

# STELLARIS

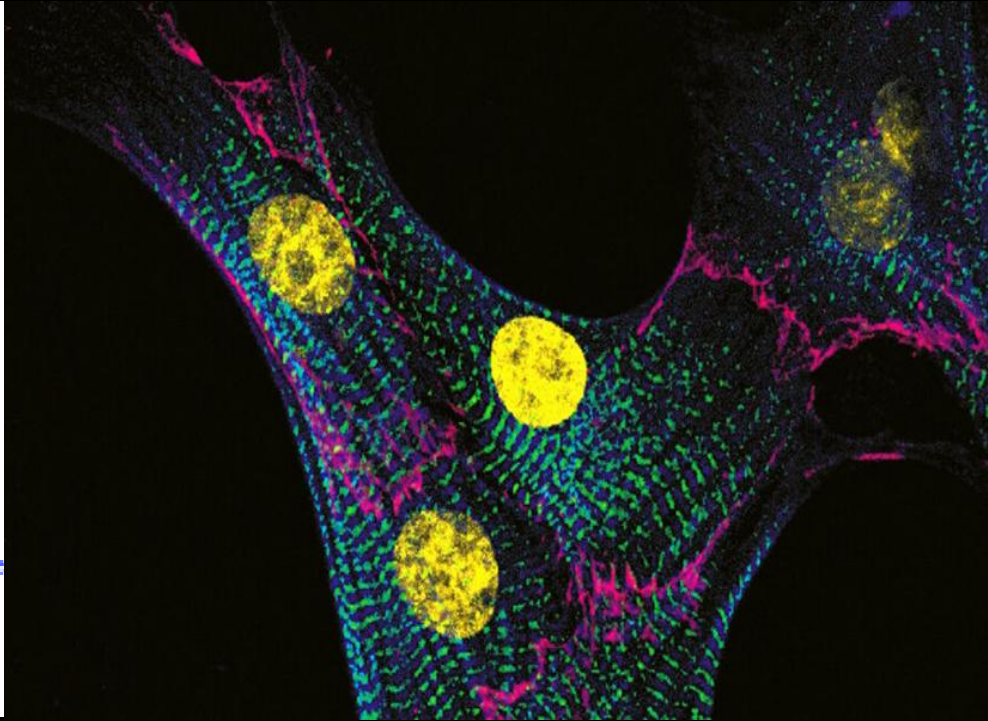
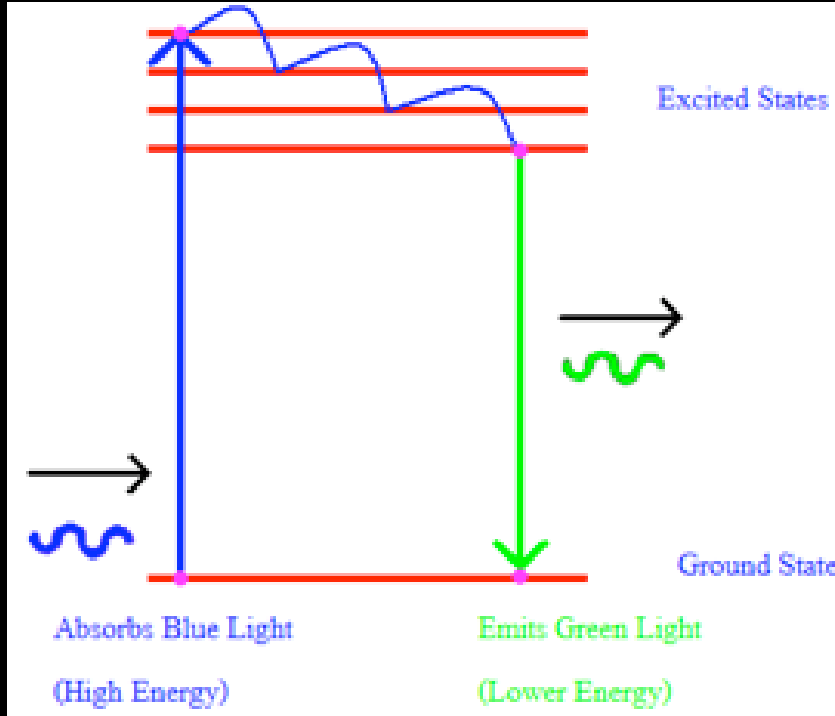
Leica New Confocal Platform



劉思嫻  
美嘉儀器股份有限公司  
[www.major.com.tw](http://www.major.com.tw)

## Confocal Image

## Fluorescence

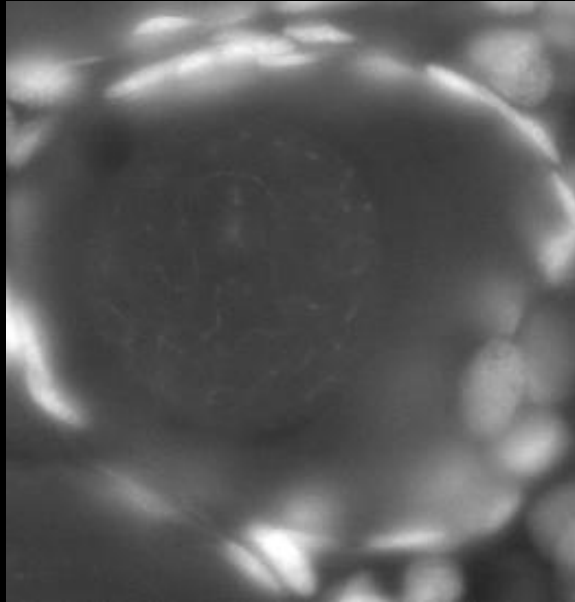


Neonatal cardiac myocyte (muscle cell)  
yellow (DAPI) indicates DNA,  
green (Cy3) myomesin,  
red (Texas Red) Cadherin,  
blue (Alexa 633) actin

**Fluorescence Microscope**



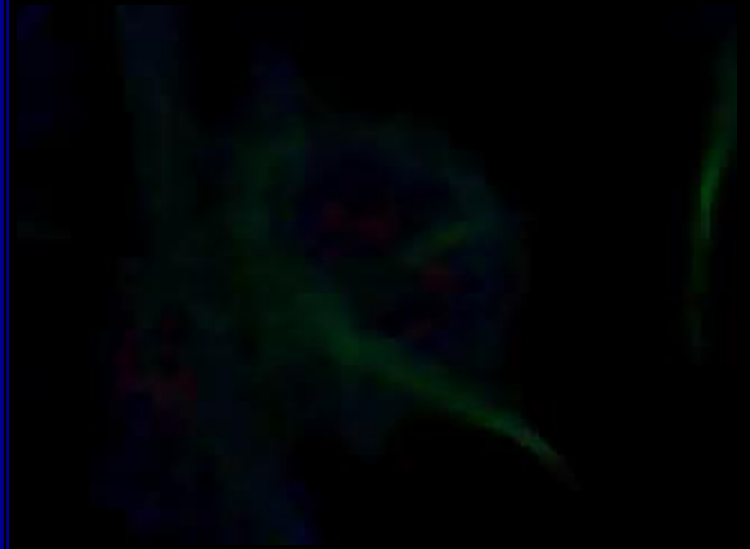
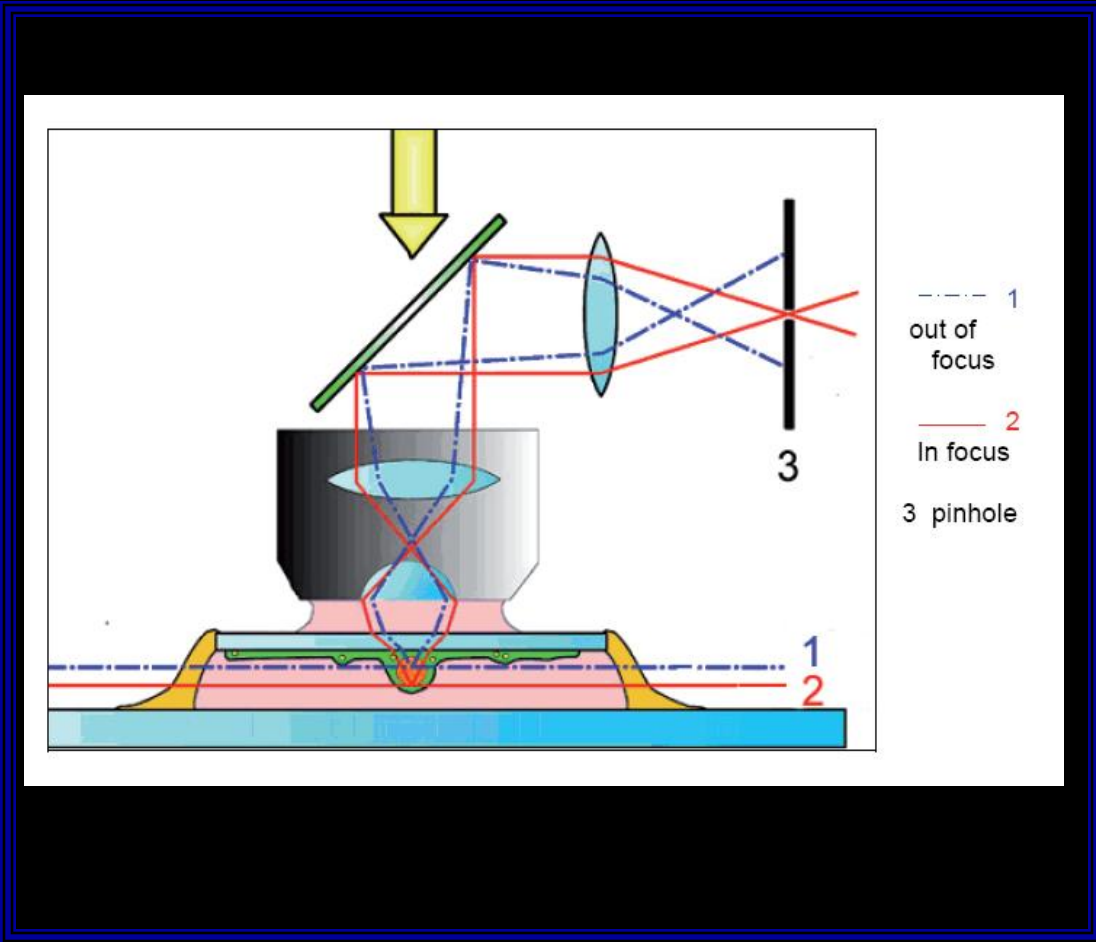
**Confocal System**



**Confocal Microscopes can optically remove all information that is from outside the depth of focus.**

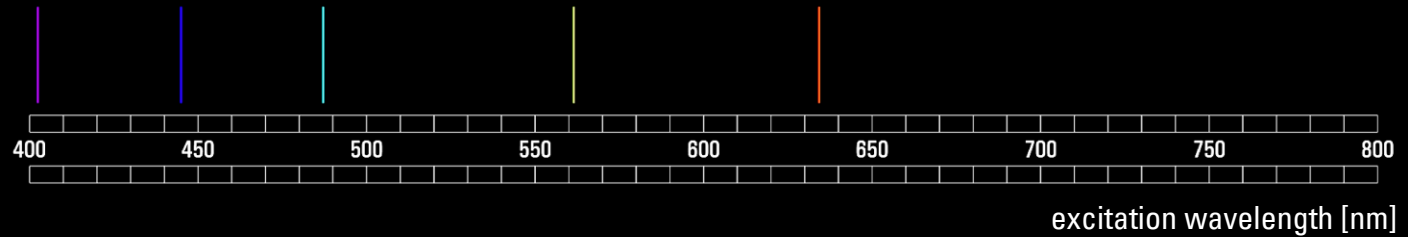
**The consequence is a sharp optical section.**

# Conventional Microscope → Confocal ?

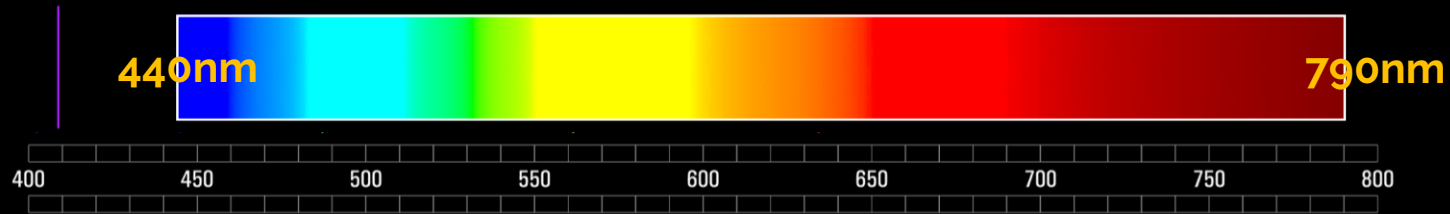


# The Second Key Innovation: The next-generation White Light Lasers

## Traditional Confocal



## STELLARIS 8



- > A single laser to do the work of many.  
Up to 8 single excitation lines  
from 440 nm to 790 nm can be used simultaneously
- > New optics design: detection range from 410nm to 850nm

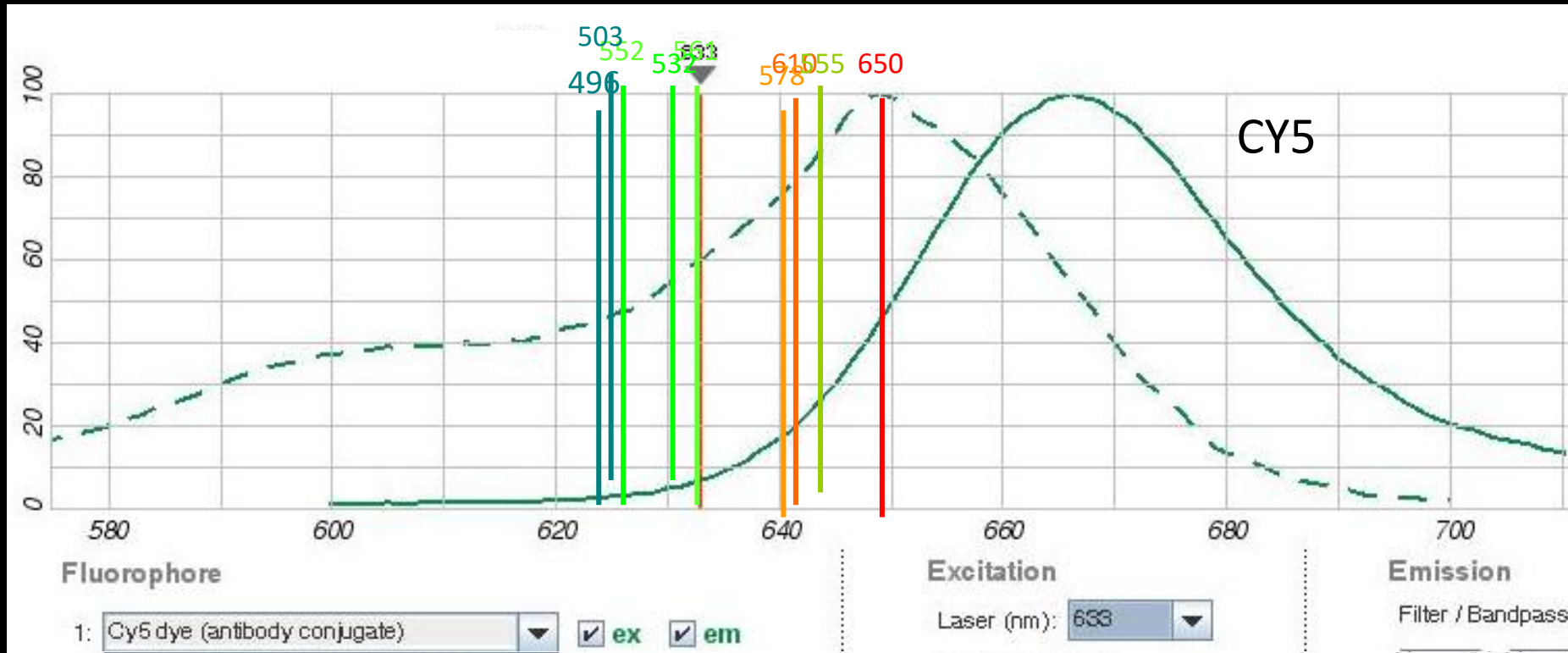
## Traditional Excitation wavelength

458nm, 476nm, 488nm, 496nm, 514nm, 543nm, 561nm, 594nm, 633nm

## LEICA STELLARIS Confocal Microscope

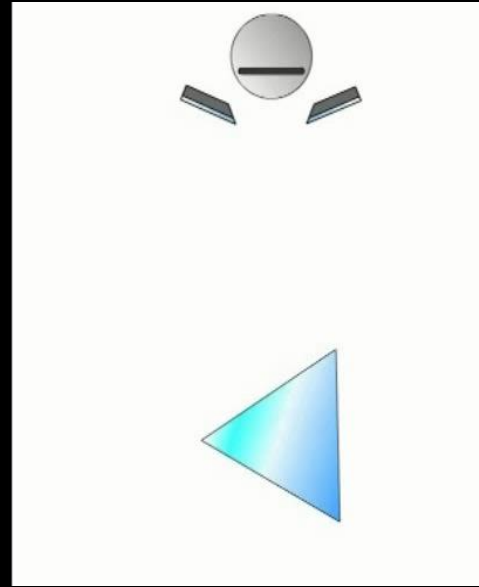
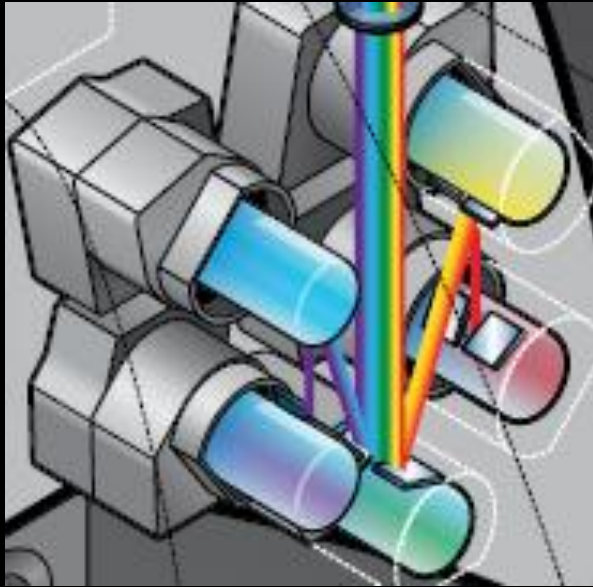
### Excitation wavelength

440nm - 790nm, 351 ex. Lines, no limitation



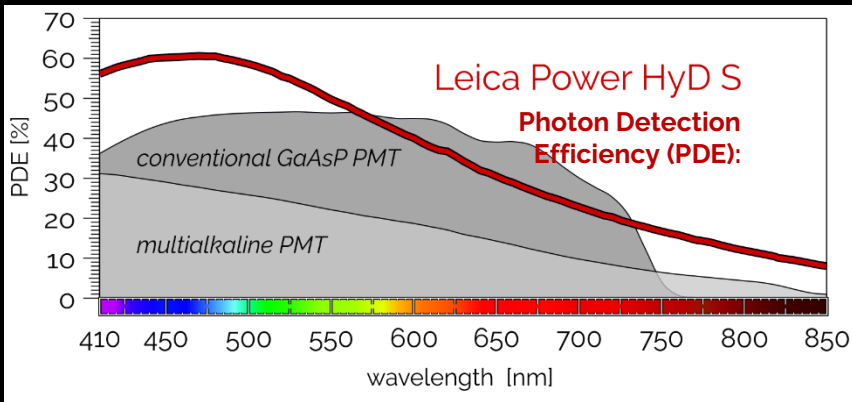


# Spectral Imaging Detector -- Fit For Purpose

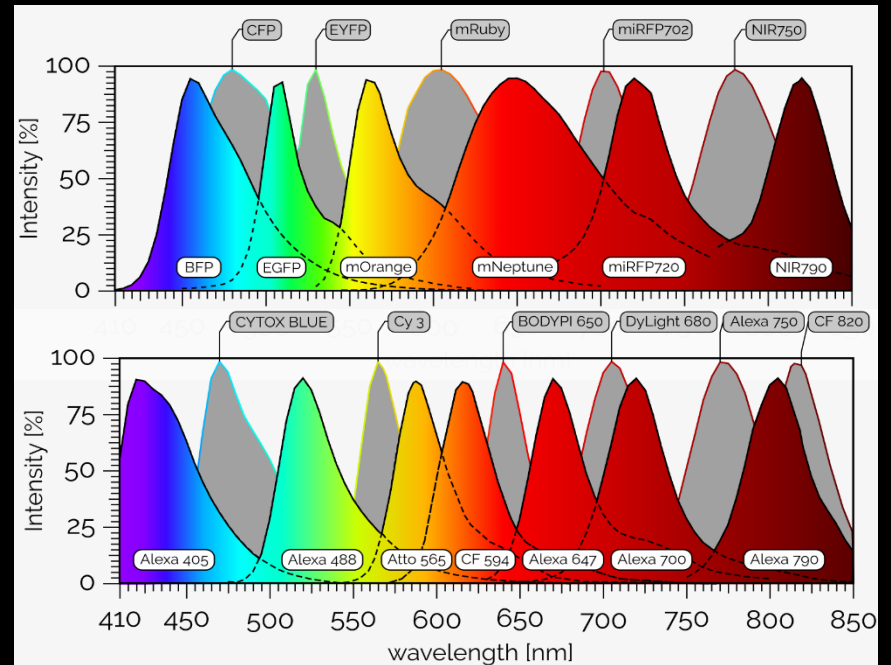


# Enhanced Spectral Freedom: STELLARIS

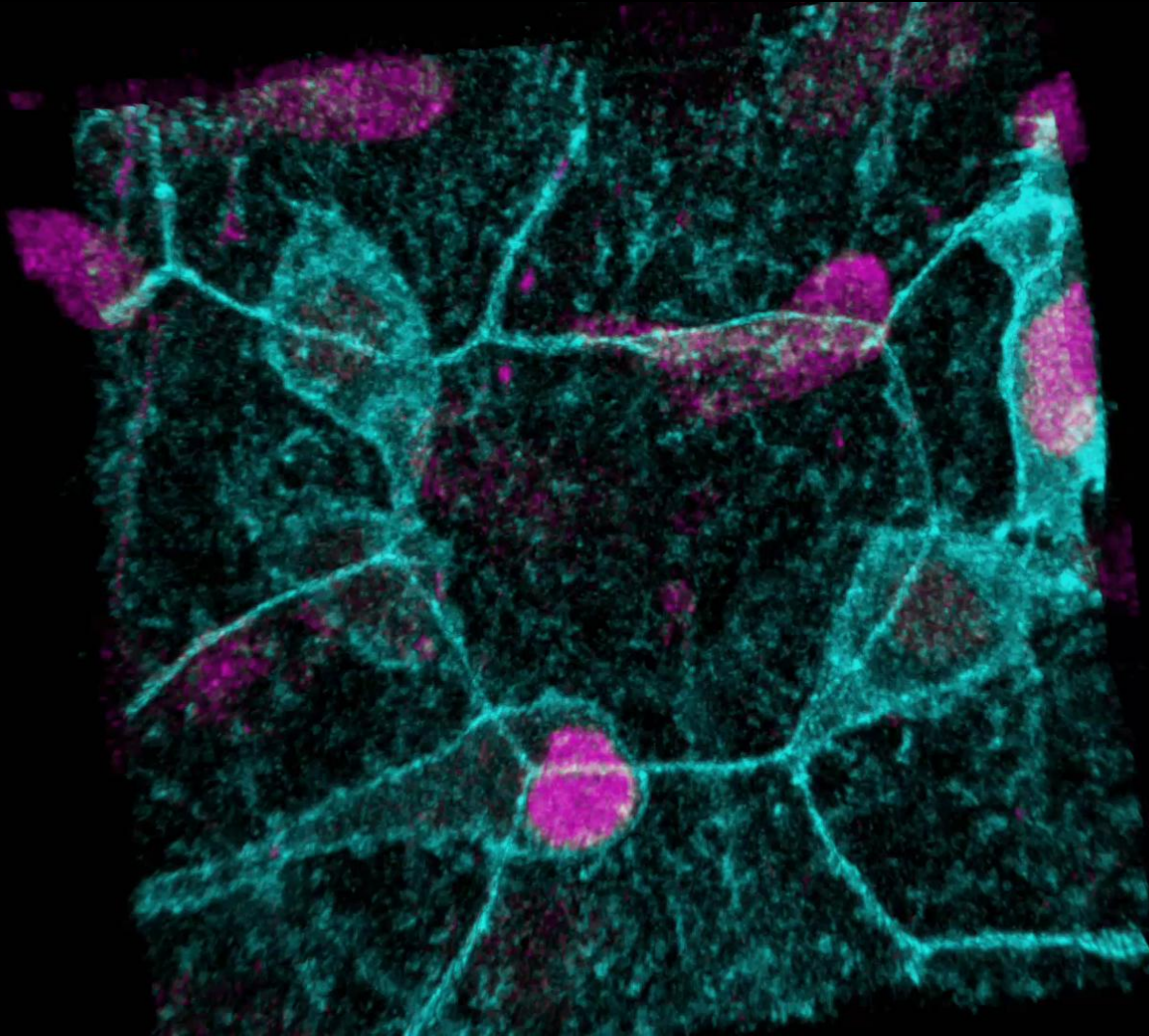
## The Power HyD S Is The New All-rounder Detector For Confocal Applications



- No more PMT or GaAsP detectors
- Detection range: 410nm-----850nm  
(normal confocal: 400nm-750nm)



# Gentle Live-Cell Imaging



- Perform imaging for longer periods, since both excitation as well as detection are optimally tuned
- Preserve sample integrity with efficient signal acquisition, at the lowest needed power

Zebrafish posterior lateral line primordium migration.

Cyan: Membranes, GFP, Magenta: Nuclei, tdTomato

Sample Courtesy: Jonas Hartmann, Gilmour Group, EMBL Heidelberg.

# The Red Extended Benefits Of Our Next Generation WLLs

- > Enhance multiplexing capabilities by adding up to 3 more fluorophores in the NIR range

## Some >685 nm excitable dyes:

---

ATTO 740

ATTO 700

CF680

CellBrite NIR750

Alexa 750

CellBrite NIR680

CF700

CF750

MitoView720

CellBrite NIR770

BioTracker NIR750

Alexa 680

Alexa 700

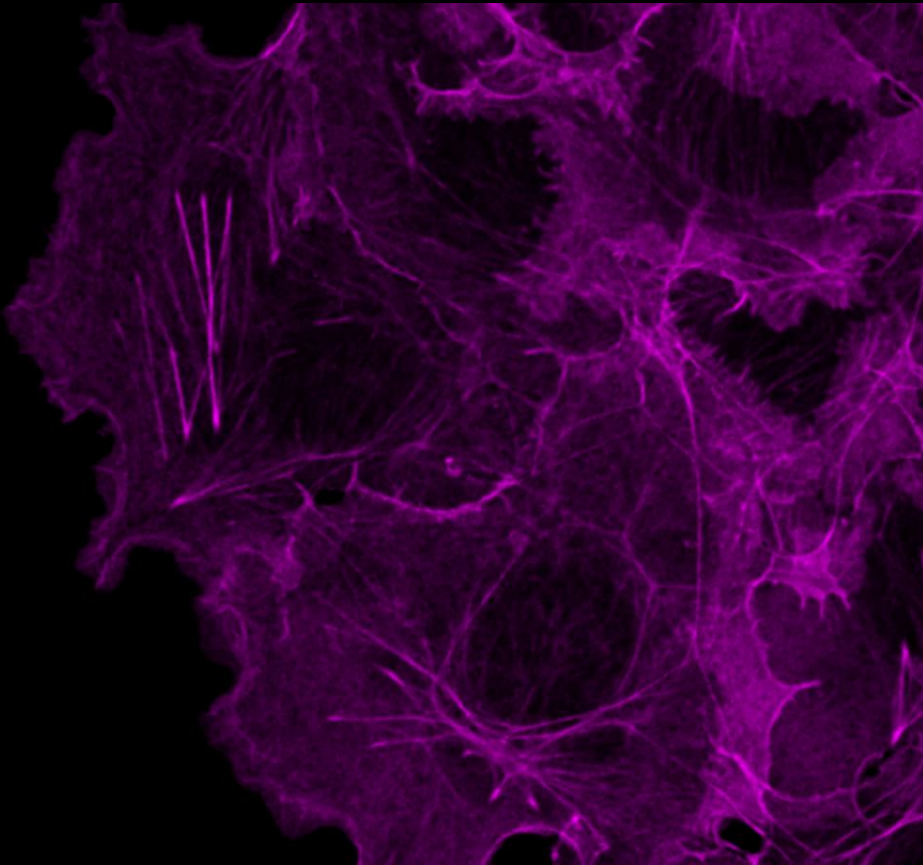
ATTO 680

CellBrite NIR700

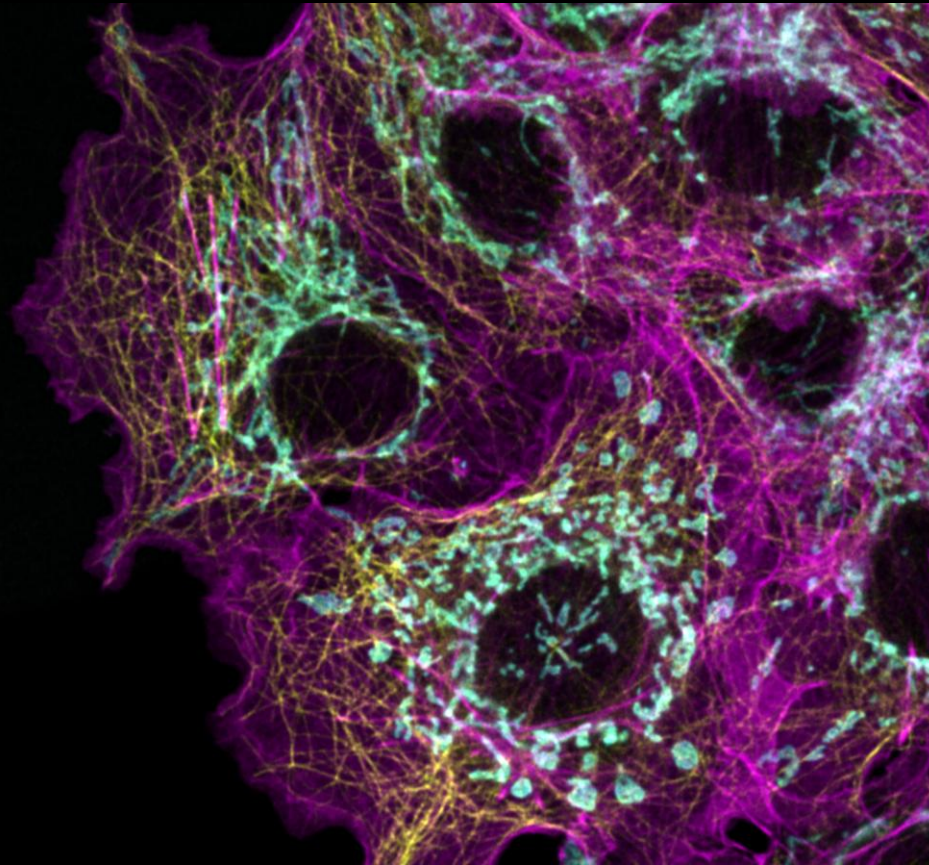
ATTO 725

# STELLARIS Delivers Expanded Multicolor Flexibility

Traditional Confocal



STELLARIS

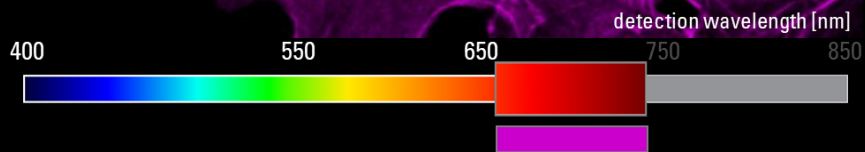
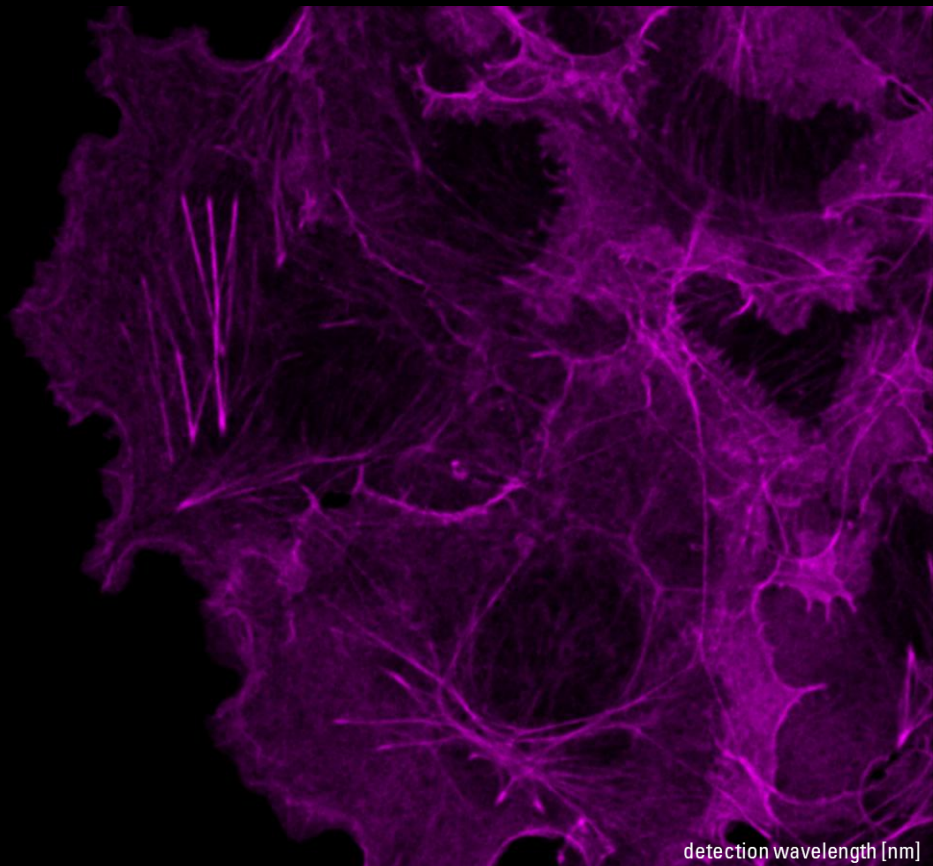


COS7 cells. Actin (magenta, SiR-Actin 657-740 nm), Mitochondria (cyan, AF750 760-790 nm), Microtubules (yellow, AF790 810-850 nm)  
Sample Courtesy: Jana Döhner, Urs Ziegler, University of Zurich

*Leica*

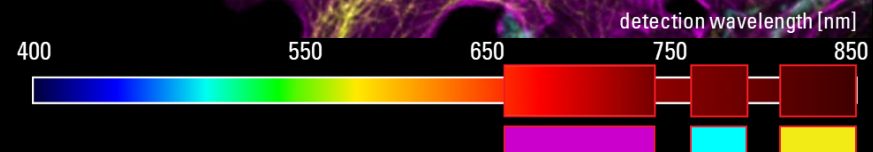
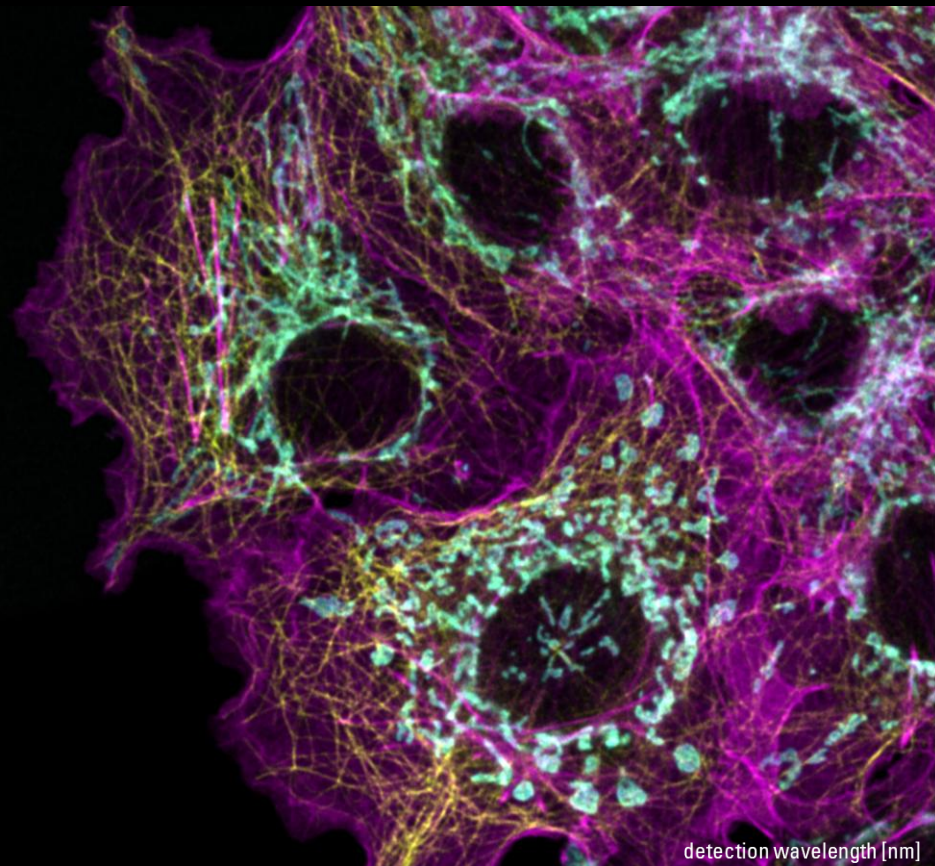
# STELLARIS Delivers Expanded Multicolor Flexibility

## Traditional Confocal



1 channel

## STELLARIS



3 channels extended to NIR

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

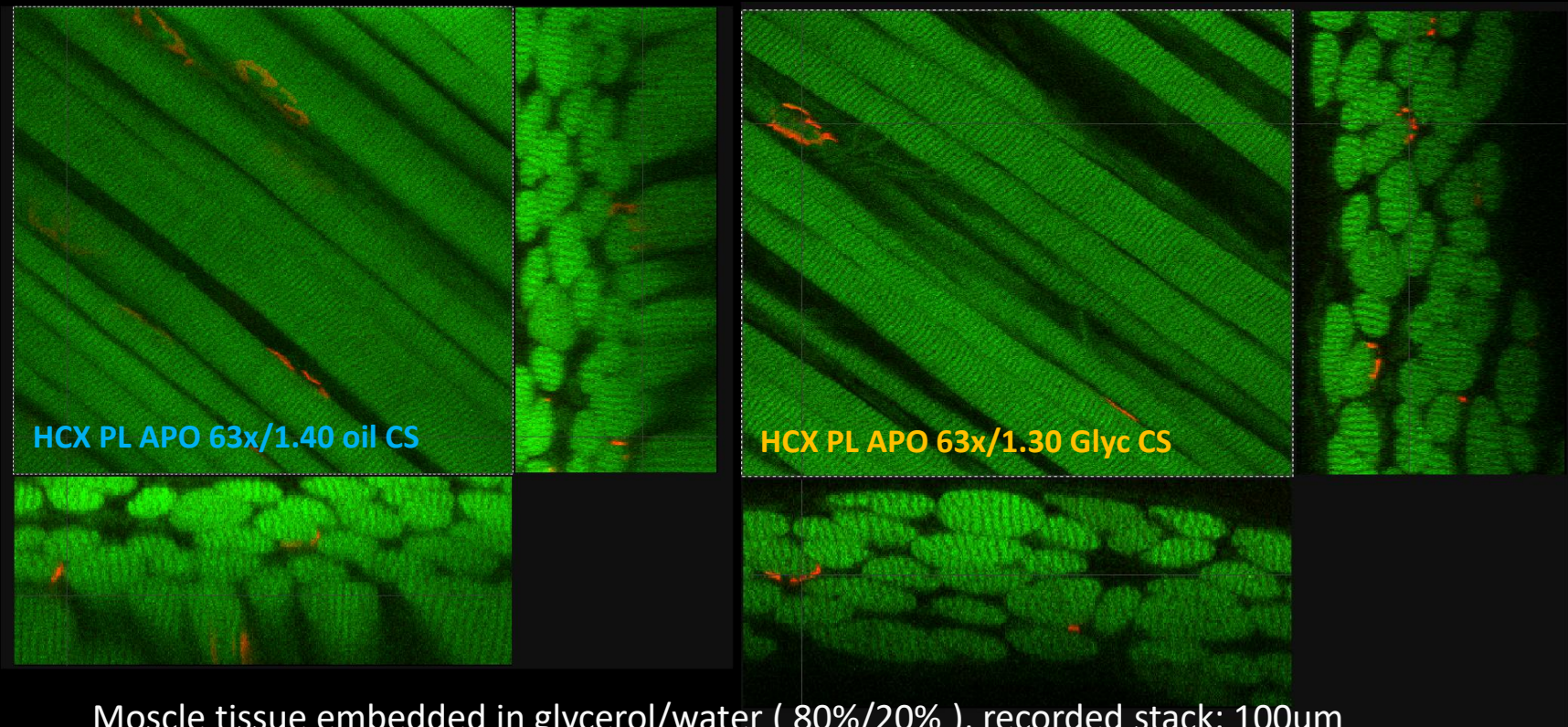
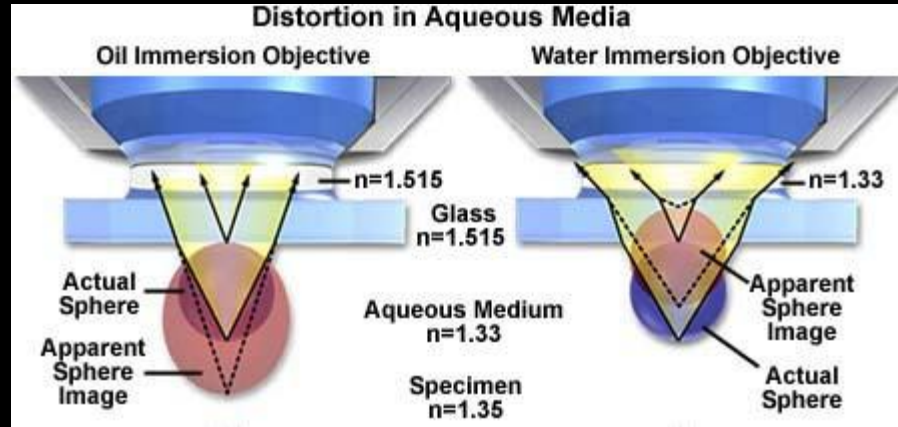
*Leica*

20X/0.75 IMM  
Water, Glyc, Oil

40X/1.25 Glyc

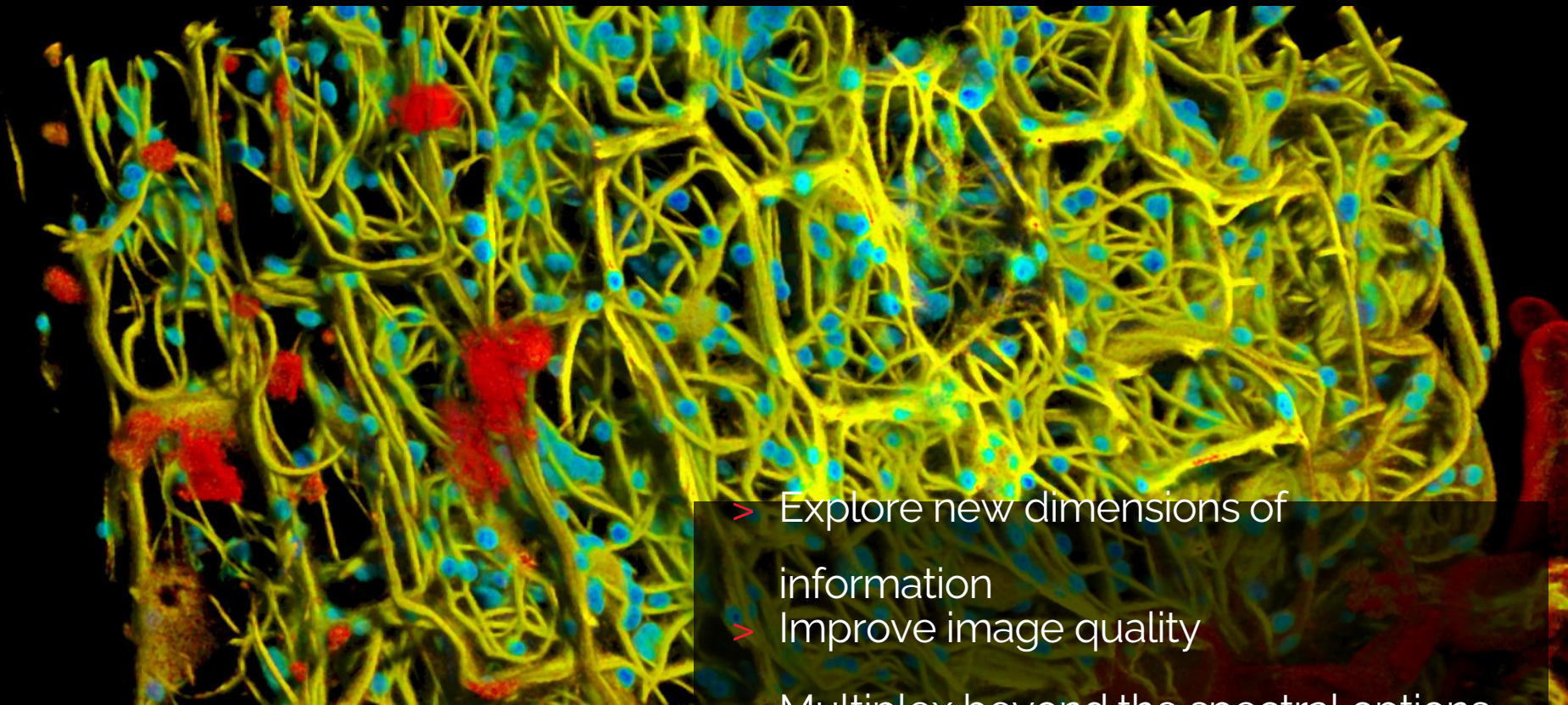


# - Reflective Index Match -



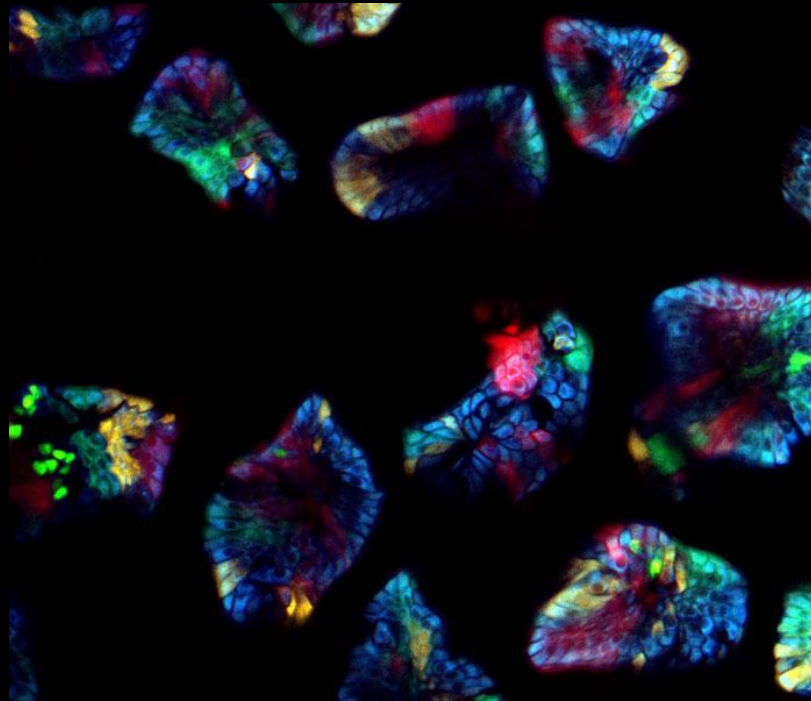
# POTENTIAL

DISCOVER MORE



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

# Fluorescence imaging focus on spectral contrast....



Confetti Mouse Small Intestine. CFP, GFP, YFP and RFP. Acquired with SP8 DIVE  
Sample courtesy of Jacco van Rheenen,  
University of Utrecht, the Netherlands

... fluorescence contains much more information

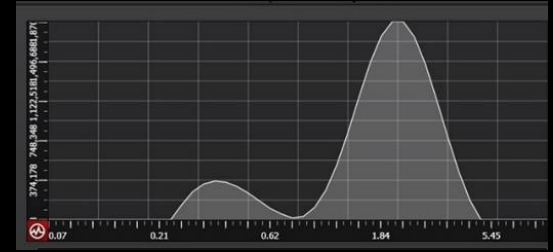
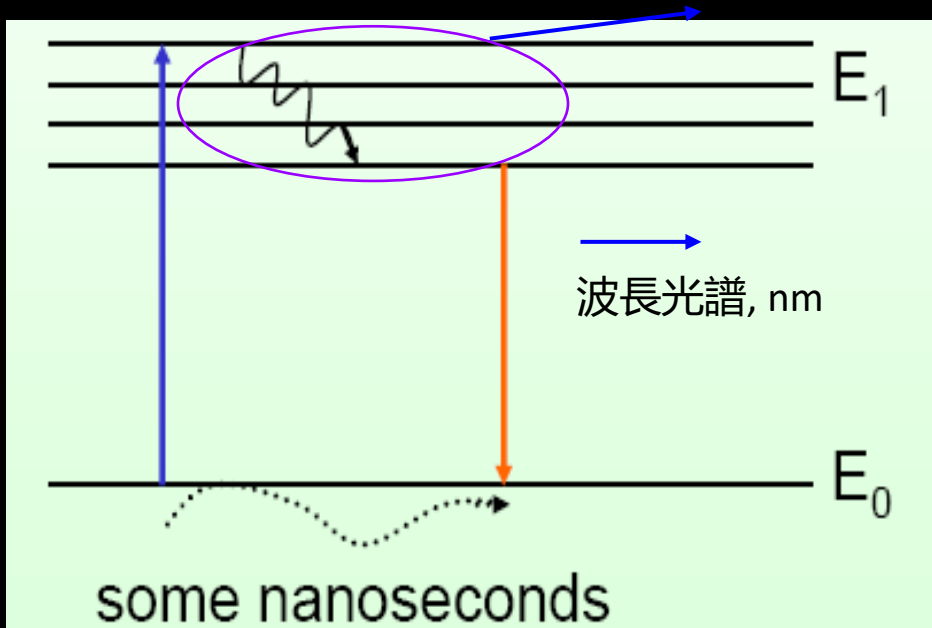
# 時脈頻譜(TauSense)技術的應用概念來自螢光生命週期影像技術

## Fluorescence Lifetime Imaging Microscopy (FLIM)

### Fluorescence lifetime

Average time that molecules stay in their excited state

生命週期 (時脈頻譜, ns)



Fluorophore	Ex. Max.	(X) Em. Max.
AF 488	494nm	519nm
GFP	498nm	516nm

Fluorophore	Ex. Max.	Em. Max.
Fluo3 w. Ca2+	490nm	520nm
Fluo3 w/o Ca2+	490nm	520nm

## TauSense™

- > Set of tools based on **fluorescence lifetime**



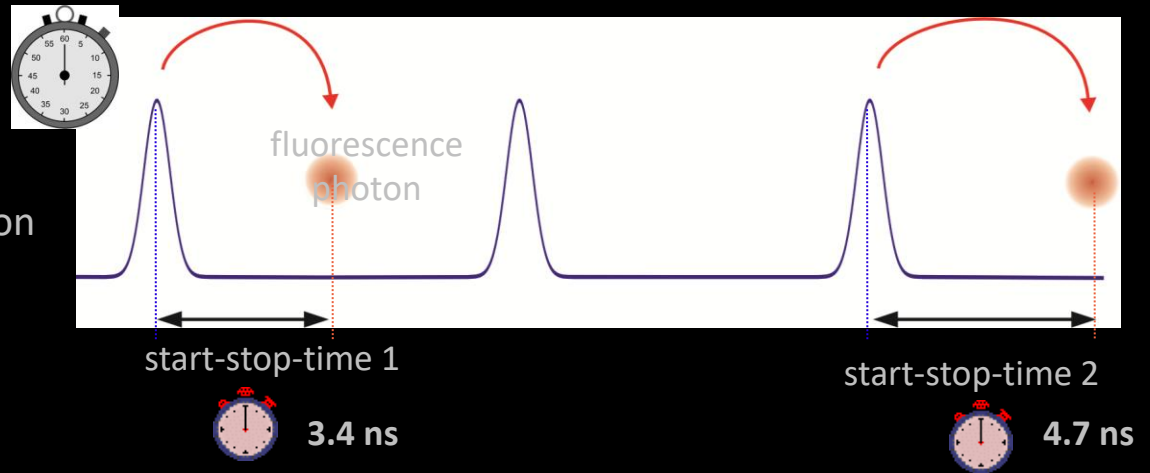
Leica STELLARIS首度將時脈頻譜  
技術 TauSense technology 整合於  
共軛焦顯微平台上

# How to Measure the Time?

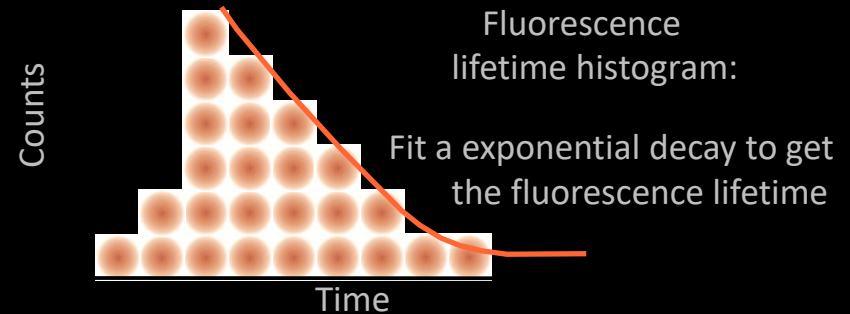
In principle with a stop watch:

1. Start the clock with a laser pulse.
2. Stop the clock with the first photon that arrives at the detector.
3. Reset the clock and wait for next start signal.

It is a statistical process!

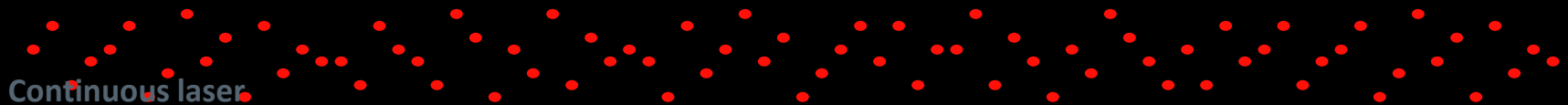


- Repeat this time measurement very often and count “how many photons have arrived after what time”
- Sort the photons within a histogram into time bins according to their arrival times

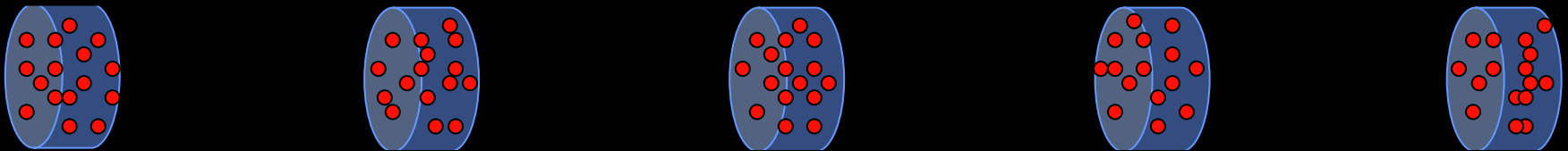


# What we need for TauSense or FLIM?

- A pulsed laser



Leica STELLARIS white light laser is pulsed laser 440nm-790nm



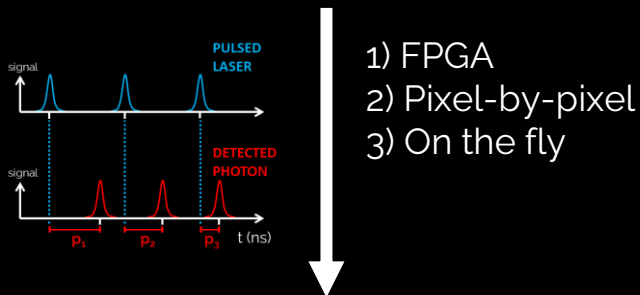
Pulsed laser

- Photon counting detector

-- Leica STELLARIS power HyD detectors are all photon counting detectors  
Normal PMT or GaAsP is not suitable for photon counting

# The Technology Behind TauSense

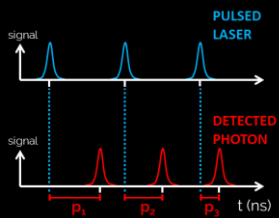
- > Fluorescence Intensity ( $N_{\text{photons}}$ )
- > Photon Arrival Time (ns)



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

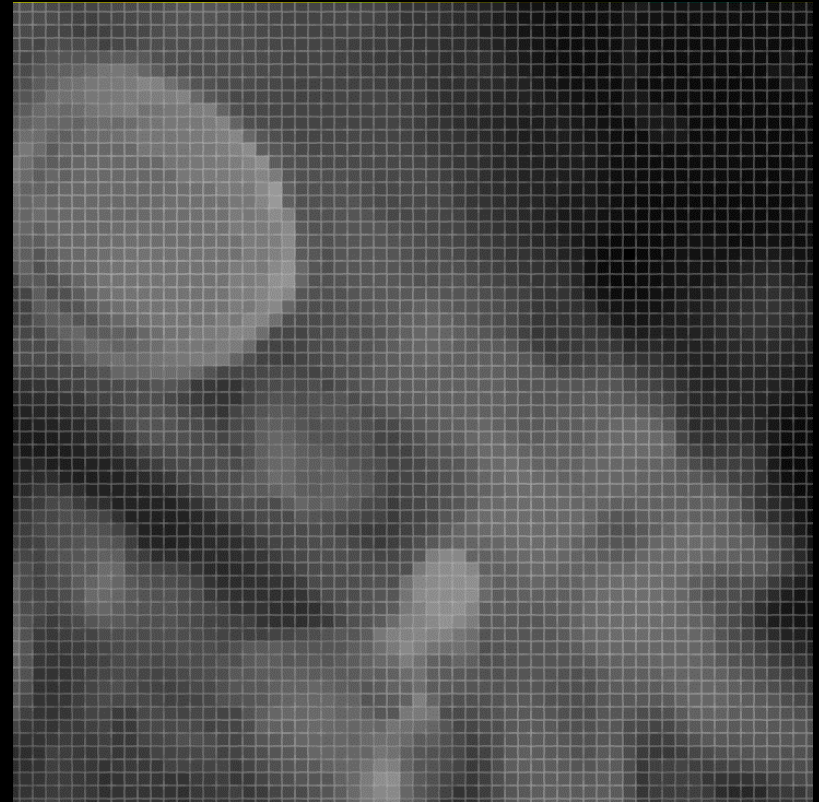
# The Technology Behind TauSense

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



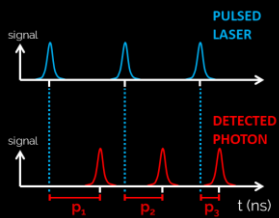
- 1) FPGA
- 2) Pixel-by-pixel
- 3) On the fly

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



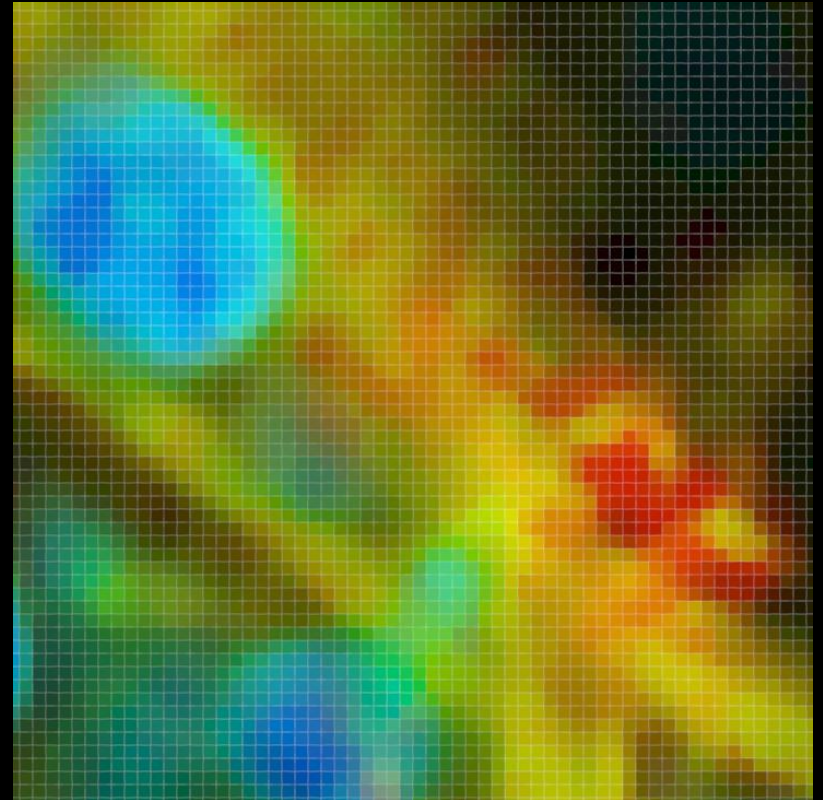
# The Technology Behind TauSense

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



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

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



## Alexa Dyes

Alexa Fluor Dye *	Ex (nm)	Em (nm)	$\tau$ (ns) ‡
Alexa Fluor 488	496	519	4.1 §
Alexa Fluor 532	532	553	2.5
Alexa Fluor 546	556	573	4.1
Alexa Fluor 555	555	565	0.3
Alexa Fluor 568	578	603	3.6 §
Alexa Fluor 594	590	617	3.9 §
Alexa Fluor 647	650	665	1.0
Alexa Fluor 660	663	690	1.2 **
Alexa Fluor 680	679	702	1.2
Alexa Fluor 700	702	723	1.0
Alexa Fluor 750	749	775	0.7

Measurements were made on free succinimidyl ester derivatives in aqueous solutions. † For Alexa Fluor 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594 and Alexa Fluor 647 dyes, QY measurements were made in PBS (50 mM potassium phosphate, 150 mM NaCl, pH 7.2) at 22°C relative to fluorescein in 0.01 M NaOH (QY = 0.92). For Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700 and Alexa Fluor 750 dyes, QY measurements were made in PBS (50 mM potassium phosphate, 150 mM NaCl, pH 7.2) at 22°C relative to Alexa Fluor 647 succinimidyl ester in PBS (QY = 0.33). ‡ Except for the footnoted values, lifetime measurements were made in water at 22°C, data provided by ISS Inc. (Champaign, IL). § Lifetime measurements were provided by the SPEX Fluorescence Group, Horiba Jobin Yvon Inc. \*\* Lifetime measurement was made in pH 7.5 buffer at 20°C by Pierre-Alain Muller, Max Planck Institute for Biophysical Chemistry, Göttingen.

## Atto Dyes

Dyes	Ex (nm)	Em (nm)	$\tau$ (ns)
Atto 465	453	508	2.2
Atto 488	501	523	3.2
Atto 495	495	527	2.4
Atto 514	511	533	3.0
Atto 520	516	538	3.8
Atto 532	532	553	3.8
Atto Rho6G	535	560	4.1
Atto 550	554	576	3.2
Atto 565	563	592	3.4
Atto Rho3B	565	592	1.5
Atto Rho11	571	595	4.0
Atto Rho12	576	601	4.0
Atto Thio12	579	609	2.0
Atto Rho101	586	610	4.2

Dyes	Ex(nm)	Em(nm)	$\tau$ (ns)
Atto 590	594	624	3.7
Atto 594	601	627	3.5
Atto Rho13	600	625	3.9
Atto 610	615	634	3.3
Atto 620	619	643	2.9
Atto Rho14	625	646	3.7
Atto 633	629	657	3.2
Atto 647	645	669	2.3
Atto 647N	644	669	3.4
Atto 655	663	684	1.9
Atto Oxa12	663	684	1.8
Atto 665	663	684	2.9
Atto 680	680	700	1.8
Atto 700	700	719	1.5
Atto 725	729	752	0.5
Atto 740	740	764	0.6

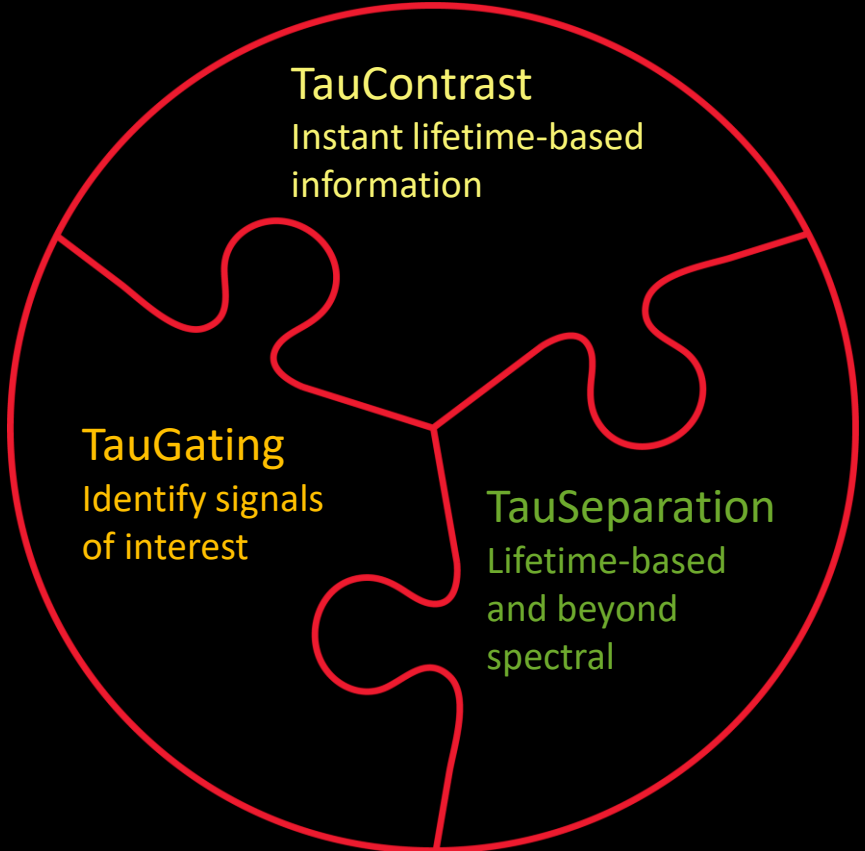


Dye	Ex (nm)	Em (nm)	$\tau$ (ns)
Cy3	548	562	0.3
Cy3.5	581	507	2.6
Cy5	646	664	1.0
Cy5.5	675	695	1.0
FITC	494	518	4.1
Oregon Green 488	493	520	4.1
Oregon Green 500	402	522	2.18
Rhodamine 6G	525	555	4.08
Rhodamin B	562	583	1.68
Texas Red	589	615	4.2
TOTO-1	514	533	2.2

## Fluorescent Protein

Fluorescent Protein	Ex (nm)	Em (nm)	$\tau$ (ns)
ECFP	434	477	3.0
EGFP	488	507	2.6
EYFP	513	527	3.1
mRuby	558	605	2.6
mScarlet	569	594	3.9
mCherry	587	610	1.4
mKate2	588	633	2.5

# STELLARIS: Ready to Discover with TauSense ( 時脈頻譜技術 )



ALL Leica STELLARIS series confocal with WLL offer TauSense technology (lifetime-based information) to discover more

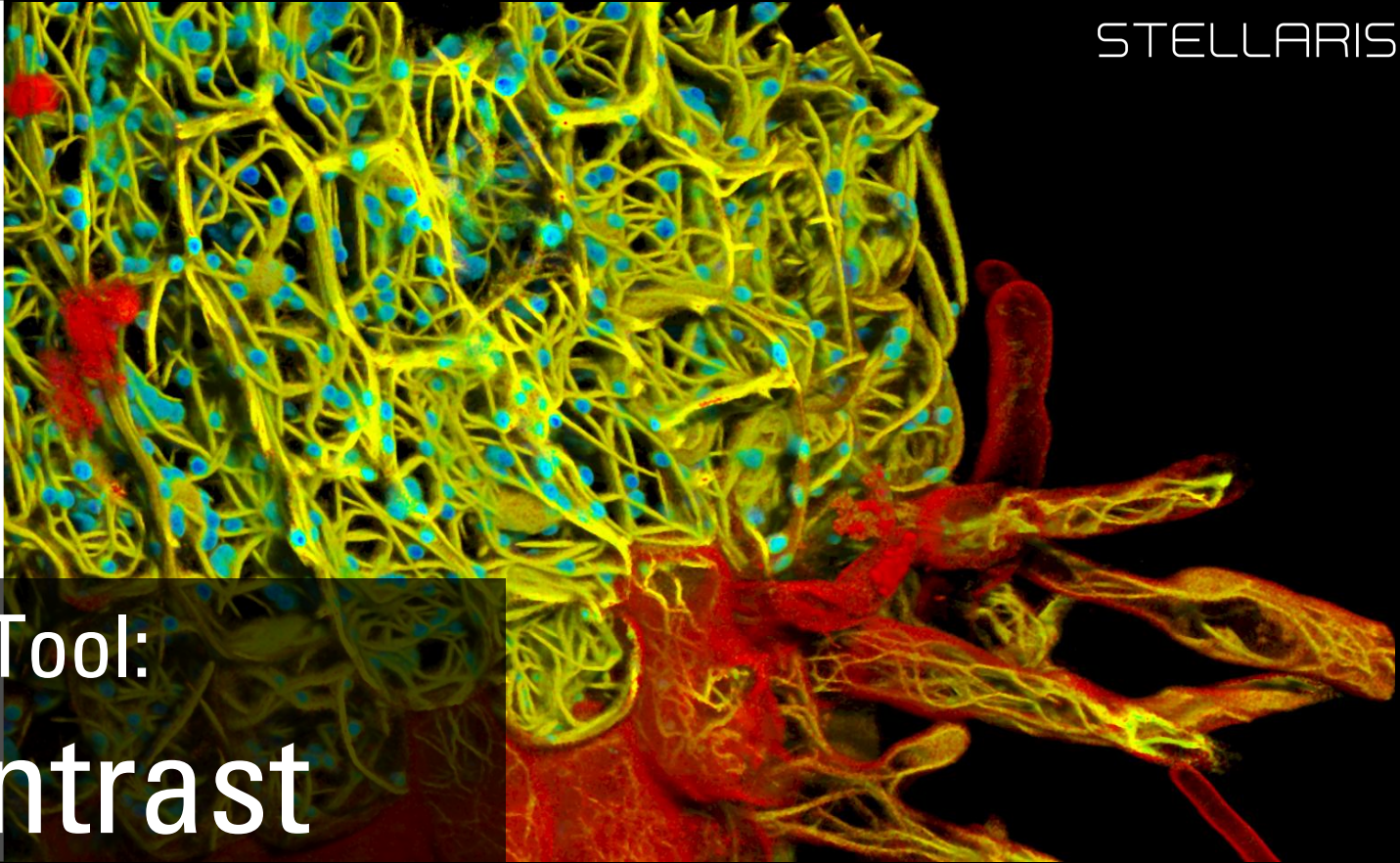


Explore A New Dimension Of Information

Traditional Confocal

STELLARIS

TauSense Tool:  
TauContrast



# Explore New Dimensions Of Information



Root-hypocotyl-junction of Arabidopsis thaliana (Era et al. Plant Cell Physiol., 2009). Chlorophyll, Life-Act Venus, IProp. Sample courtesy: Dr. Krebs, COS, University of Heidelberg.

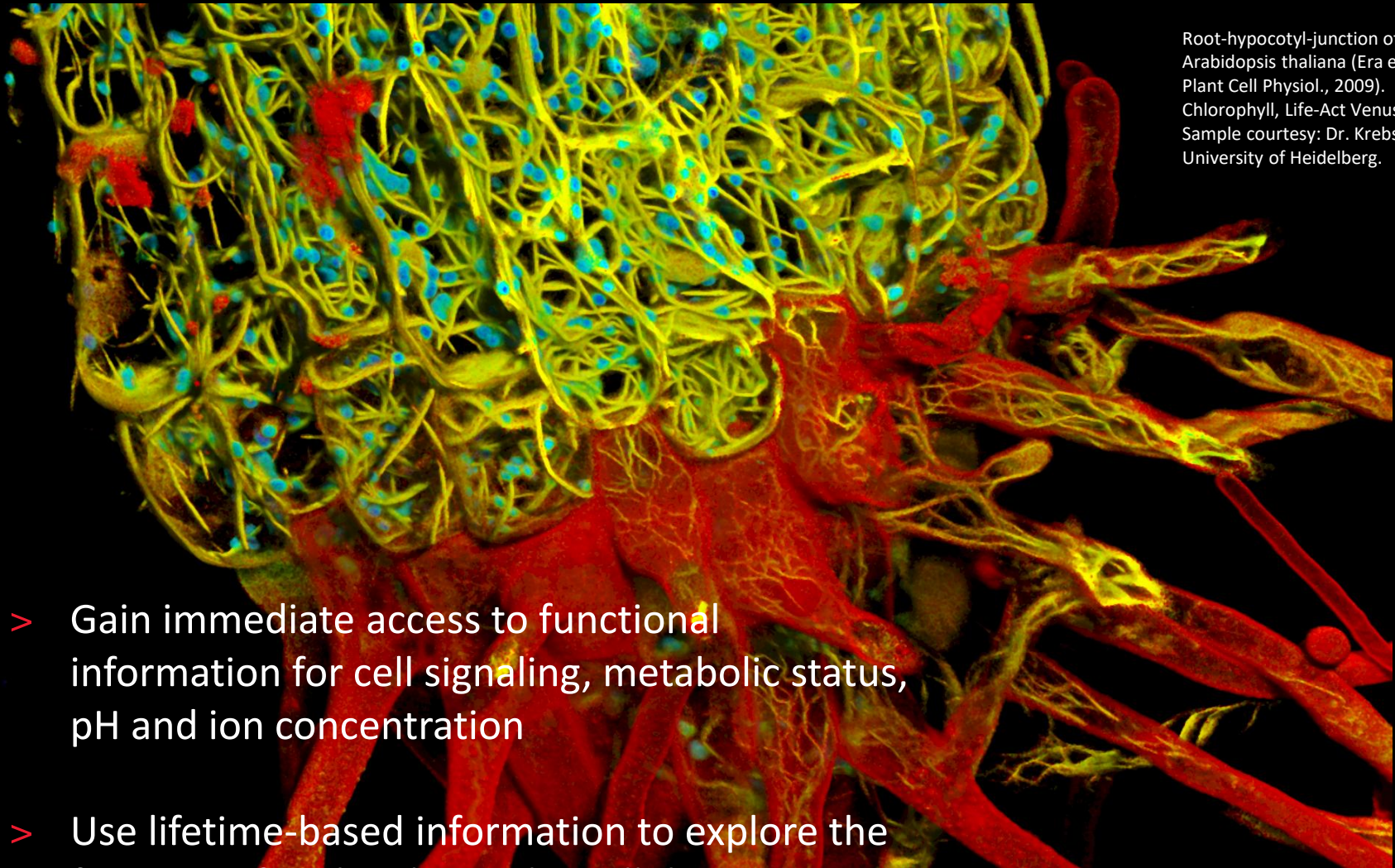
... go beyond today's limits

... see the unseen

... acquire more accurate and reliable data to prove your hypothesis



# Explore New Dimensions Of Information - TauContrast



Root-hypocotyl-junction of  
Arabidopsis thaliana (Era et al.  
Plant Cell Physiol., 2009).  
Chlorophyll, Life-Act Venus, IPProp.  
Sample courtesy: Dr. Krebs, COS,  
University of Heidelberg.

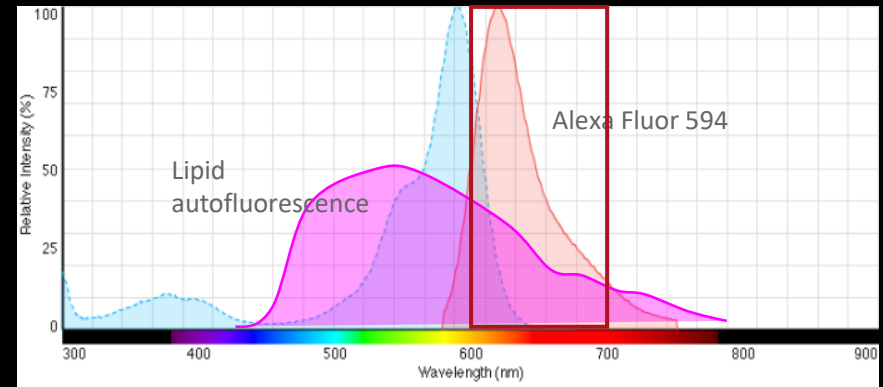
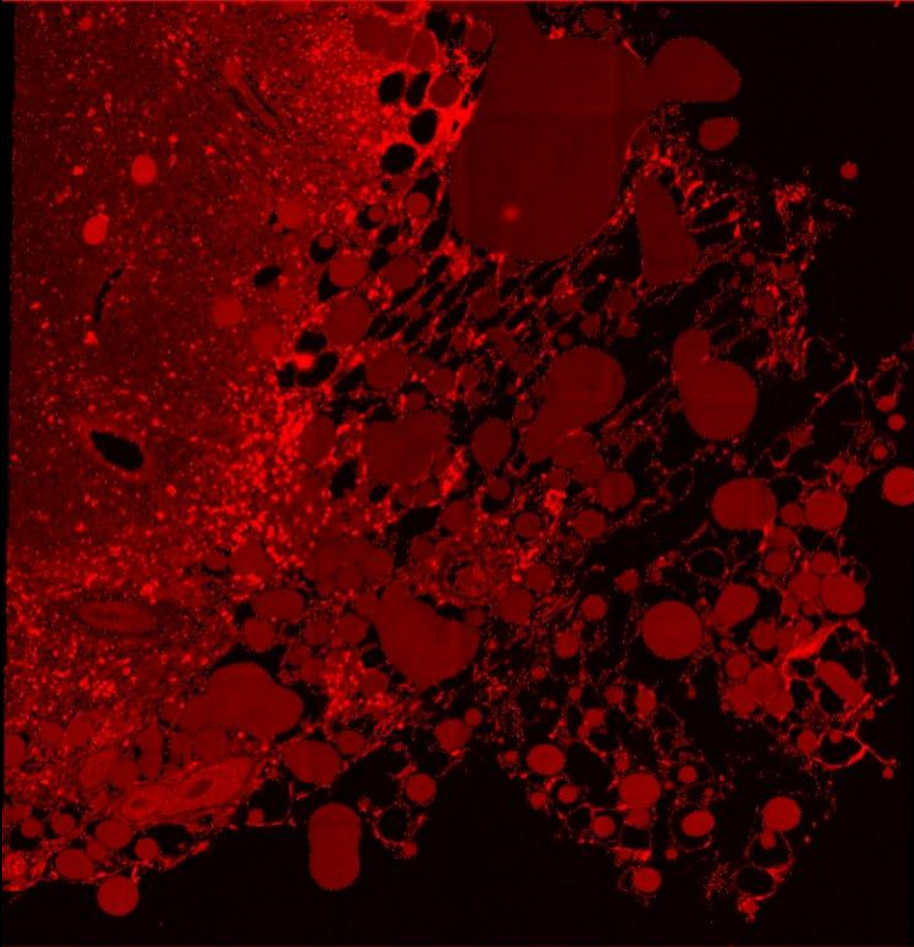
- > Gain immediate access to functional information for cell signaling, metabolic status, pH and ion concentration
- > Use lifetime-based information to explore the function of molecules in the cellular context

**TO GET CLOSER TO THE TRUTH**

*Leica*

# TauContrast – autofluorescence- T cell

Excitation : 588nm, detection : 615-705nm

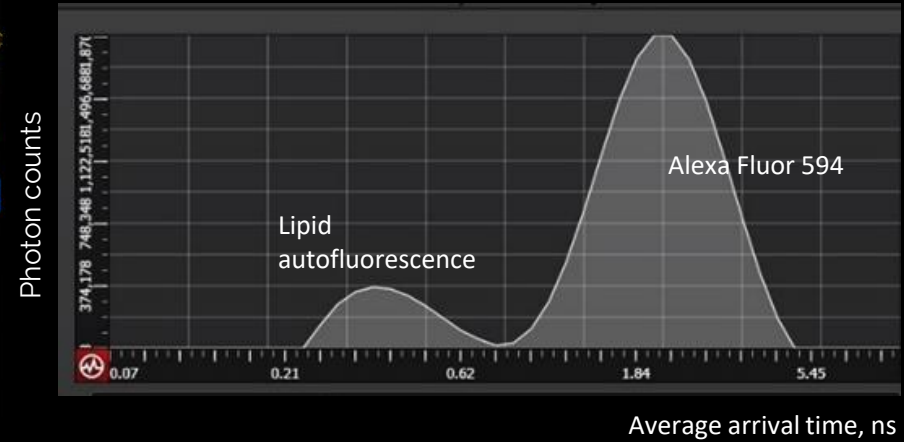
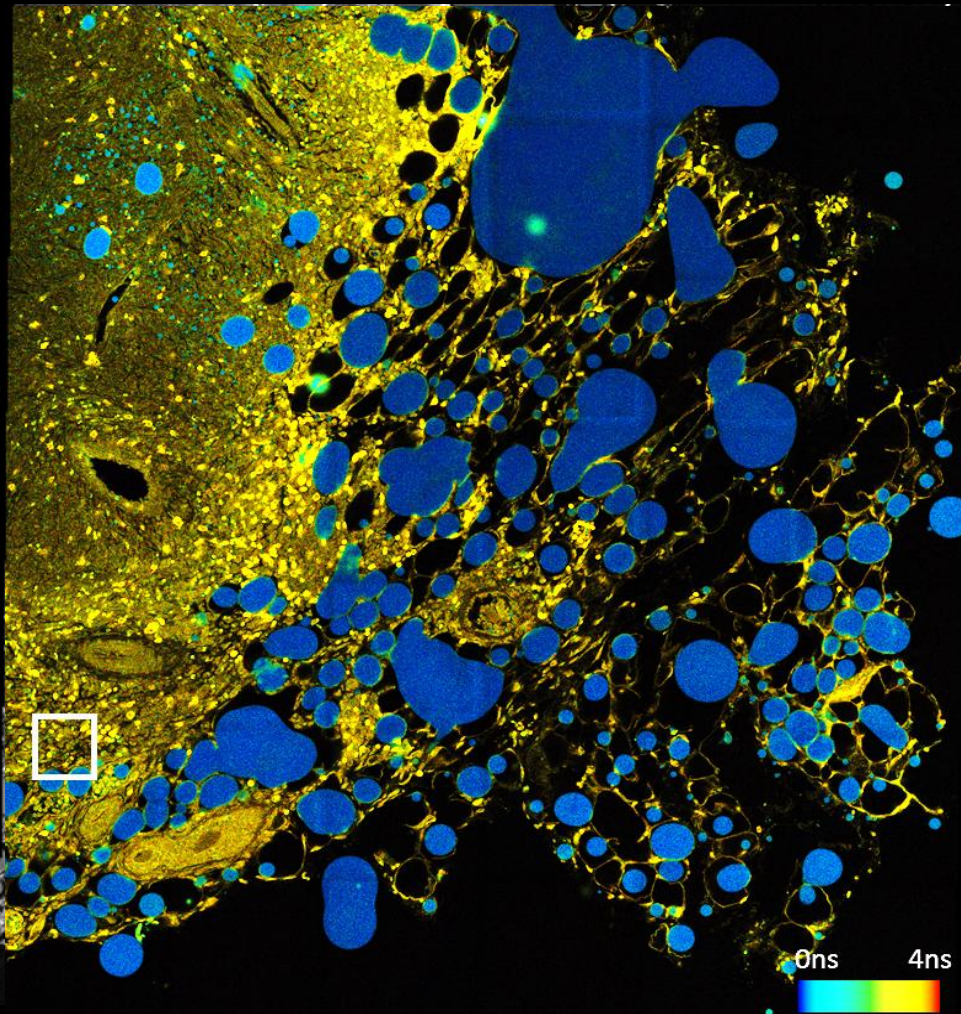


Subcutaneous BRAF600E mouse melanoma with surrounding fat tissue. Cytotoxic T cells stained with anti-CD8a Alexa Fluor 594. Tissue shows high autofluorescence which interferes with Alexa Fluor 594 signal. Fluorescence lifetime information (fast FLIM) enables to distinguish CD8a+ T cells (longer lifetime, yellow) from the autofluorescence of the lipid droplets (short lifetime in blue) and of other cells (green in Fast FLIM images)

Courtesy of Dr. Jan Boettcher, Institute of Molecular Immunology, TU Munich

# TauContrast – autofluorescence- T cell

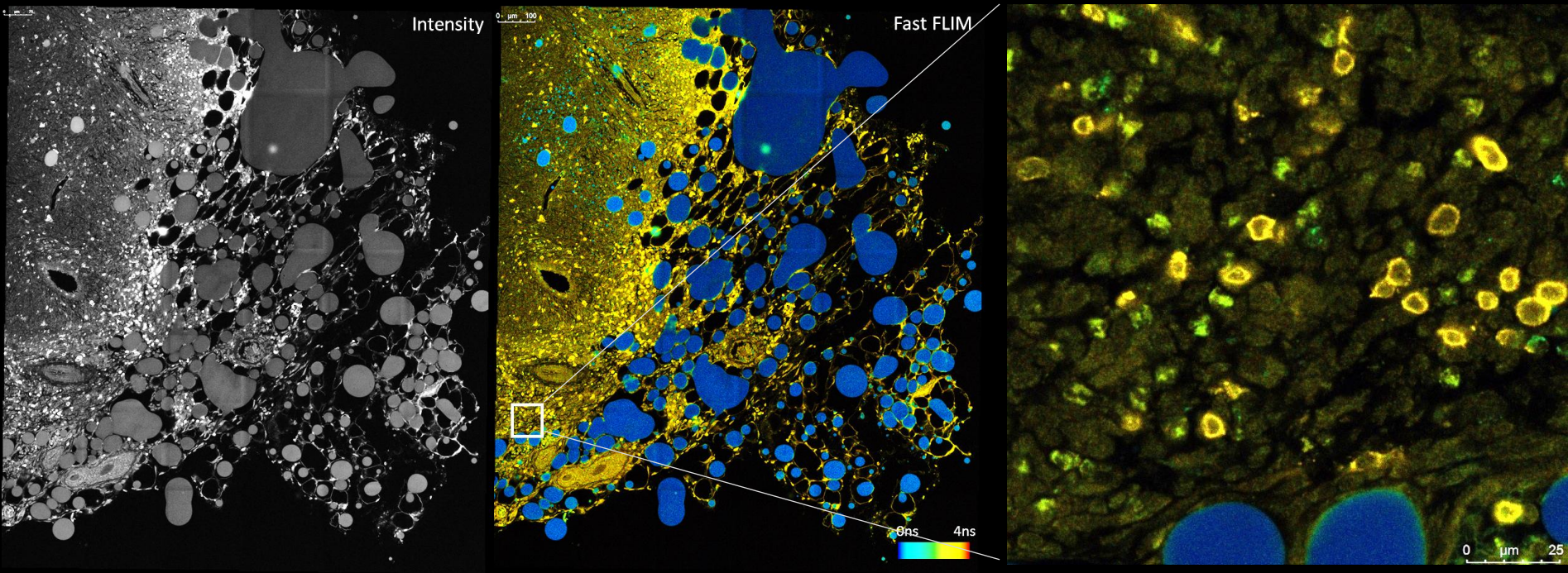
Excitation : 588nm, detection : 615-705nm



Subcutaneous Brafv600E mouse melanoma with surrounding fat tissue. Cytotoxic T cells stained with anti-CD8a Alexa Fluor 594. Tissue shows high autofluorescence which interferes with Alexa Fluor 594 signal. Fluorescence lifetime information (fast FLIM) enables to distinguish CD8a+ T cells (longer lifetime, yellow) from the autofluorescence of the lipid droplets (short lifetime in blue) and of other cells (green in Fast FLIM images)

Courtesy of Dr. Jan Boettcher, Institute of Molecular Immunology, TU Munich

# TauContrast – autofluorescence- T cell

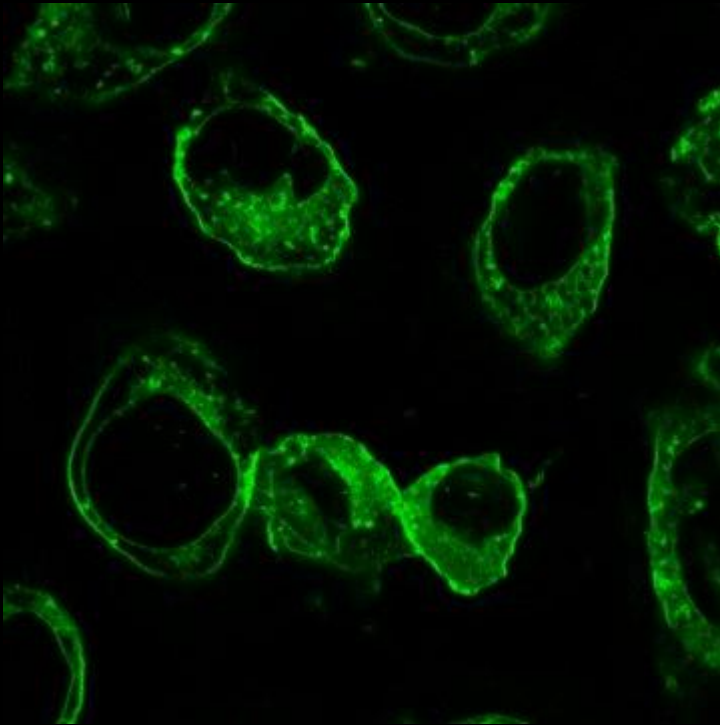


Subcutaneous BRAF600E mouse melanoma with surrounding fat tissue. Cytotoxic T cells stained with anti-CD8a Alexa Fluor 594. Tissue shows high autofluorescence which interferes with Alexa Fluor 594 signal. Fluorescence lifetime information (fast FLIM) enables to distinguish CD8a+ T cells (longer lifetime, yellow) from the autofluorescence of the lipid droplets (short lifetime in blue) and of other cells (green in Fast FLIM images)

Courtesy of Dr. Jan Boettcher, Institute of Molecular Immunology, TU Munich

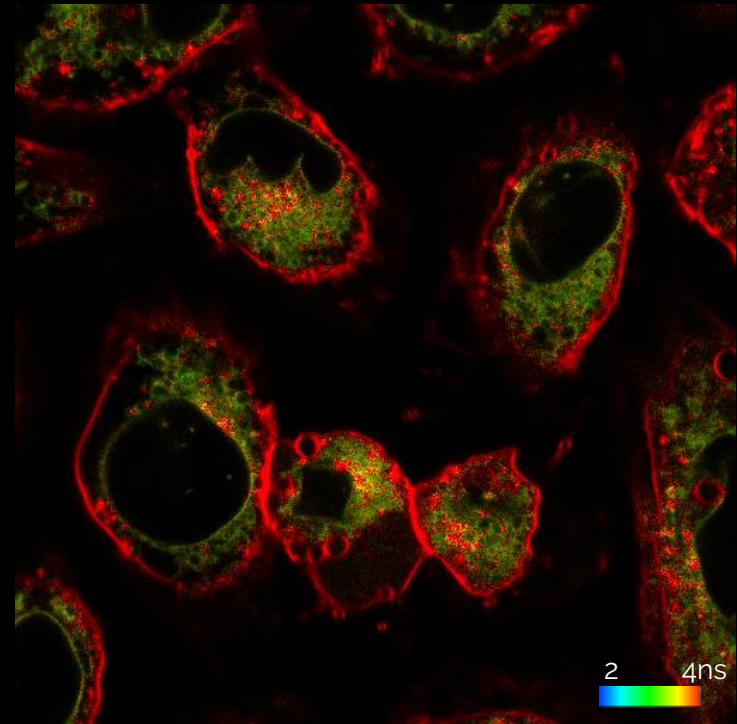
# Membrane tension visualization with FlipperTR<sup>®</sup>

Excitation : 488nm, detection : 575-625nm  
Intensity



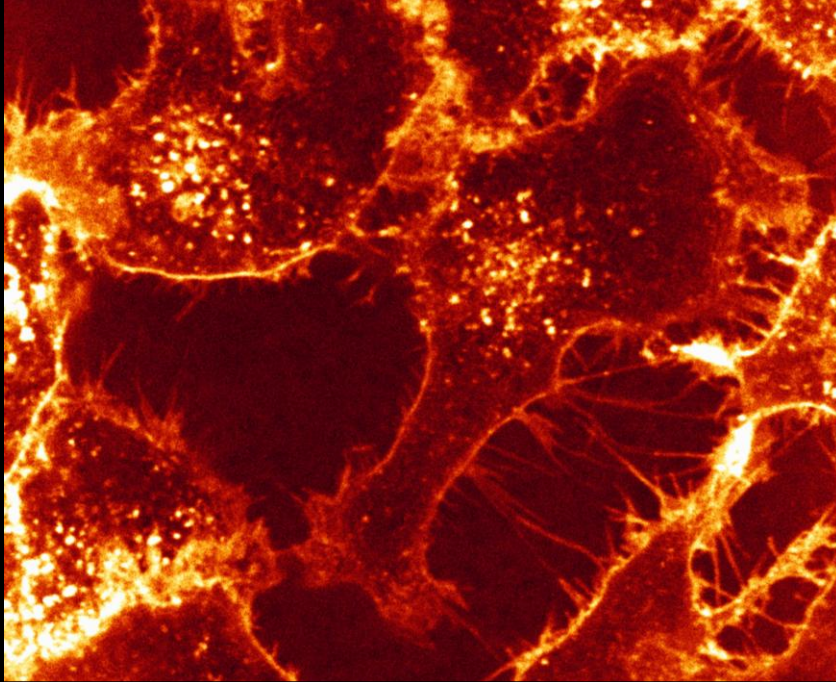
U2OS cells labeled with Flipper TR,

Tau Contrast



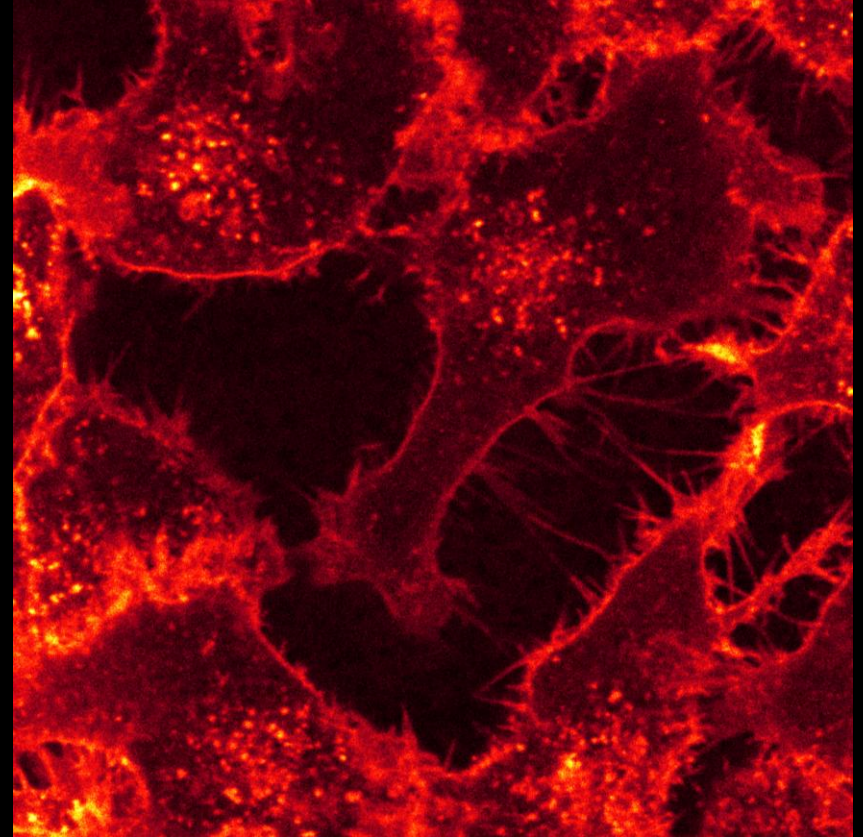
# Improve Image Quality

Traditional Confocal

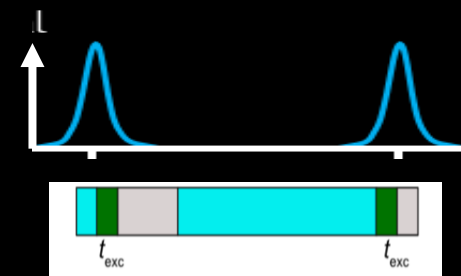
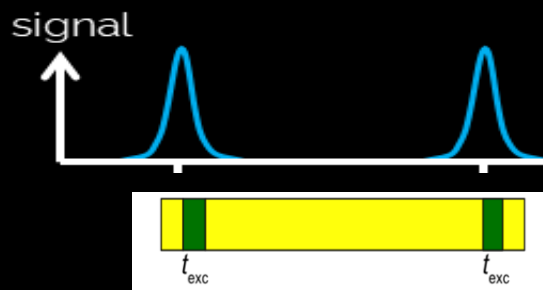
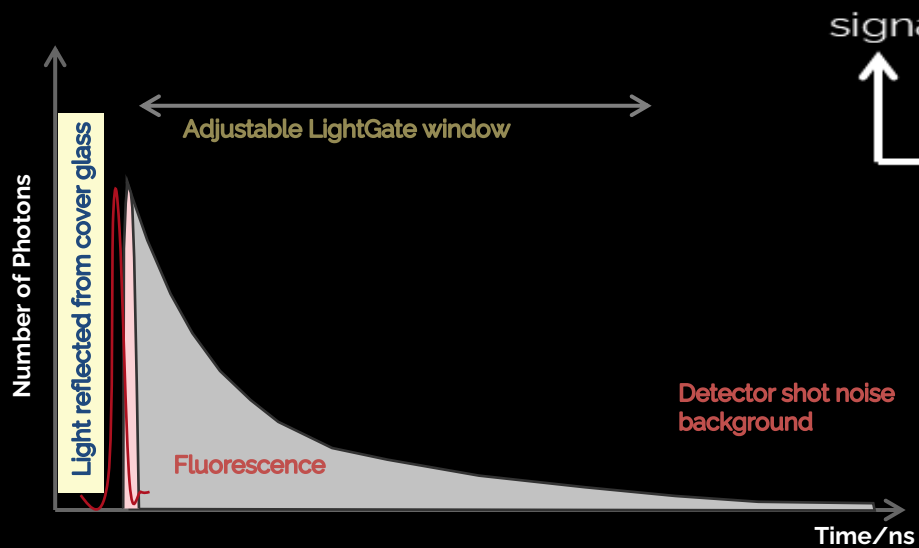


TauSense Tool:  
**TauGating**

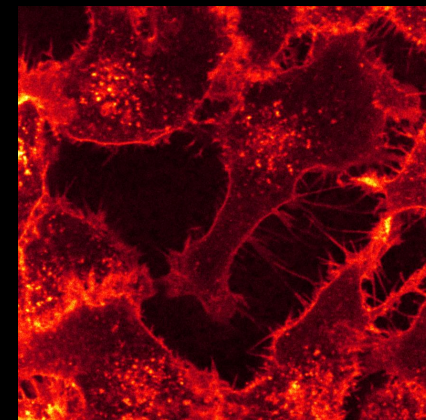
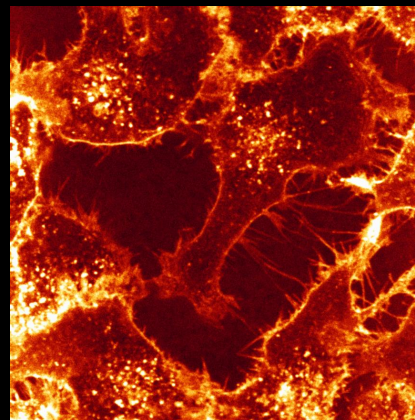
STELLARIS



# The Technology Behind TauGating

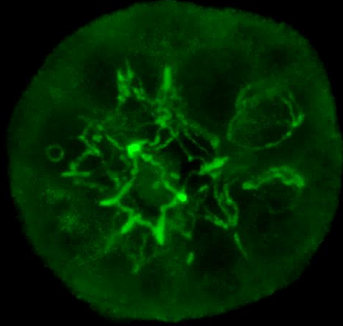


> Digital Gate Channels  
(Intensity,  $N_{photons}$ )

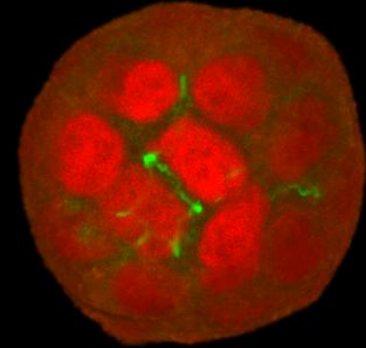
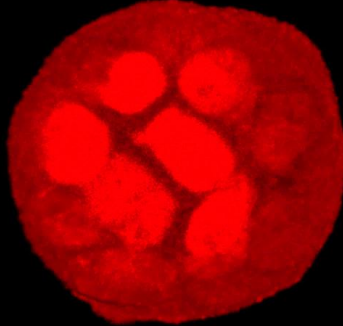


# TauGating - Improve Image Quality

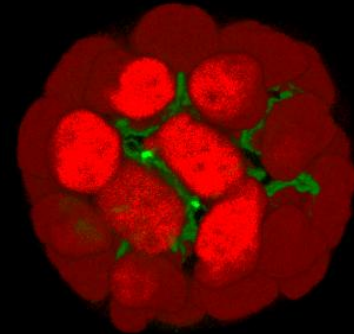
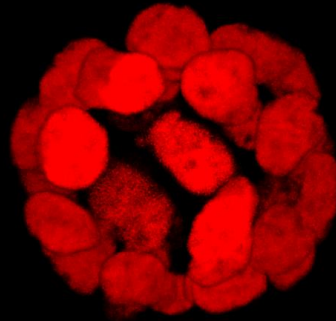
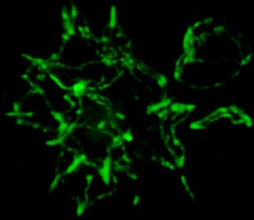
## Traditional Confocal



3D cell culture

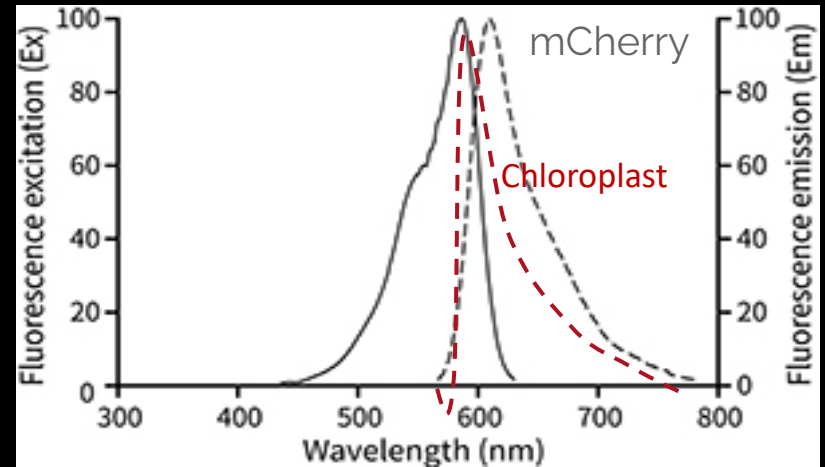
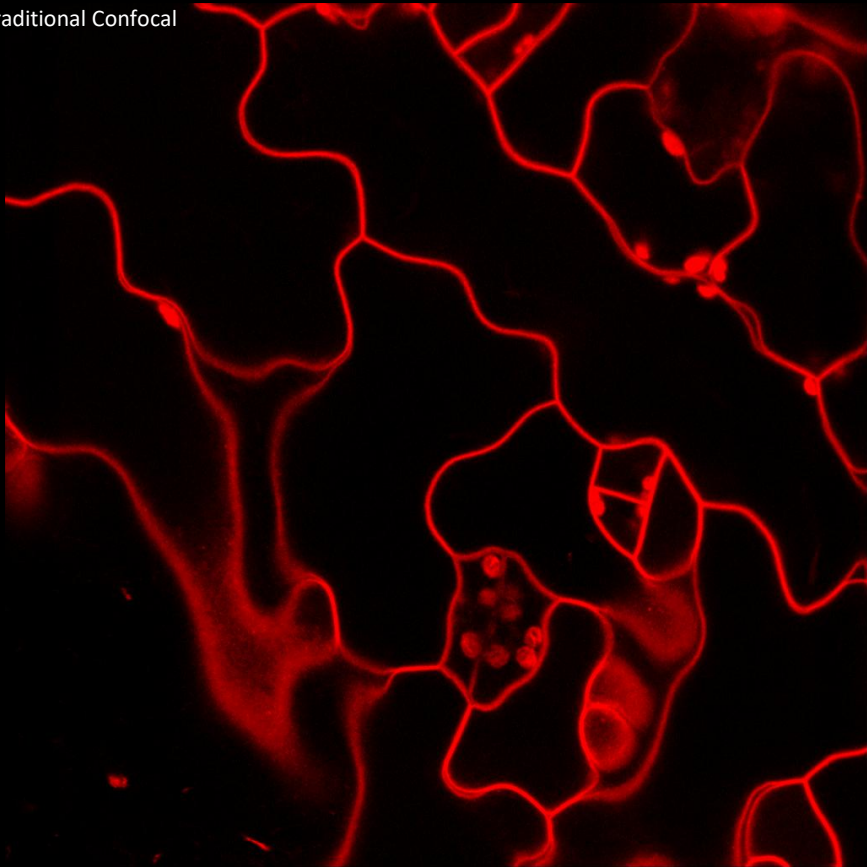


## STELLARIS TauGating

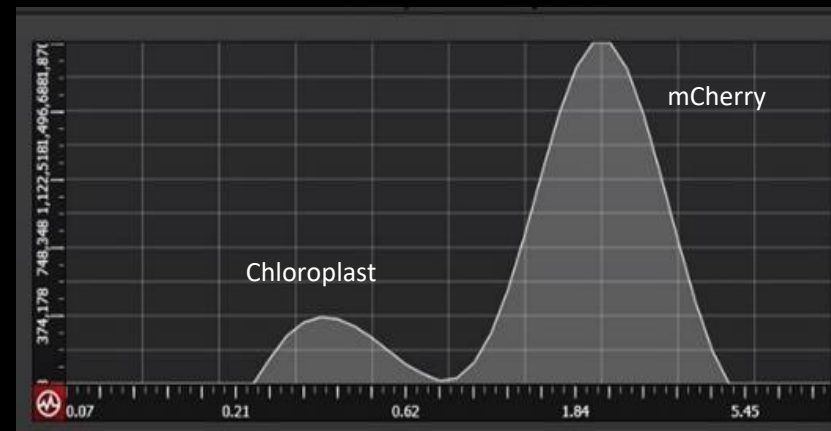


# mCherry + Chloroplast

Traditional Confocal



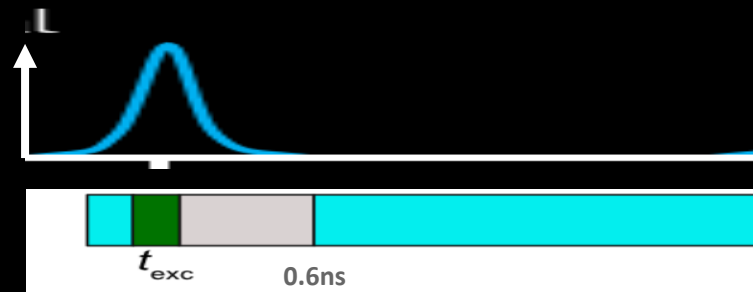
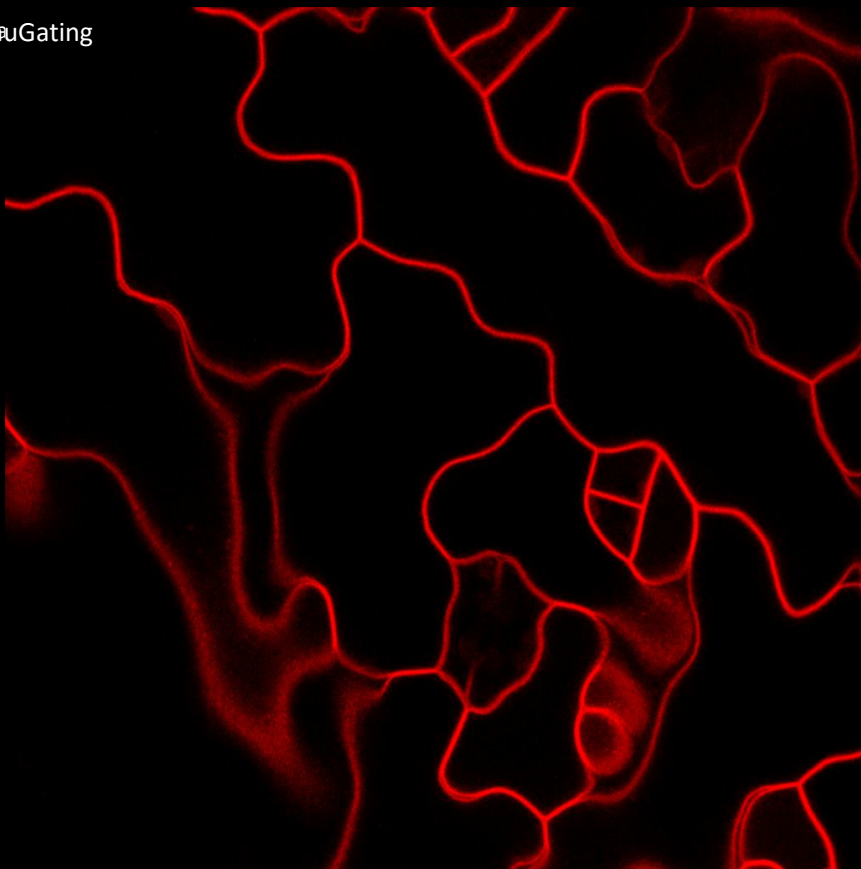
Photon counts



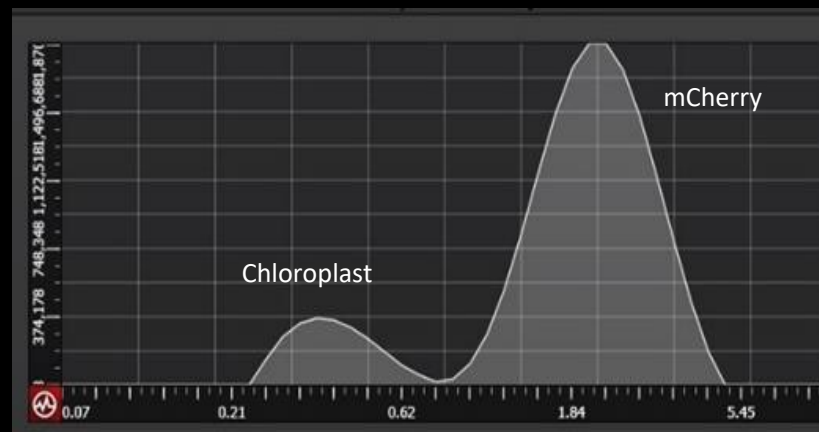
Average arrival time, ns

# mCherry + Chloroplast

TauGating



Photon counts

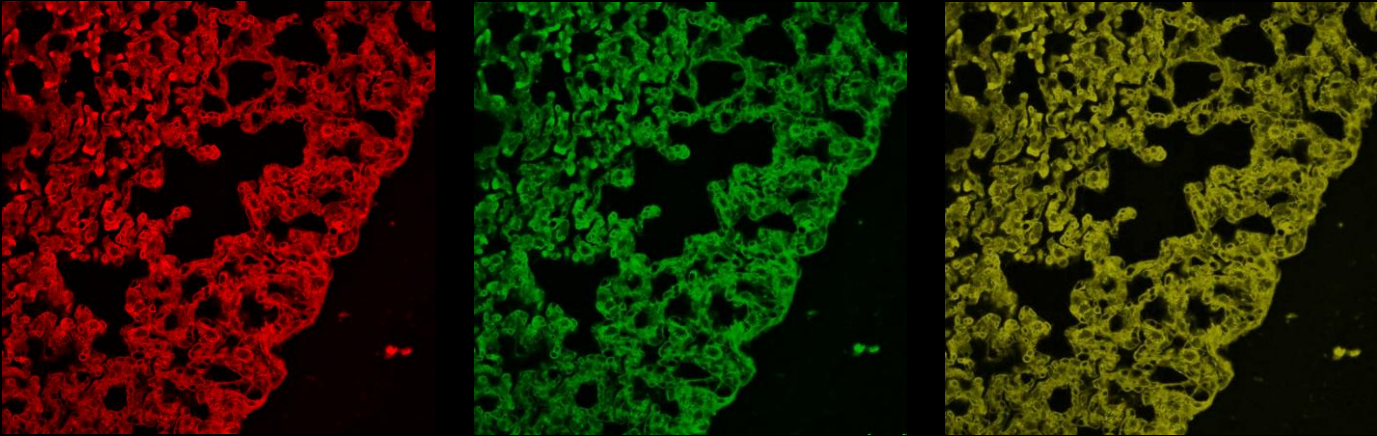


Average arrival time, ns

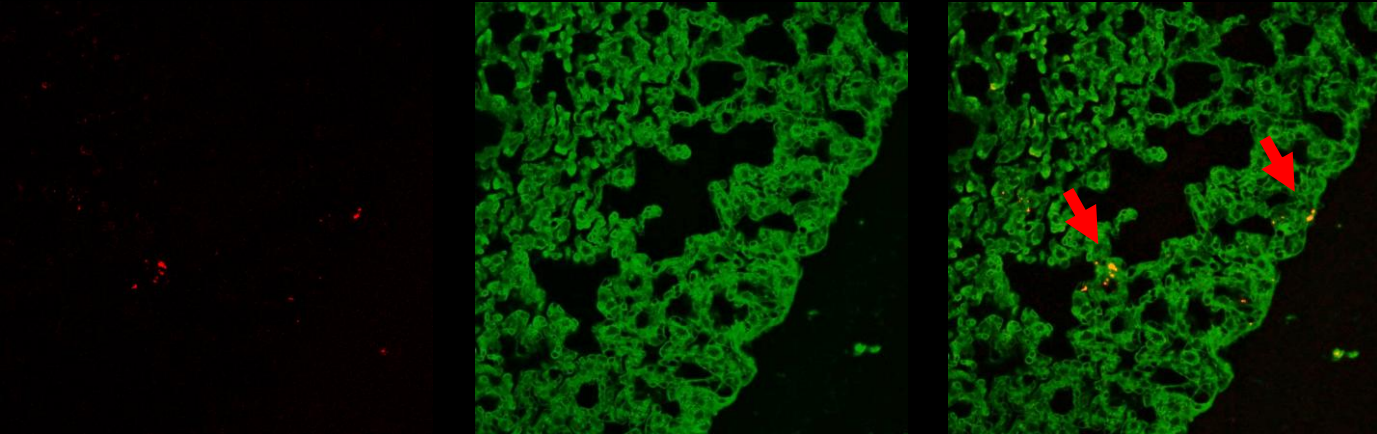
# TauGating - Improve Image Quality

## Tissue & nano diamond

Traditional Confocal

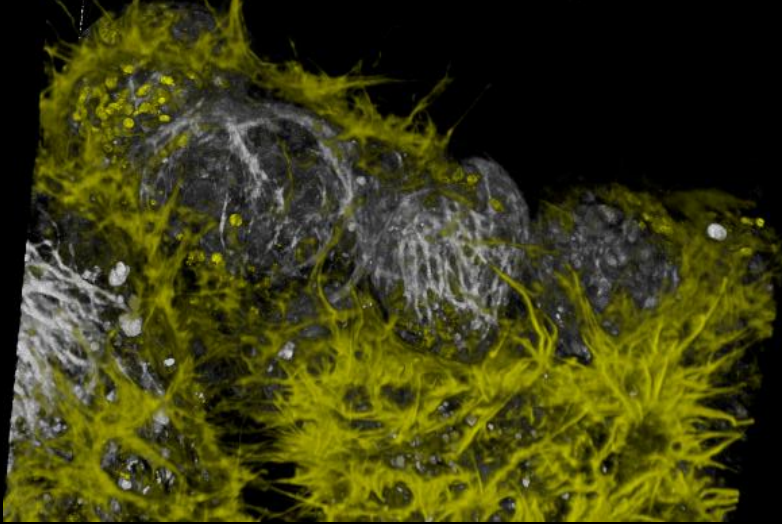


Tau gating

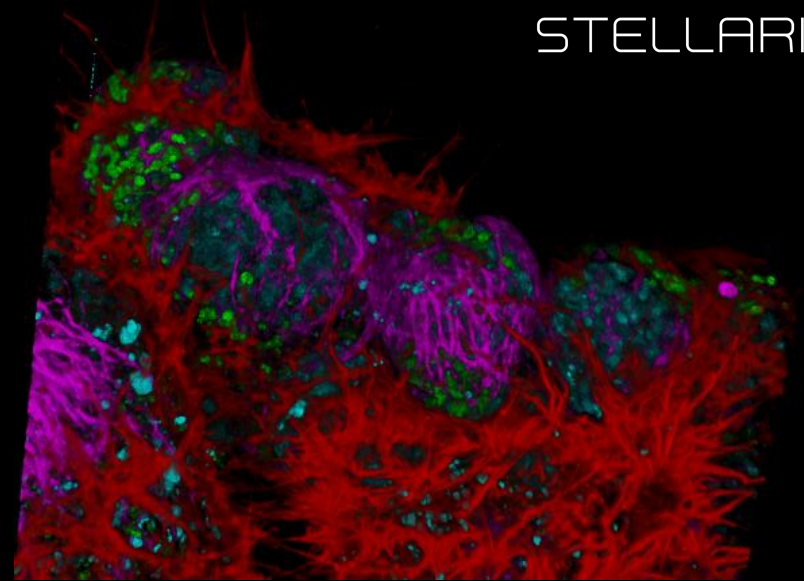


# Multiplex Beyond The Spectral Options

Traditional Confocal



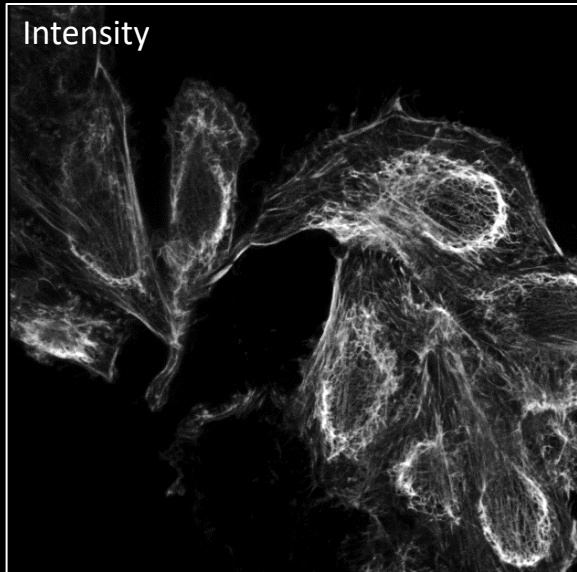
STELLARIS



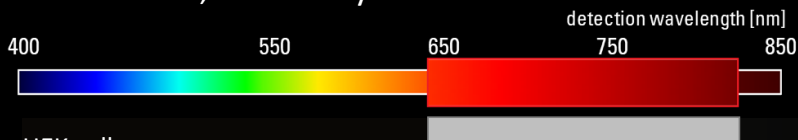
TauSense Tool:  
**TauSeparation**

# Application Example

## Species Separation using TauSense



1 detector, 1 intensity channel

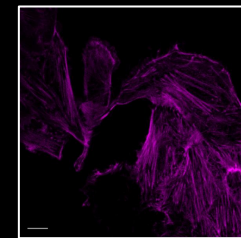
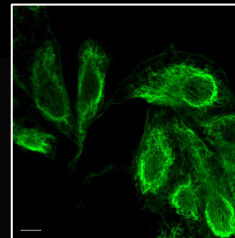
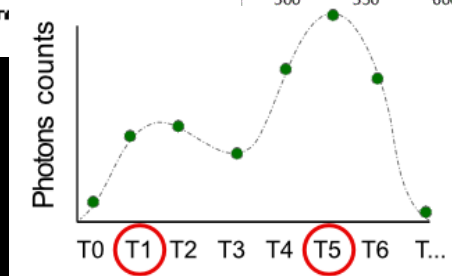
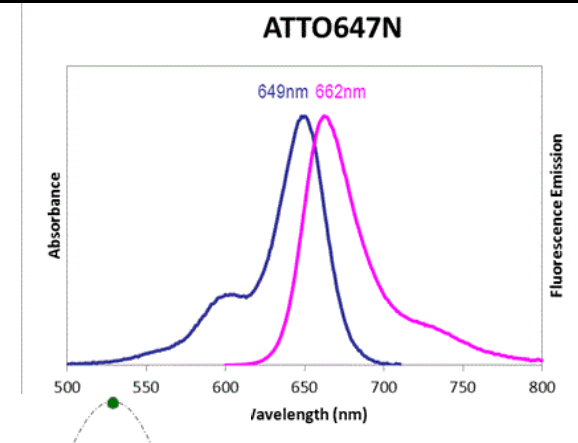
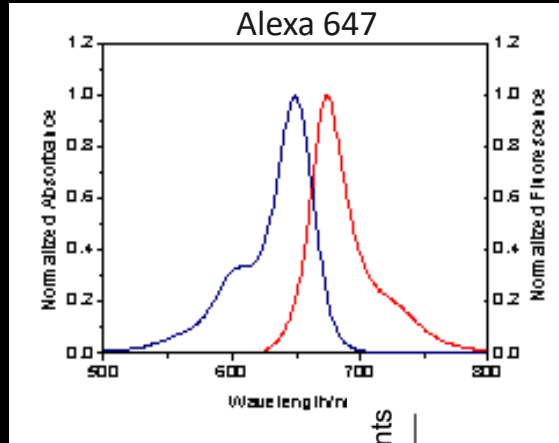


HEK cells.

Vimentin (Alexa 647 IF), actin (ATTO647N-phalloidin)

Sample Courtesy: Sebastian Hänsch,

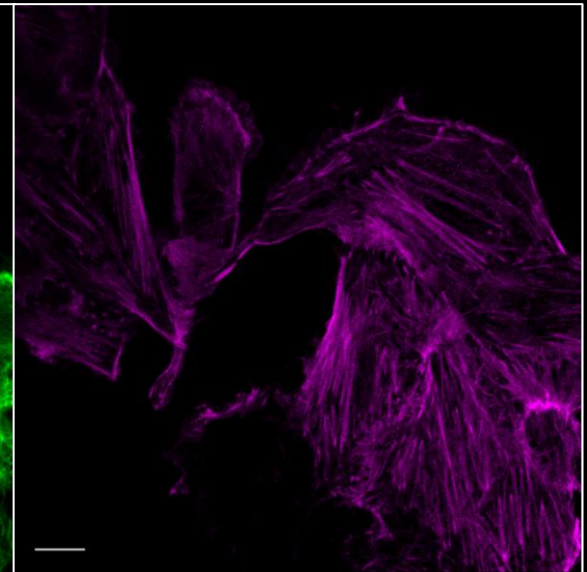
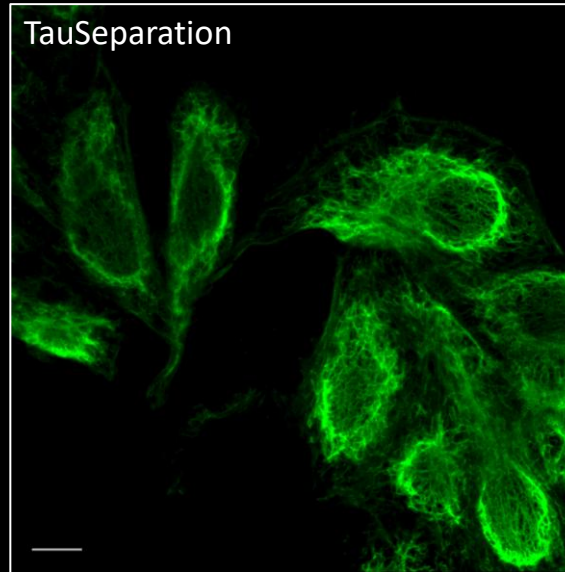
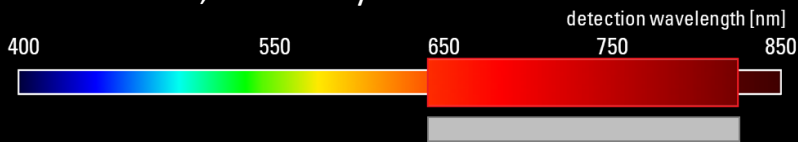
Stephanie Weidtkamp-Peters, CAI, Düsseldorf.



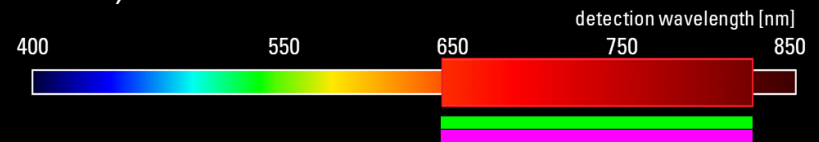
# Species Separation using TauSense



1 detector, 1 intensity channel



1 detector, 2 lifetime-based channels

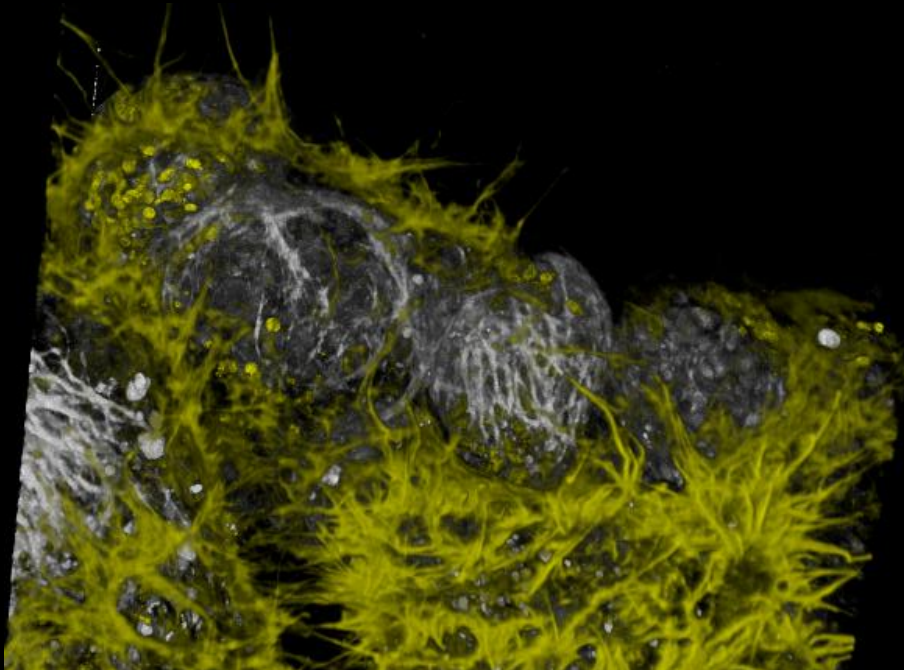


HEK cells. Vimentin (left: gray, Alexa 647 IF), actin (left: gray, ATTO647N-phalloidin). TauSeparation separates the signals coming to the detector according to the lifetime components distribution generated online at the FPGA level (right: green, Vimentin; right: magenta, Actin). Scale bar 10  $\mu$ m.

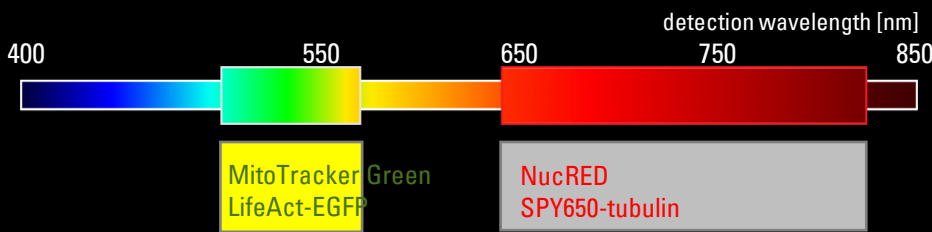
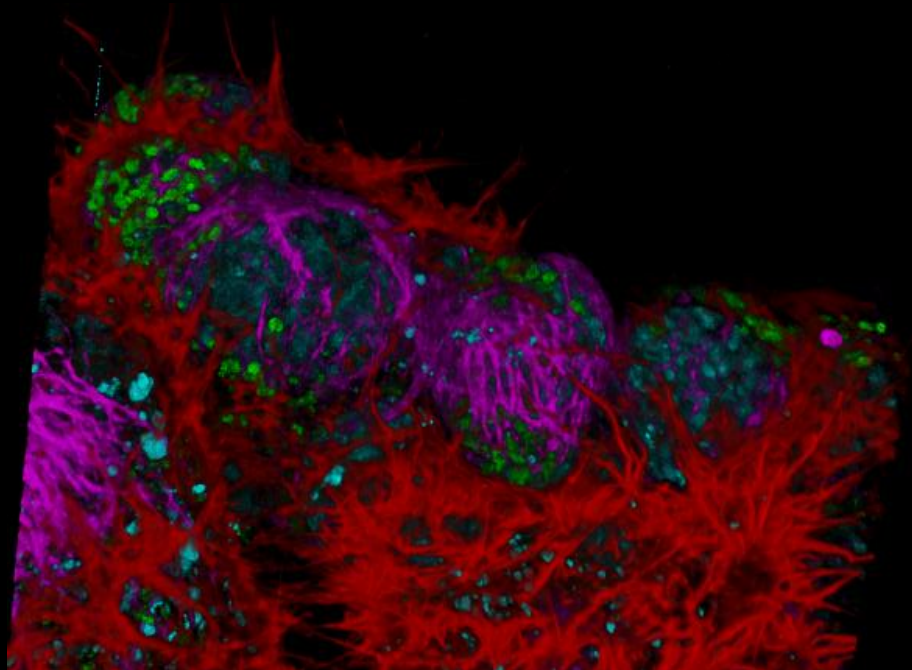
Sample Courtesy: Sebastian Hänsch, Stephanie Weidtkamp-Peters, CAI, Düsseldorf.

# Separate Species Beyond The Spectral Options

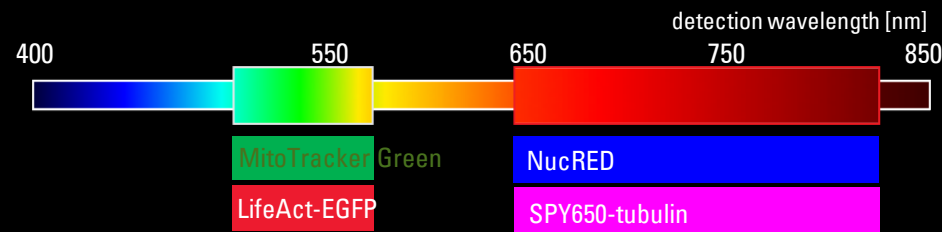
## Traditional Confocal



## STELLARIS



2 detectors, 2 intensity chnnels

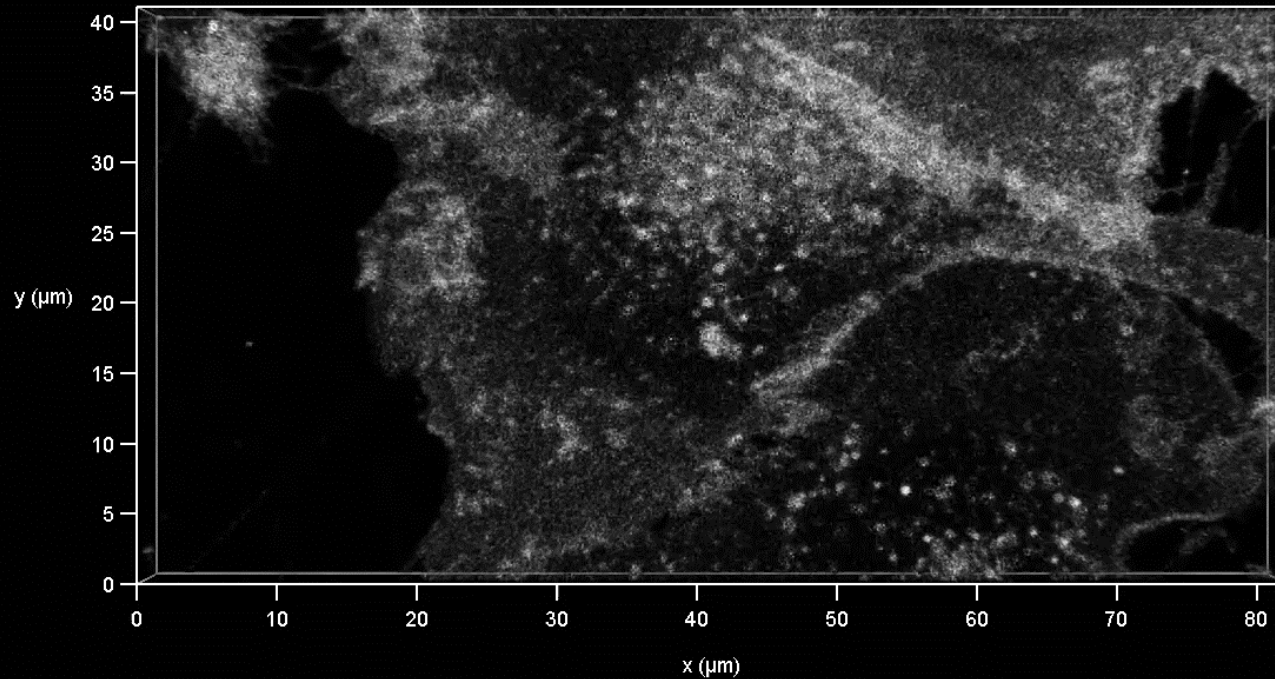


2 detectors, 4 lifetime-based channels

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



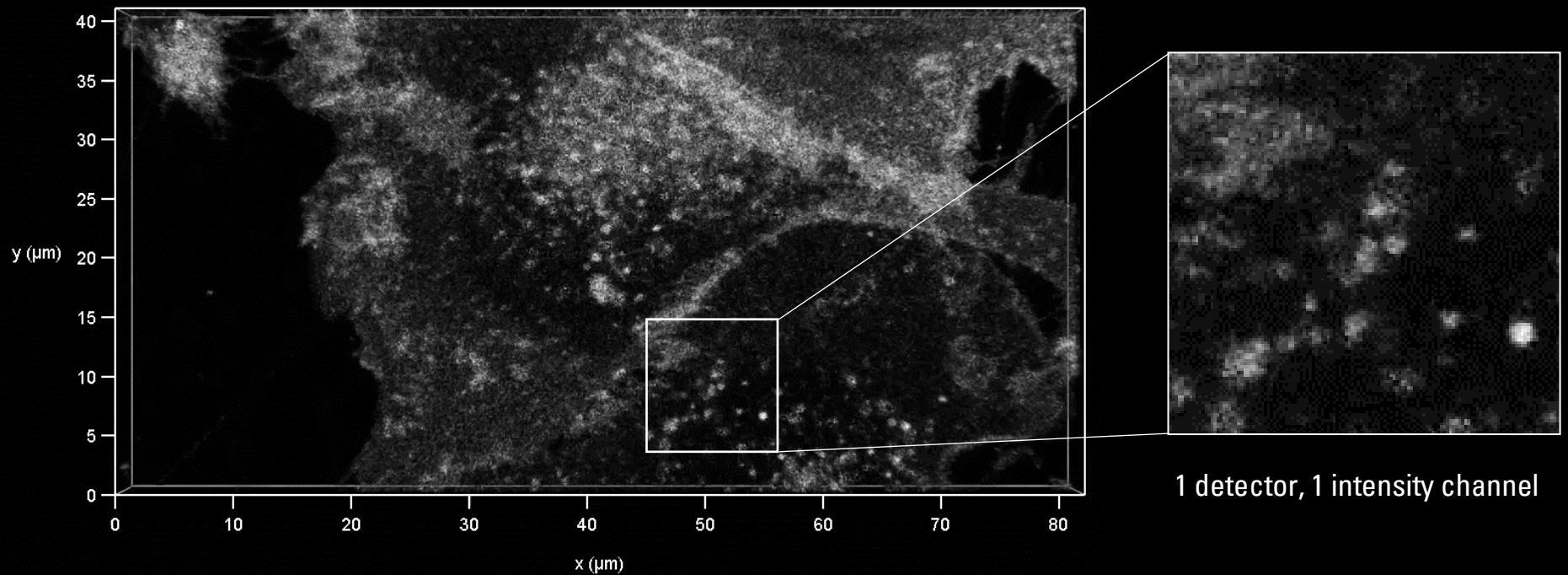
# Visualize vesicle transport in living cells



Endosome maturation  
pH changes (7 --> 4)  
early to late endosomes and lysosomes

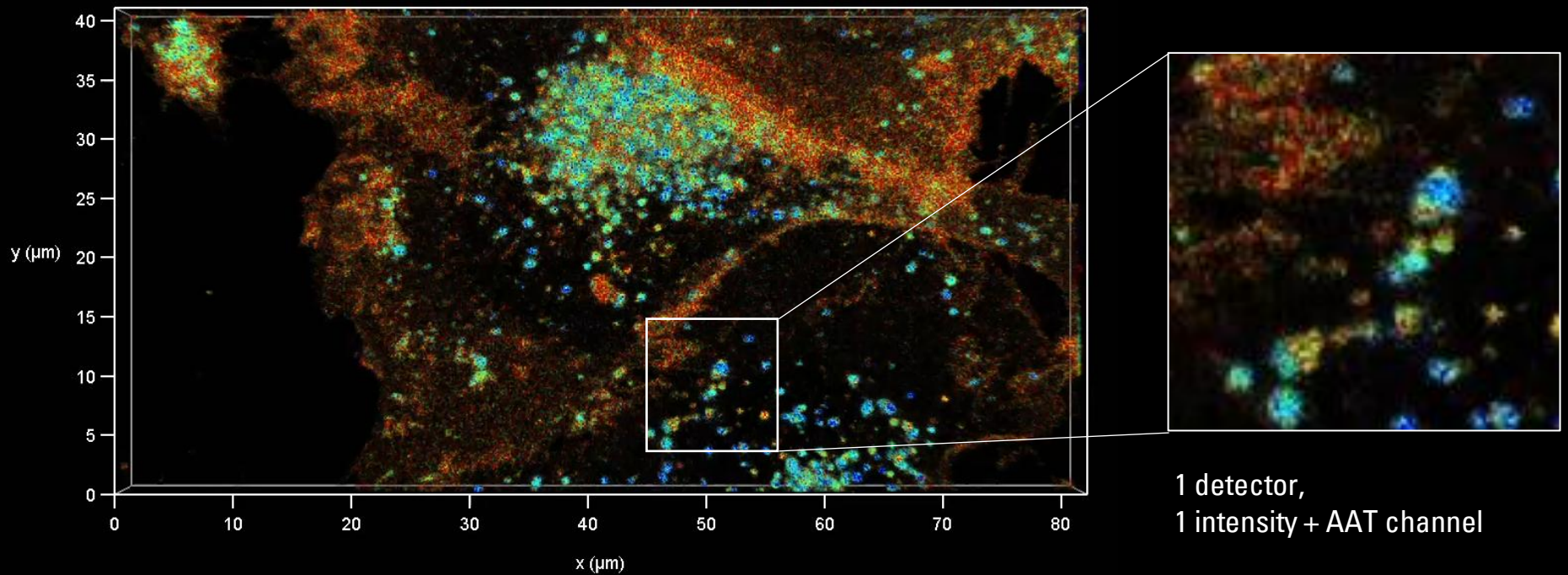
Vesicle transport in mammalian cells labeled with a NIR membrane stain, imaged @1 volume every 1,82s.

# Visualize vesicle transport in living cells



Vesicle transport in mammalian cells labeled with a NIR membrane stain, imaged @1 volume every 1,82s.

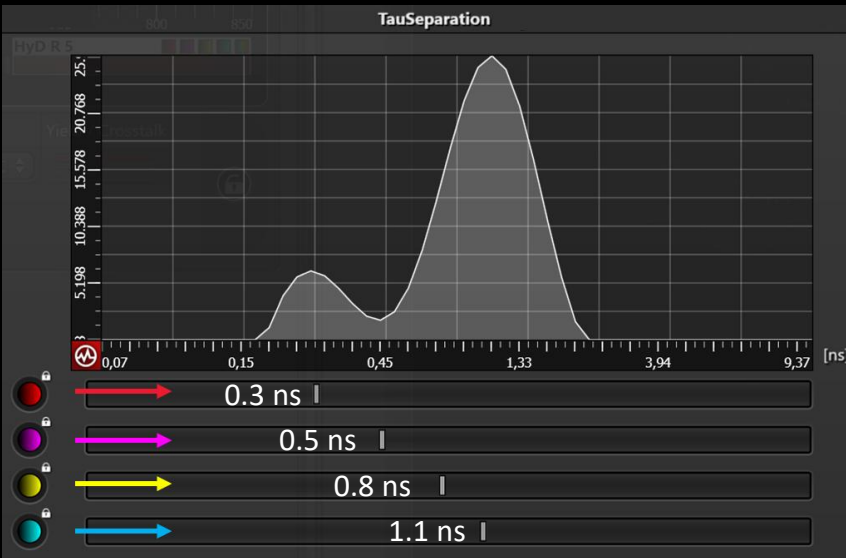
# TauContrast: Average Arrival Times show effect of pH



Vesicle transport in mammalian cells labeled with a NIR membrane stain, imaged @1 volume every 1,82s.

# Visualize pH-related changes with TauSeparation

TauSeparation: lifetime components divided into 4 distinct channels



Fluorescence lifetime for NIR stain shortens @lower pH.

Early endosomes: cyan / yellow.

Late endosomes and lysosomes: magenta / red.

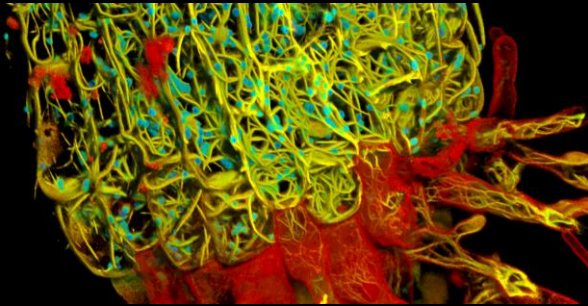
# Application Example Species Separation using TauSense



U2OS cells. nuclear counterstain (cyan, Sytox green), tubulin (magenta, AF 555).

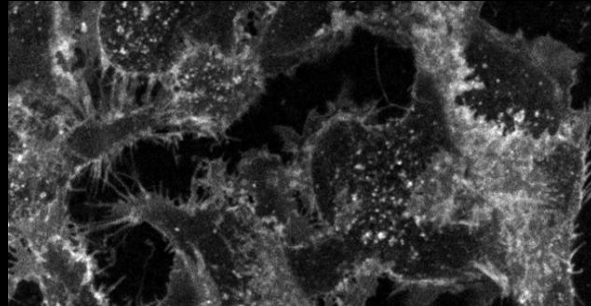
#CONFOCALREIMAGINED

# What is TauSense Good For?



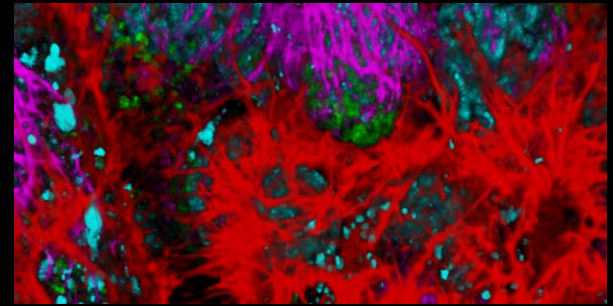
## TauContrast

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



## TauGating

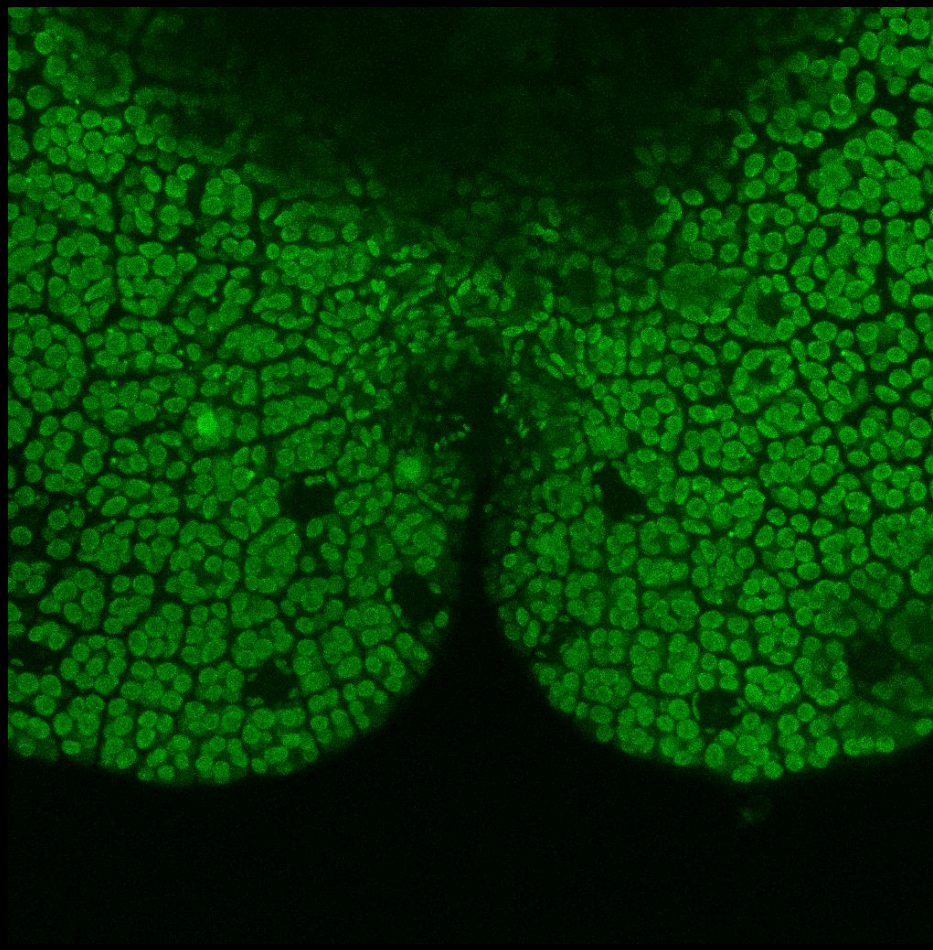
- Explore sample with gates
- Remove reflections
- Remove unwanted fluorescence contributions



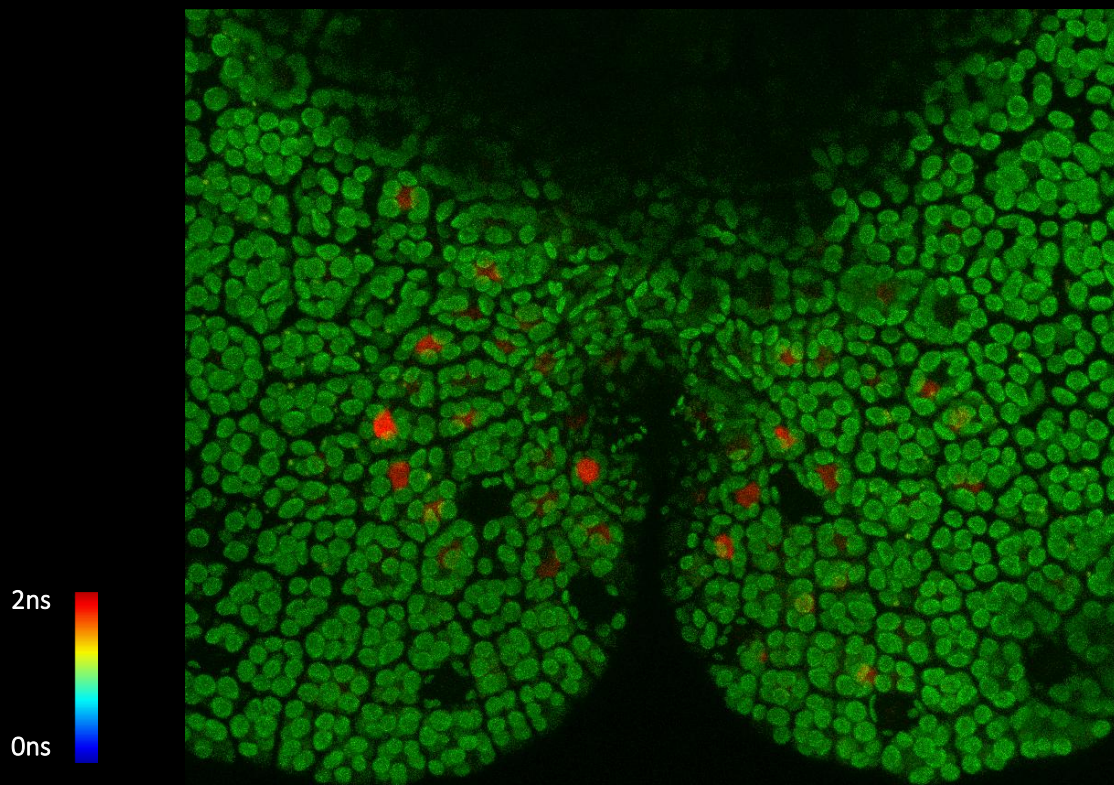
## TauSeparation

- Separate species with different lifetimes

Traditional Confocal - intensity

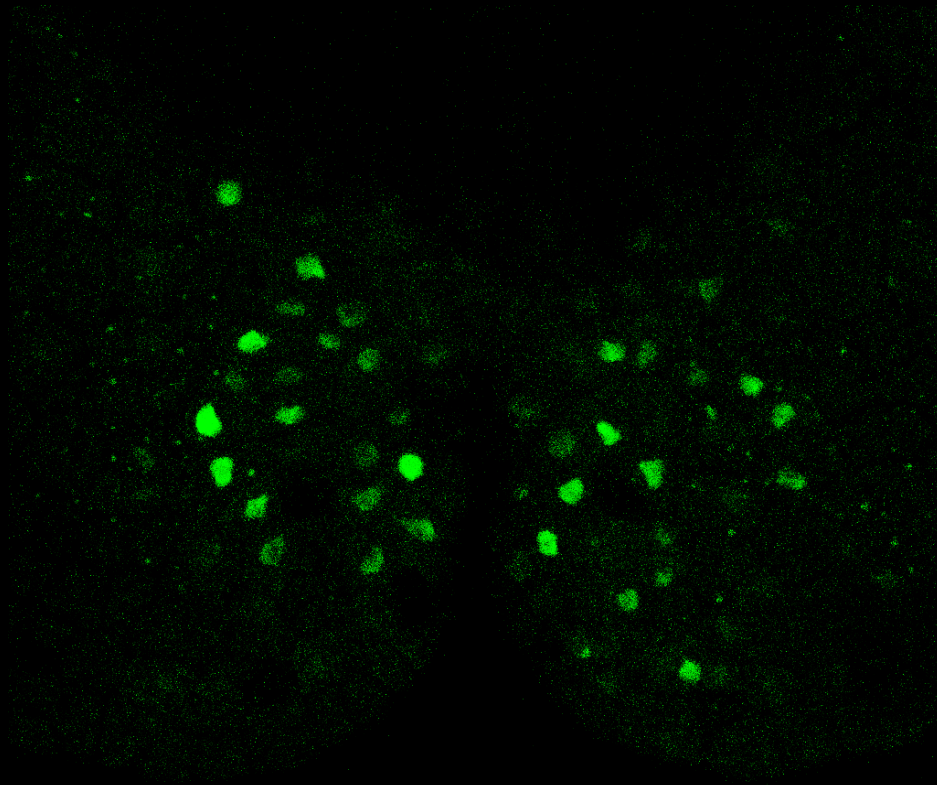


# Tau Contrast

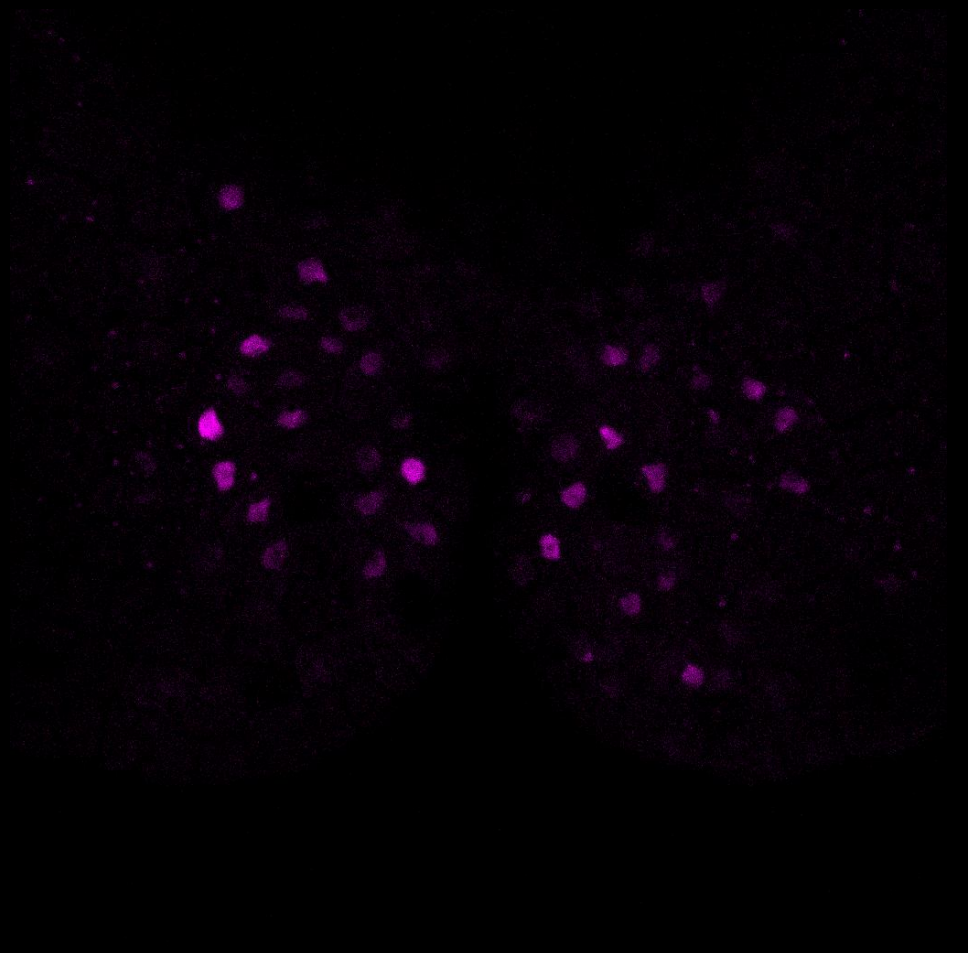
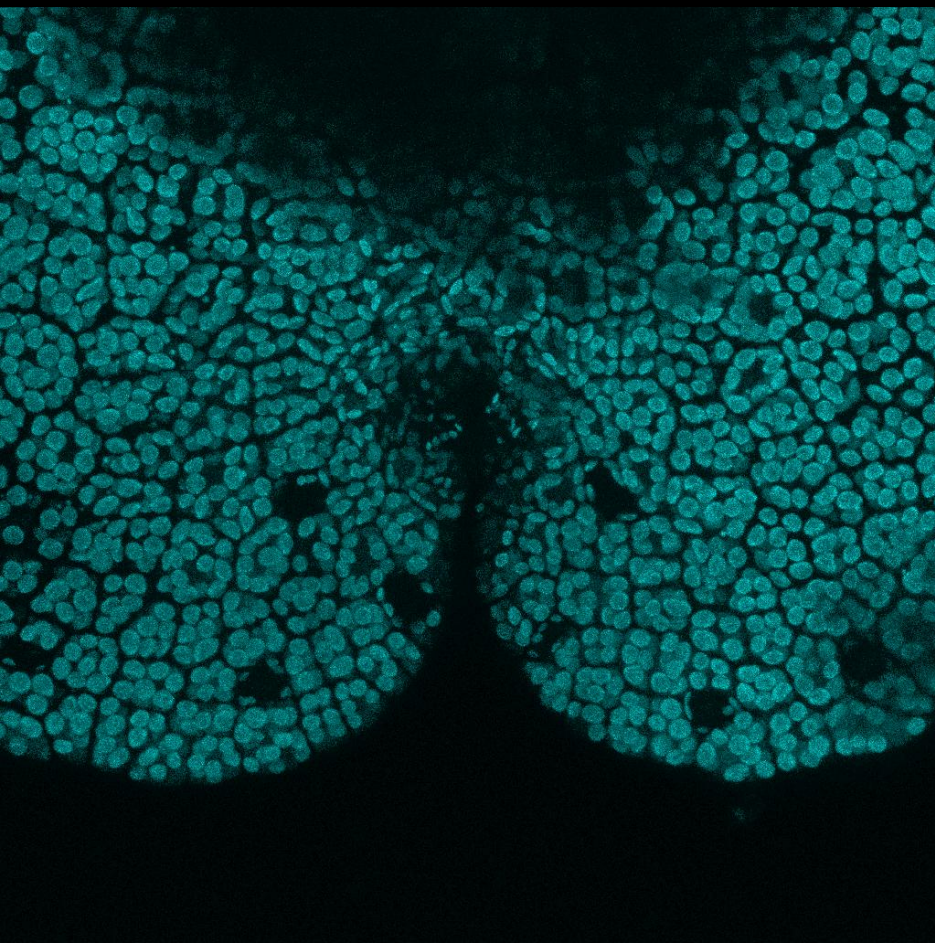


## Tau Gating

Gating : 0.5-6 ns



## Tau Separation

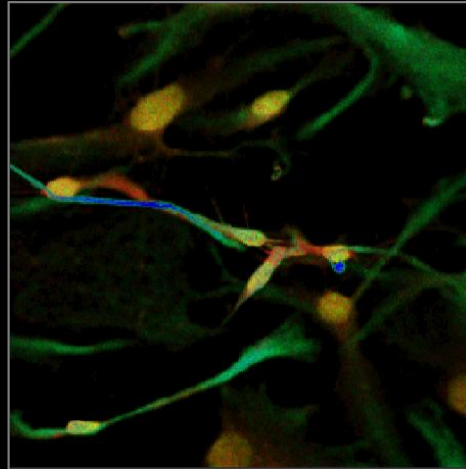


# FALCON: Synergies and integrated workflows

- Component Separation

Parameters to fit	
Parameter	Fit
Decay Time 1	<input type="checkbox"/>
Decay Time 2	<input type="checkbox"/>
Decay Time 3	<input type="checkbox"/>
Amplitude 1	<input checked="" type="checkbox"/>
Amplitude 2	<input checked="" type="checkbox"/>
Amplitude 3	<input checked="" type="checkbox"/>
Tail Offset	<input checked="" type="checkbox"/>
IRF Background	<input type="checkbox"/>
IRF Shift	<input type="checkbox"/>

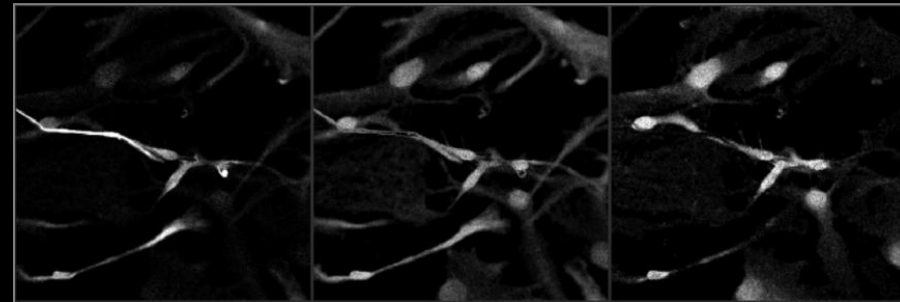
FLIM Image Fit  
3 Components



0.75 ns

1.84 ns

6.30 ns

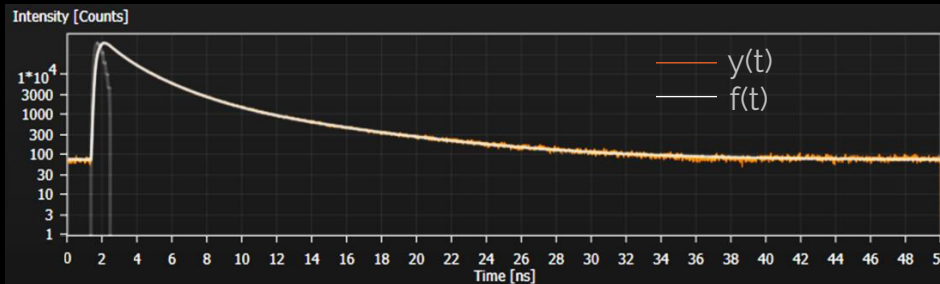


# FALCON: FASt Lifetime CONtrast

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



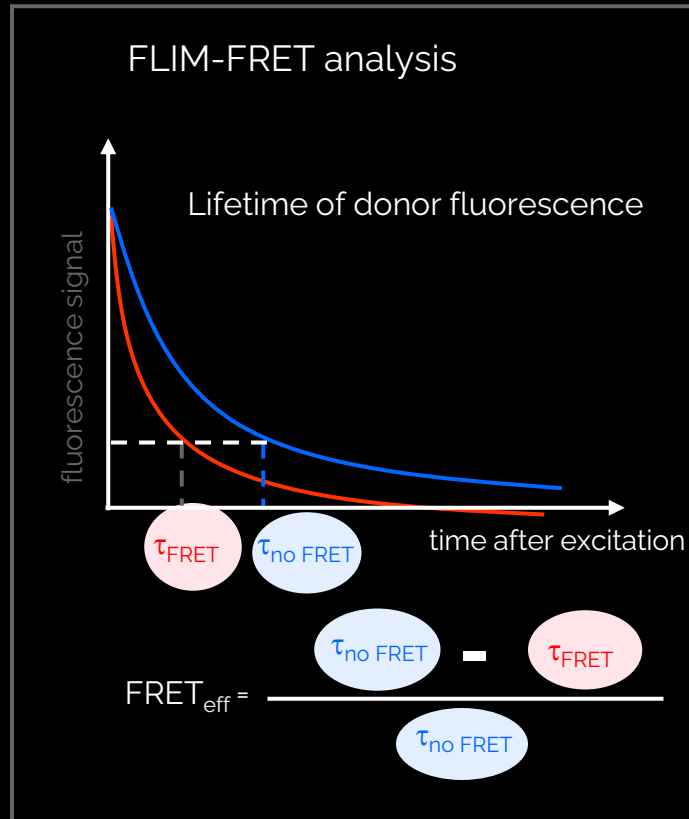
Maximum-Likelihood Estimator (Poissonian distribution)

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

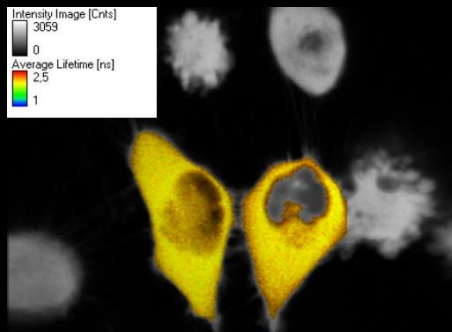
# FLIM-FRET?

- **Donor lifetime shortens**

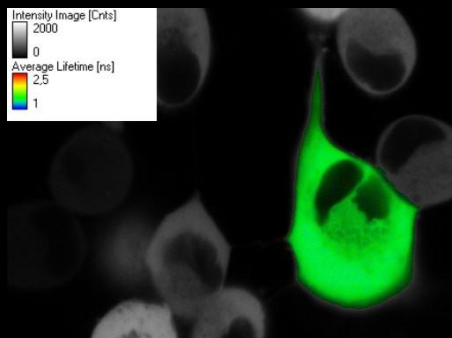
FRET efficiency is calculated from the difference between arising fast component in donor lifetime in the presence of the acceptor and original lifetime in the absence of the acceptor



## 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

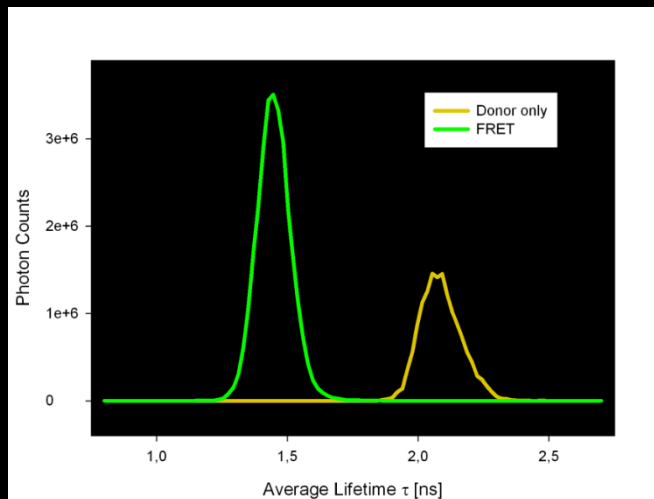
**Data Analysis:** The coloured region has been used for analysis. Colours represent intensity modulated fluorescence lifetimes.

**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:

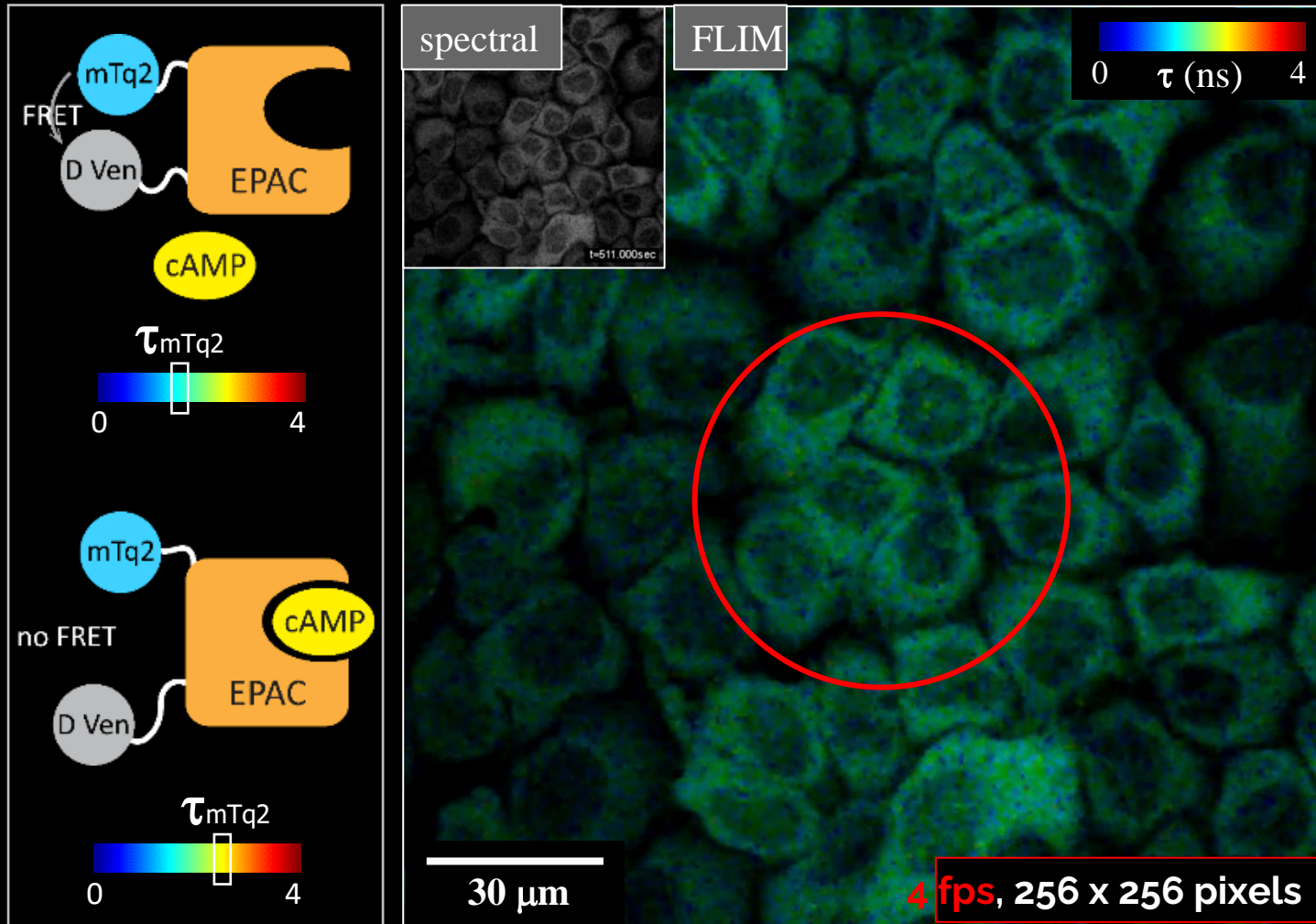
- average lifetime of the donor is: 2.1 ns.
- donor lifetime of the FRET construct is: 1.4 ns.
- FRET efficiency is:  $E = 30\%$ .

Computation of FRET Efficiency:

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

# Key application: Molecular interaction

## cAMP signalling on-the-fly with a FLIM-FRET sensor

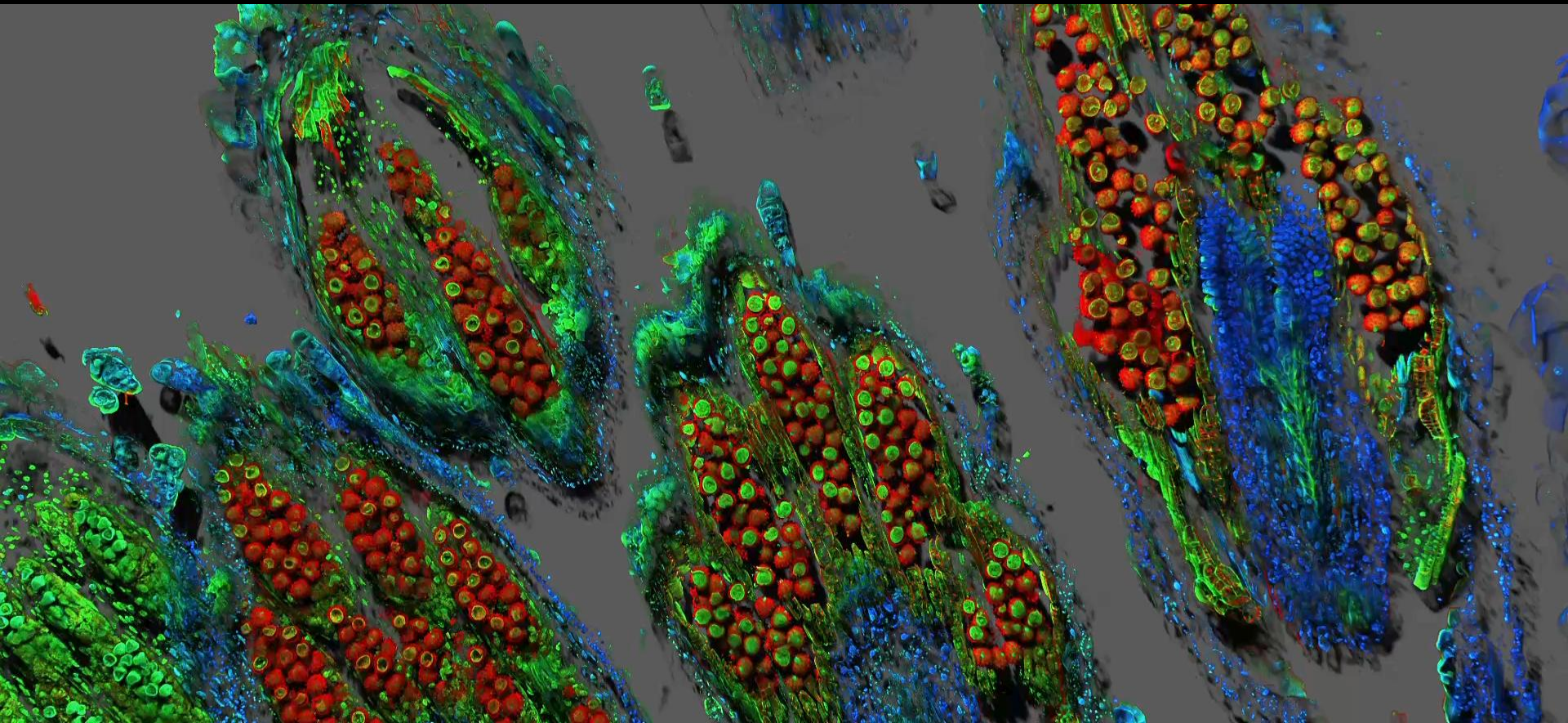


Caged cAMP in HeLa cells expressing EPAC mT2-dVenus FRET sensor.  
EPAC response to UV-mediated cAMP uncaging.


Courtesy Kees Jalink, Bram van den Broek, NKI Amsterdam.

# PRODUCTIVITY

DO MORE



# Simple, Even For Complex Experiments



The screenshot displays the Leica Stellaris B software interface, which is divided into several functional areas:

- Left Panel (Acquisition Settings):** Contains controls for Acquisition Mode (xyz), Format (512 x 512), Speed (600), Bidirectional X, Zoom Factor (3.91), Zoom In, Image Size (47.25 µm x 47.25 µm), Pixel Size (92.47 nm x 92.47 nm), Optical Section (0.896 µm), Pixel Dwell Time (951 ns), Frame Rate (0.231/s), Line Average, Line Accu, Frame Average, Frame Accu, Rotation (0.00), Pinhole, Unit (AU), Airy (1), Pinhole, Emission λ (580), Z-Stack controls (Begin, End, Z Position, Z Size, Re-Center, Galvo, Number of Steps, Z-Step Size, System Optimized, Z-Compensation, Galvo Flow, Travel Range).
- Top Panel (Configuration):** Shows the Objective (HC PL APO CS2 63x/1.40 Oil) and Fluo Turret (Scan-III).
- Center Panel (Fluorophore Selection):** A list of ALEXA dyes (ALEXA 633, ALEXA 635, ALEXA 647, ALEXA 647, ALEXA 660, ALEXA 680, ALEXA 680, ALEXA 700, ALEXA 750, ALEXA 7) is shown with a 'Drag and drop' interface. Below this is an 'Add Laser' button.
- Right Panel (Line Plots):** Five 'Setting' plots (Setting 1 to Setting 5) are displayed, each showing a fluorescence intensity profile across a wavelength range (HyD X.2 to HyD X.5) with a 'Trans PMT' label. Setting 1 shows a blue peak, Setting 2 a green peak, and Setting 3 a yellow-green peak.
- Bottom Panel (Status):** Includes 'Autofocus', 'Live', 'Fast Live', a date/time stamp (18/03/2020 12:34:25), and 'Capture Image' and 'Start' buttons.

Annotations on the right side of the interface include a search icon and a zoom level of 98%.

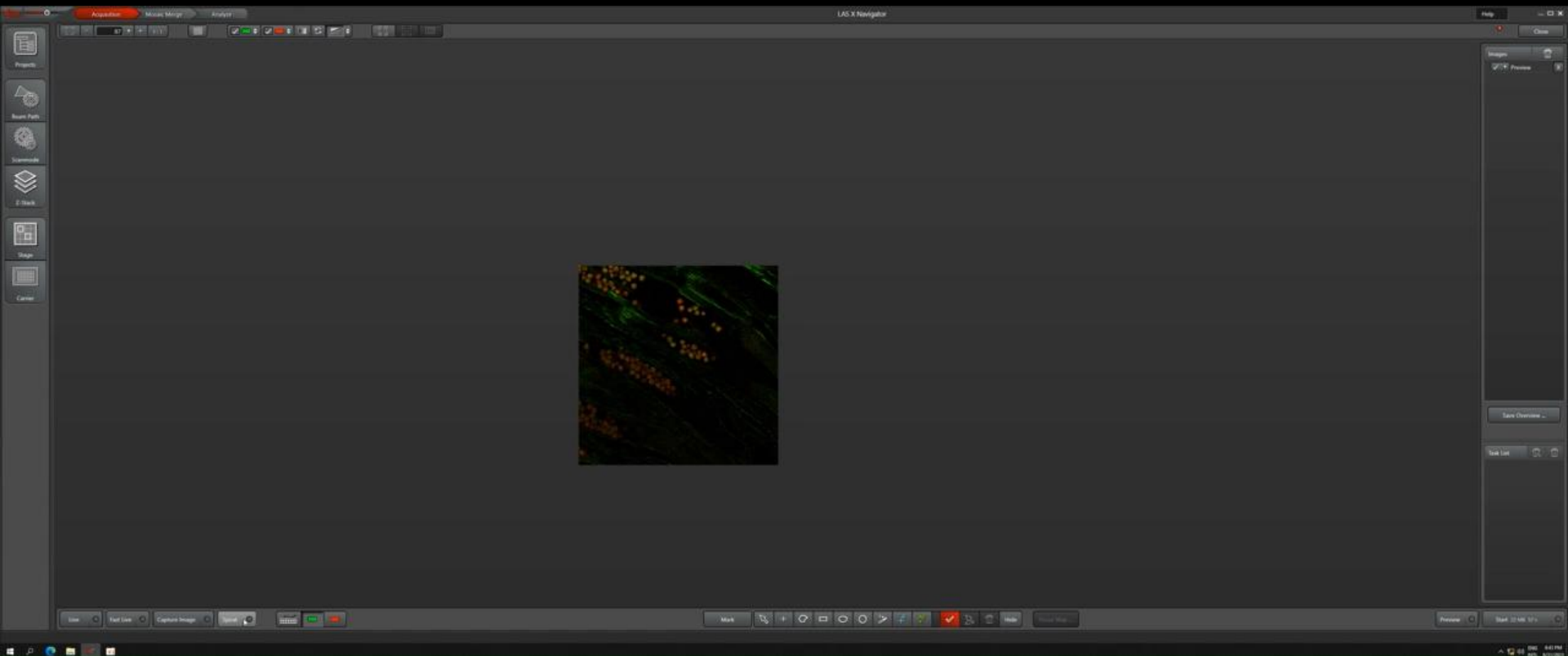
- > “Drag and drop” to add fluorophores
- > Automatic optimization of excitation and detection

# Simple, Even For Complex Experiments – Image Compass



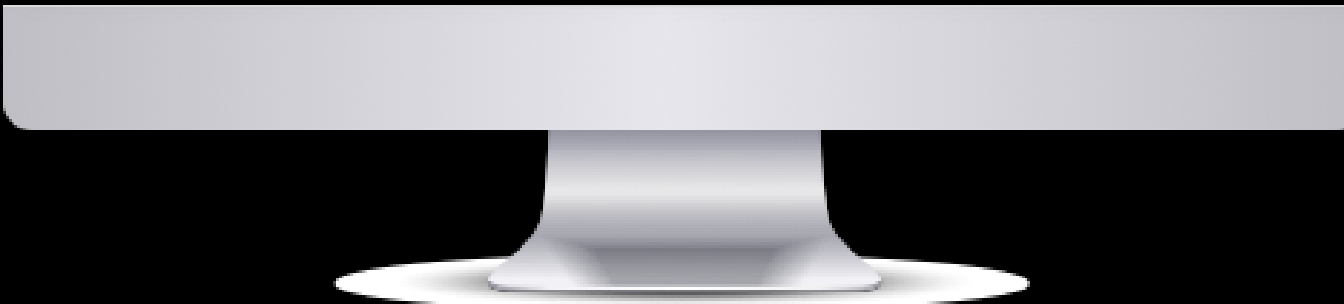
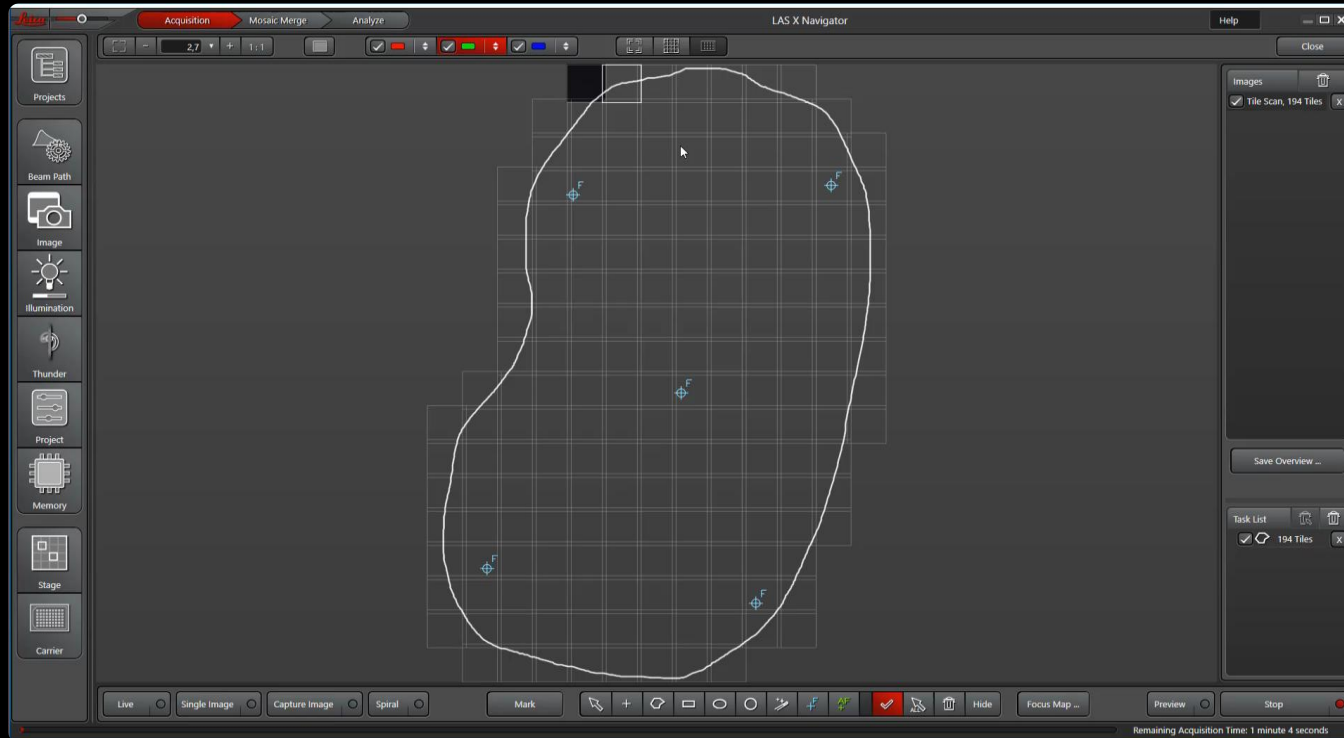
# Leica Navigator

## Spiral scan – preview – High magnification

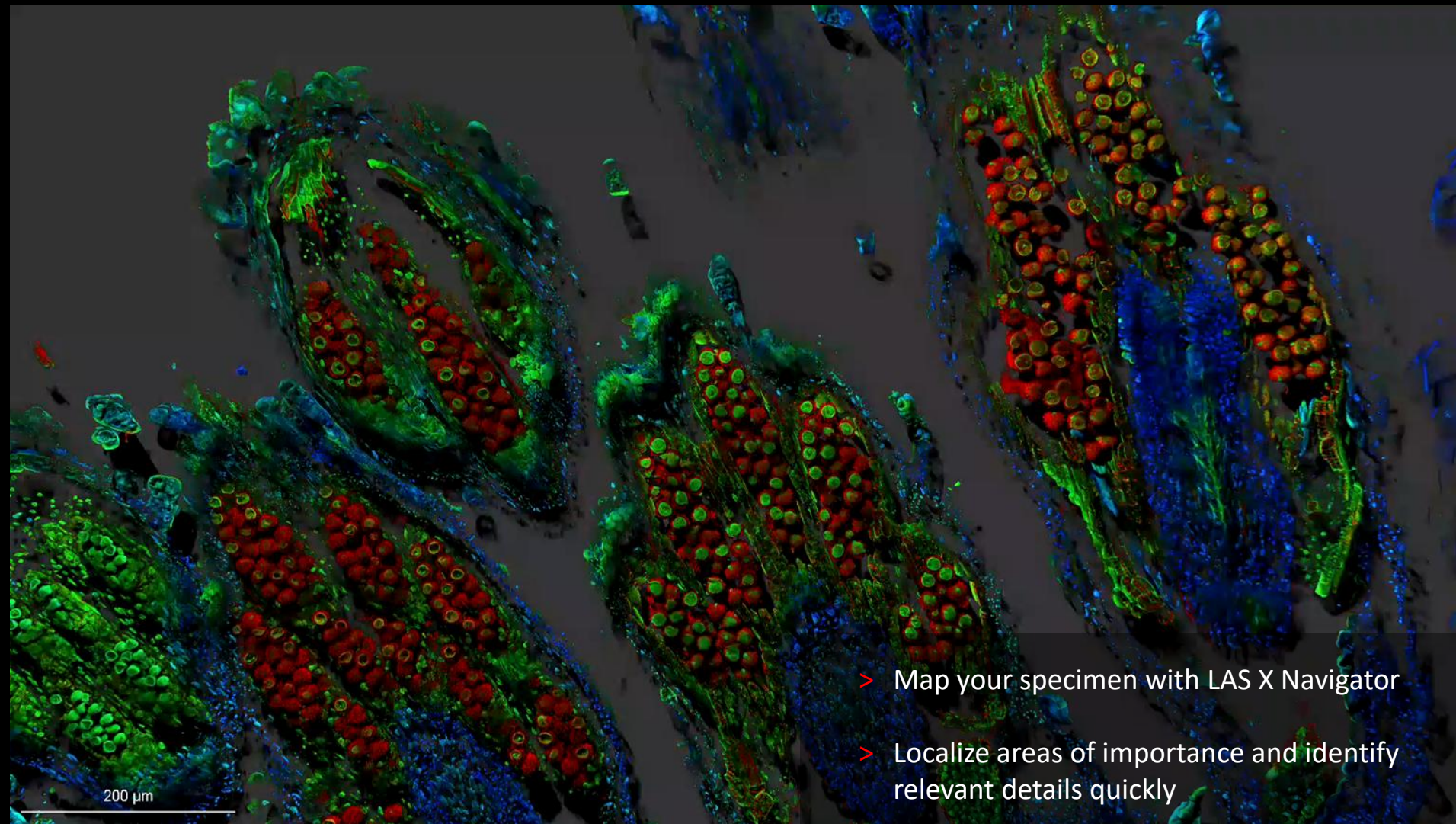


# LAS X software - Navigator

Flexible setting for tile scan and multiposition



# Relevant Details Instantly Identified



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

Daisy pollen. Image acquired with TauContrast and LAS X Navigator.

# Tandem Scanner



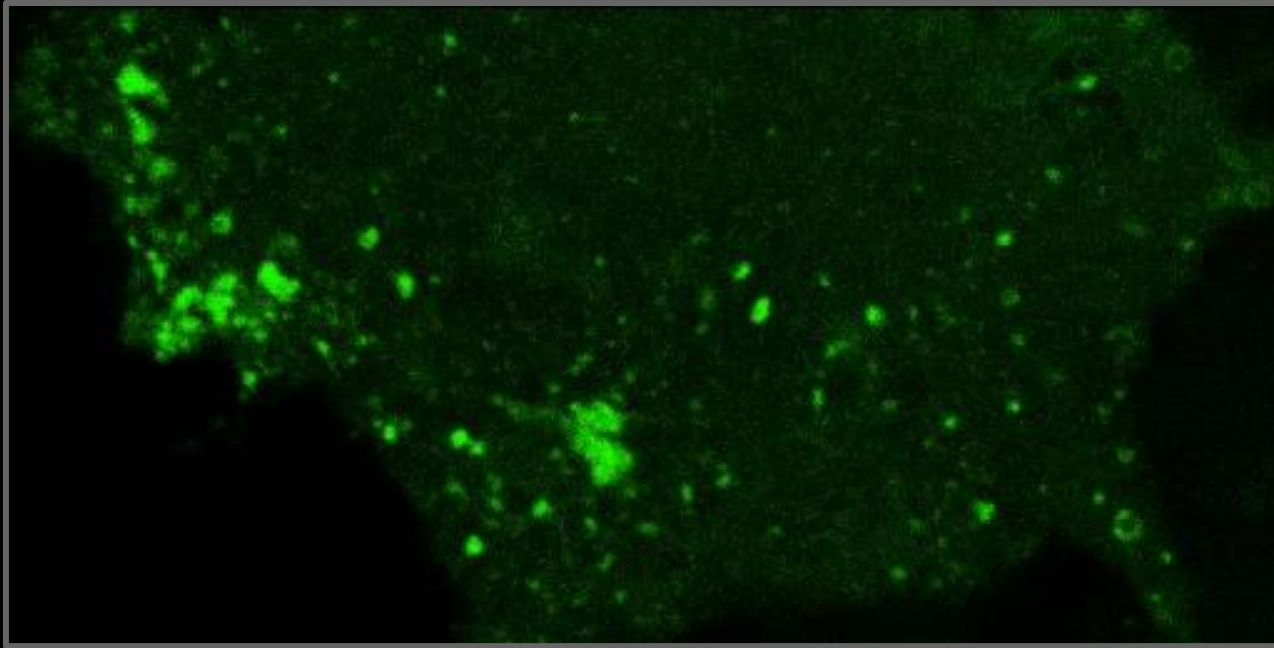
- Tandem Scanner with 8 kHz



- Fits in well with
  - SuperZ
  - Galvoflow
  - Power HyD

Scan format	8 kHz [fps]
512 x 512	28
512 x 32	145
512 x 16	286

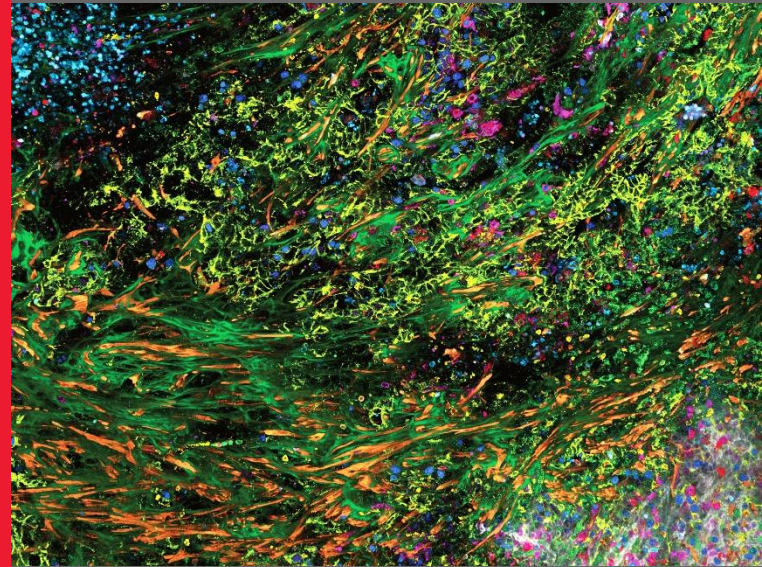
## 50 fps, Resonant Scanner



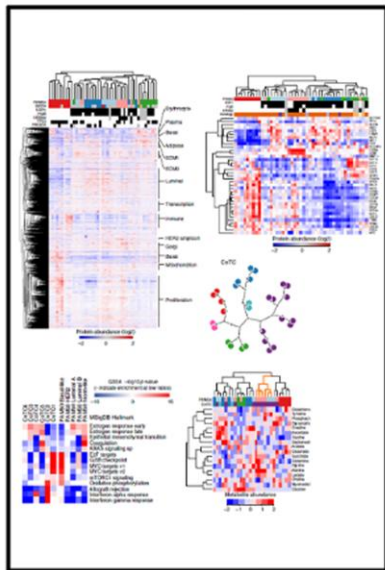
Hrab5a\_GFP, vesicle dynamics in live cell imaging, Sample Courtesy of Dr. Sandra Ritz, Microscopy & Histology Core Facility, Institute of Molecular Biology gmbH (IMB), Mainz, Germany. Transfection of EGFP by Marino Zerial

# STELLARIS SpectraPlex:

Discover the power of  
3D high-plex imaging across scales

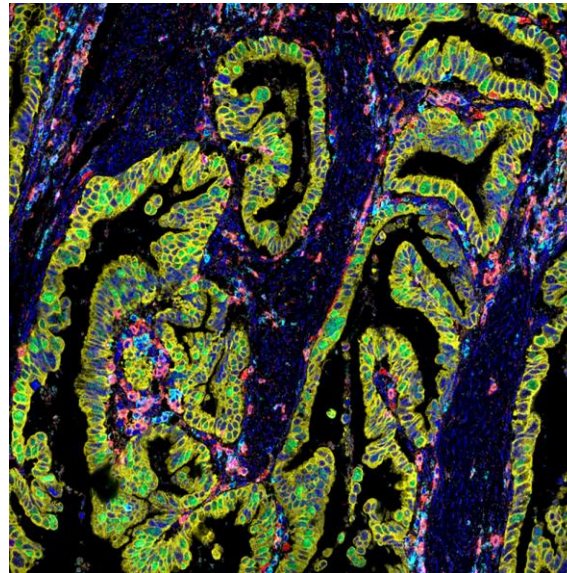


From Eye to Insight



Multi-Omics

2000s



Cervix cancer tissue showing 9 biomarkers with STELLARIS.

Spatial Biology

Cancer Research  
Immunology  
Neuroscience

Discover  
new cell types

Identify cell types  
and states

Map functional  
relationships

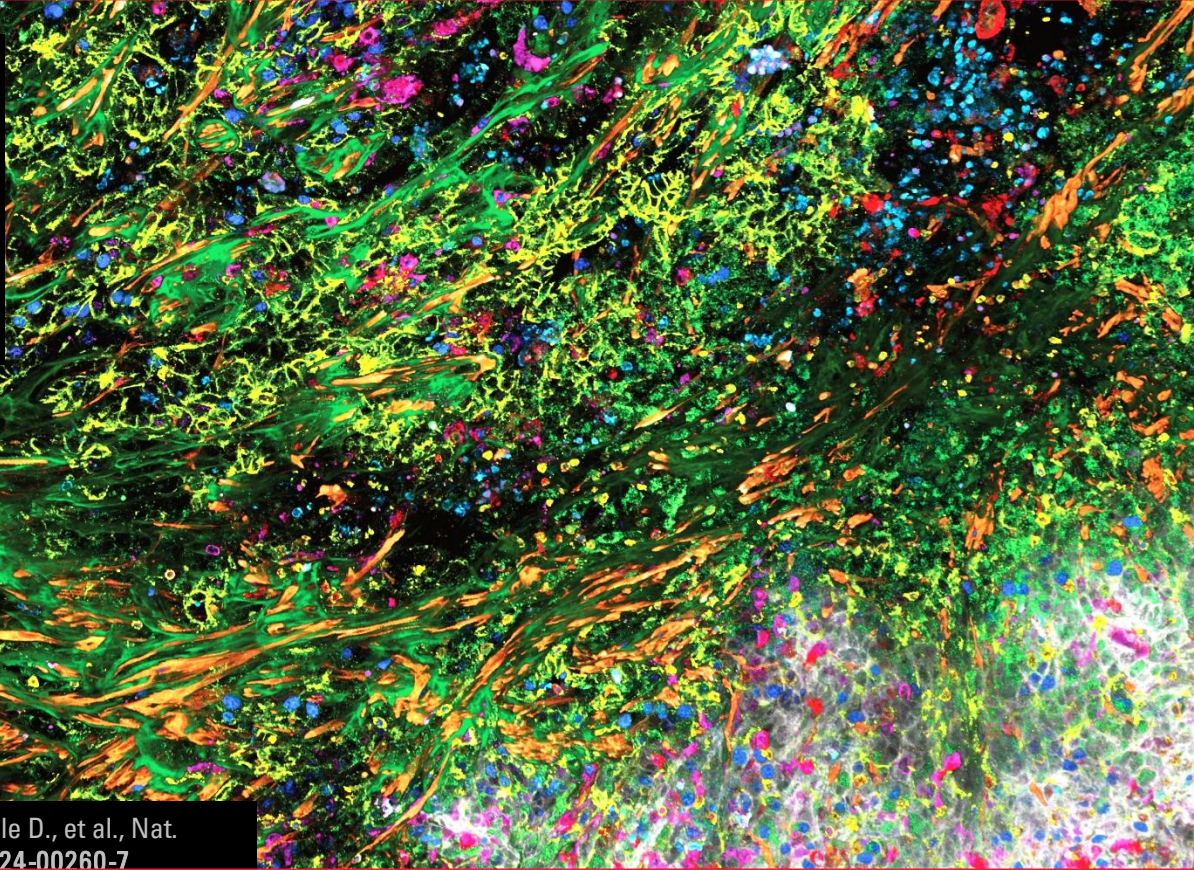
Spatial Phenotyping

# How Is SpectraPlex Integrated in STELLARIS?



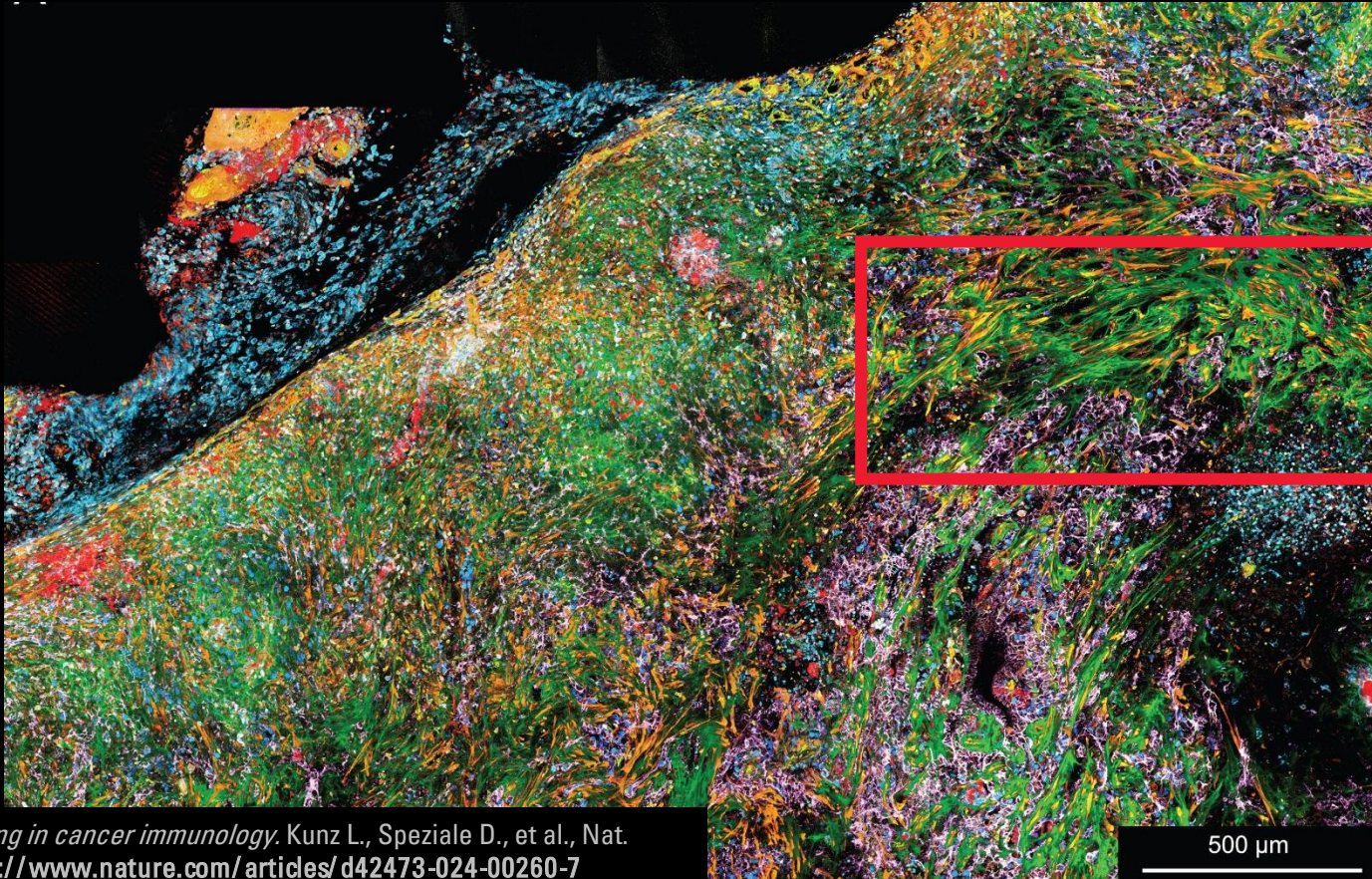
# Your Journey to Spatial Discoveries Begins Here

Adenocarcinoma mouse model  
70  $\mu\text{m}$  tissue section  
15-plex simultaneous labeling  
3D high-plex imaging in one go



*3D multiplexing imaging in cancer immunology.* Kunz L., Speziale D., et al., Nat. Methods (2024). <https://www.nature.com/articles/d42473-024-00260-7>

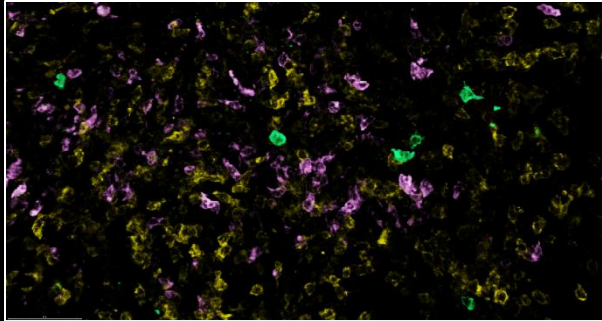
# Your Journey to Spatial Discoveries Begins Here



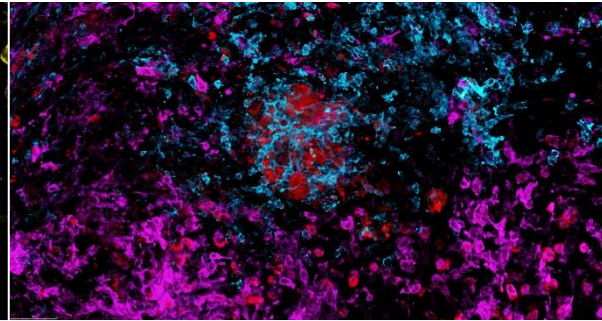
*3D multiplexing imaging in cancer immunology.* Kunz L., Speziale D., et al., Nat. Methods (2024). <https://www.nature.com/articles/d42473-024-00260-7>

500 μm

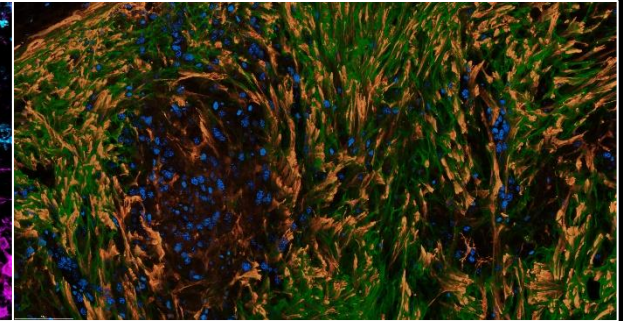
# Your Journey to Spatial Discoveries Begins Here



Lymphoid cells (B and T cells)

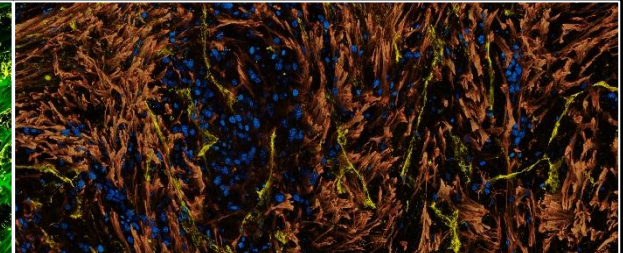
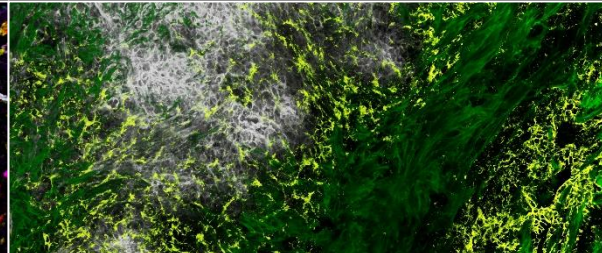
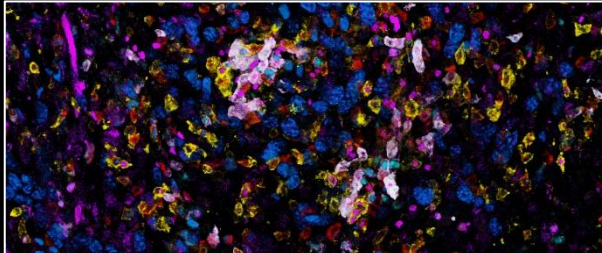


Lymphoid cells fighting tumor growth  
(TCF1 and PD1 expression)



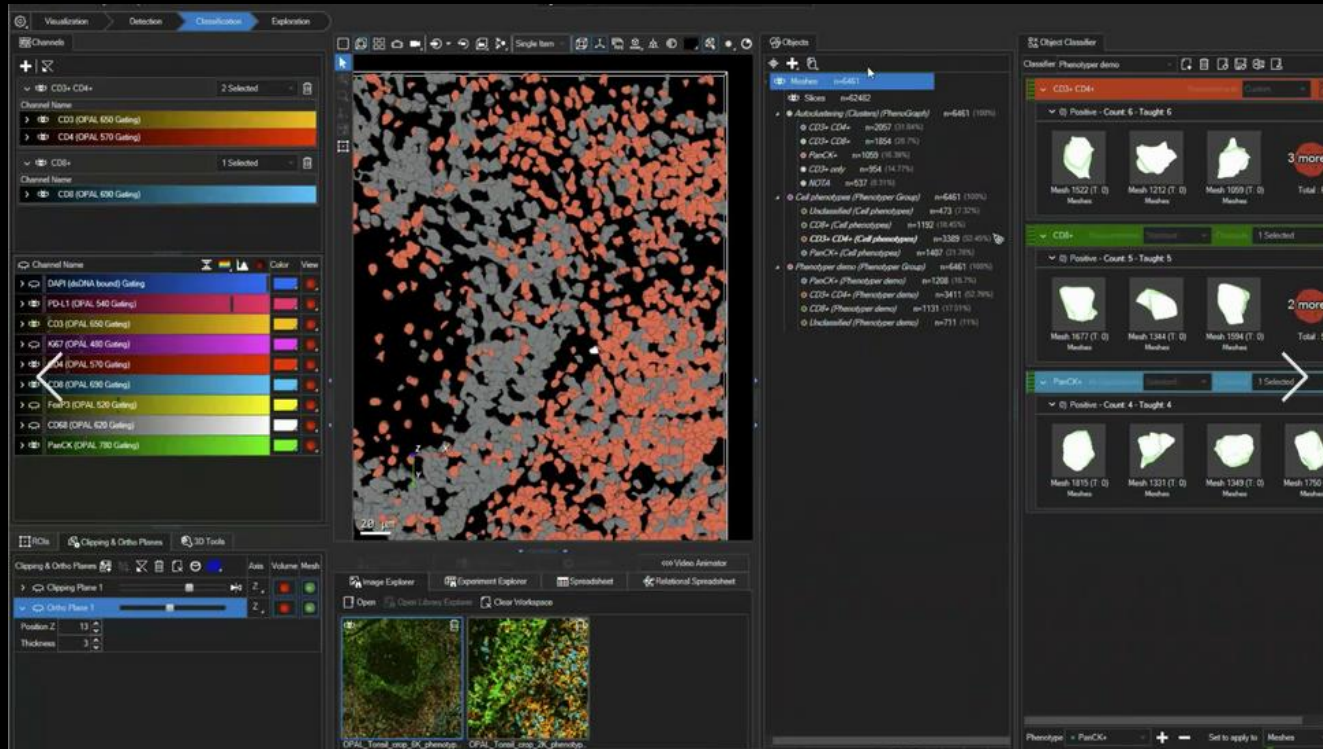
Myeloid cells

Cancer, stromal and extracellular matrix markers decorating regions of epithelial and endothelial cells



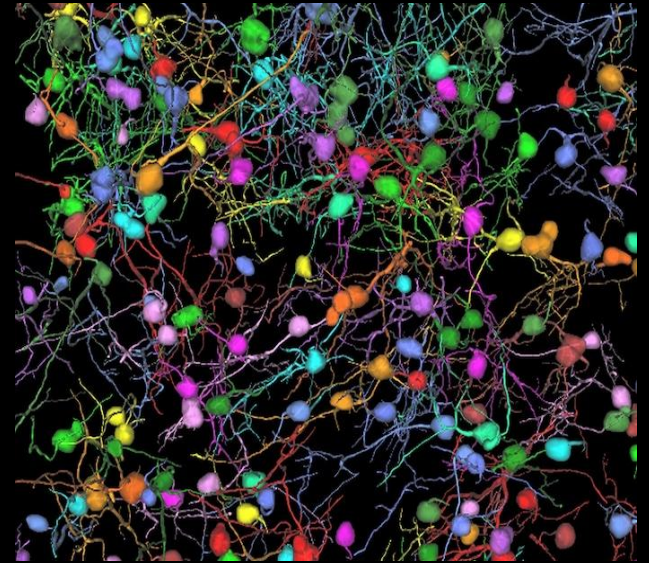
SpectraPlex enables a functional description of the tissue

# Benefit From a Workflow from Imaging to Analysis with Aivia

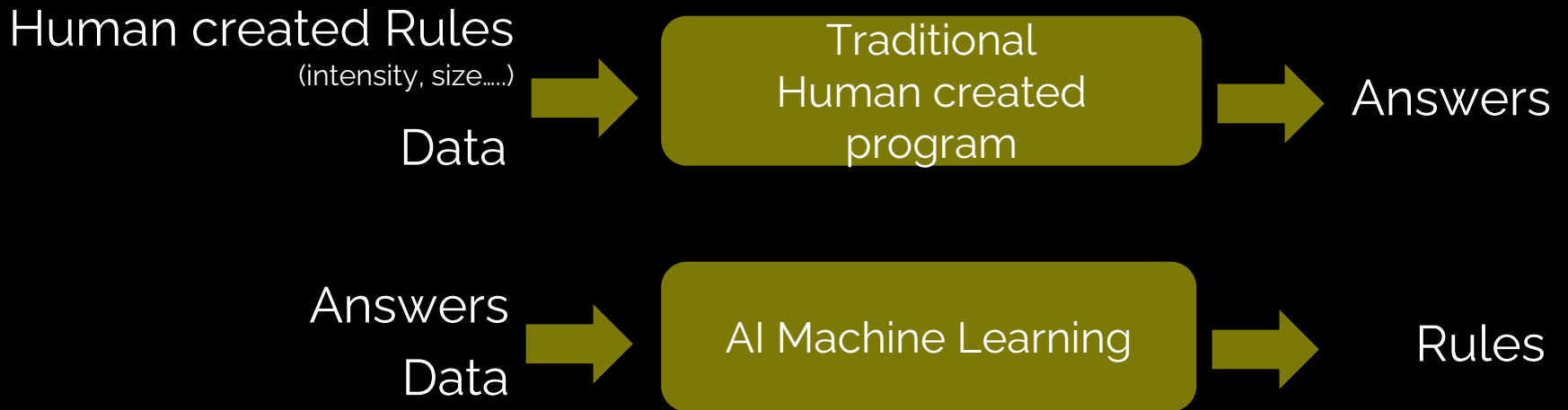


- Transfer your results from STELLARIS to Aivia and benefit from the expert- and data-driven approach for cell clustering and phenotyping.

AIVIA –  
AI based Imaging analysis software



# Traditional vs AI based image analysis (Provide examples)



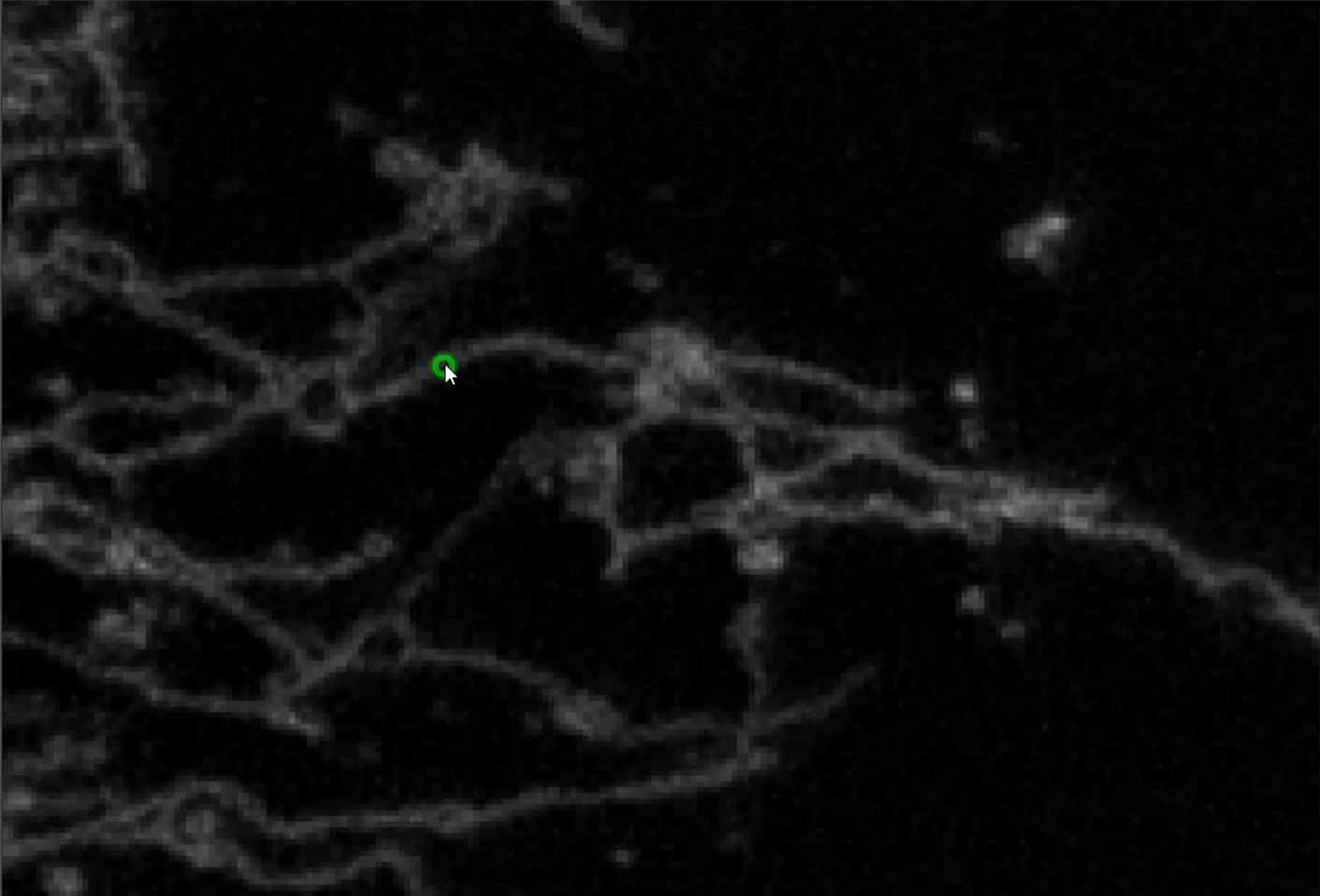
# Pixel classification\_AI Mitochondria analysis

Aivia - Update Available

File Edit View Analysis Help

Search or enter a command (Ctrl + F)

1:1 500.0%



Channel Settings

- Channel Name
  - Gray
  - Want
- Object Set Settings

Analysis Tools

Recipe Console Image Enhancement Tool Charts

Classifier (1) - 2D

Input & Training

Drawing

- Classifier (1)
  - Select pixel class
    - Want
    - Background

# Machine Learning in Microscopy (AIVIA AI image analysis software)

Channel Name

Channel Name	Color
> red	red
> green	green
> blue	blue

Object Groups

Recipe Console Image Enhancement Tool Charts Pixel Classifier

Classifier

Training Images Input/Output

Classifier

Select pixel class	Color	Output
Want	yellow	<input checked="" type="checkbox"/>
Background	gray	<input checked="" type="checkbox"/>

> Advanced Options

Teach

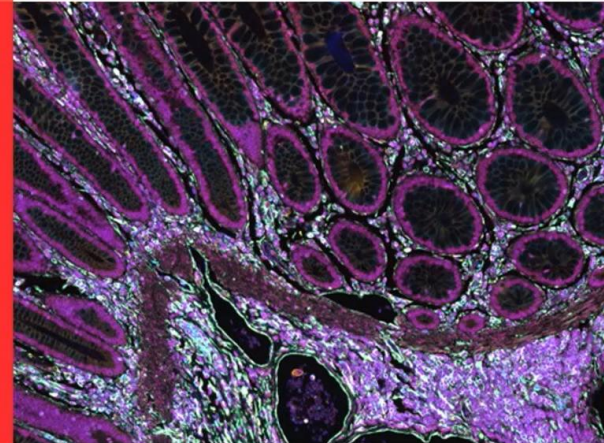
Image Explorer Annotations Outline Editor Track Editor Spreadsheet

1 Uncalibrated/Pixel Bbit : 2040x1536 Breast\_invasive\_scmhou... X: 228.0px Y: 670.0px Z: 0 T: 0 Intensity: (161, 71, 21)

# Highlight video: Complete Spatial Biology Workflow

*Leica*

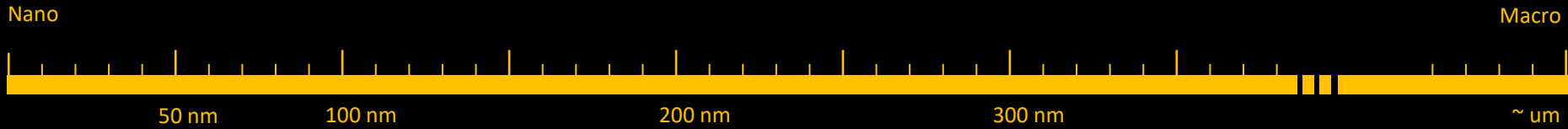
Full workflow for 2D / 3D spatial biology



From Eye to Insight



# LEICA STELLARIS



30 nm

140 nm

180 nm

**775STED+gSTED**

**Confocal SR**

**Confocal microscopy**

**Digital light sheet**

This block contains two microscopy capability cards. The first card, titled "775 pulsed STED", shows a "Confocal" image of red filaments and a "Gated STED" image of a red spot, with a red circular icon below. The second card, titled "Lightning", shows a 3D cell reconstruction and logos for X-S, NVIDIA, and CUDA.

This block contains a "DLS" (Digital Light Sheet) microscopy capability card, featuring a 3D reconstruction of a biological structure and an image of the DLS objective lens.

**STELLARIS**

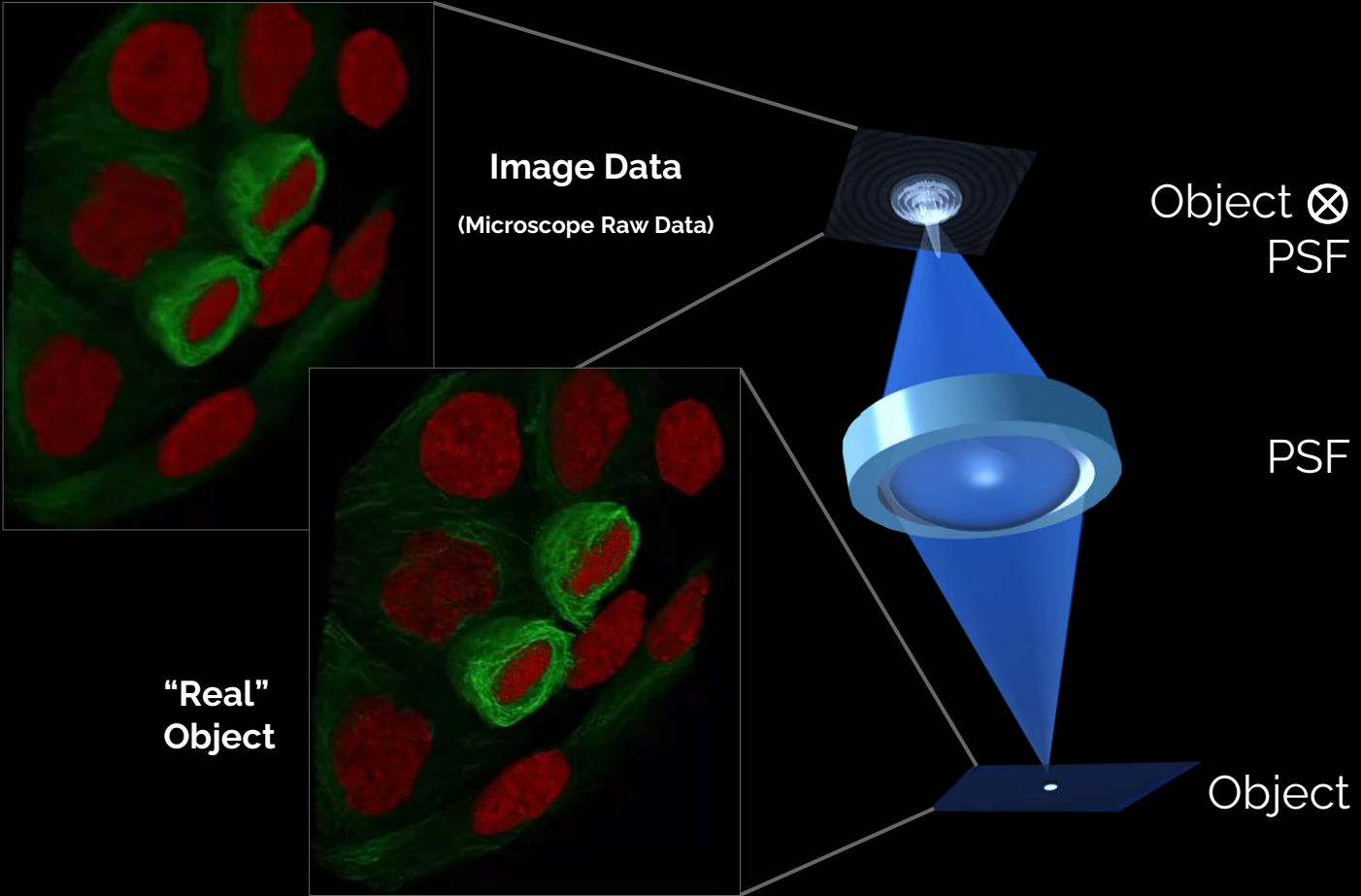


# LIGHTNING

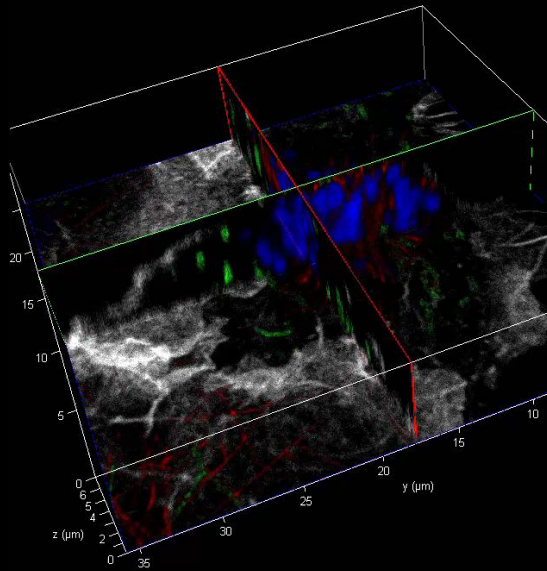
Image Information Extraction



# Microscopy Image Formation

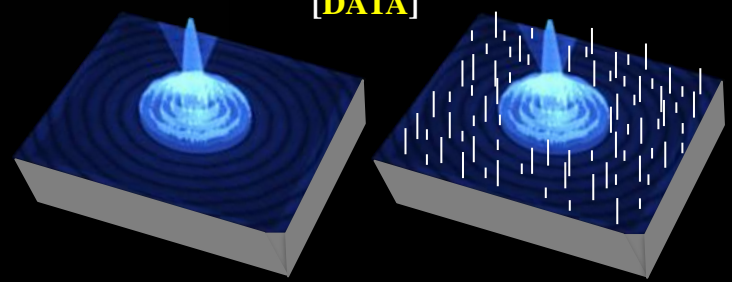
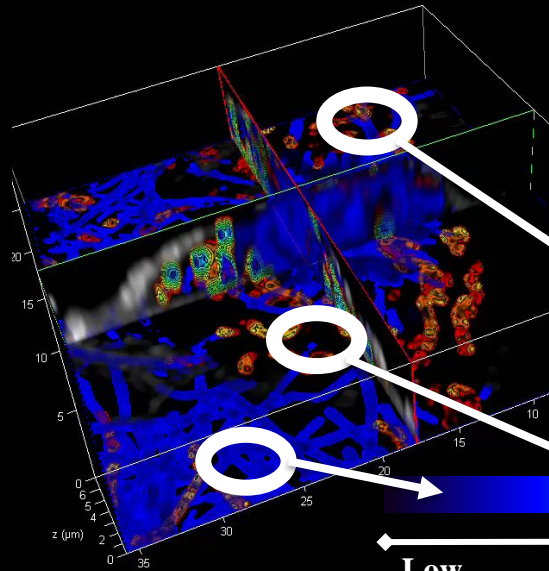


Raw data

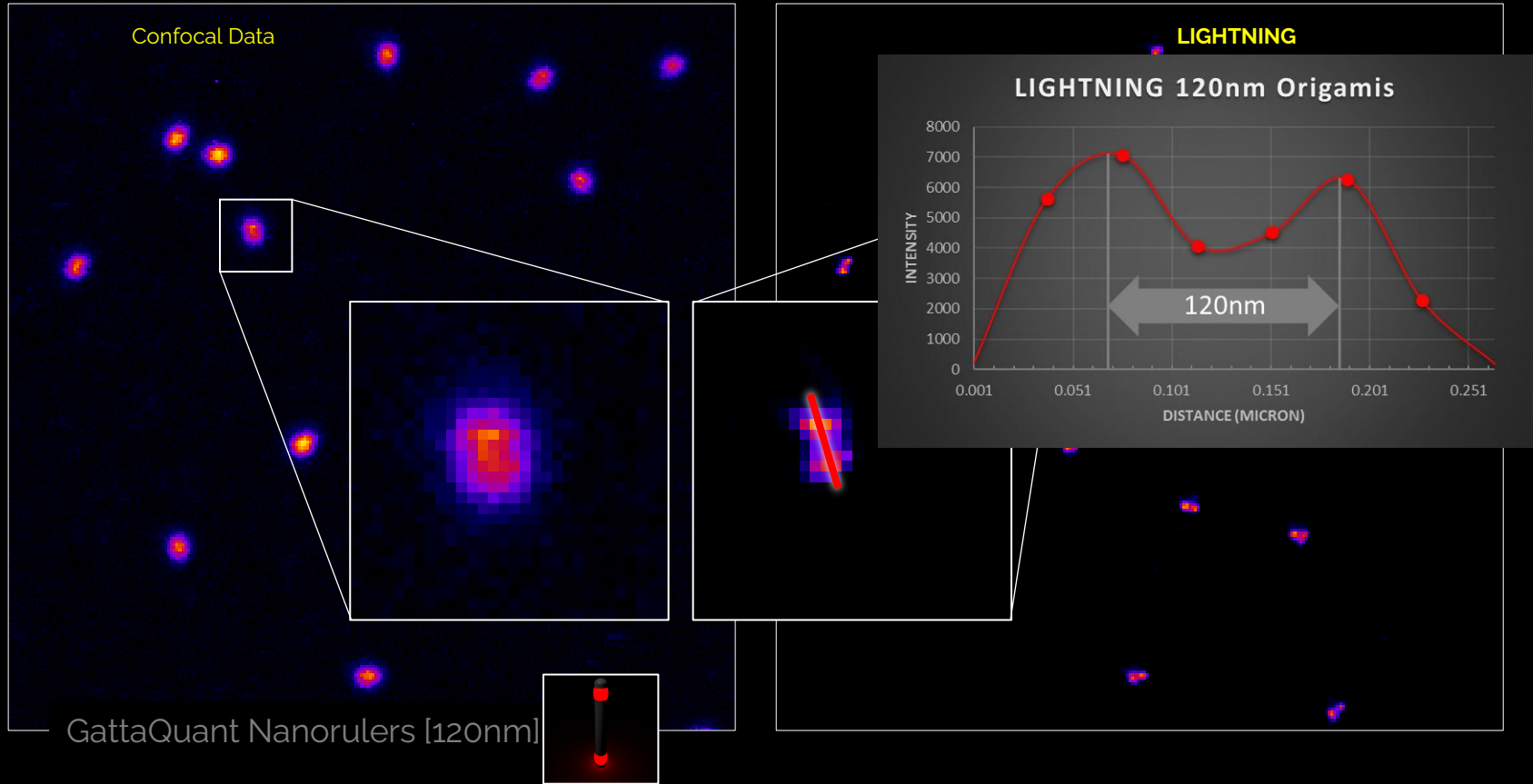


GATTA cells

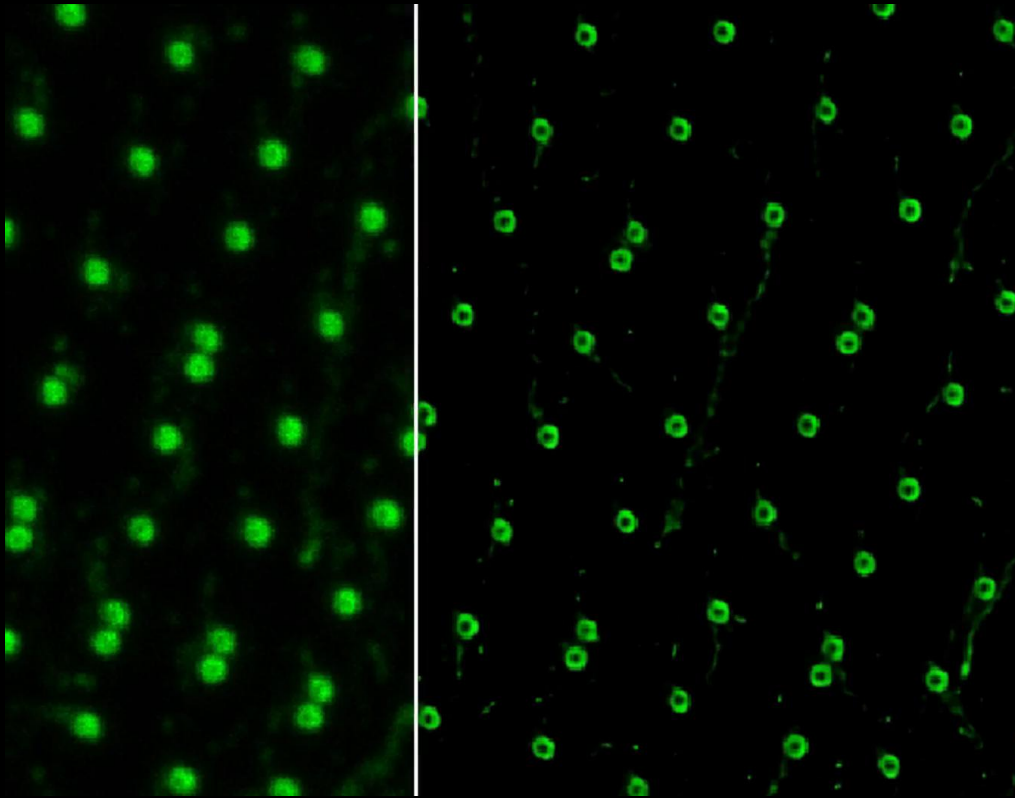
Decision Mask  
Position dependant image quality



# Accessing Super-Resolution



# LIGHTNING: Accessing The True Nature Of Image Data

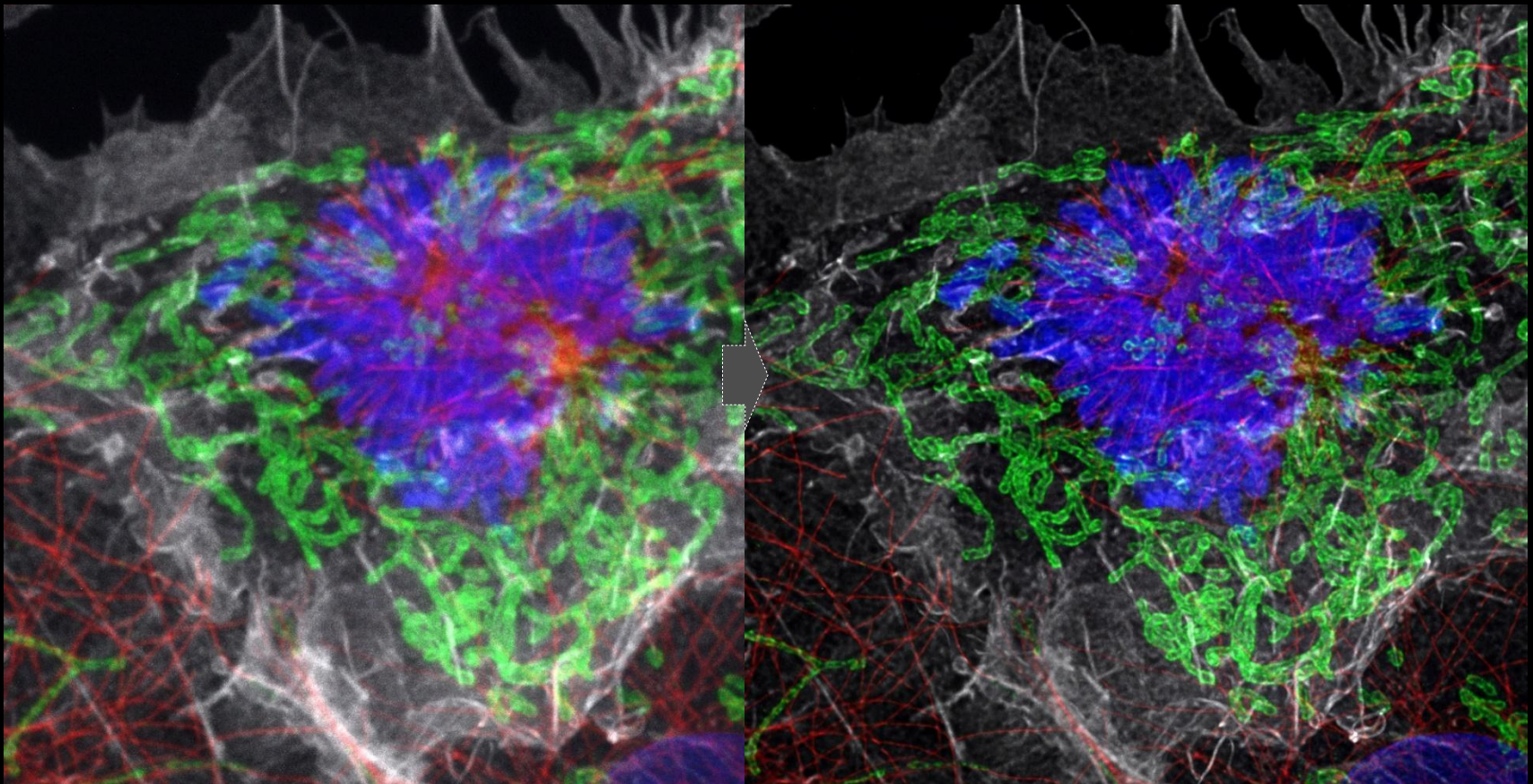


**Confocal | MP | gated STED**  
Including every imaging modality



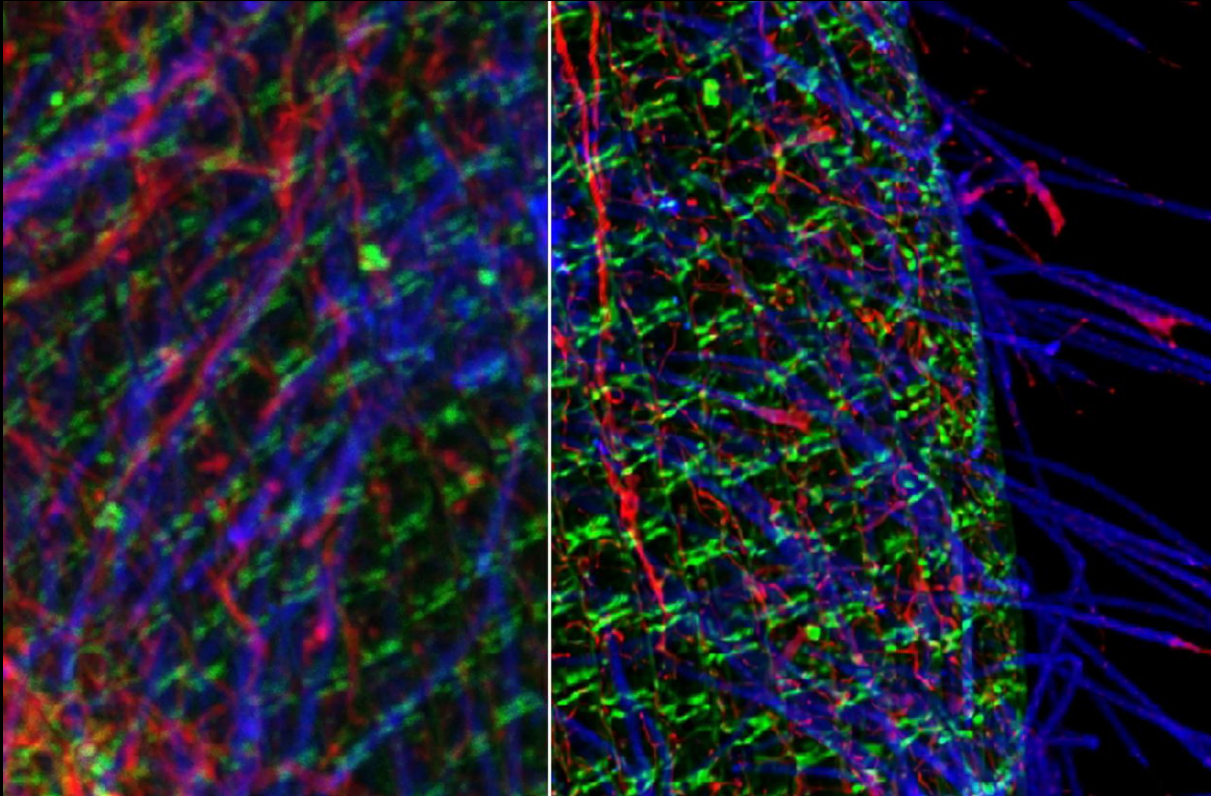
# LIGHTNING: Adaptive Multicolor Super-Resolution

## Adaptive Deconvolution



Gatta cells

# LIGHTNING: Adaptive Multicolor Super-Resolution



**Confocal | MP | gated STED**  
Including every imaging modality



# STELLARIS



*Leica*