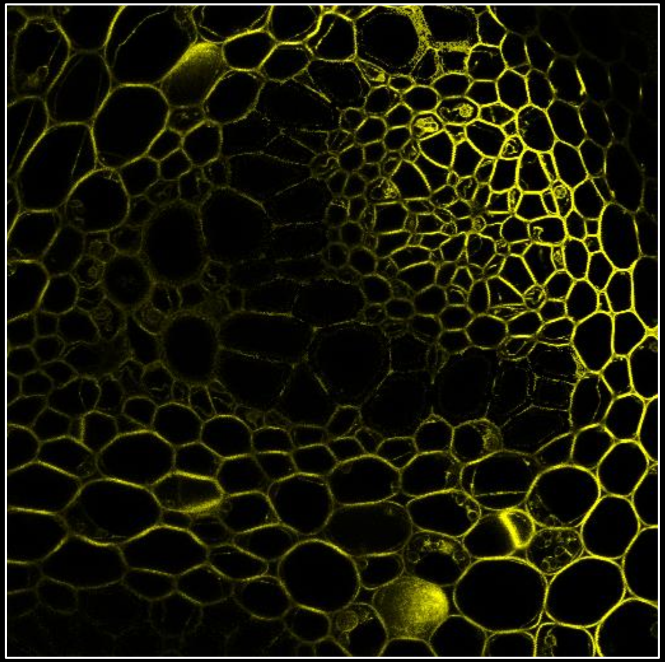


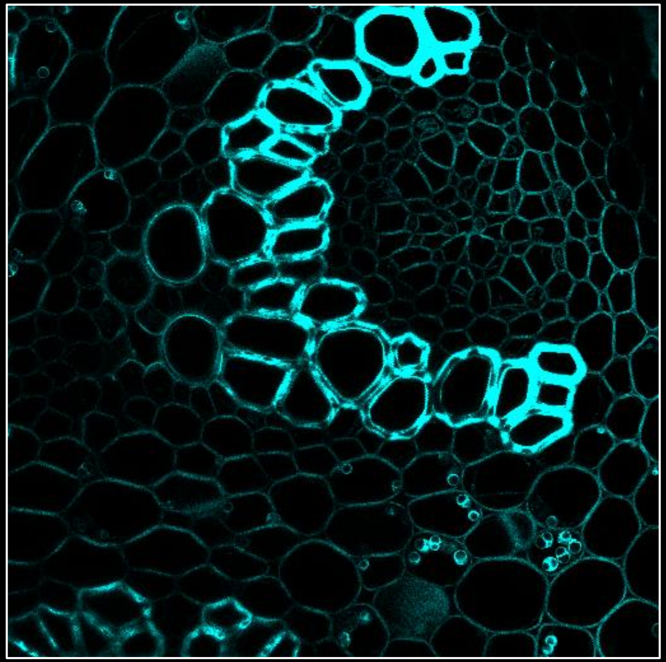
The Technology Behind Separation





> Lifetime-based Separated Channels (Intensity, Nphotons)



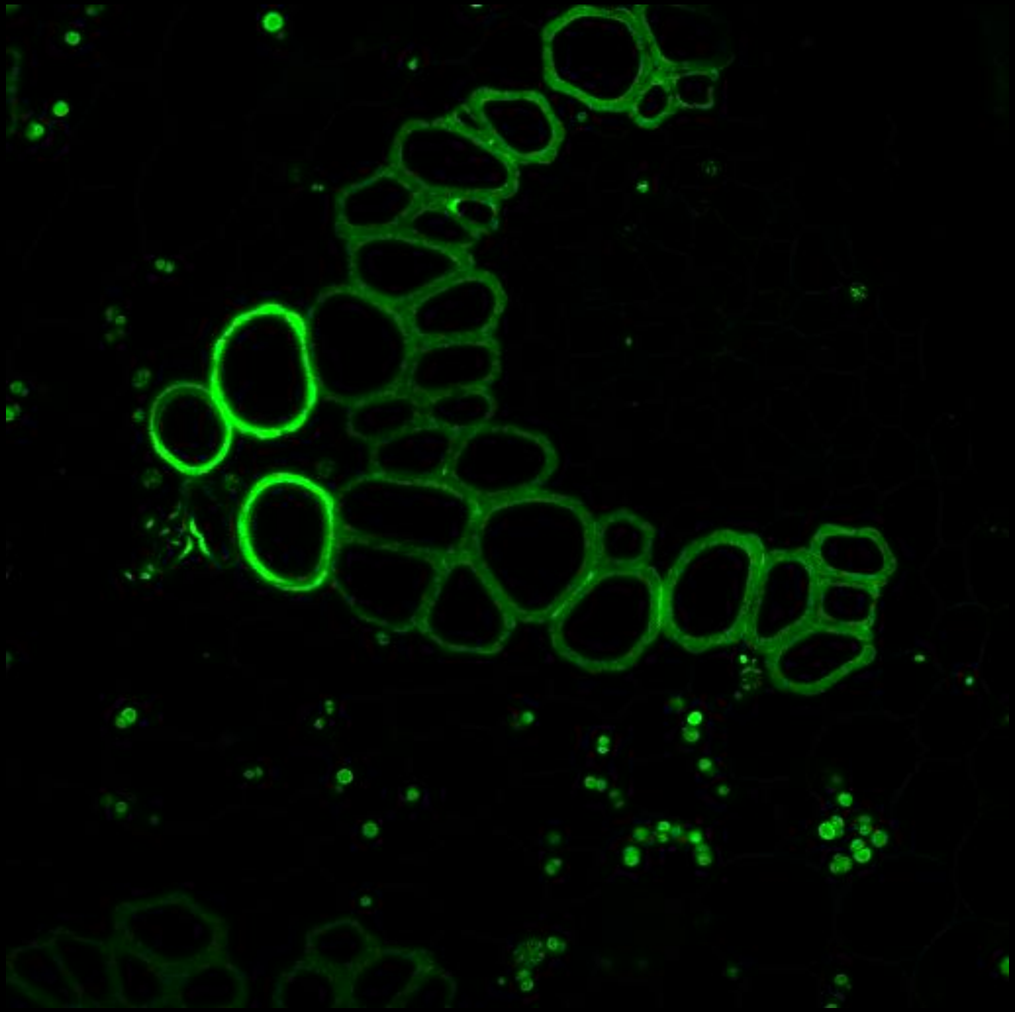


Detector 1

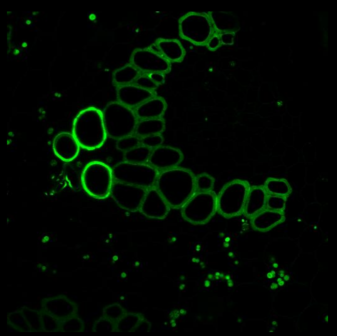
Lifetime-based Channel 1 Lifetime-based Channel 2

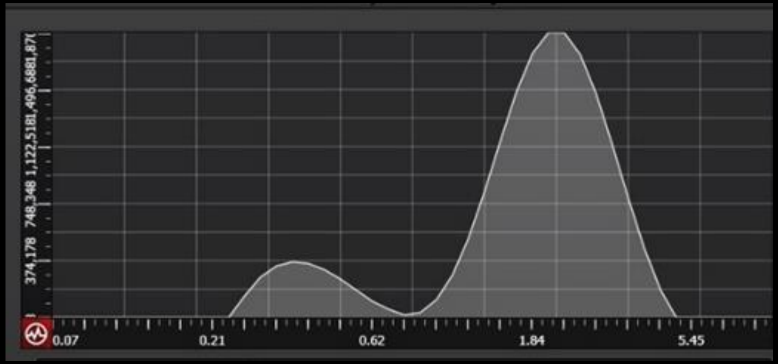
Traditional Confocal -- Intensity

Detector 2
Ex : 491nm
Em : 496-595nm

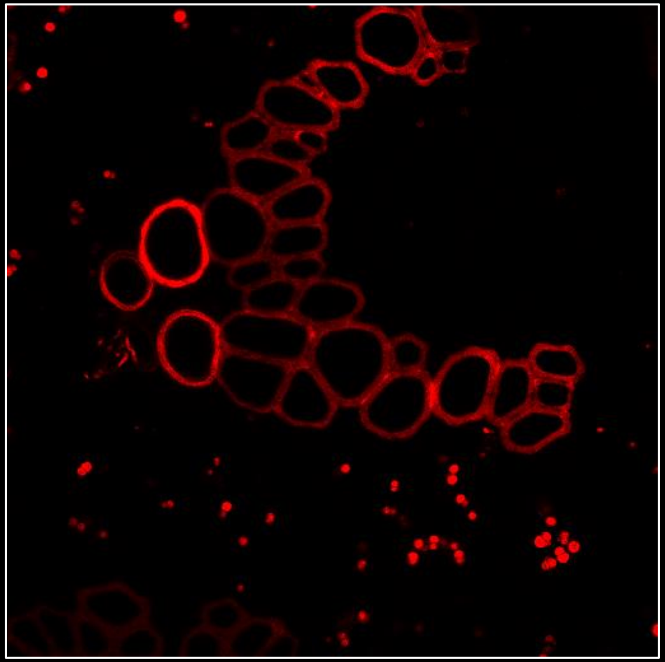


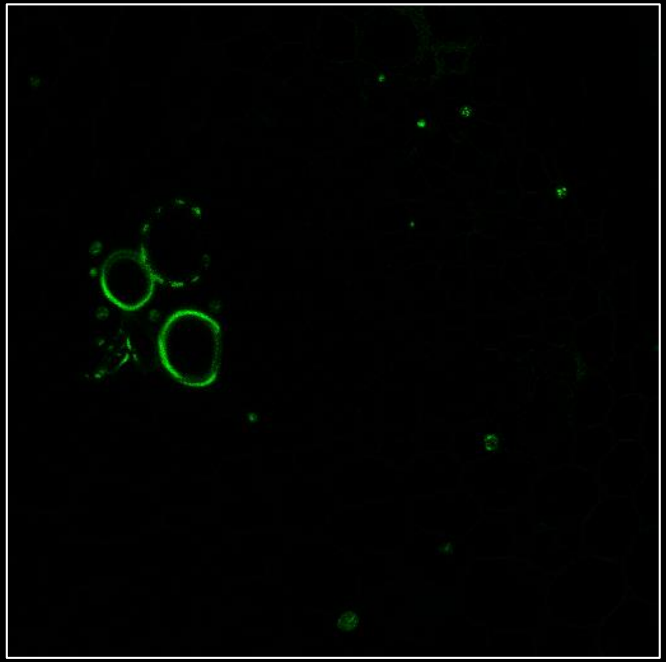
The Technology Behind Separation





> Lifetime-based Separated Channels (Intensity, Nphotons)

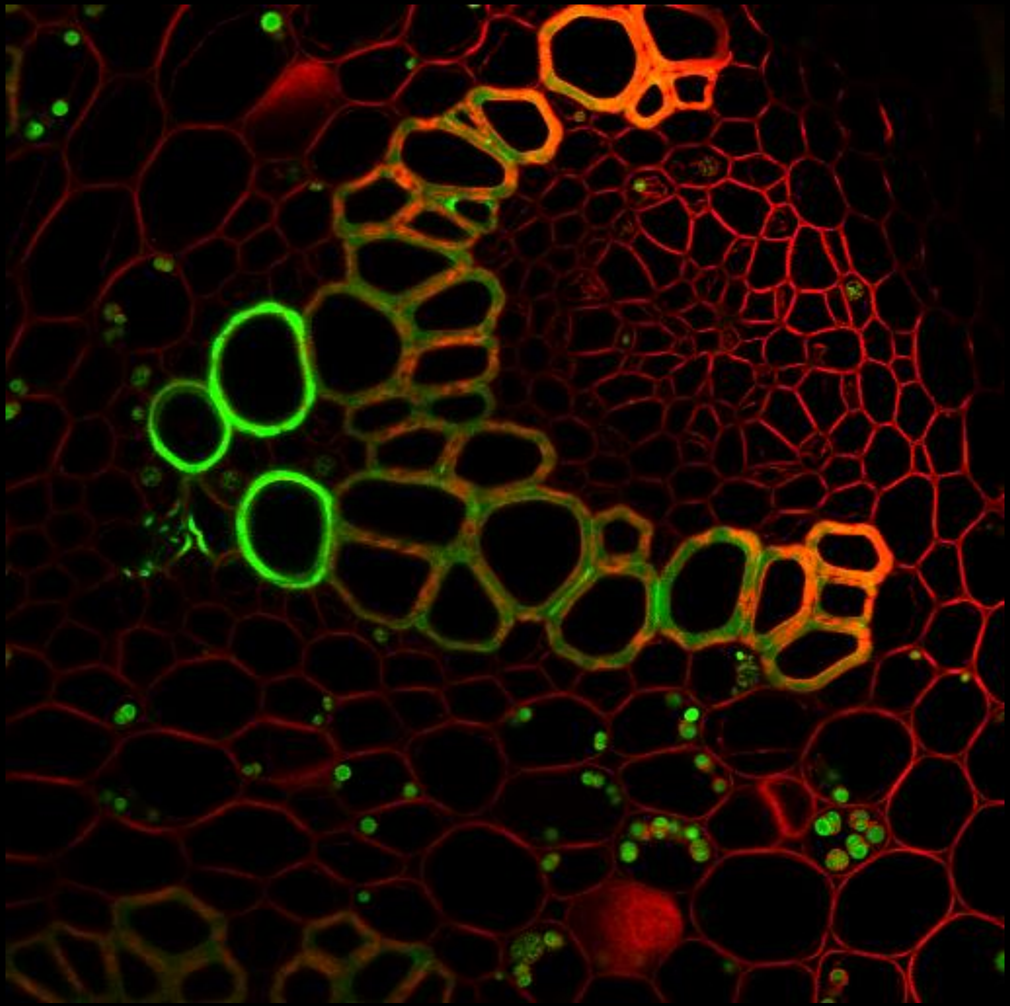




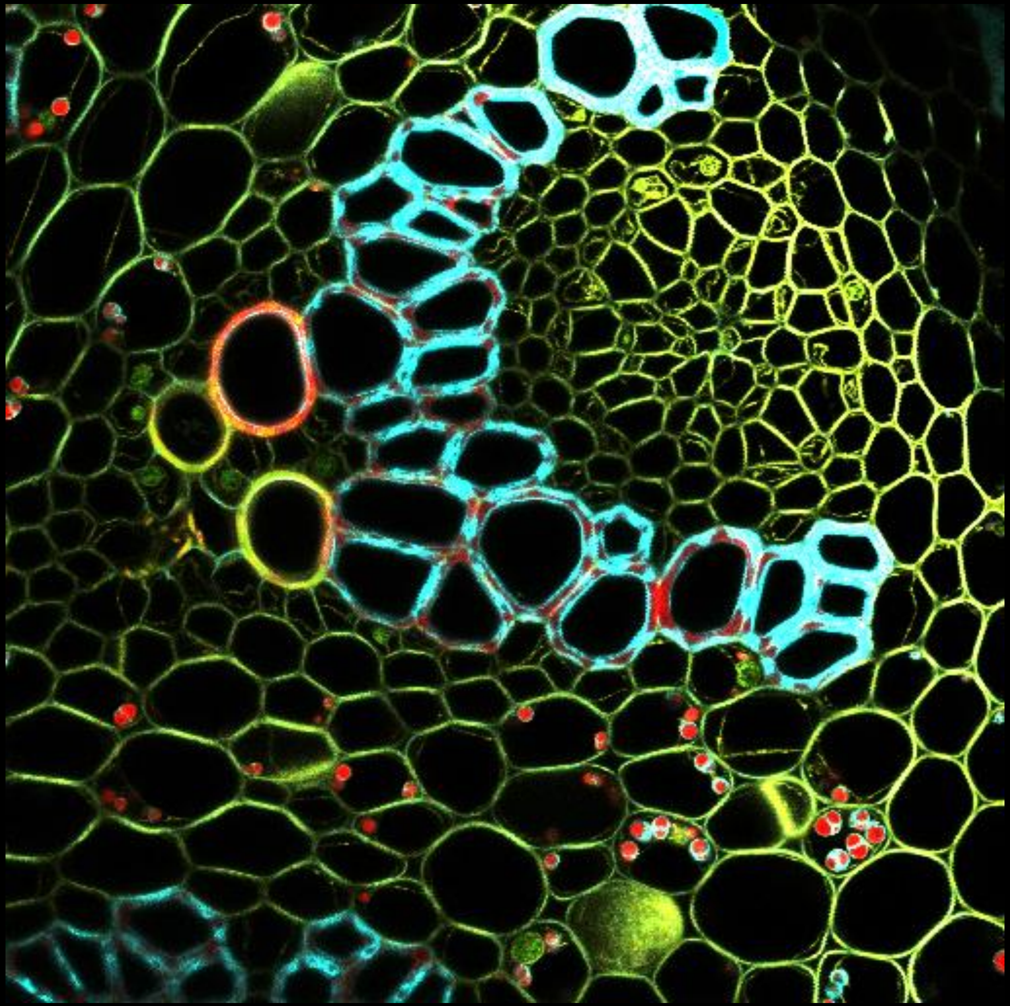
Detector 2

Lifetime-based Channel 1 Lifetime-based Channel 2

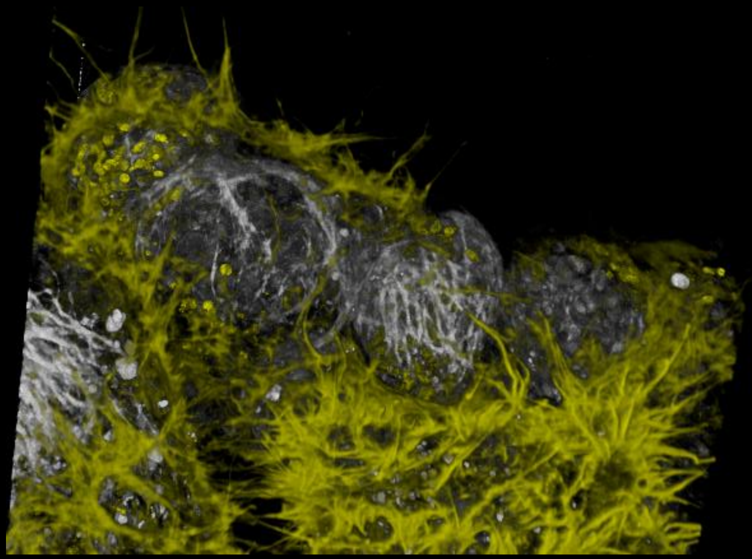
Traditional Confocal



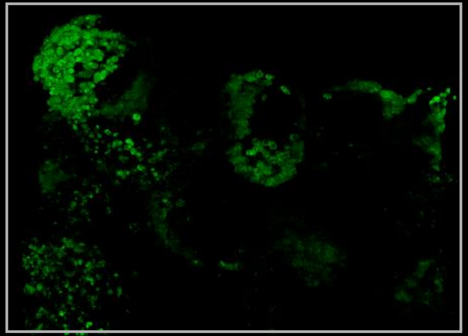
STELLARIS

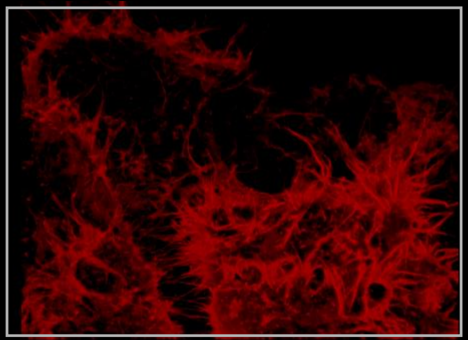


Tau Separation



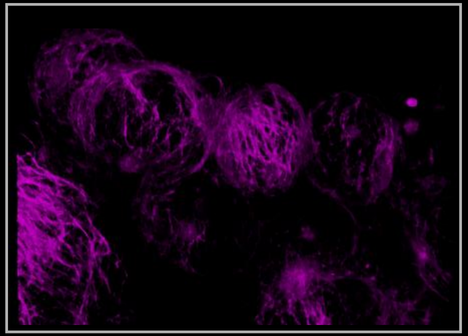
NE-115 cells.
LifeAct-mNeonGreen(left: yellow, right: red),
MitoTracker Green (left: yellow, right: green),
NUC Red (left: gray, right: blue),
and SiR-tubulin (left: gray, right: magenta).
Courtesy: Max Heydasch, University of Bern
and Spirochrome

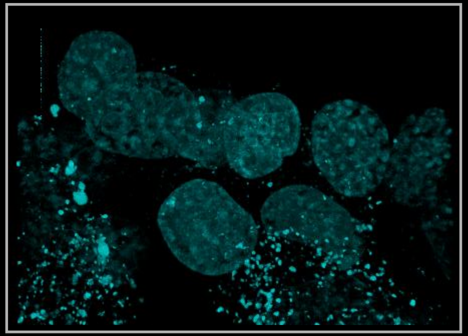




Detector 1

Lifetime-based Channel 1 | Lifetime-based Channel 2





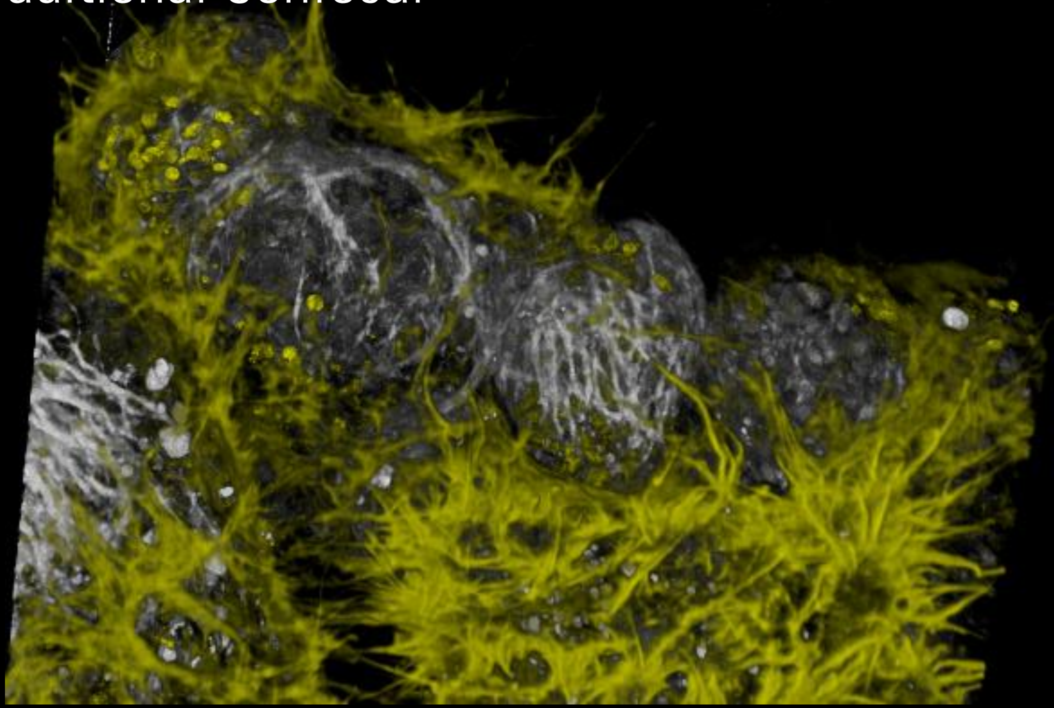
Detector 2

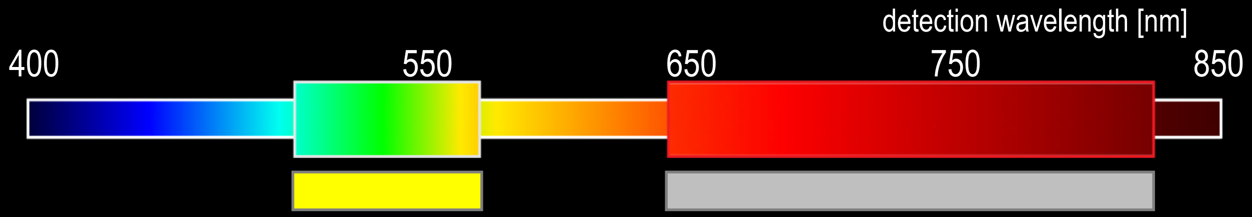
Lifetime-based Channel 3 | Lifetime-based Channel 4

TauSeparation Identifies Species With Every Image (Live/Acquired)

Multiplex Beyond The Spectral Options

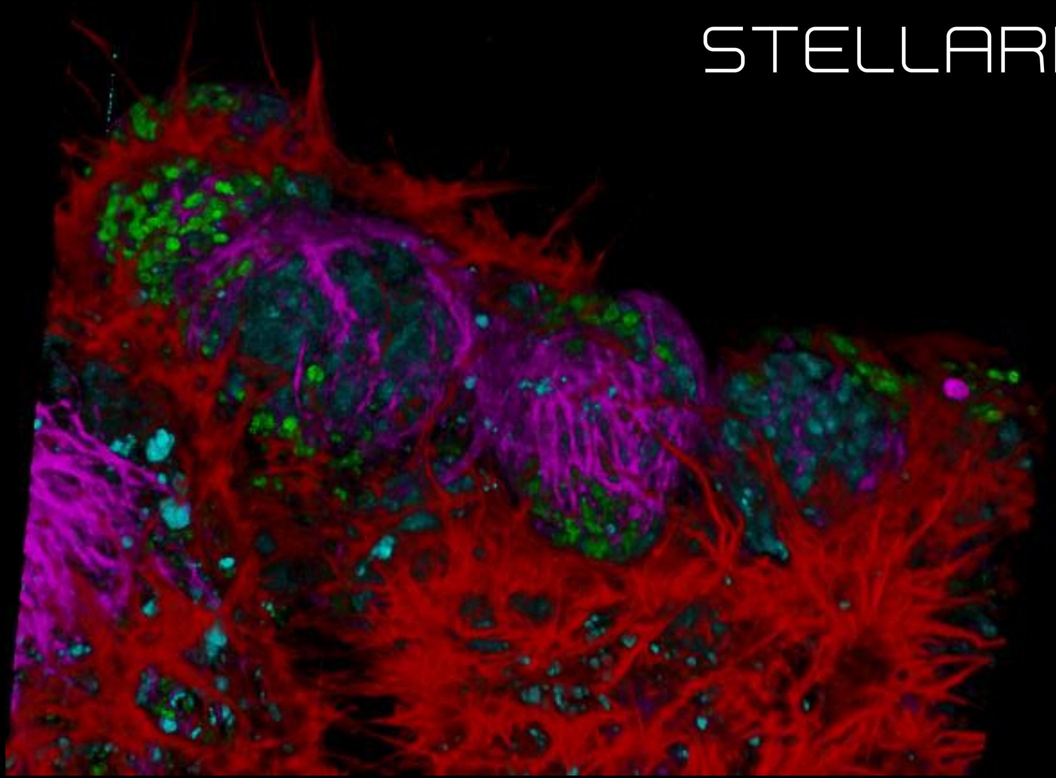
Traditional Confocal

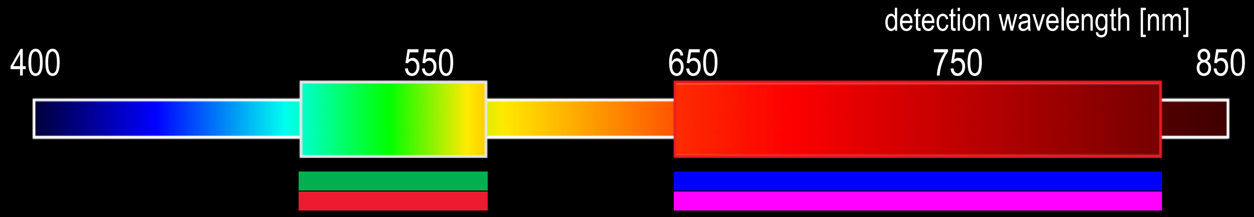




2 detectors, 2 intensity channels

STELLARIS

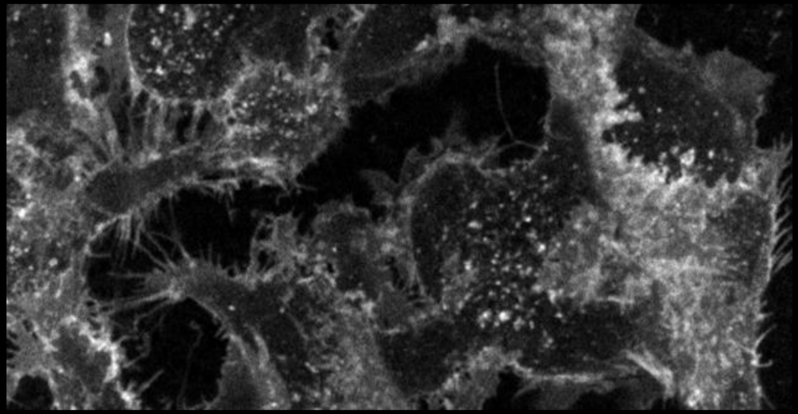


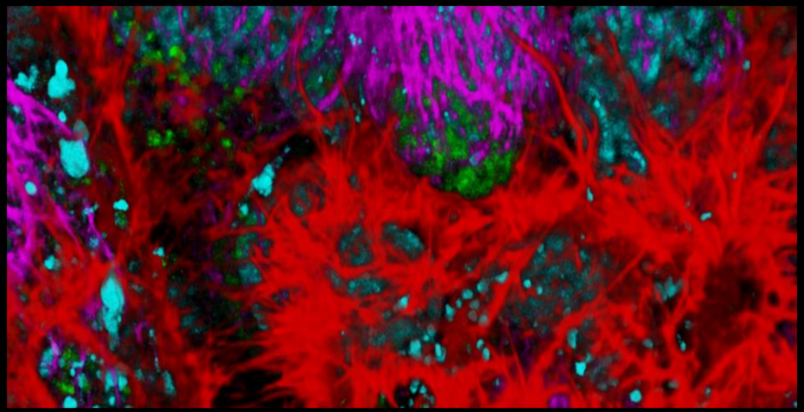


2 detectors, 4 lifetime-based channels

NE-115 cells. LifeAct-mNeonGreen (left: yellow, right: red), MitoTracker Green (left: yellow, right: green), NUC Red (left: gray, right: blue), and SiR-tubulin (left: gray, right: magenta).
Courtesy: Max Heydasch, University of Bern and Spirochrome

What is TauSense Good For?





TauContrast

- Qualitative / Semi-quantitative information
- Is there a change in microenvironment? Is FRET happening?
- Changes over time (x-fold ↑↓ compared to baseline)

TauGating

- Explore sample with gates
- Remove reflections
- Remove unwanted fluorescence contributions

TauSeparation

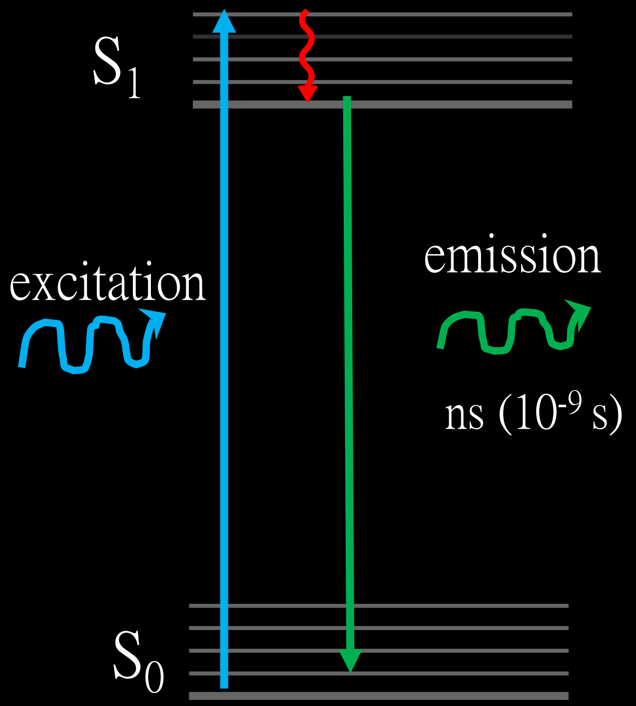
- Separate species with different lifetimes

TauSense Gives You Application-based Tools To Explore Lifetime-based Information

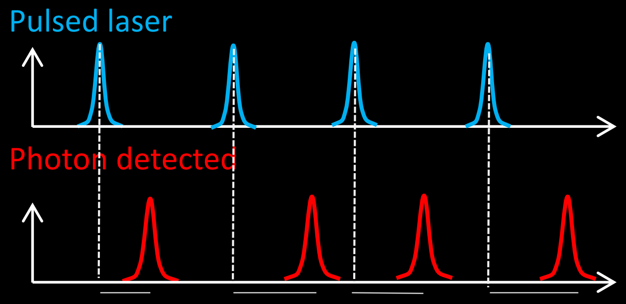
#CONFOCALREIMAGINED

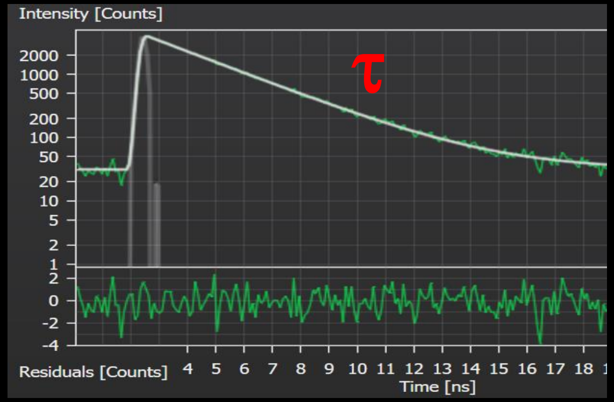
FALCON: why FLIM?

Fluorophore behavior…

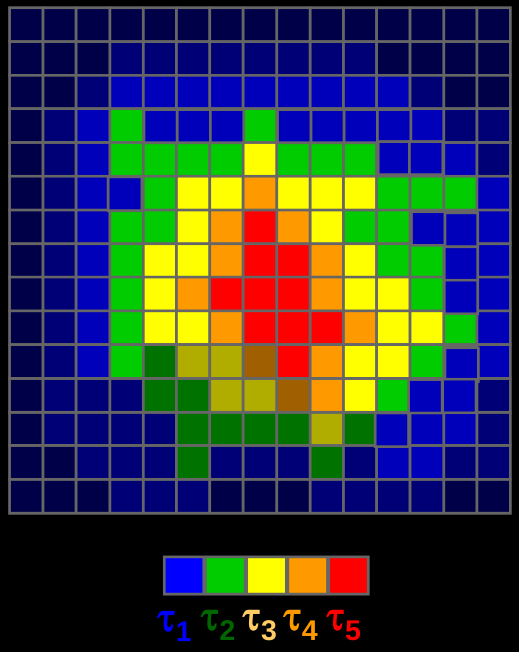


..measured as lifetime…





..generating a FLIM image



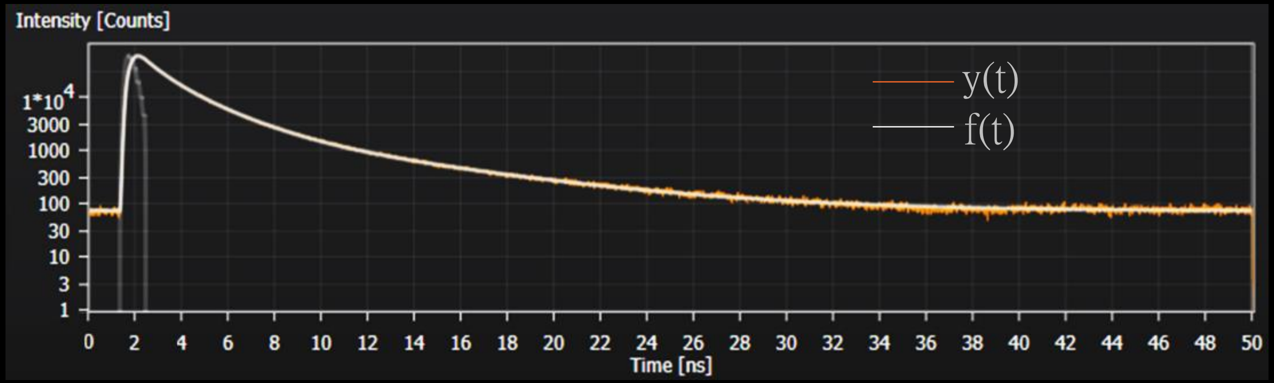
FLIM creates images with lifetime information

FALCON: IRF reconvolution algorithm

Fit Model

$$f(t) = \int_0^t IRF(x) \cdot \left\{ \sum_{i=0}^{n-1} A(n) \cdot e^{-\frac{x}{\tau(n)}} + B \right\} dx$$

$y(t)$ - Experimental data
$f(t)$ - Theoretical curve
$IRF(t)$ - Instrument Response Function
$A(n)$ - Amplitude of n-th component
$\tau(n)$ - Decay time of n-th component
B - Background



Maximum-Likelihood Estimator (Poissonian distribution)

Minimizes:

$$\chi^2_{mle} = 2 \cdot \sum_{i=1}^{N} f(i) - y(i) - 2 \cdot \sum_{i=1}^{N} y(i) \cdot ln(f(i)/y(i))$$

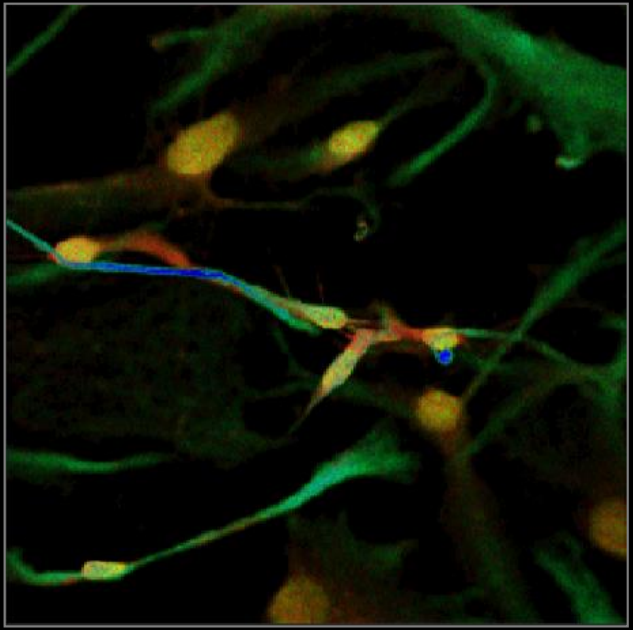
FALCON: Synergies and integrated workflows

- Component Separation

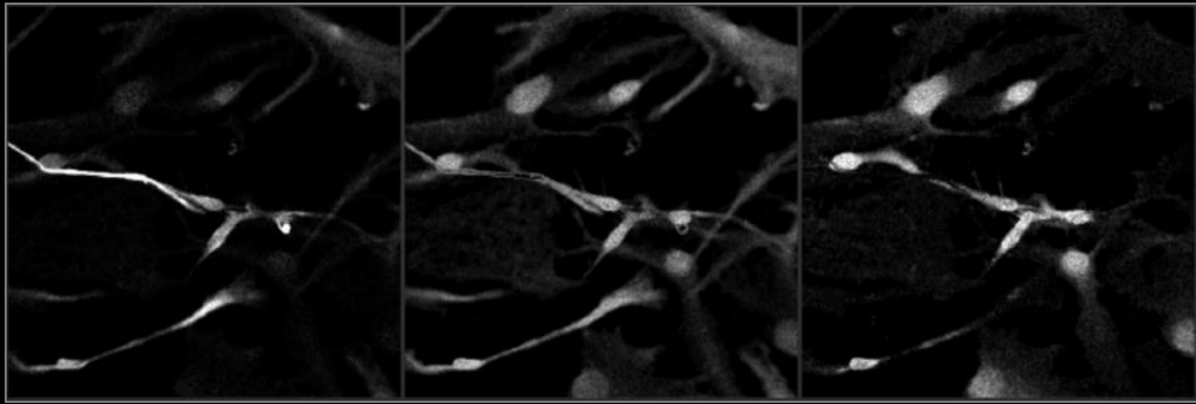
Parameters to fit

Parameter	Fit
Decay Time 1	
Decay Time 2	
Decay Time 3	
Amplitude 1	✓
Amplitude 2	✓
Amplitude 3	✓
Tail Offset	✓
IRF Background	
IRF Shift	

FLIM Image Fit
3 Components



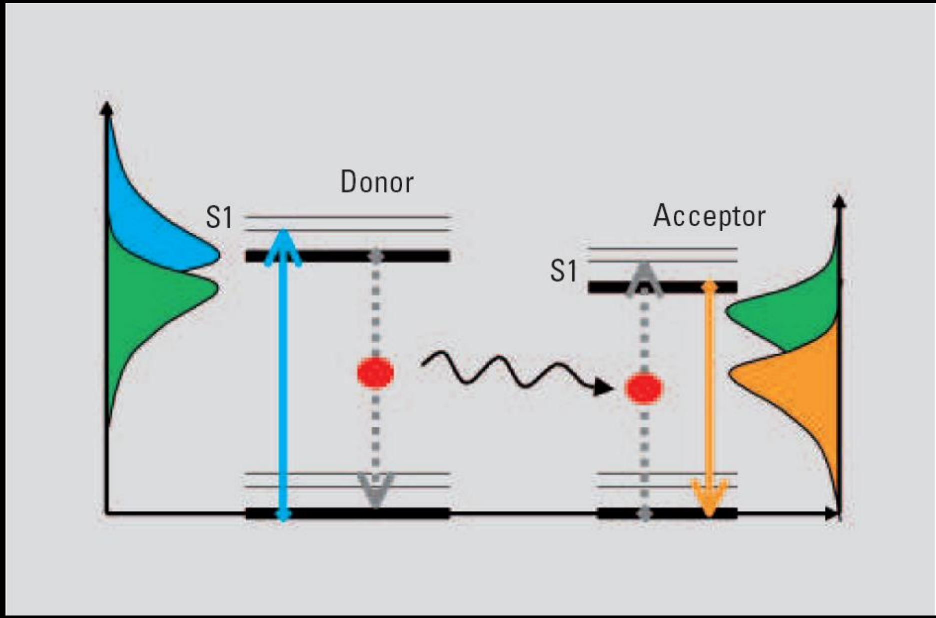
0.75 ns　　1.84 ns　　6.30 ns



FRET?

Fluorescence **R**esonance **E**nergy **T**ransfer - **FRET**

- Fluorescence based method
- Describes the non-radiative transfer of energy stored in an excited fluorescent molecule (the donor) to a non-excited different fluorescent molecule (the acceptor) in its vicinity
- Thus probes the proximity of fluorescently labelled molecules

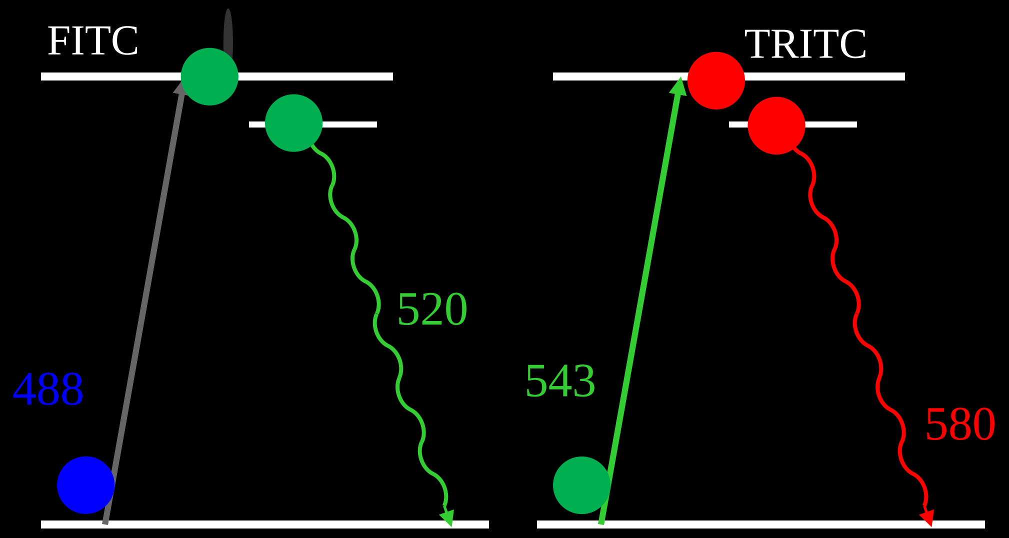


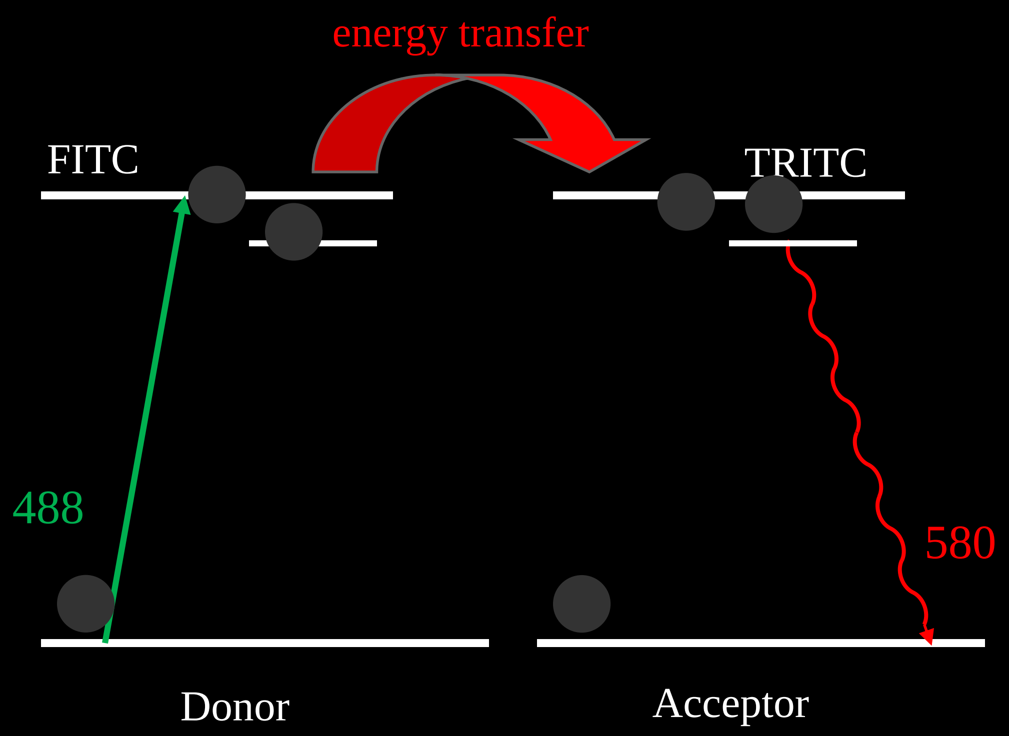
Energy transitions in FRET pair:

Light energy matching a transition in the donor molecule is absorbed (**blue arrow**).

The excited donor can relax either by fluorescence (left gray dotted arrow) or by resonance energy transfer to the acceptor molecule (**black arrow**).

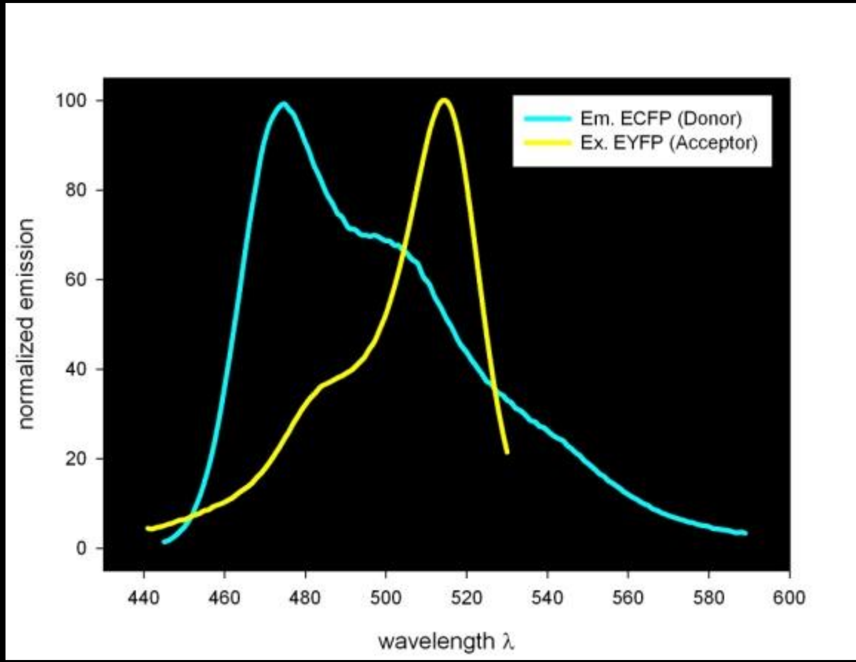
FRET Principle


FITC
TRITC
520
488
543
580



- Donor fluorescence is reduced
- Acceptor fluorescence appears (without selective excitation)

Conditions for FRET



Three conditions must be fulfilled for FRET to take place:

1. Overlap of donor emission spectrum with acceptor excitation spectrum

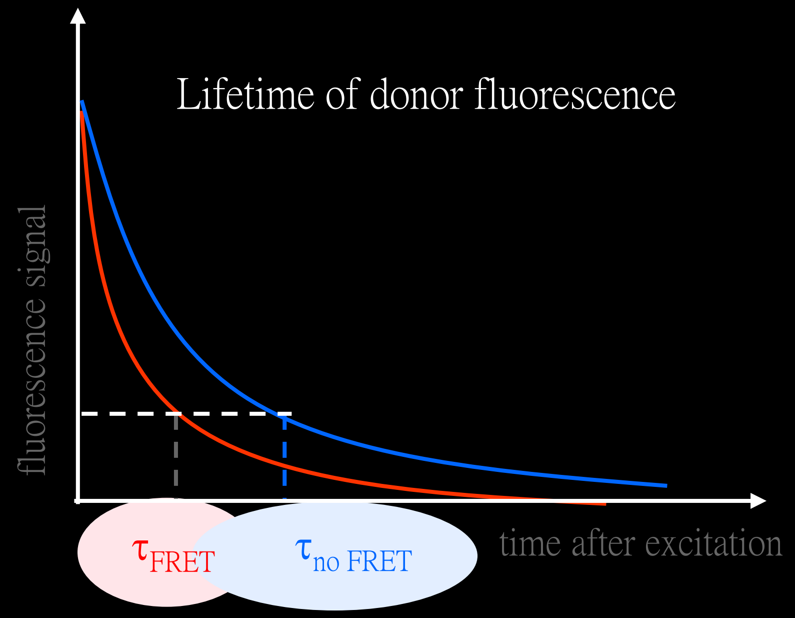
2. Molecules must be in close proximity on an Angstrom (10^{-10} m) scale.

3. Molecules must have the appropriate relative orientation.

FLIM-FRET?

- **Donor lifetime shortens**

  FRET efficiency is calculated from the difference between arising fast component in donor lifetime in the presence of the acceptor and original lifetime in the absence of the acceptor



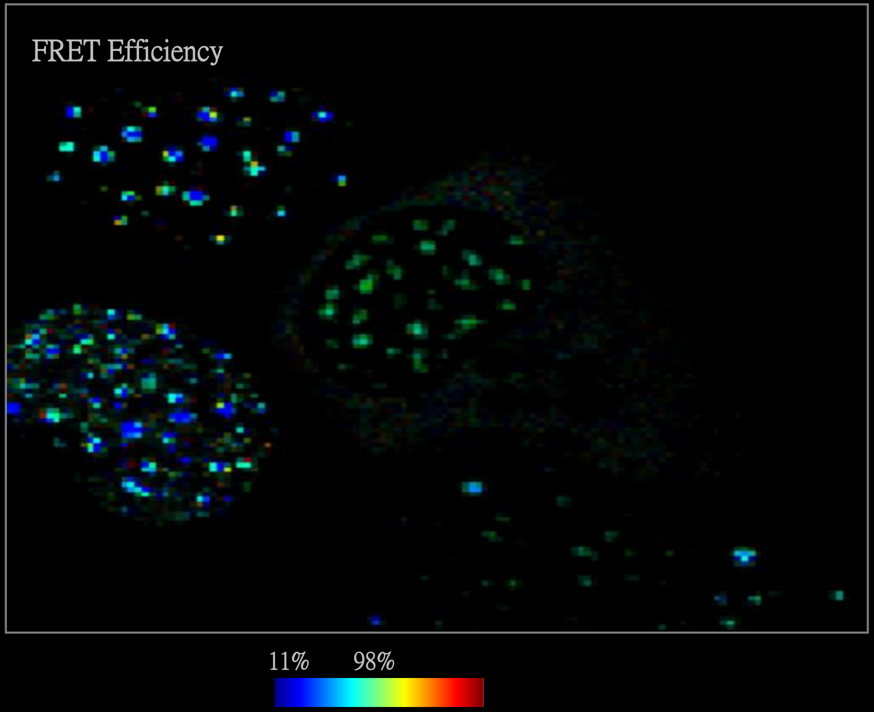
$$FRET_{eff} = \frac{\tau_{no\ FRET} - \tau_{FRET}}{\tau_{no\ FRET}}$$

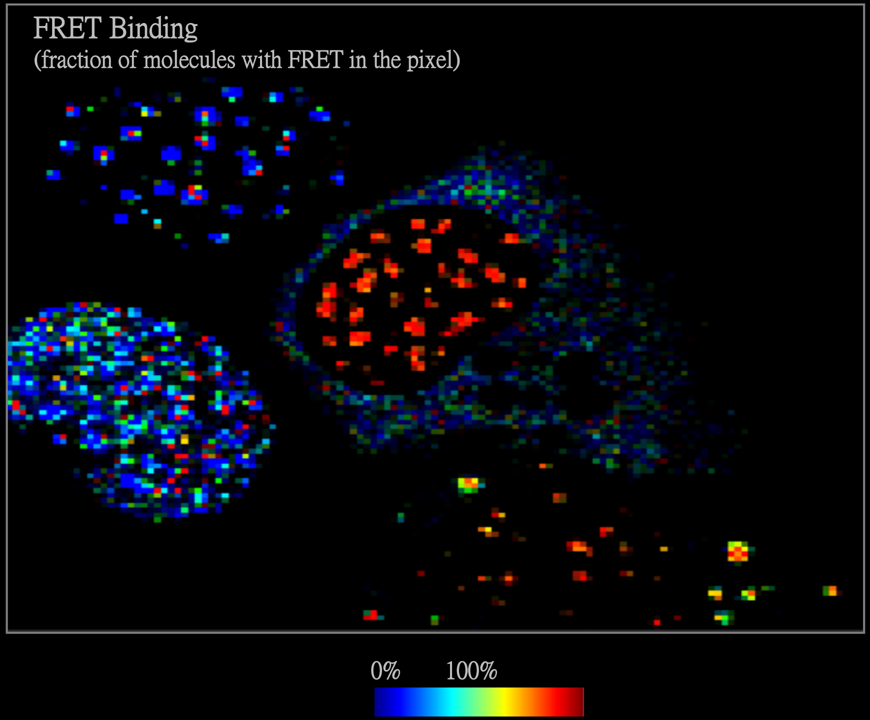
FALCON: Synergies and integrated workflows

- FLIM-FRET

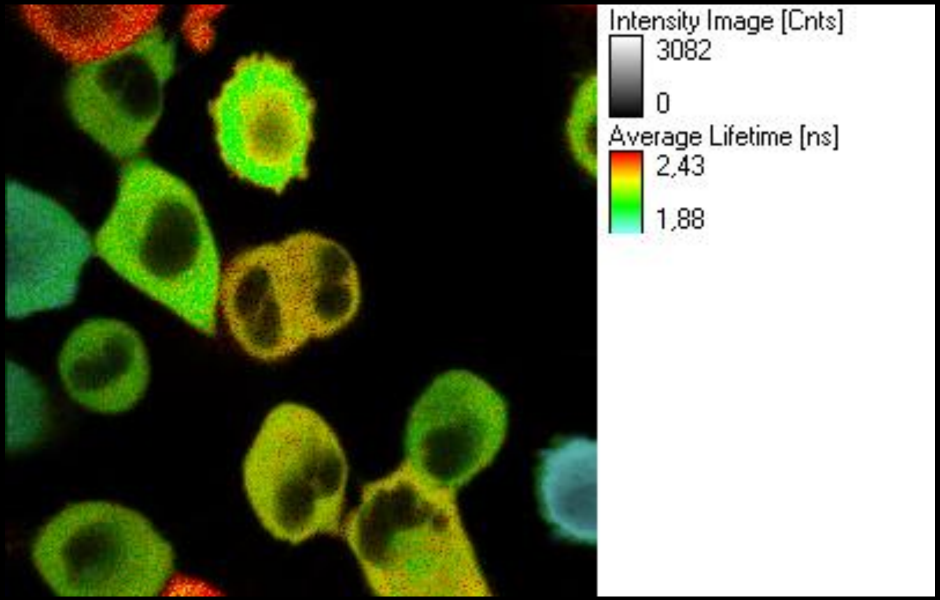
Parameters to fit

Parameter	Fit
Unquenched Donor Lifetime	
Unquenched Donor Amplitude	✓
Quenched Donor Lifetime	✓
Quenched Donor Amplitude	✓
Tail Offset	✓
IRF Background	
IRF Shift	





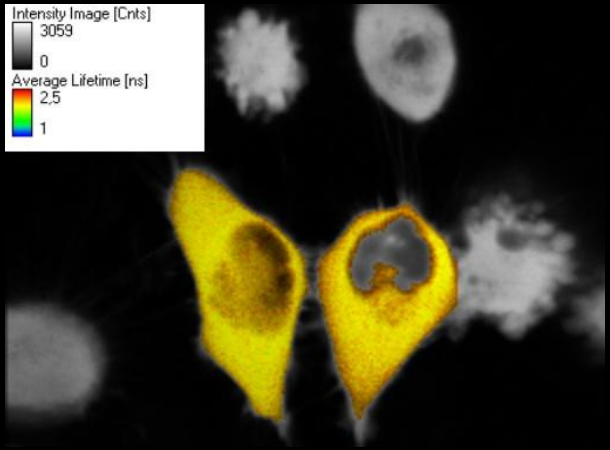
FLIM-FRET in live cells: typical FRET pairs



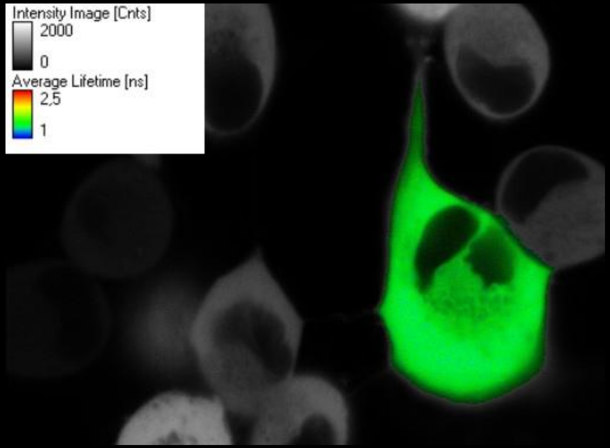
- CFP-YFP
- Sapphire-Venus
- (GFP-YFP)
- GFP-HcRed
- GFP-mCherry

Biological heterogeneity of FRET cells (CFP-YFP fusion), Courtesy: G. Hams, University of Würzburg

FLIM-FRET (CFP-YFP) in live cells



Donor only (CFP)



FRET pair (CFP-YFP tandem)

Donor lifetime images of FRET and control cells:

Sample: RBKB78 cells transfected with a CFP donor only or CFP-YFP fusion.

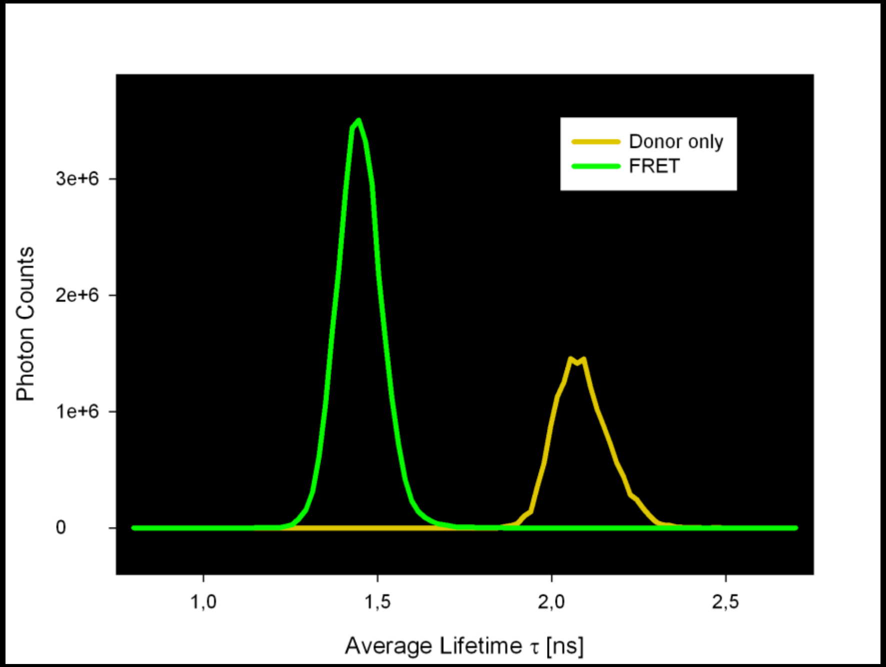
Data Acquisition: The detection band was set between 445-495 nm. Excitation @ 405 nm

Data Analysis: The coloured region has been used for analysis. Colours represent intensity modulated fluorescence lifetimes.

Result: In the presence of acceptor the donor lifetime is decreased.

Courtesy: G. Hams, University of Würzburg

FLIM-FRET (CFP-YFP) in live cells: Quantitative data analysis



Fluorescence lifetime distribution histogram of **donor only (yellow)** and **FRET (green)** samples using average lifetimes. There is a clear shift of 0.7 ns towards shorter lifetimes in the FRET sample.

From lifetime distribution histograms one obtains:

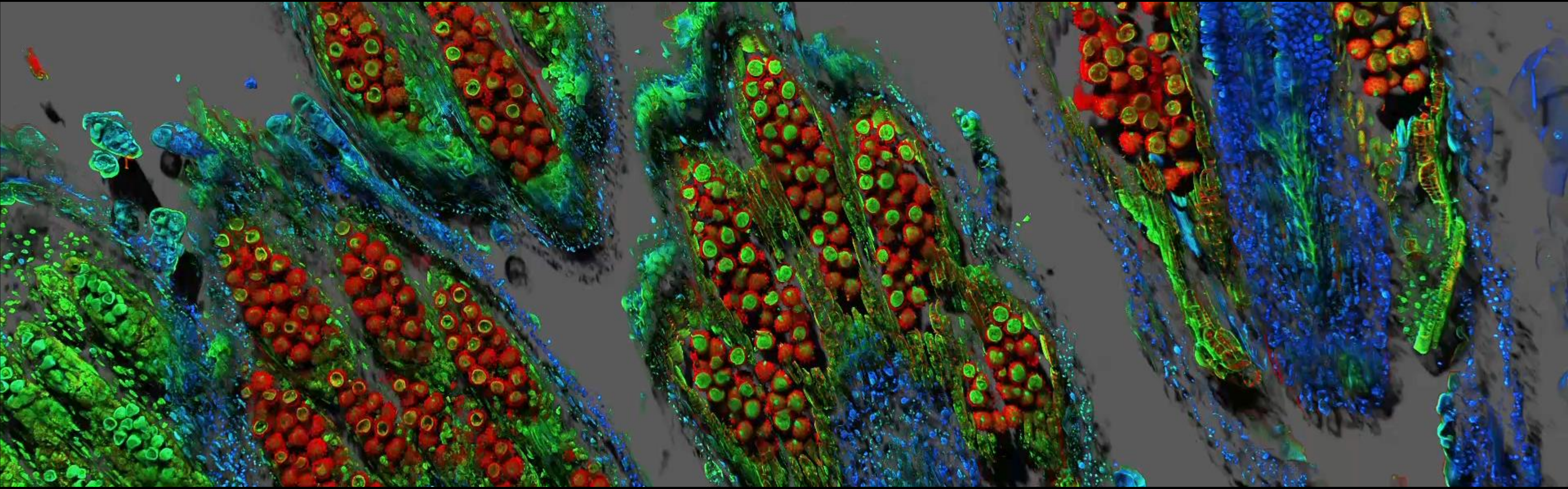
- average lifetime of the donor is: 2.1 ns.
- donor lifetime of the FRET construct is: 1.4 ns.
- FRET efficiency is: E = 30%.

Computation of FRET Efficiency:

$$E = 1 - \frac{\tau_{quench}}{\tau}$$

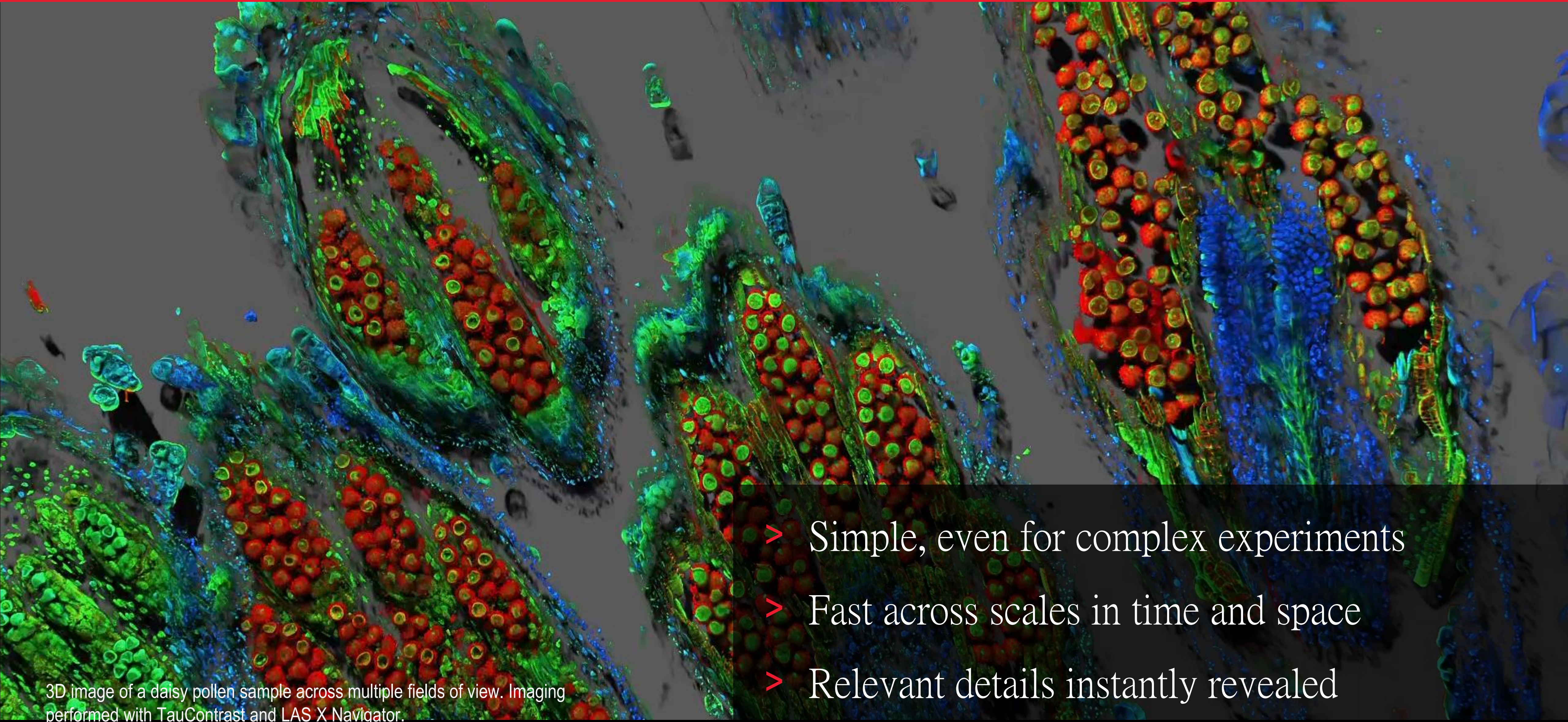
PRODUCTIVITY

DO MORE



#CONFOCALREIMAGINED

STELLARIS Gives You The Productivity To Do More

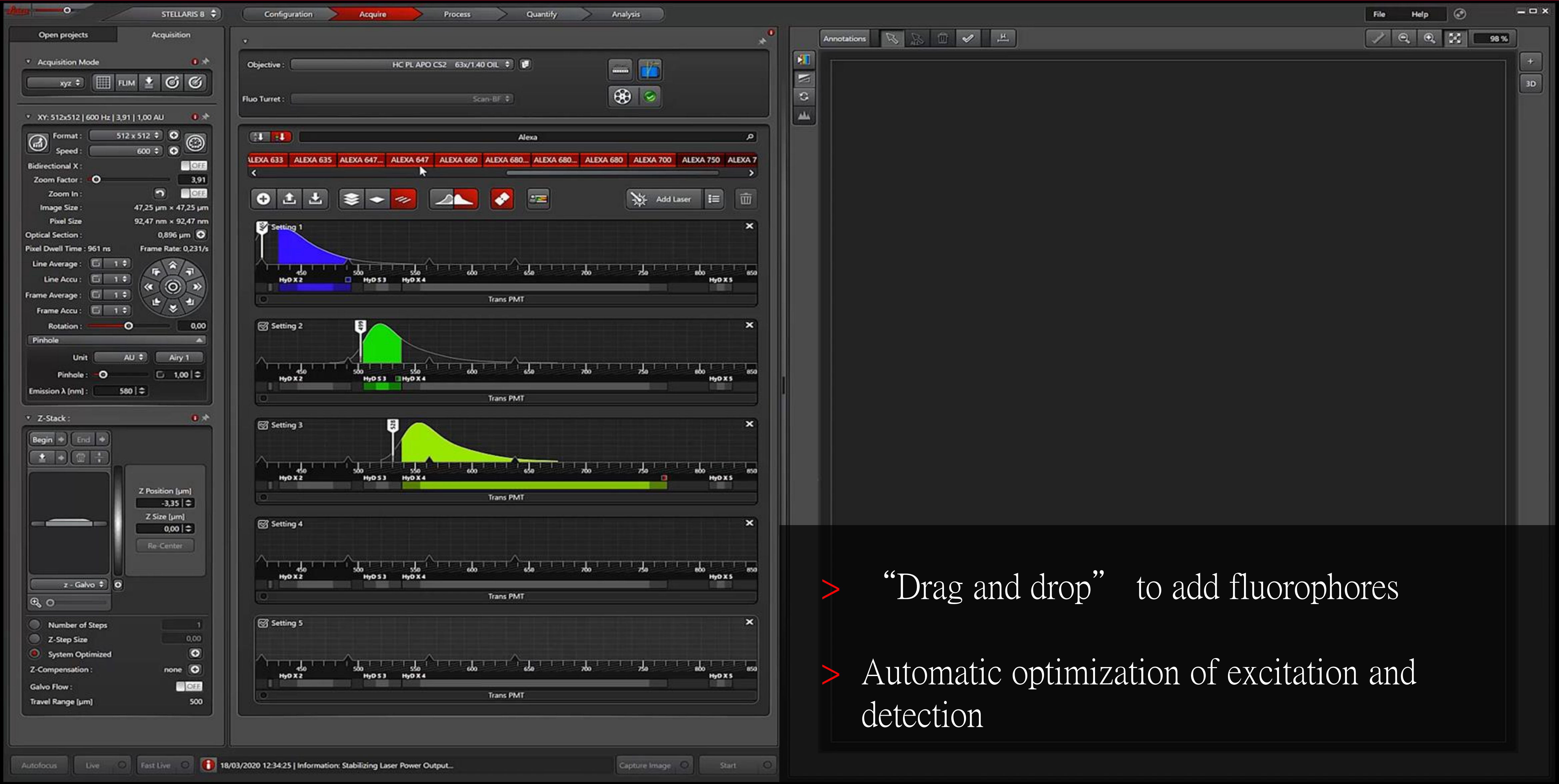


- Simple, even for complex experiments
- Fast across scales in time and space
- Relevant details instantly revealed

3D image of a daisy pollen sample across multiple fields of view. Imaging performed with TauContrast and LAS X Navigator.

#CONFOCALREIMAGINED

Simple, Even For Complex Experiments



> “Drag and drop” to add fluorophores

> Automatic optimization of excitation and detection

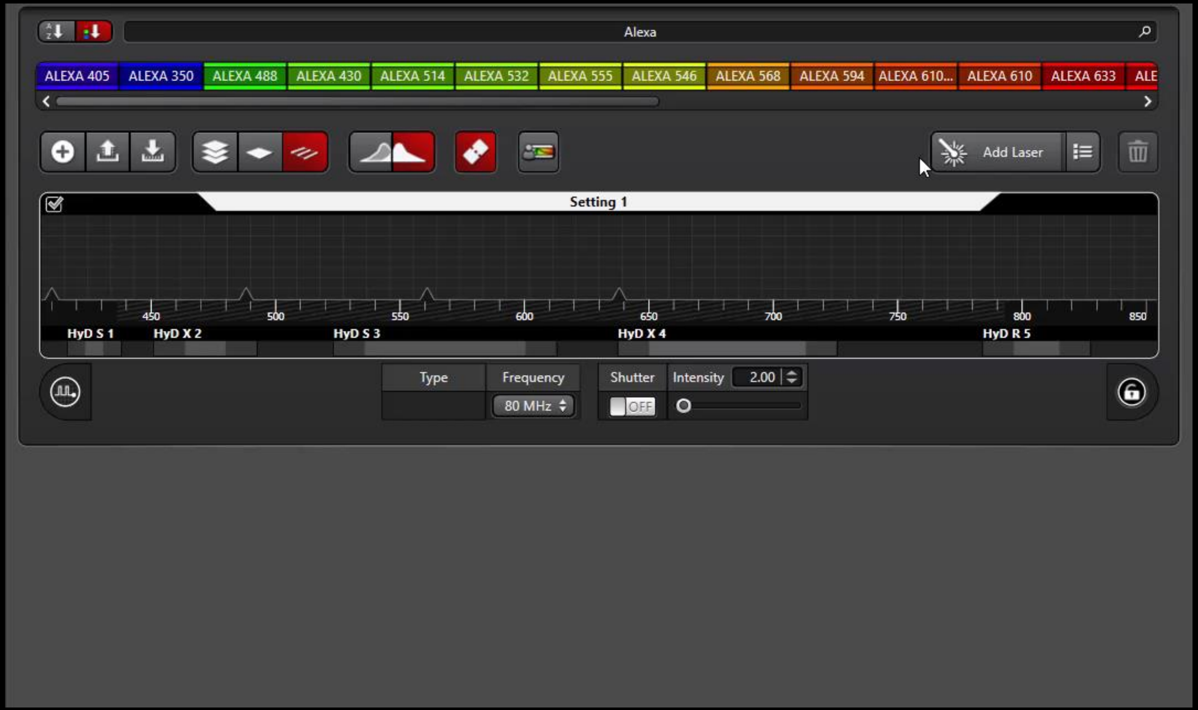
#CONFOCALREIMAGINED

Simple, Even For Complex Experiments – Image Compass

#CONFOCALREIMAGINED

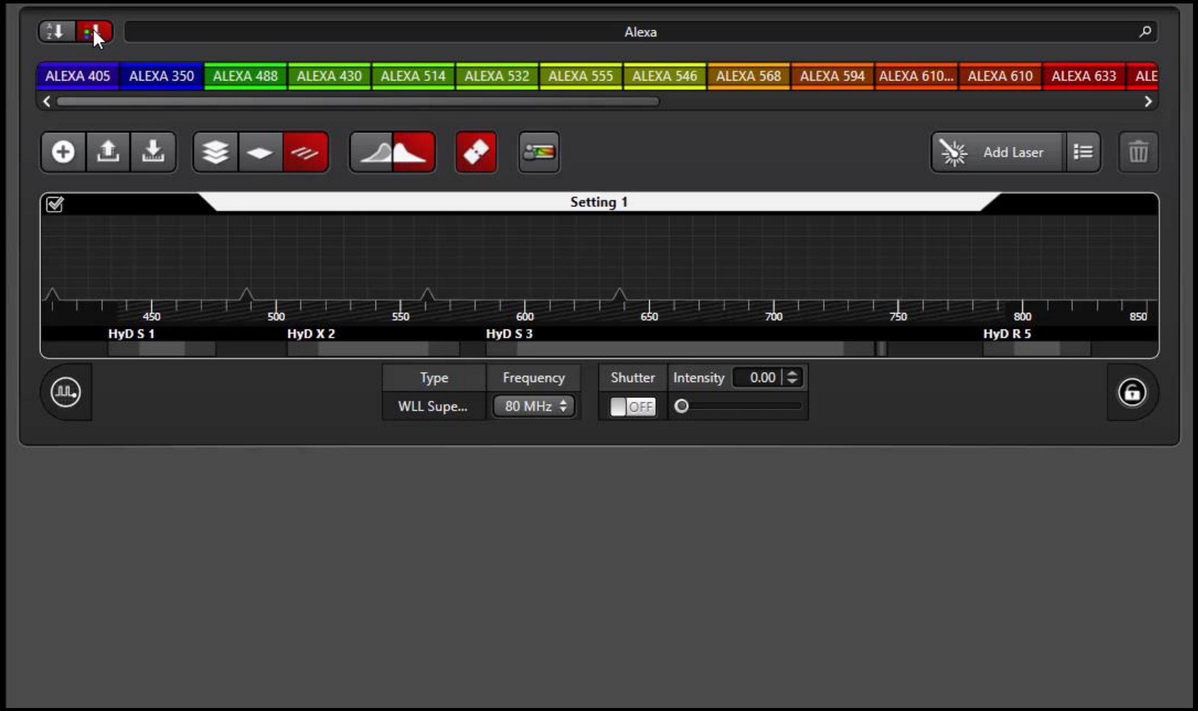
Who Is First At Getting An Image With Alexa 488 And Alexa 568?

Technology centered:



33 sec for the expert

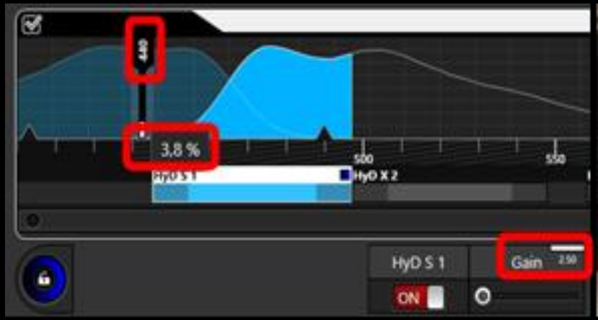
Sample centered:



10 sec for everybody

What Is Key to The **Cockpit View** Of Image Compass?

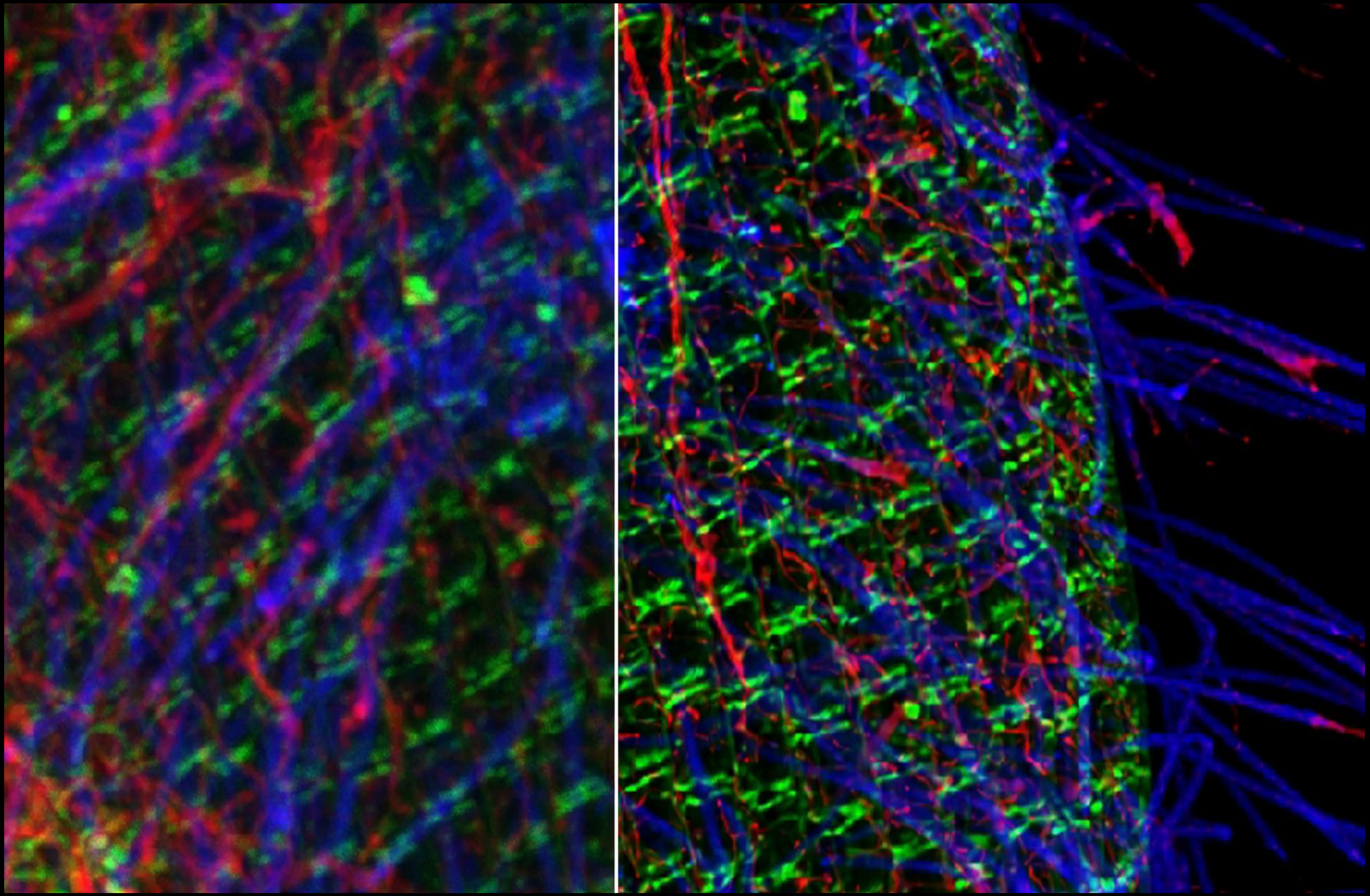
- Interact with images directly using Control panel:
- Smart Gain – Detector
- Smart Intensity – Laser



LIGHTNING

Image Information Extraction

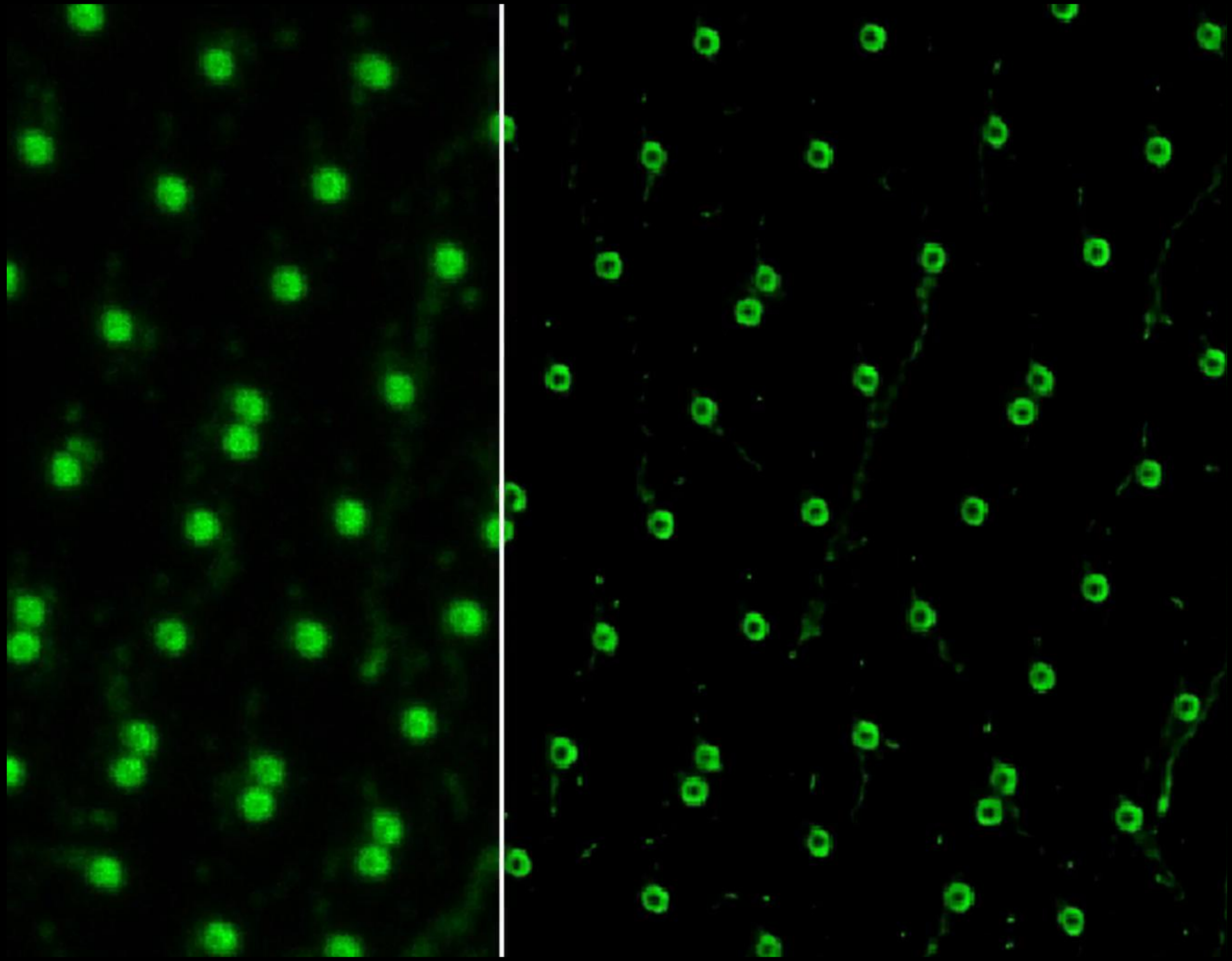
LIGHTNING: Adaptive Multicolor Super-Resolution



Confocal I MP I gated STED

Including every imaging modality

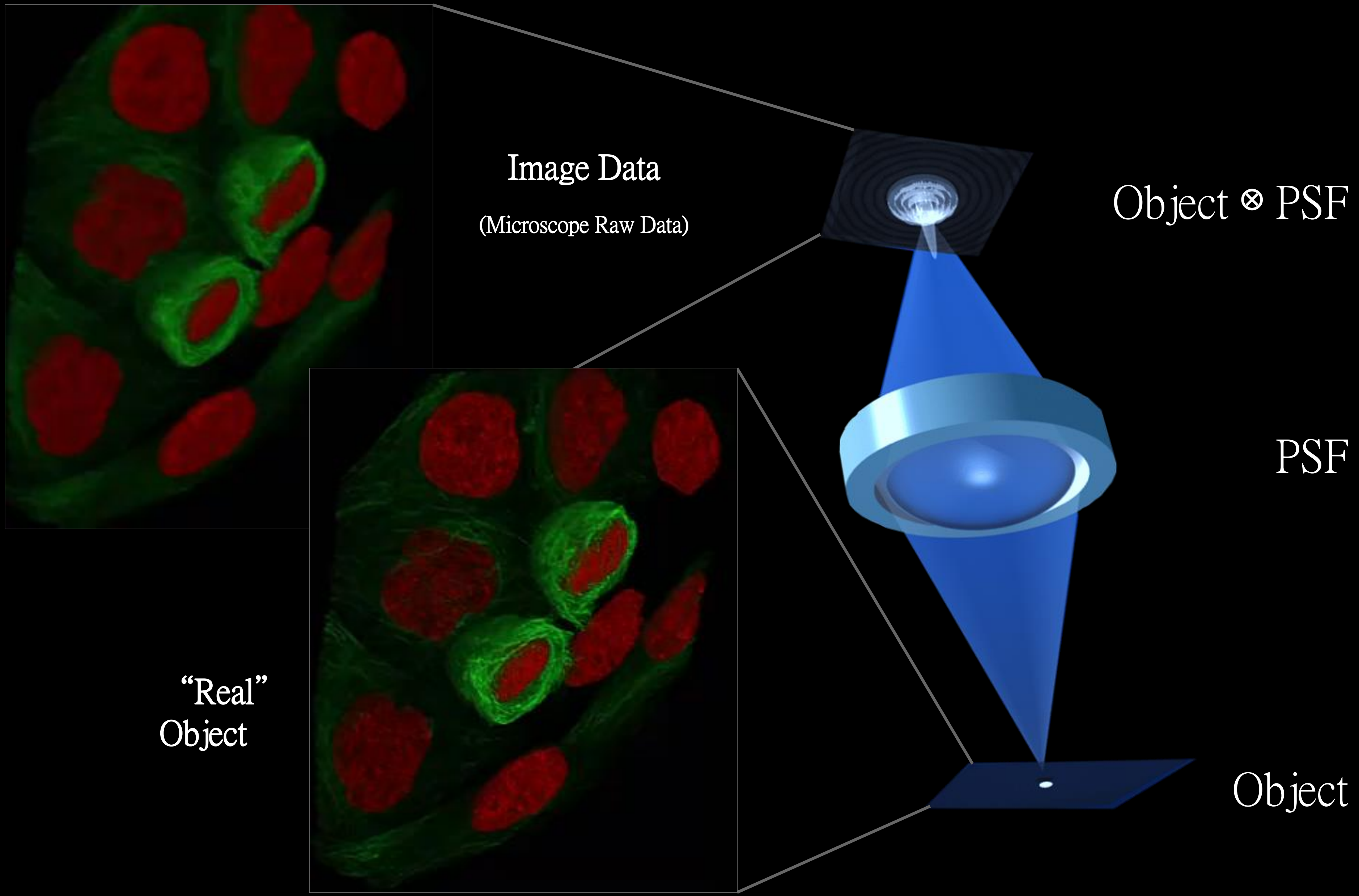
LIGHTNING: Accessing The True Nature Of Image Data



Confocal | MP | gated STED

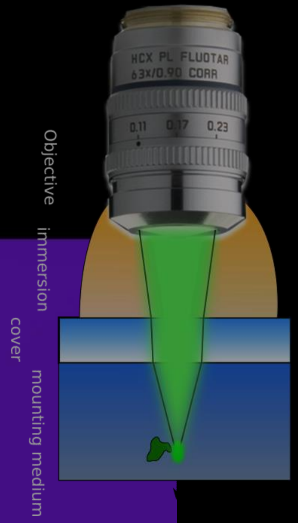
Including every imaging modality

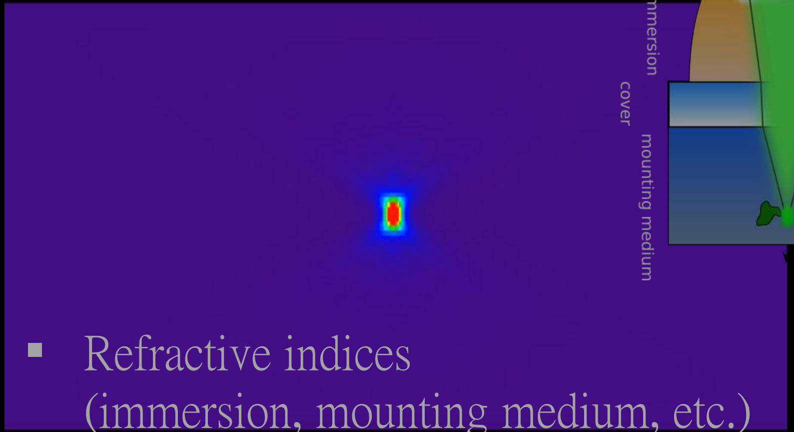
Microscopy Image Formation



PSF with varying parameters

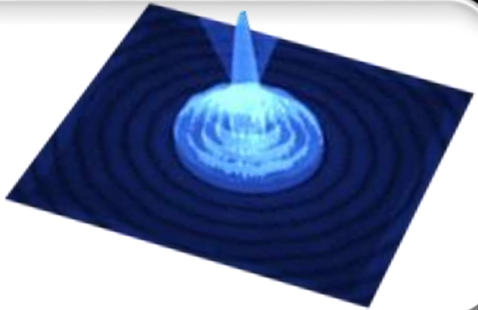
- Pinhole width
- λ_{ex} | λ_{em}

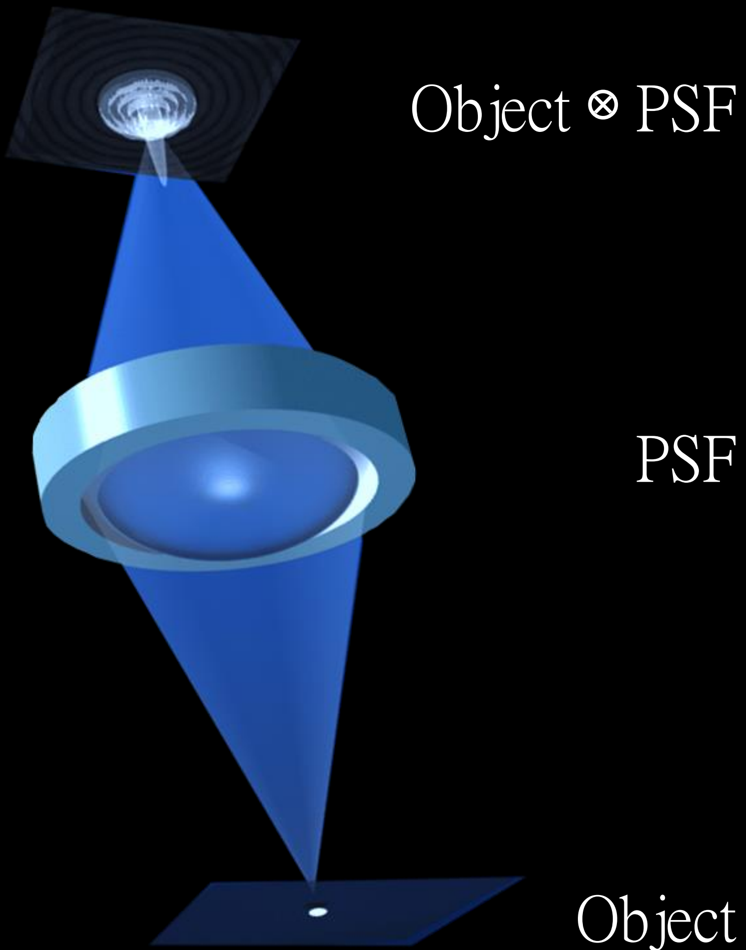




- Refractive indices (immersion, mounting medium, etc.)
- Objective lens (NA, magnification, etc.)

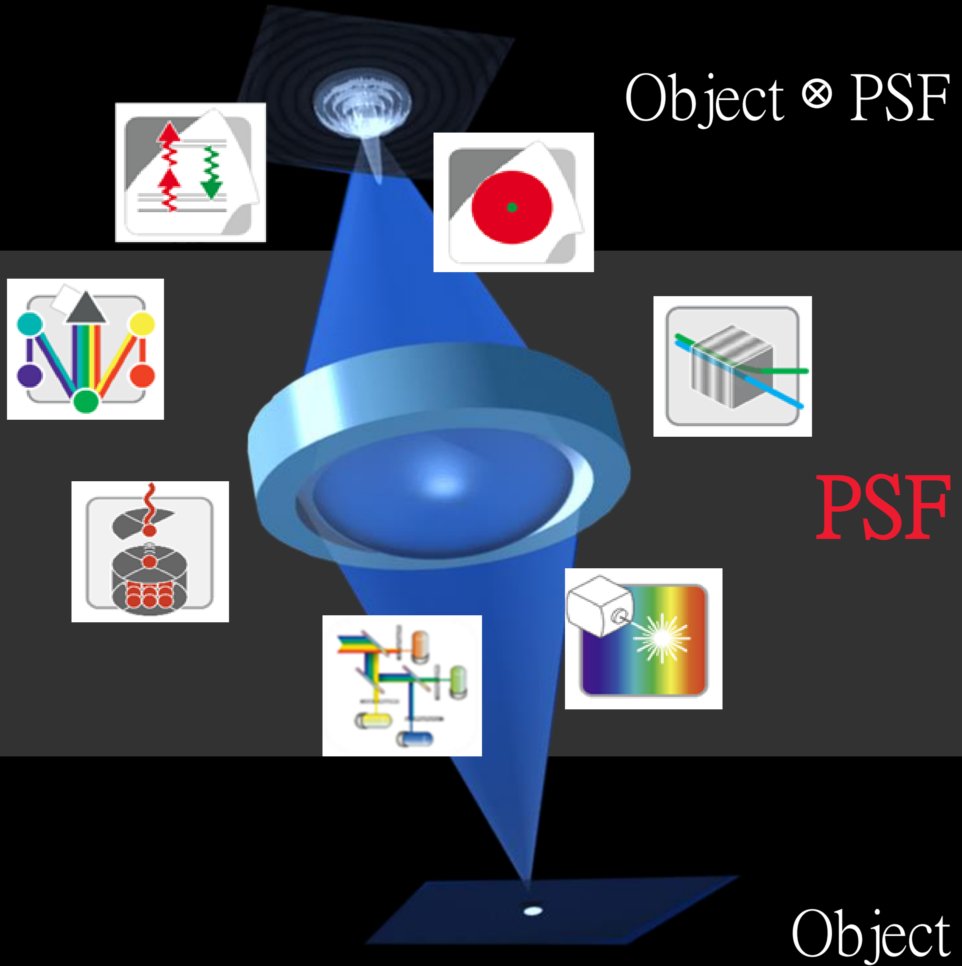
Diffraction



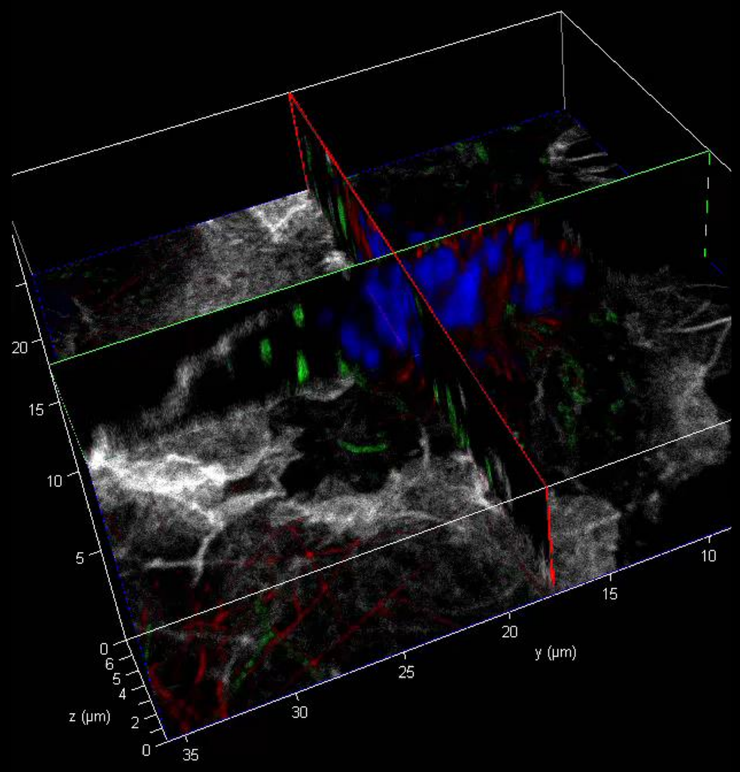


Integrating Microscope Characteristics

Accessing the intrinsic microscope characteristics and acquisition parameters is indispensable

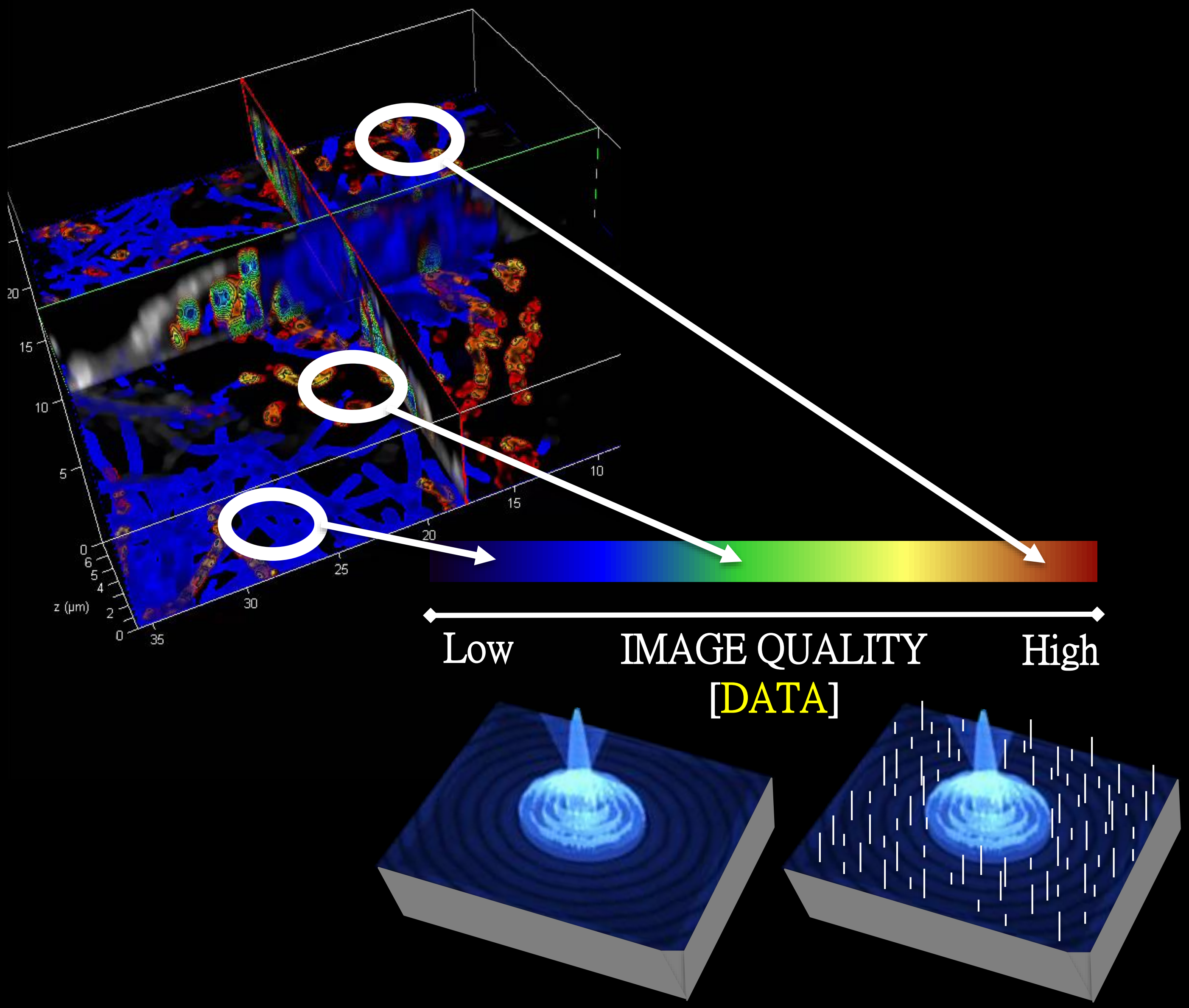


Raw data

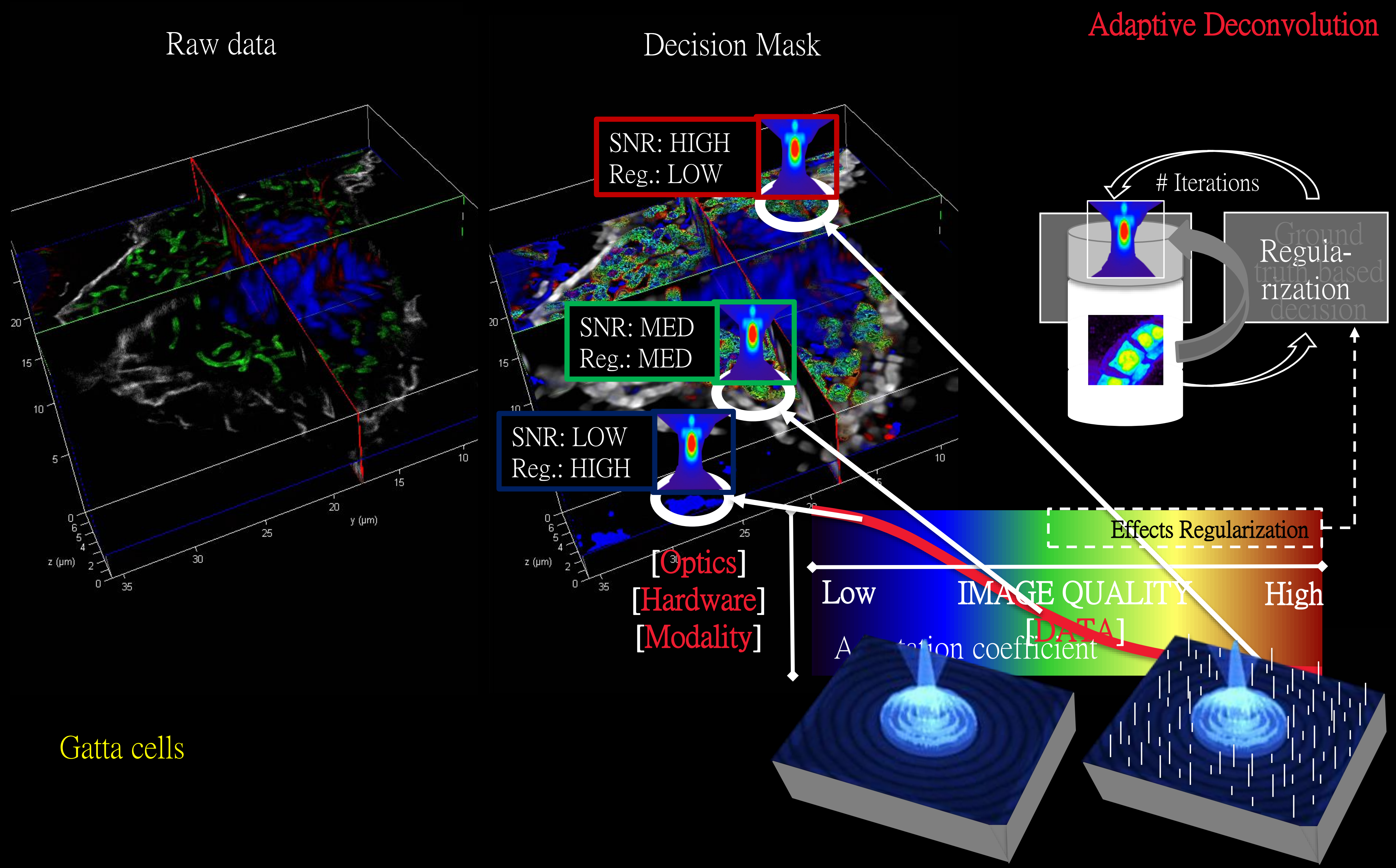


Decision Mask

Position dependant image quality



GATTA cells

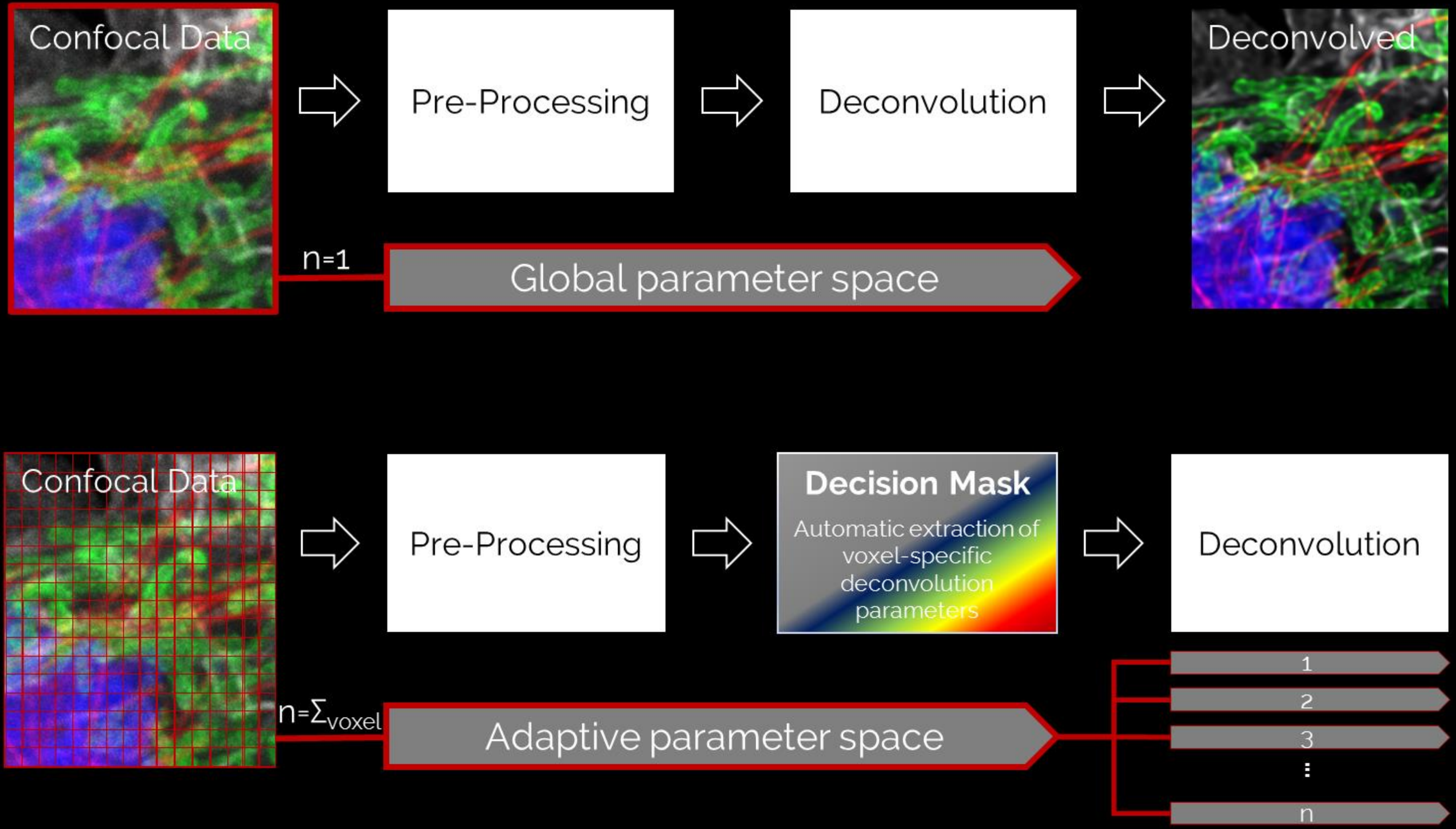


Raw data
Decision Mask
Adaptive Deconvolution
SNR: HIGH
Reg.: LOW
SNR: MED
Reg.: MED
SNR: LOW
Reg.: HIGH
Iterations
Regula-
rization
Effects Regularization
Low
IMAGE QUALITY
High
[DATA]
[Optics]
[Hardware]
[Modality]
z (µm)
y (µm)
Gatta cells

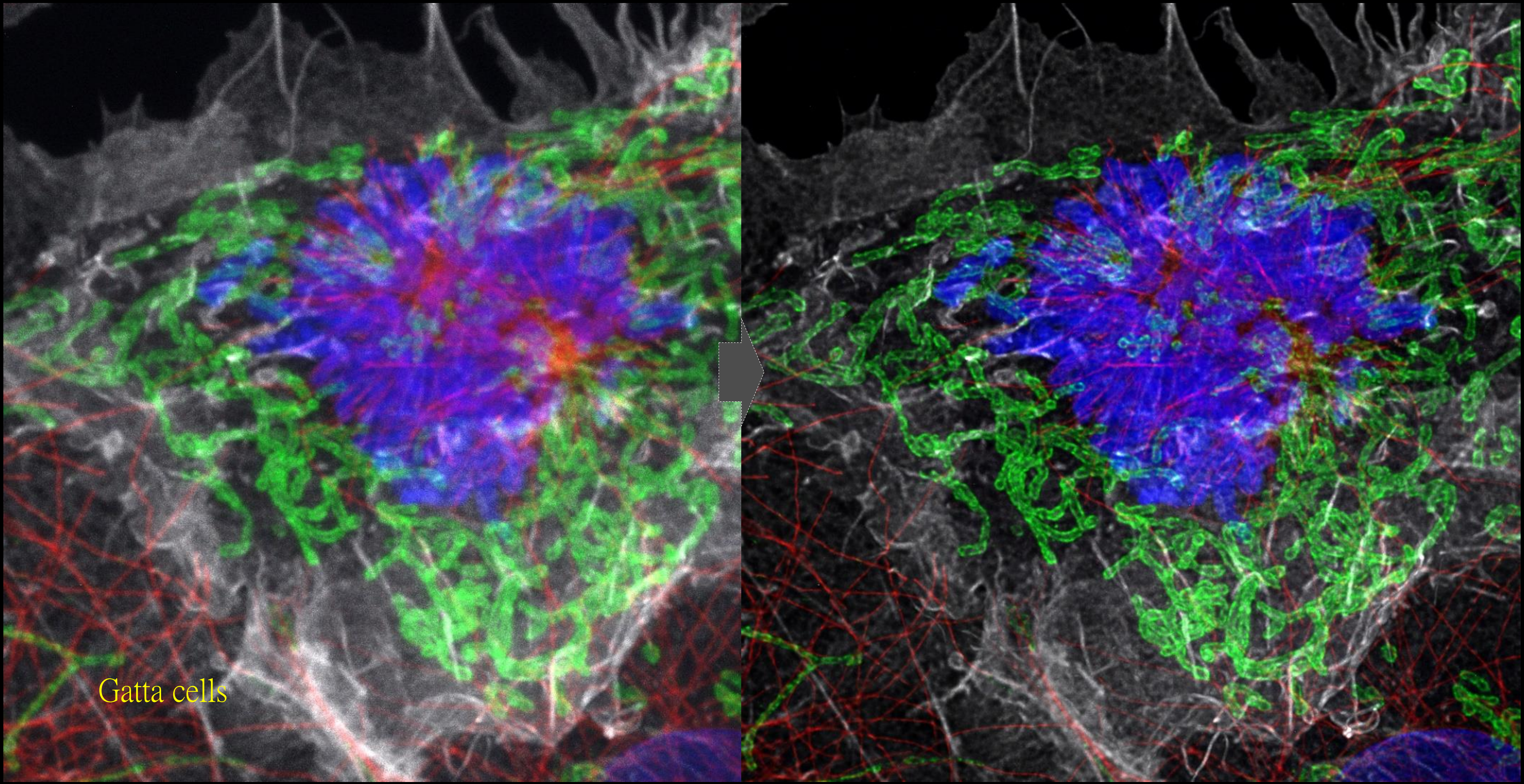
Adaptive Deconvolution

Classical Deconvolution

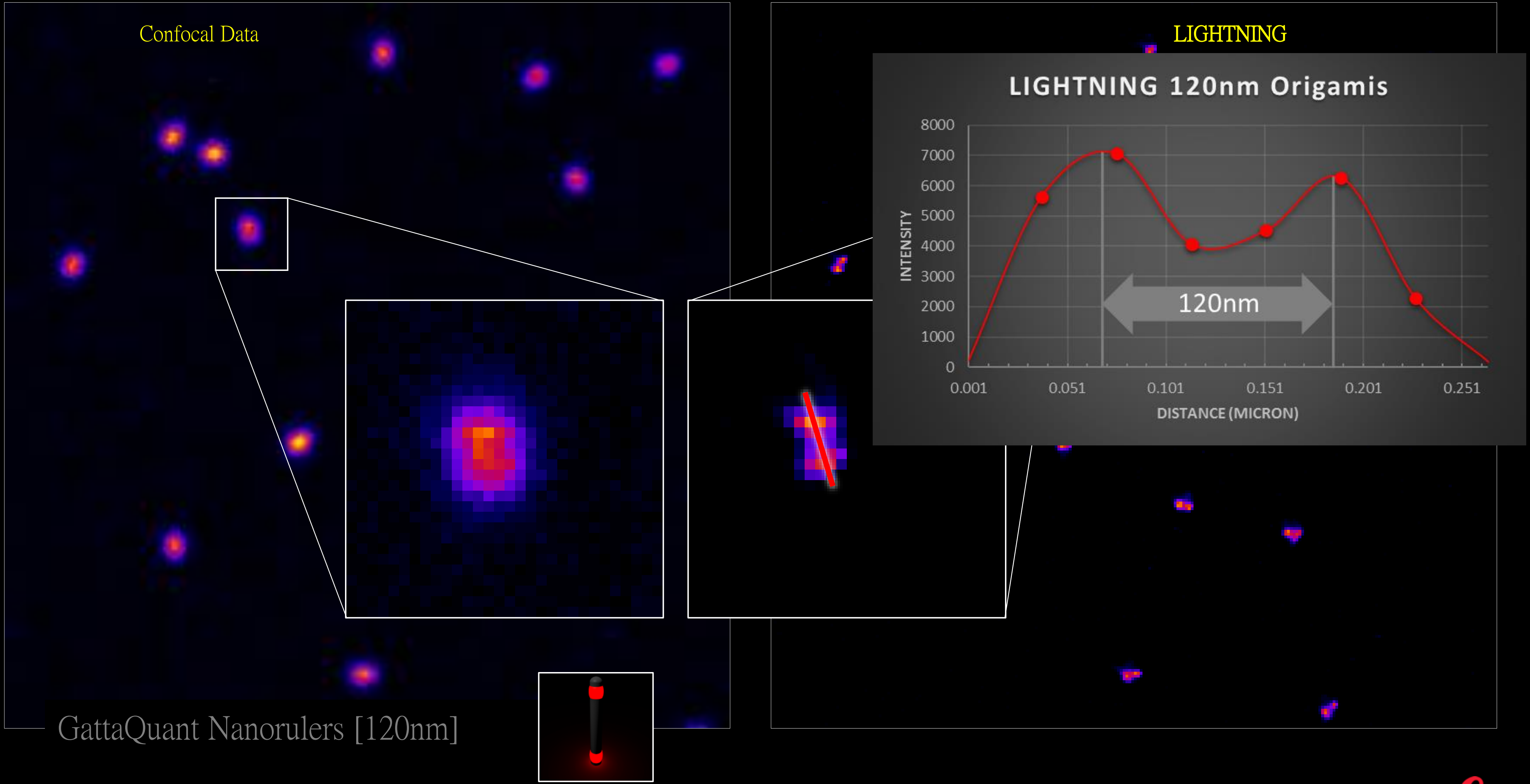
Adaptive Deconvolution



Adaptive Deconvolution

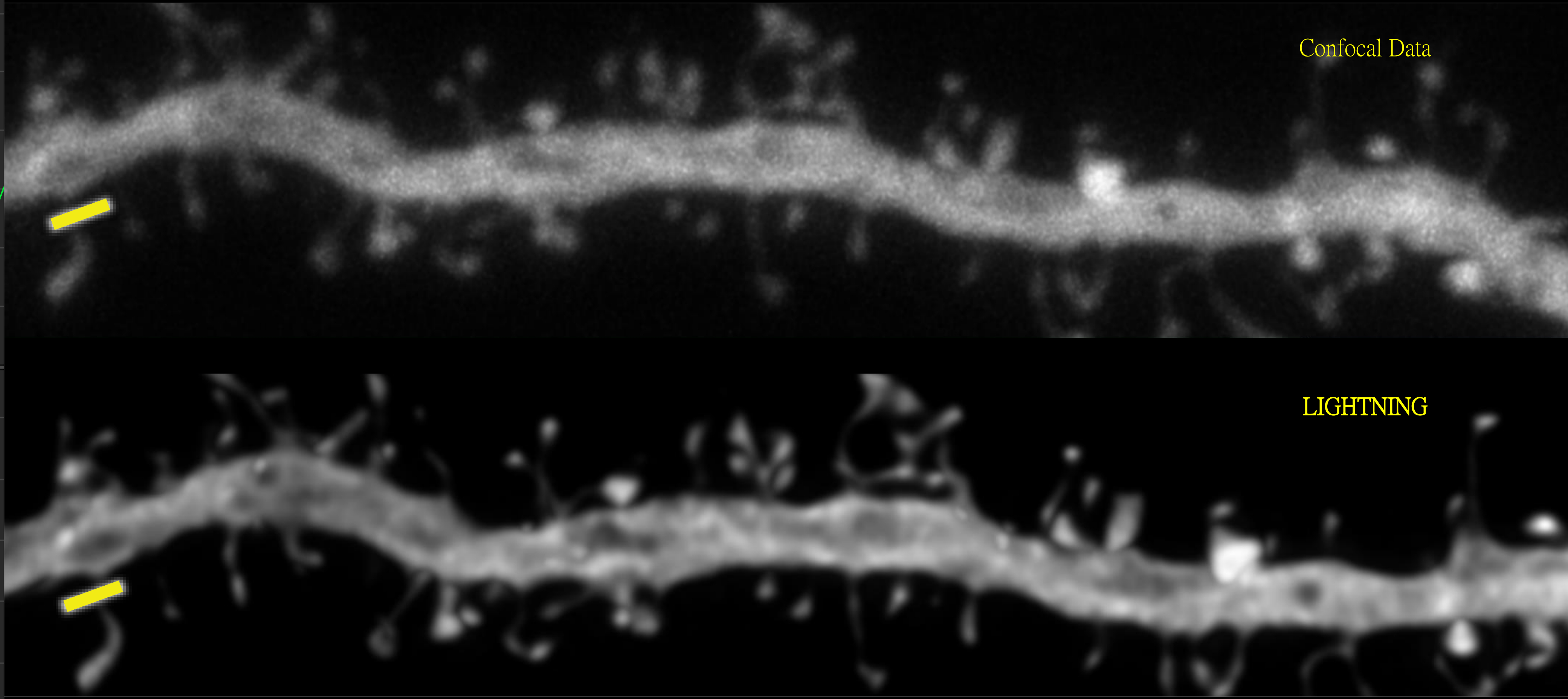


Accessing Super-Resolution



Accessing Structural Information

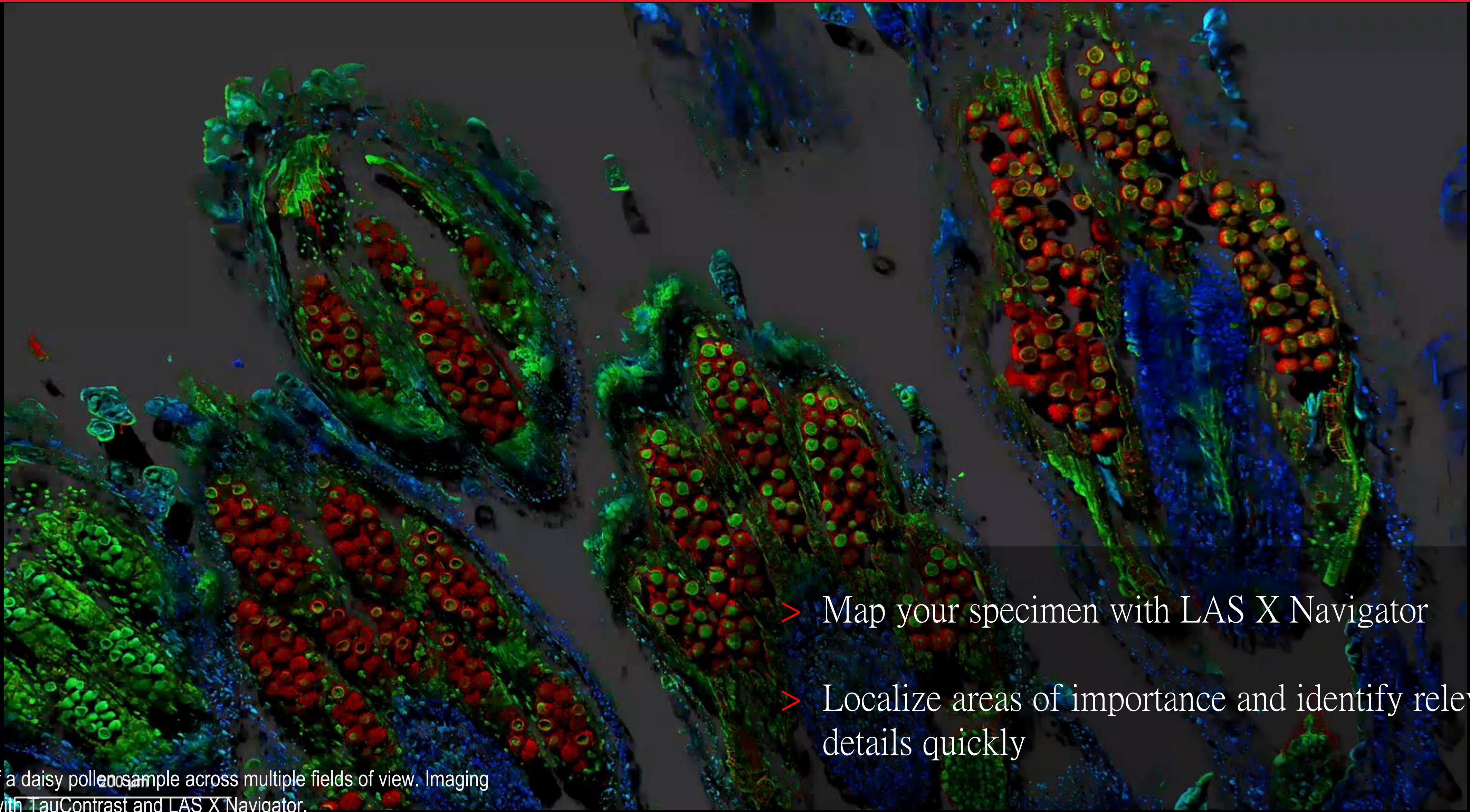
MAX projection



Sample: courtesy of Dani Dumitriu, PhD, MD

Neuroscience, Icahn School of Medicine, New York, US

Relevant Details Instantly Identified



3D image of a daisy pollen sample across multiple fields of view. Imaging performed with TauContrast and LAS X Navigator.

- Map your specimen with LAS X Navigator
- Localize areas of importance and identify relevant details quickly

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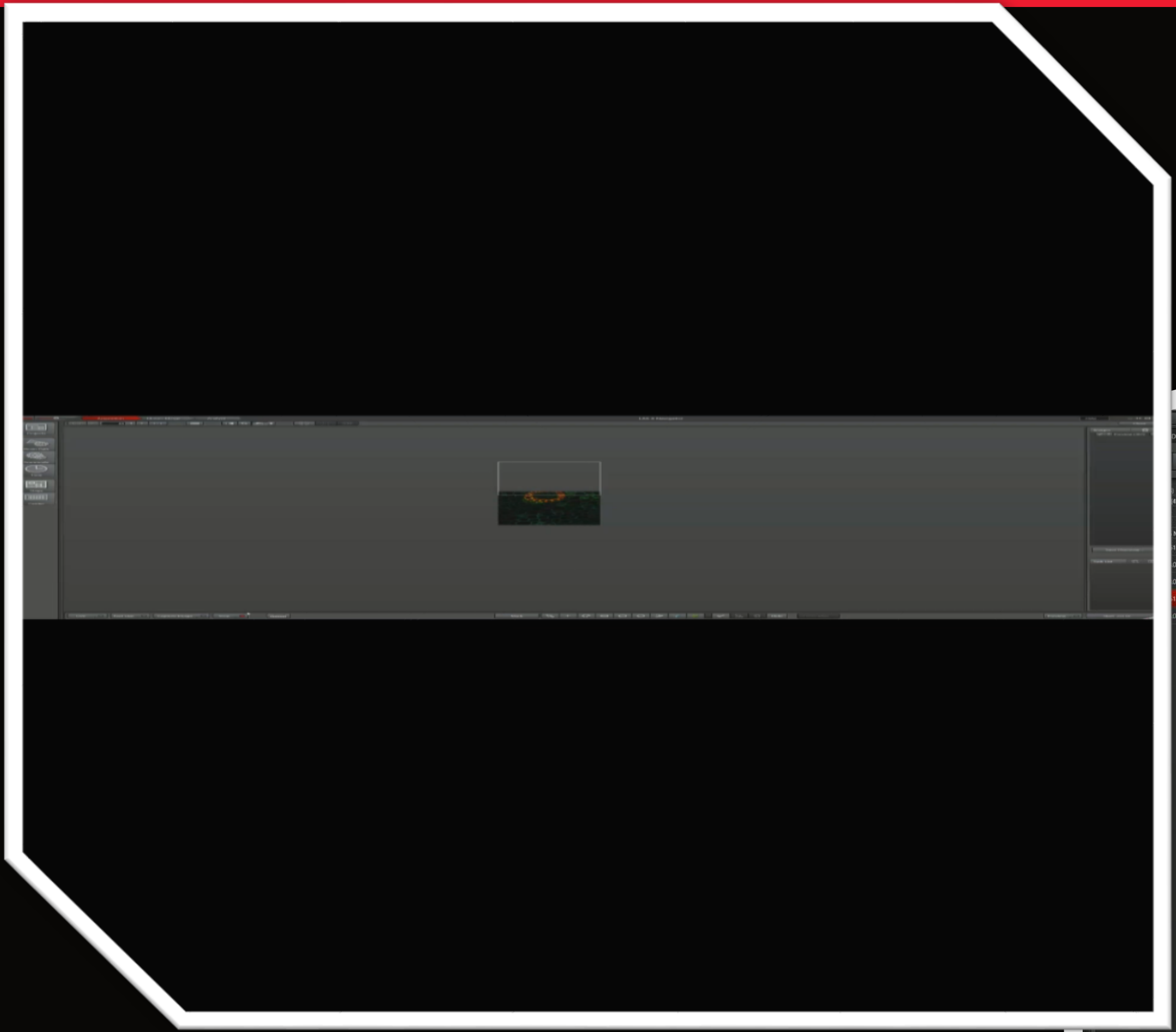
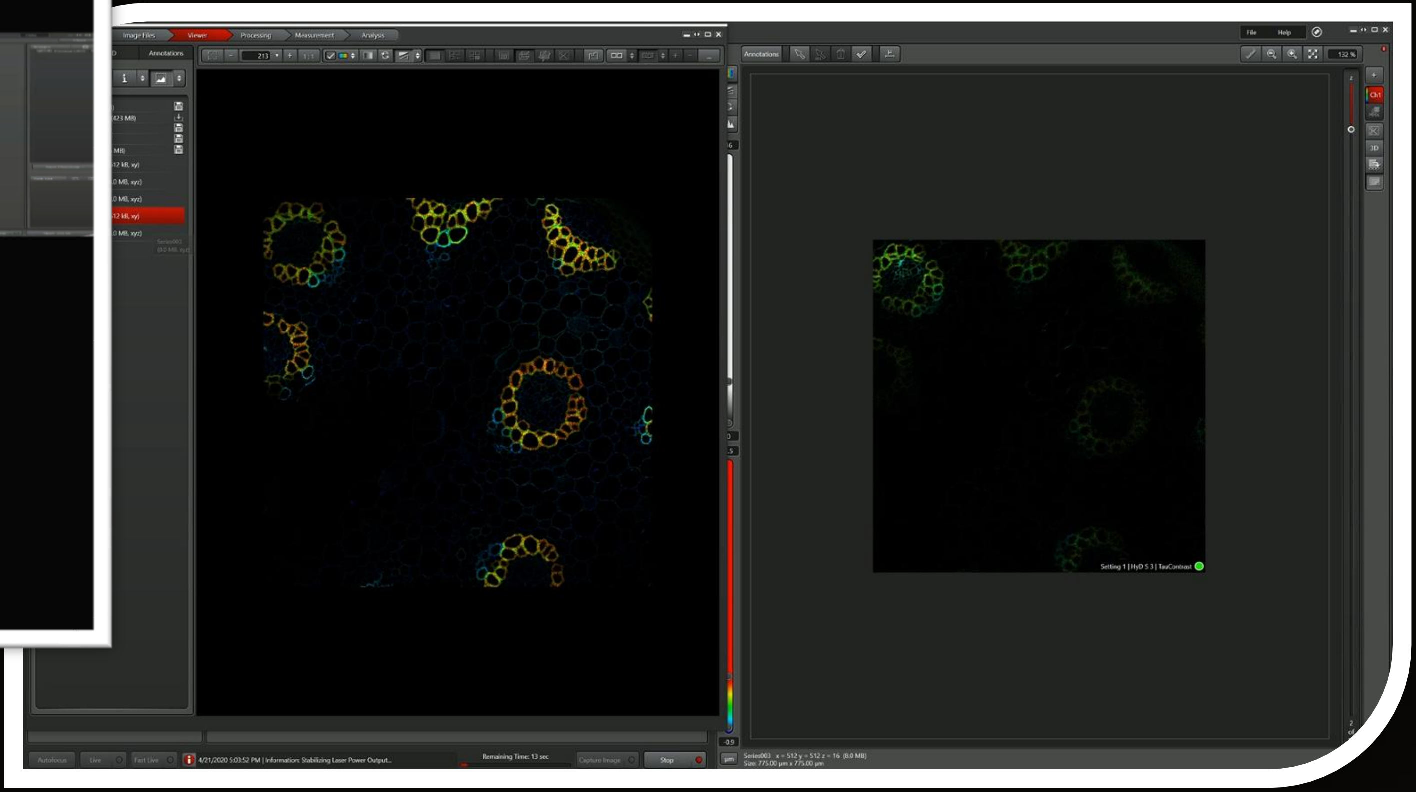
Navigator and 3D Viewer With TauSense





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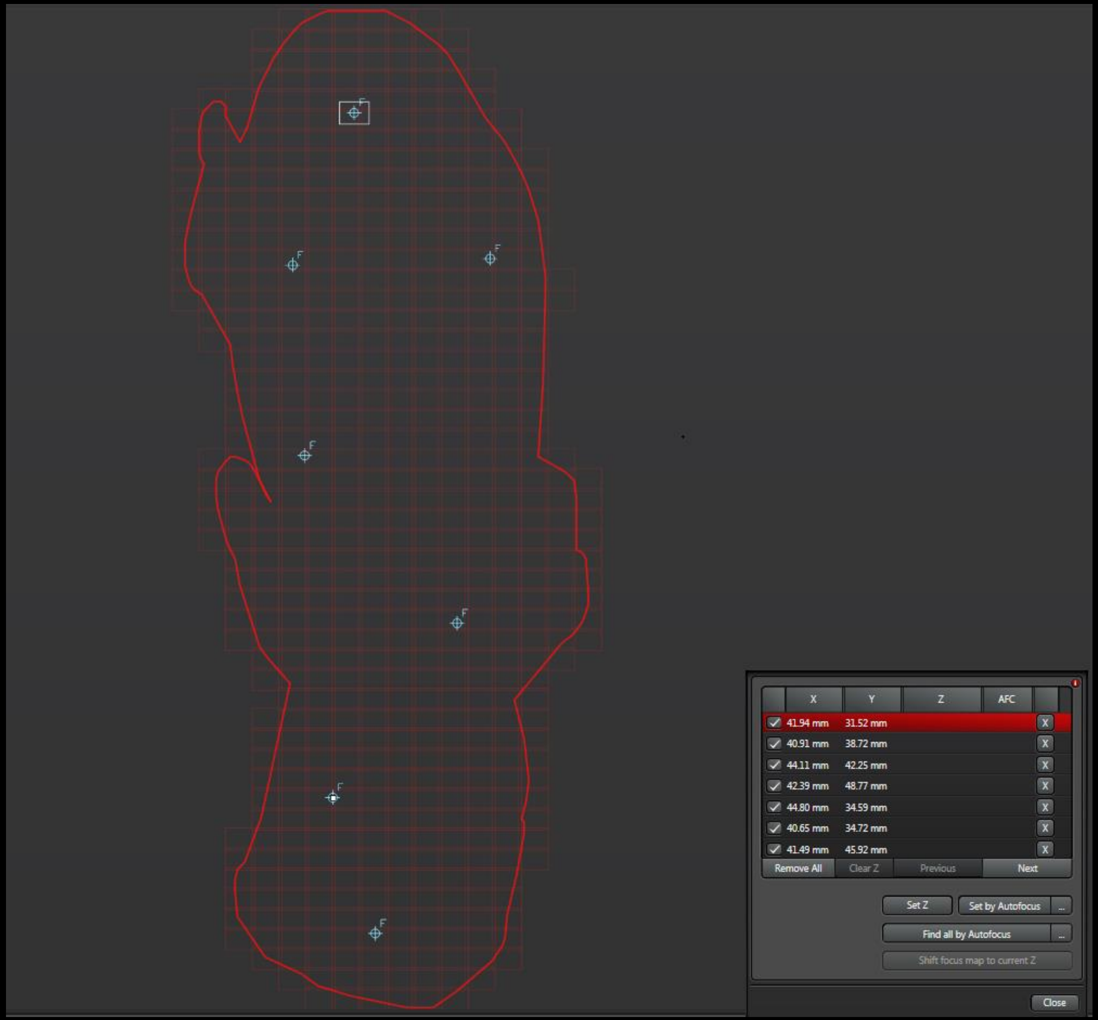
Define Regions And Stay In Focus

- Focus map to keep sample in focus
- Use software based autofocus or Adaptive Focus Control (AFC) to compensate for drift



What do we have in IPMB ?

STELLARIS | 8

Gain more knowledge about the specimenfrom different angles

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DMi 8 Inverted Microscope

Fully Auotomatic:

Filter cubes selection

DAPI 、CFP、GFP 、YFP、Rhodamine

Objectives

Scanning Stage :

For Multiposition experiments

AFC : Adaptive Focus Control
For longterm timelapse experiments

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Objectives

Objectives	DRY/IMM	Sample Types
HC PL APO 10x/0.4 CS2	DRY	0.17 mm cover glass
HC PLAN APO 20x/0.75 IMM CS2	WaterGlycerin/Oil	0.17 mm cover glass
HC PLAN APO 40x/1.10 W CS2	Water	0.17 mm cover glass
HC PL APO 63x/1.2 W CS2	Water	0.17 mm cover glass

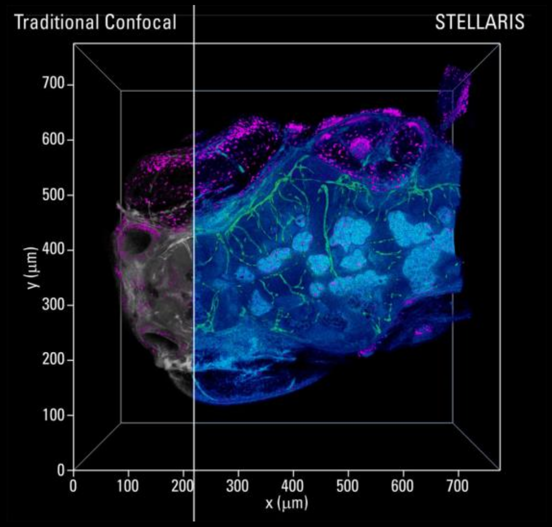
Lasers

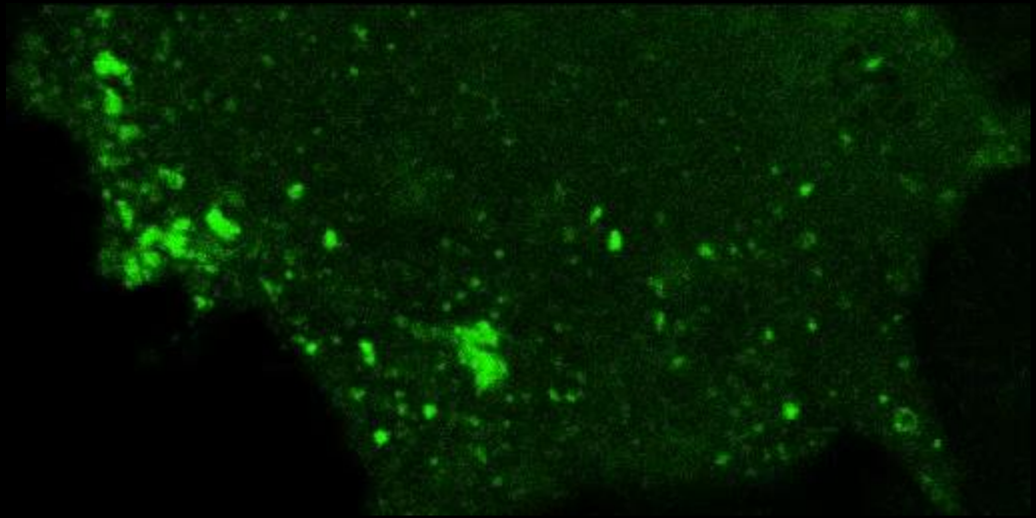
	Wavelength	Fluorochrome
Violet	405nm	DAPI,Hoechst, BFP···
White Light Laser	440-790nm tunable	Visible to NIR

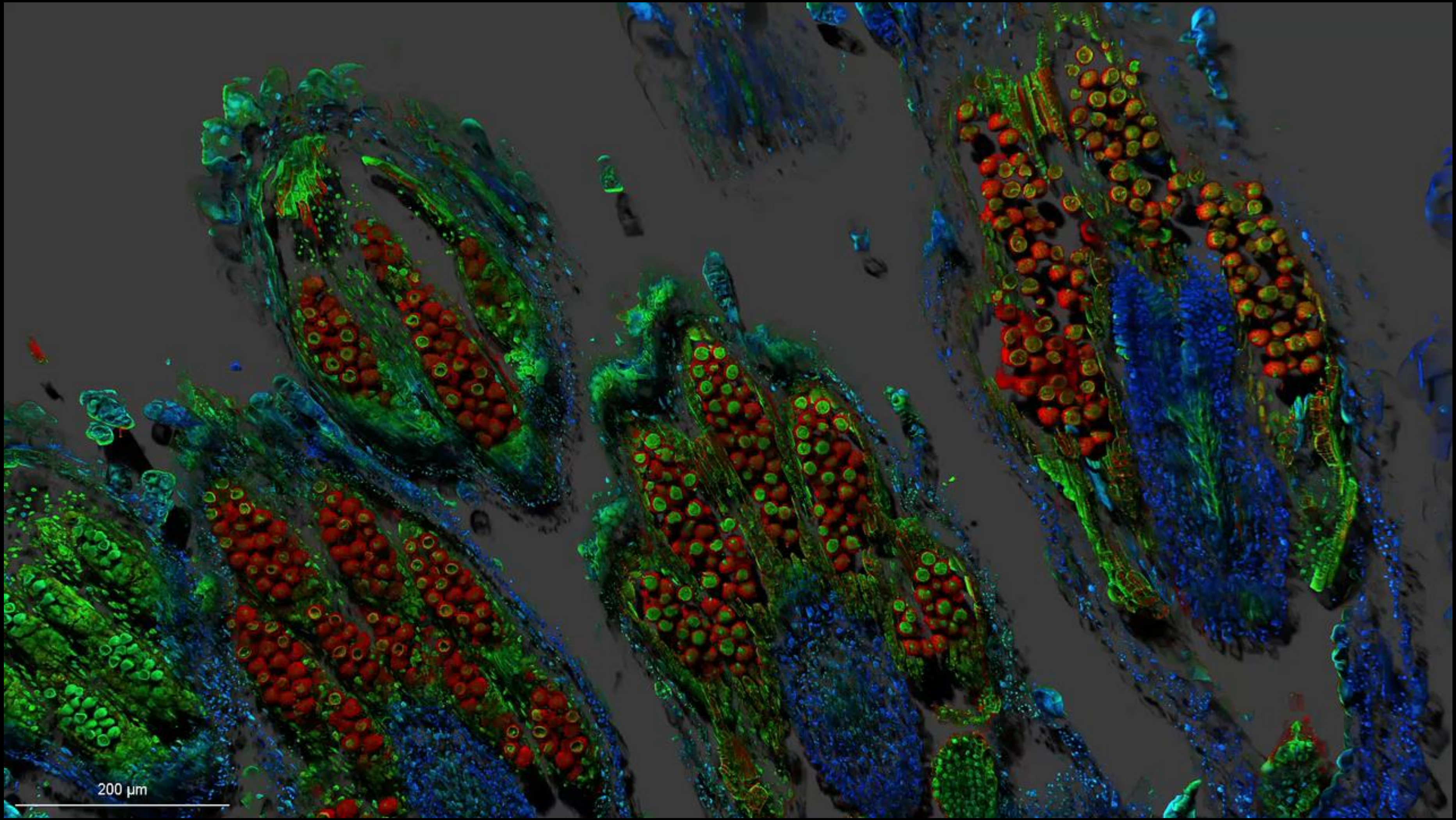
Detectors

Power HyD S	3 HyD S
Power HyD X	Upgradable
Power HyD R	Upgradable

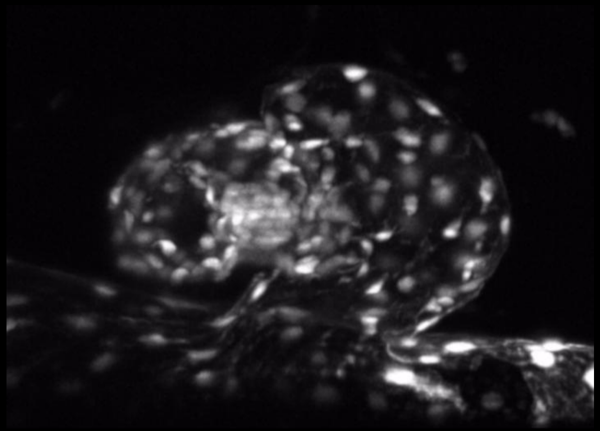
Software	
Tau sense	Tau gating Tau contrast Tau seperation
Navigator	
Image compass	
Lightning	
FALCON	

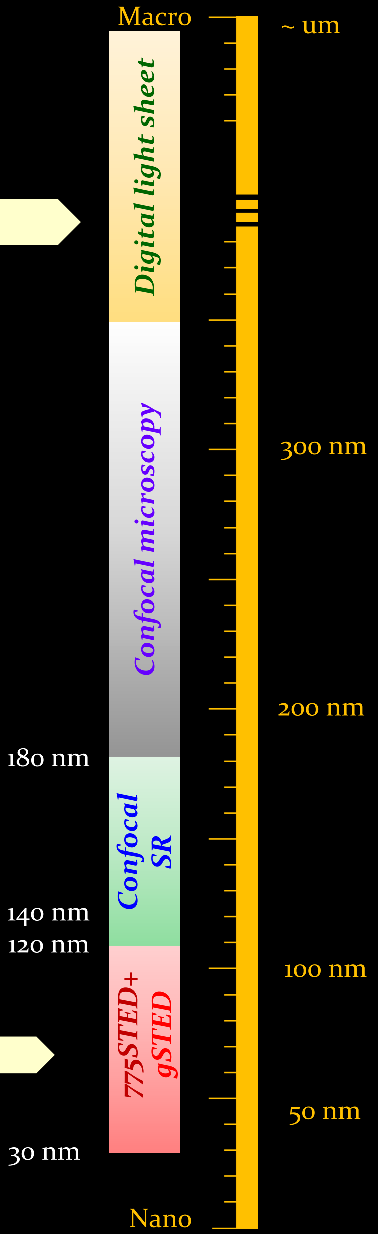


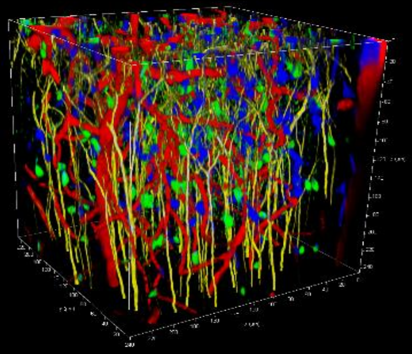



200 µm

Stellaris Platform – Ready to grow



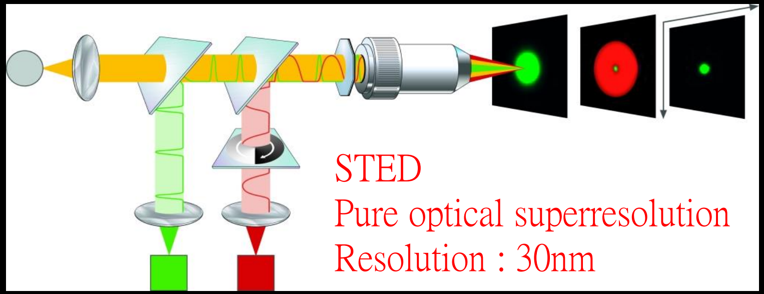




DIVE
Depth FREEDOM

LIGHTNING
New Confocal SR

Leica Stellaris 8 Platform



STELLARIS

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