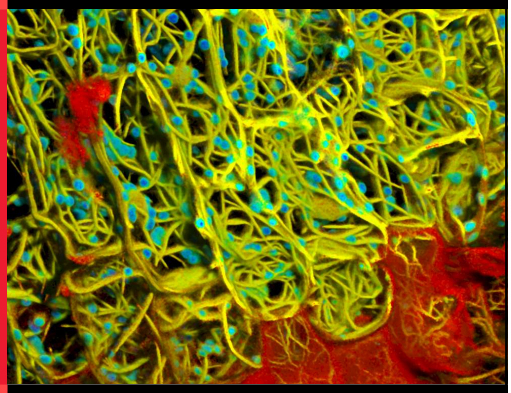
STELLARIS

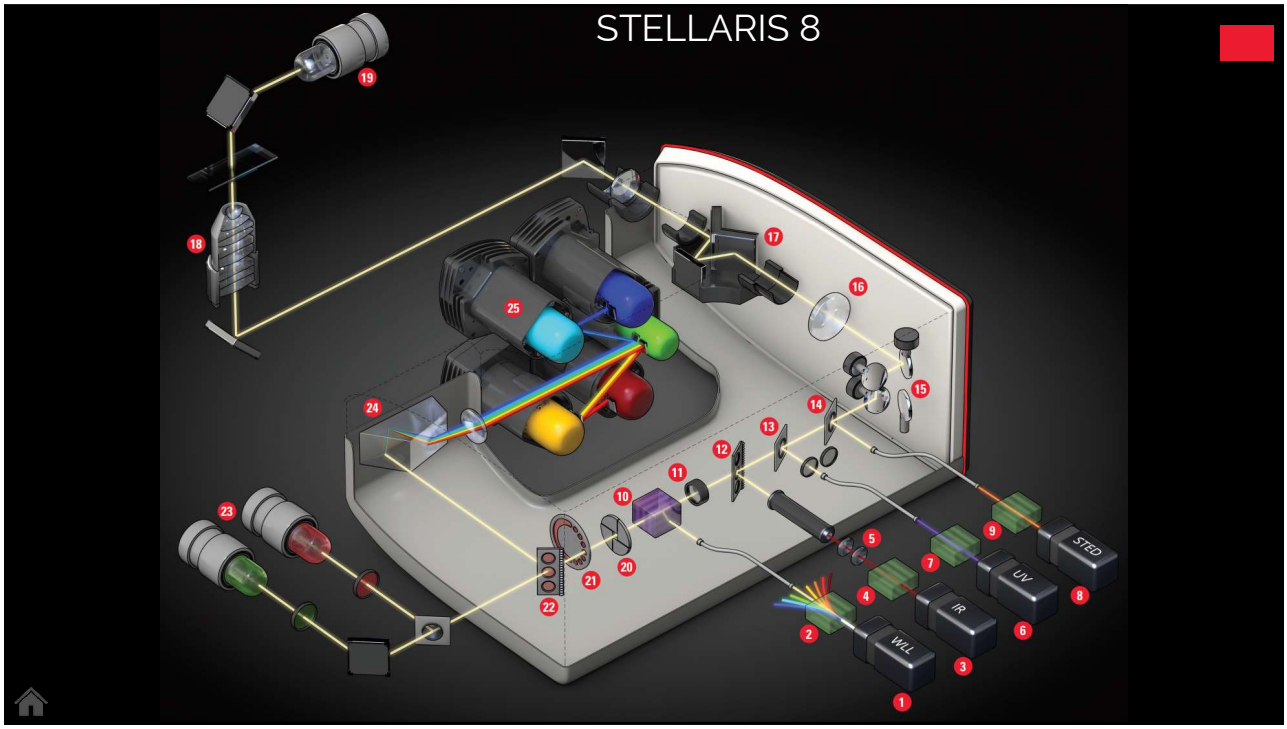
#CONFOCALREIMAGINED

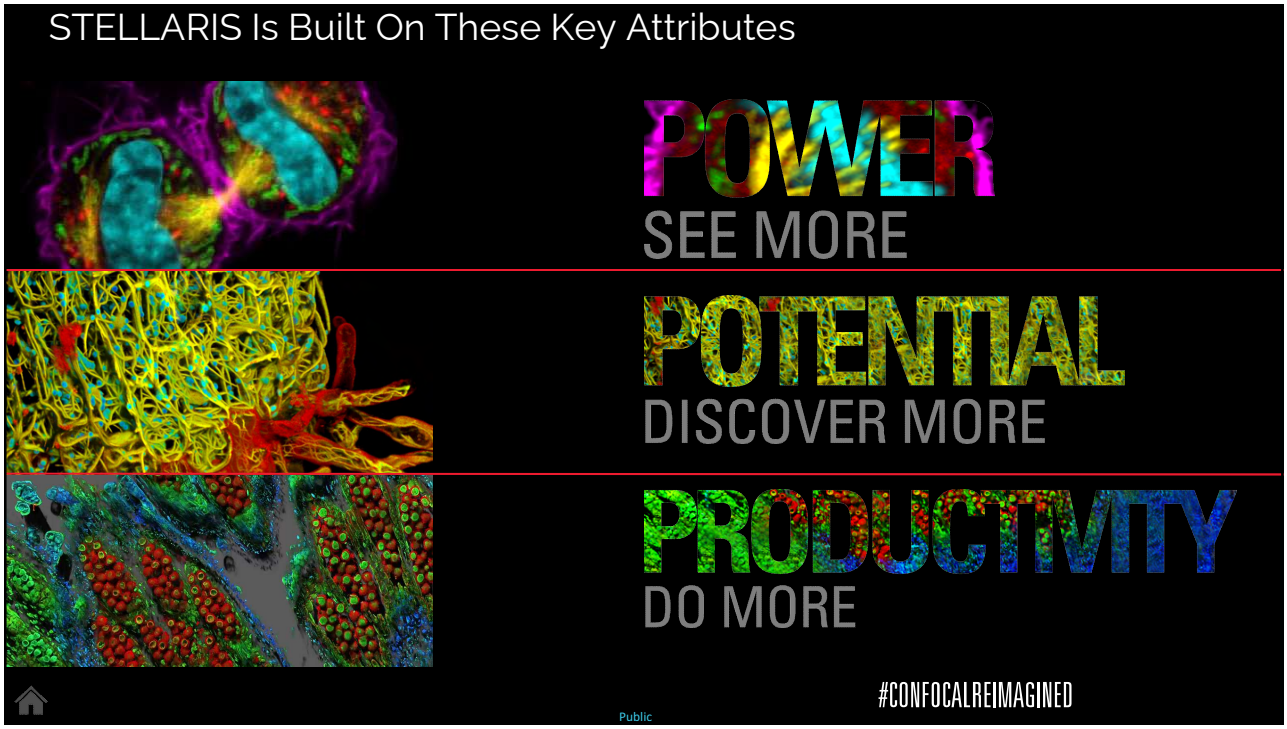
Qing Tang, Ph.D., Advanced Workflow Specialist - Confocal
Leica Microsystems Inc



Leica STELLARIS 8 Training Agenda @ UARK

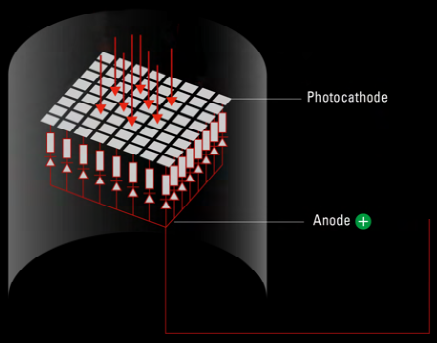
- STELLARIS 8 Confocal: Unique Features
  - WLL / AOBS / Prism / Detectors
  - Lifetime τ
  - LAS X ImageCompass and Navigator (Stitching)
- LAS X Software Preview
  - Dos and Don'ts
  - Multiple (Color) Channels / XY λ (Using ImageCompass)
  - Z Stack / Time Lapse / Live Cell Incubation
  - TauSense Tools: TauContrast; TauGating; TauSeparation etc.
  - LAS X Navigator (Stitching)


STELLARIS 8
19
18
17
16
25
24
15
14
13
12
11
10
23
9
STED
5
7
UV
8
21
20
4
IR
6
22
2
WLL
3
1

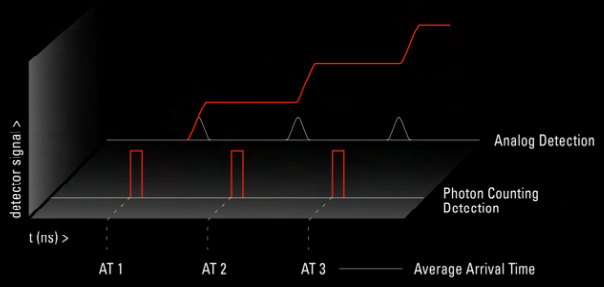

STELLARIS Is Built On These Key Attributes
POWER
SEE MORE
POTENTIAL
DISCOVER MORE
PRODUCTIVITY
DO MORE
#CONFOCALREIMAGINED
Public

Power HyD S: The New Standard For STELLARIS

Silicon-Based,
Multi-Pixel Photon Counter (MPPC) Technology



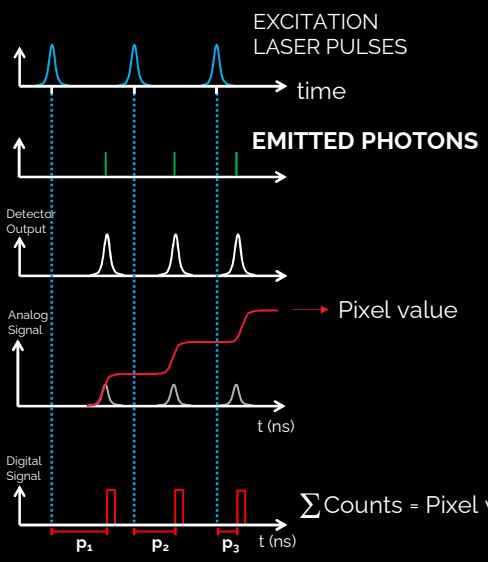
ANALOG MODE
> The default mode
> High dynamic range imaging



PHOTON COUNTING MODE
> Facilitates quantification
> Fluorescence lifetime-based imaging

Exceptional performance achieved with:
> High-end, proprietary electronics & optomechanics
> Efficient cooling to suppress dark noise

Analog And Photon Counting Modes For HyD S



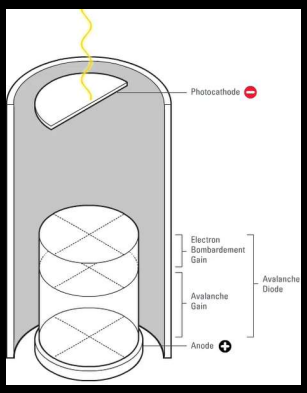
ANALOG DETECTION

PHOTON COUNTING

ANALOG DETECTION
- High dynamic range
- Traditional mode for confocal

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

Power HyD X and Power HyD R



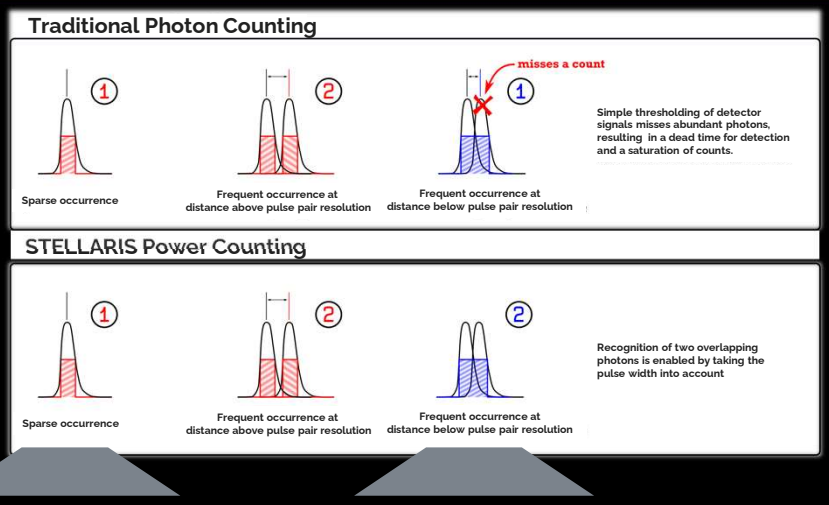
HyD X: GaAsP-Based Hybrid Technology

HyD R: Extended red-GaAsP Hybrid Technology

PHOTON COUNTING

- Extremely low dark noise to capture the faintest signals
- Efficient cooling scheme to suppress dark noise
- Facilitates quantification
- Fluorescence lifetime-based imaging

Power Counting: The New Photon Counting In STELLARIS



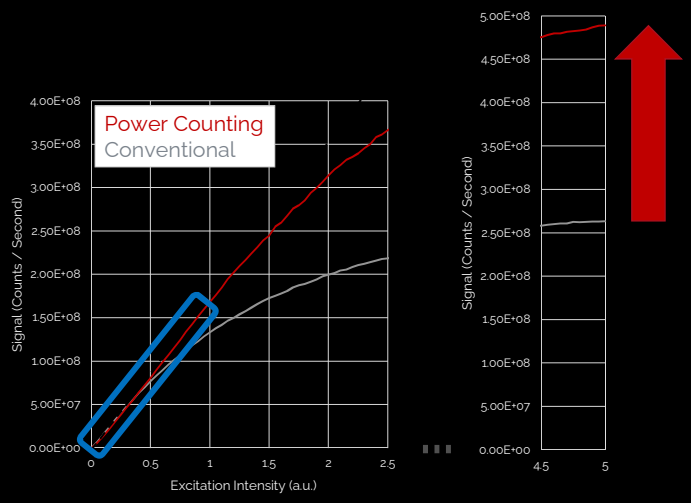
Low signal intensities:
Power counting and traditional counting results match

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

What Is The Benefit Of Power Counting?

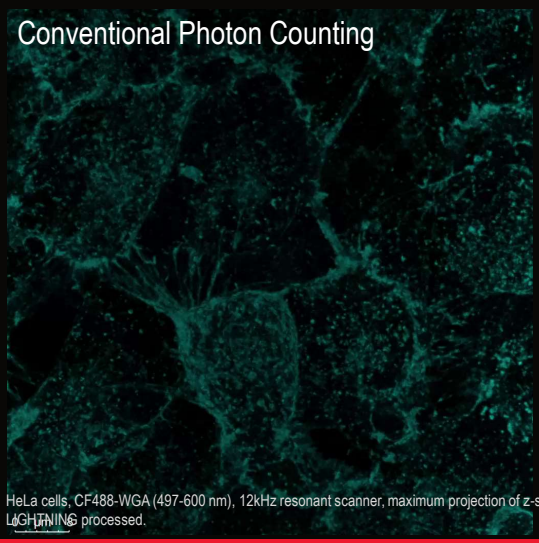
5 photons/pulse →
4 photons/pulse →
3 photons/pulse →
2 photons/pulse →
1 photon/pulse →

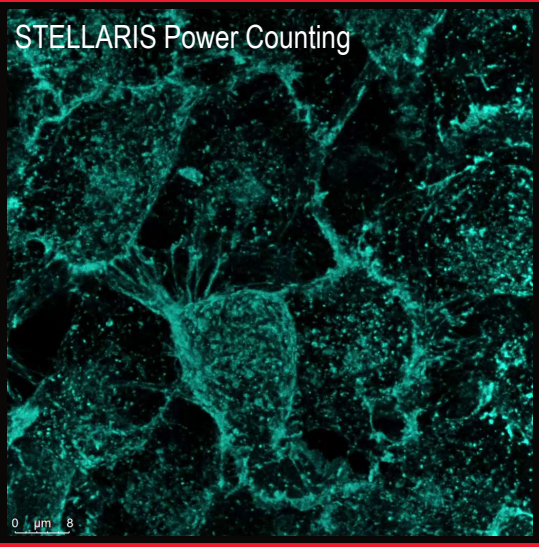
*at 80 MHz repetition rate



Power Counting Extends Significantly Dynamic Range And Linearity

What Is The Benefit Of Power Counting?

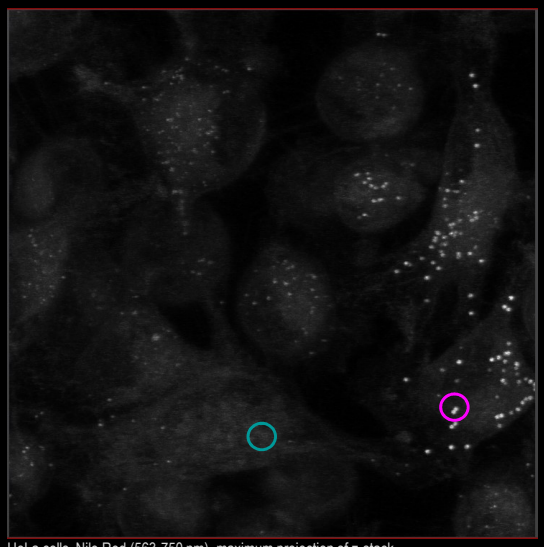




Power Counting Extends Signifiantly Dynamic Range And Linearity

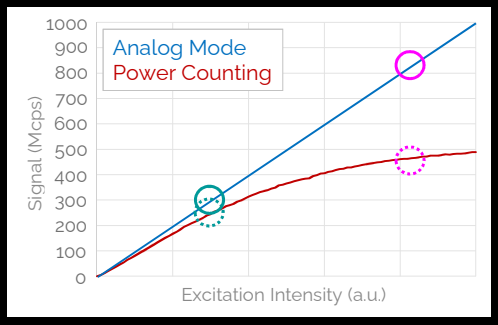
#CONFOCALREIMAGINED

Analog Mode: Enhanced Linear Dynamic Range



HeLa cells, Nile Red (563-750 nm), maximum projection of z-stack

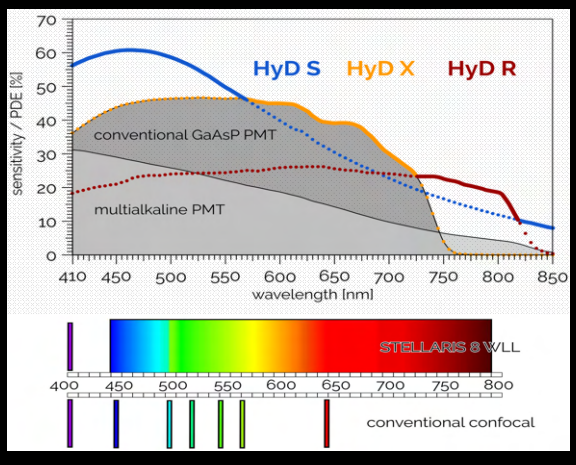
- Signal flux from vesicles approaching 1 Gcps!
- Beyond the linear range of photon counting

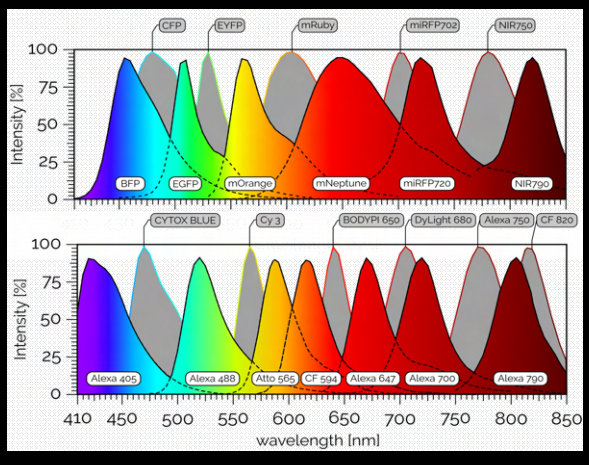


#CONFOCALREIMAGINED

Enhanced Spectral Freedom: STELLARIS 8

Photon Detection Efficiency (PDE):





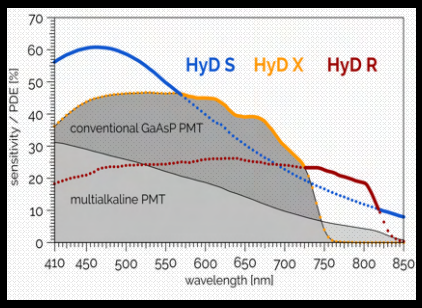
The Power HyD Family Covers The Needs Of Applications Throughout The Spectrum

Why do we specify PDE?

Quantum Efficiency (QE): Probability to convert incident photons into electrons in the photocathode.

Photon Detection Efficiency (PDE): Probability to convert an incident photon into a detected signal.

We provide PDE, since it is the quantity that counts!



PDE = QE * (Fill factor) * (Probability to trigger an avalanche).
Fill factor = (active detector area)/(total detector area).

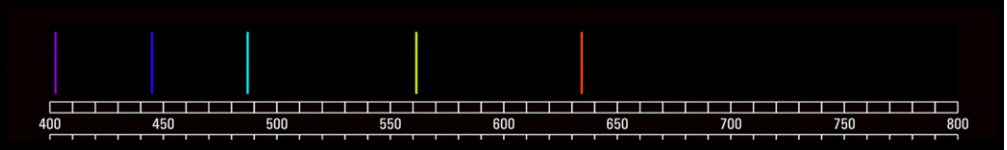
QE is always equal or larger than the specified PDE

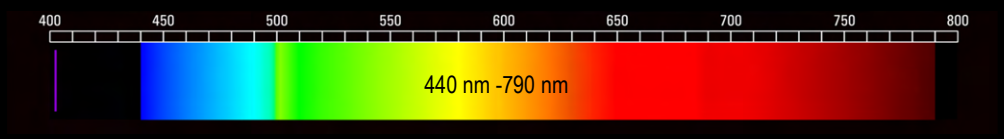
HyD X and HyD R:
Fill factor = 1; Probability to trigger an avalanche = 1, → QE = PDE

HyD S:
Multi-cell architecture → Fill factor < 1 → QE > PDE

Expanded Excitation Flexibility:STELLARIS 8

STELLARIS





- Expanded Multicolor Flexibility!
- Combine CW & Pulsed lasers

Laser 405 DMOD
Laser 405 AOTF

Also available: 355 nm
Also available: 730 nm

VIS solid state laser lines
Laser 448 nm (**V**iolet)
Laser 488 nm (**B**lue)
Laser 514 nm (**T**urquoise)
Laser 561 nm (**G**reen)
Laser 638 nm (**R**ed)

#CONFOCALREIMAGINED

The Red Extended Benefits Of Our Next Generation WLLs

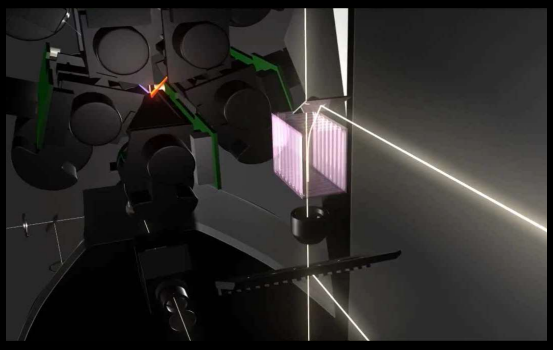
- Excite each fluorophore optimally at its excitation peak
- Enhance multiplexing capabilities by adding up to 3 more fluorophores in the NIR range
- Broad range of new fluorophores becomes accessible to STELLARIS 5 and STELLARIS 8

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

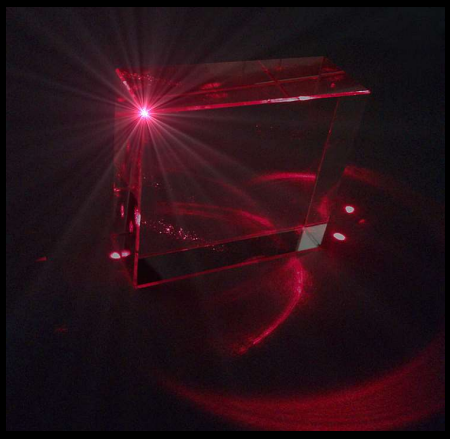
What Is Behind The White Light Laser Technology?

- The White Light Laser emits white light composed of all available wavelengths
- Tunability is achieved with the Acousto Optical Beamsplitter (AOBS)
- Up to 8 wavelengths can be picked simultaneously
- Microsecond switching time for line sequential acquisiton
- Free choice of wavelength with nm precision across the spectrum



Benefits of the Leica Acousto Optical Beam Splitter (AOBS)

- Highly transparent
  - Increase sample lifetime
  - Cell viability
- Flexible
  - links in with WLL, diode lasers
  - more diverse experiments
- Ease of use
  - Save training time
  - Better turnover (low failure rate)
- Supports new dyes
  - Future-proof



The Leica AOBS Maximizes Signal Collection

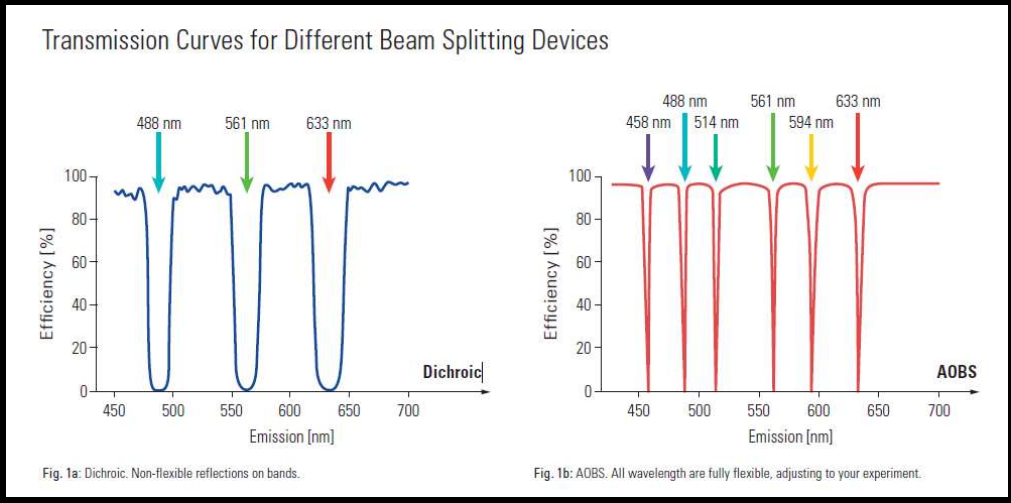
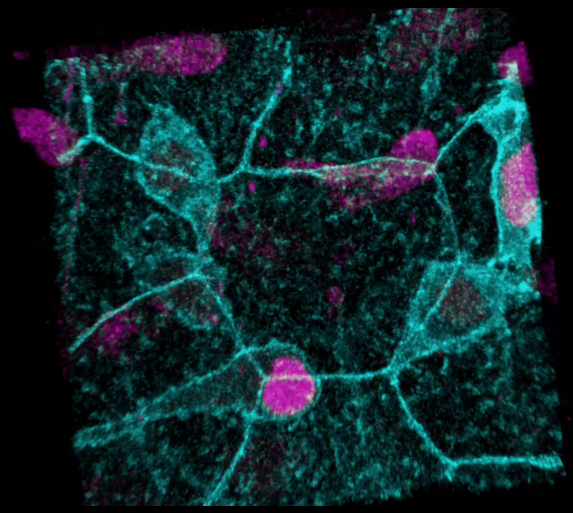


Fig. 1a: Dichroic. Non-flexible reflections on bands.

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 Gives You Gentle Live Cell Imaging



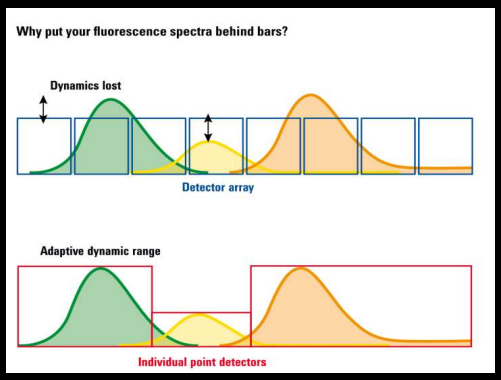
Zebrafish posterior lateral line primordium migration. Cell membrane (cyan), Nuclei (magenta)
Sample Courtesy: Jonas Hartmann, Gilmour Group, EMBL Heidelberg.

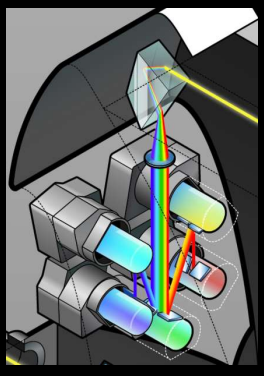
- Perform imaging for longer periods, since both excitation as well as detection are optimally tuned
- Preserve sample integrity through efficient signal acquisition at the lowest required levels of illumination
- Possible thanks to redesigned optics for optimized transmission

#CONFOCALREIMAGINED

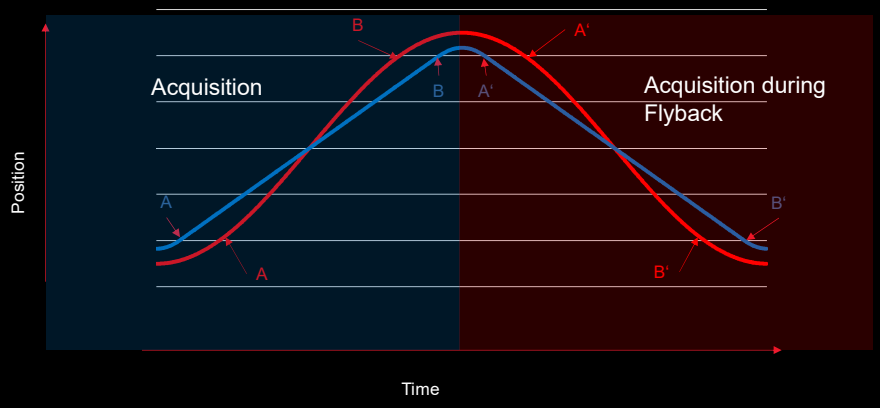
Prism-based spectral detection

- Bandwidth freely tunable, band-Edges freely tunable
- Arbitrary 1 nm step size to precisely cover emission spetra of each fluorophore
- Unmixing not mandatory. It is possible, of course, if desired.





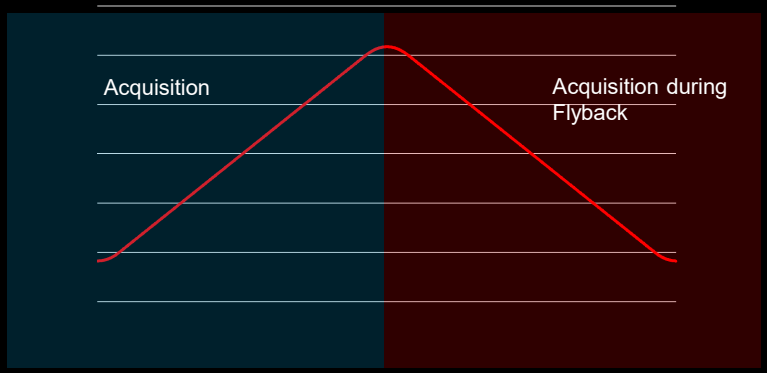
Movement of Line Galvanometer



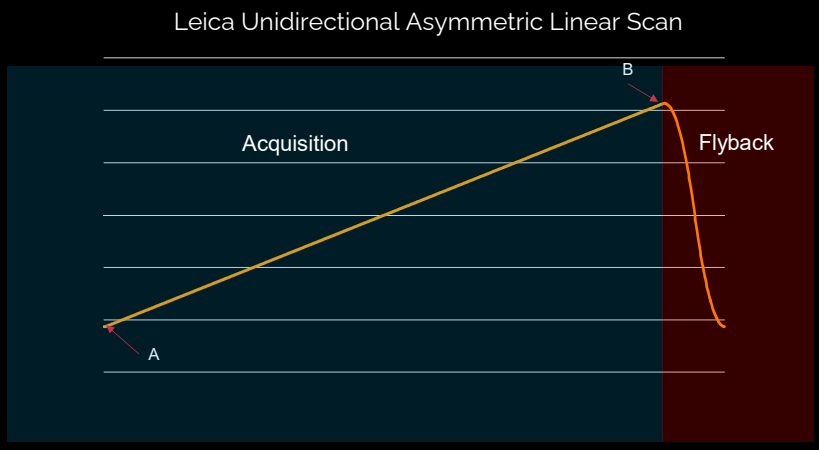
comparison sinusoidal and linear

Movement of Line Galvanometer

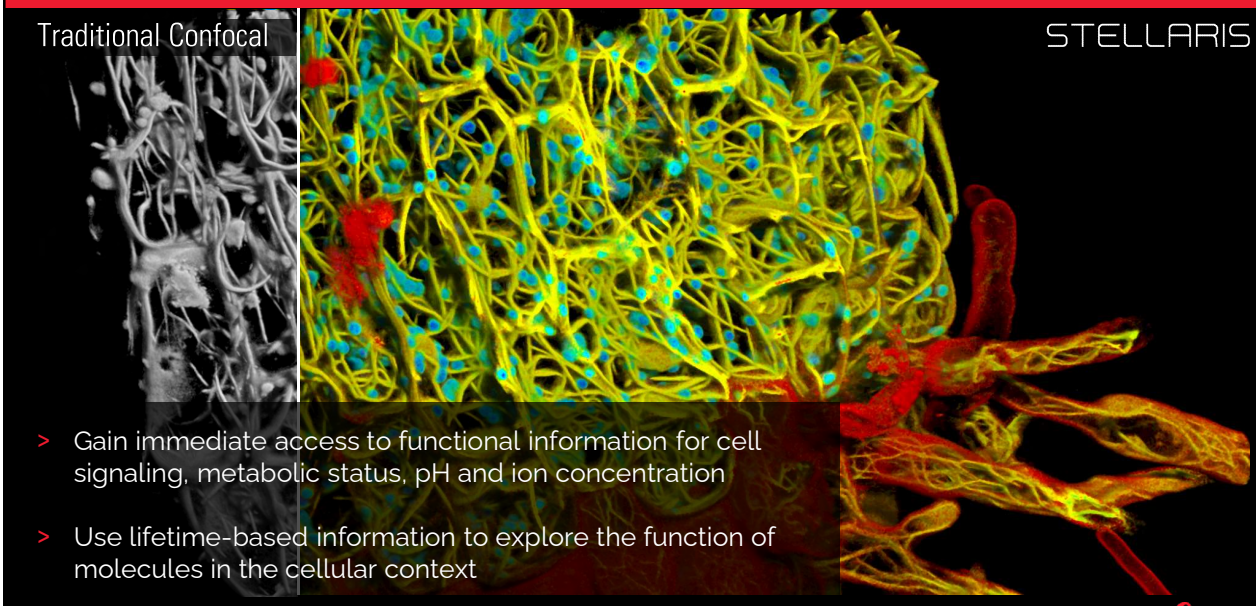
Leica Bidirectional Linear Scan



Unidirectional Scanning



Explore A New Dimension Of Information

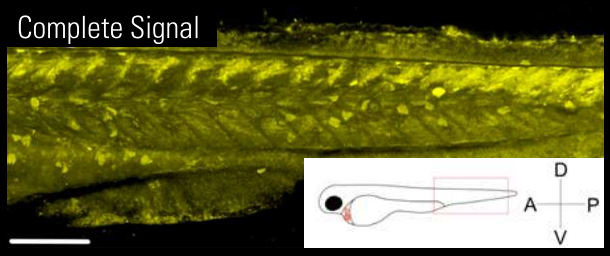


- Gain immediate access to functional information for cell signaling, metabolic status, pH and ion concentration
- Use lifetime-based information to explore the function of molecules in the cellular context

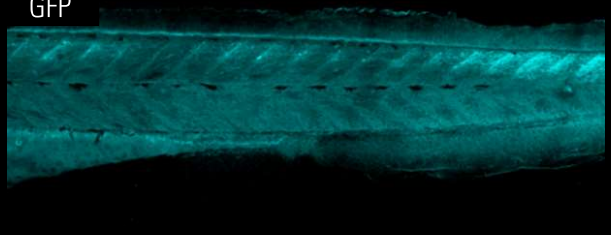
#CONFOCALREIMAGINED

Improve Image Quality

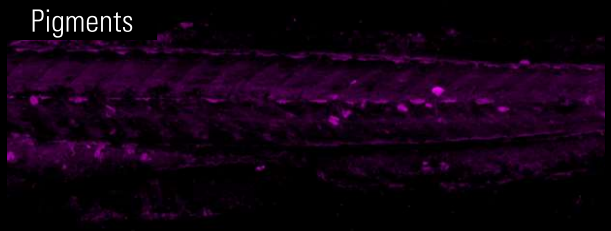
Complete Signal



GFP



Pigments



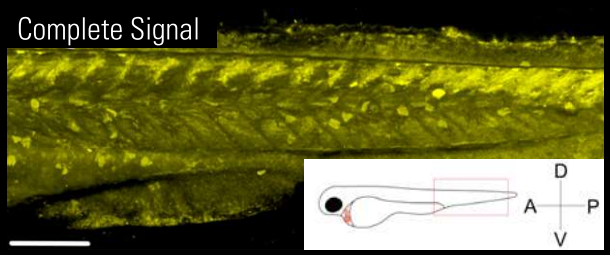
Zebrafish 4xGTIIC:d2GFP line exhibiting native pigments. GFP fluorescence provides a readout of Yap1/Taz-Tead activity and is used here to visualize the striated muscle of the trunk at 55 hpf. The signal of interest (cyan, long ATs) is singled-out from endogenous pigment contributions (magenta, short ATs). Scale bar: 200 µm.

Sample courtesy: Julien Vermot, IGBMC, Strasbourg.

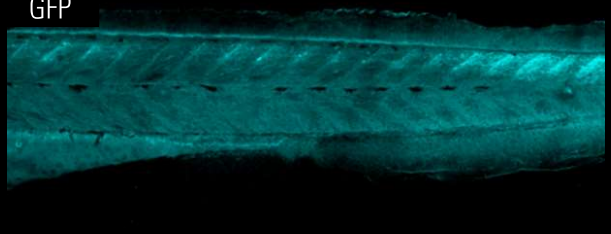
#CONFOCALREIMAGINED

Improve Image Quality

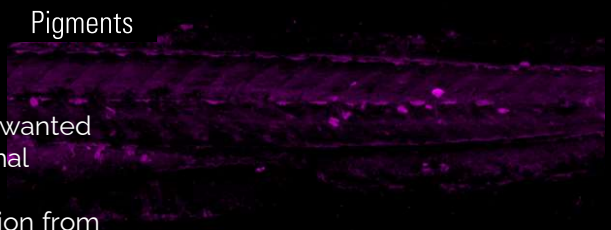
Complete Signal



GFP



Pigments



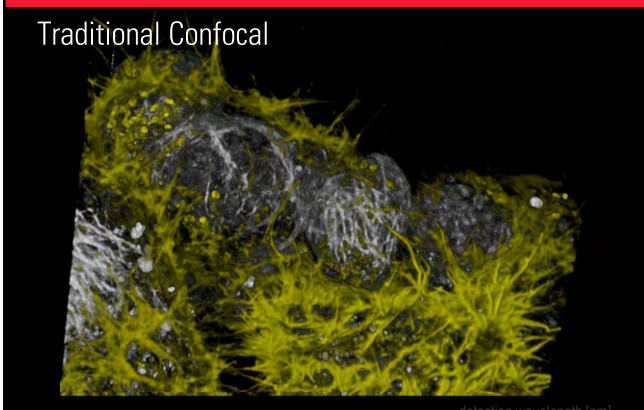
- Maximize detection efficiency by removing unwanted contributions while preserving the desired signal
- Enjoy the ease of identifying relevant information from intrinsic contributions

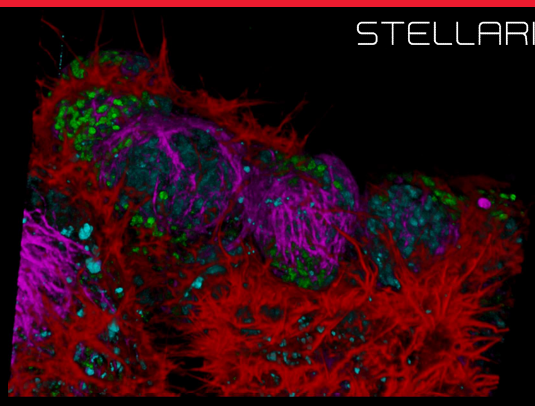
ica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

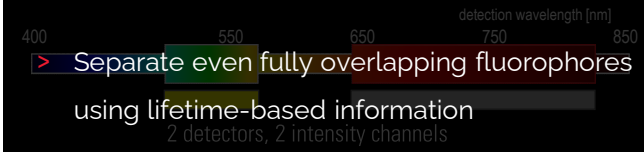
Multiplex Beyond The Spectral Options

Traditional Confocal

STELLARIS







> Separate even fully overlapping fluorophores using lifetime-based information

2 detectors, 2 intensity channels

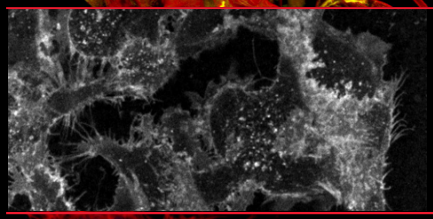
NE-115 cells. LifeAct-mNeonGreen (left: yellow, right: red), MitoTracker Green (left: yellow, right: green), NUC Red (left: gray, right: blue), and SiR-tubulin (left: gray, right: magenta). Courtesy: Max Heydasch, University of Bern and Spirochrome

STELLARIS Potential Delivers The Following Benefits

POTENTIAL
DISCOVER MORE

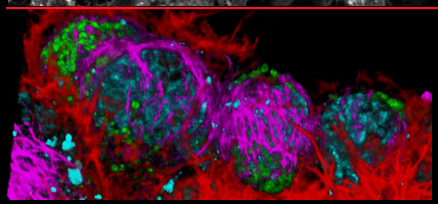
Explore a new dimension of information

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



Improve image quality

> TauGating removes undesired signal contributions
> Maximize detection efficiency



Separate species beyond the spectral options

> TauSeparation separates even fully overlapping fluorophores with lifetime-based information

#CONFOCALREIMAGINED

The Technology Behind STELLARIS Potential

What Is TauSense?

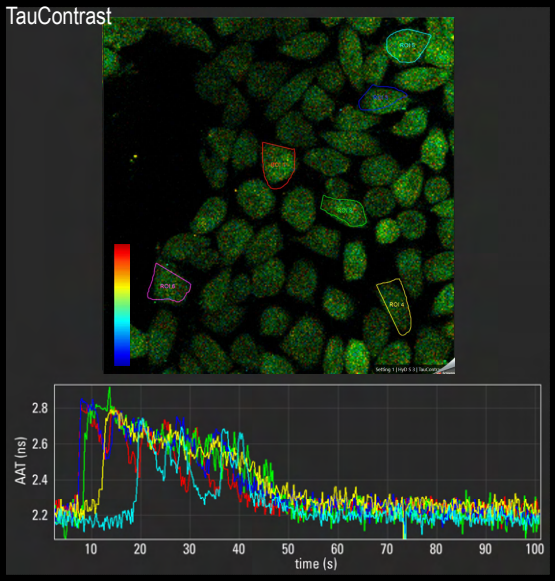
- A new, straightforward way to generate images using lifetime-based information.
- Access an extra dimension of information:
  - Qualitative: reveal contrast worth exploring
  - Quantitative: quantify relative changes happening within the sample
- Understand molecular function within the cellular environment, increase image quality, expand the number of probes that can be visualized in a specimen.
- Set of tools for distinct applications:
  TauConstrast, TauGating, TauScan and TauSeparation (+TauSTED for STED).
- Smaller data size and computational load compared to classical approach (FLIM).

Explore A New Dimension Of Information

Explore A New Dimension Of Information

Application Example: TauContrast

Monitoring Calcium Oscillations Using TauSense

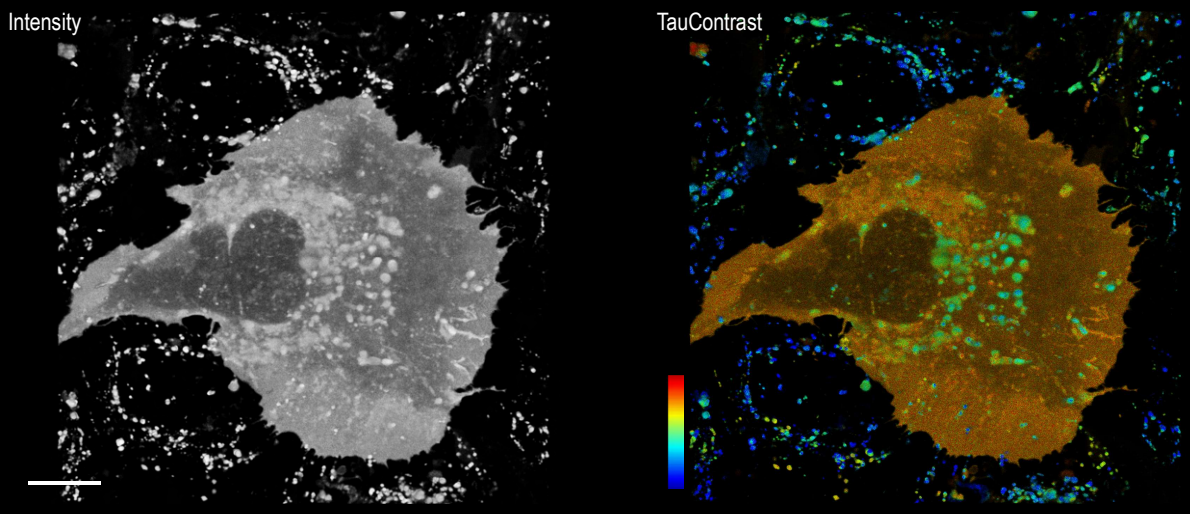


Calcium oscillations after mechanical stimulation in mammalian cells loaded with Oregon Green 488-BAPTA. The response in individual cells is recorded as a change in TauContrast.

Time series acquired at 4.5 fps, TauContrast traces in different cells (ROI selected in different color).
Image size: 256 x 256 pixels. LUT (TauContrast): 0-4 ns

Application Example: TauContrast

pH Sensing In Live Cells Using TauSense

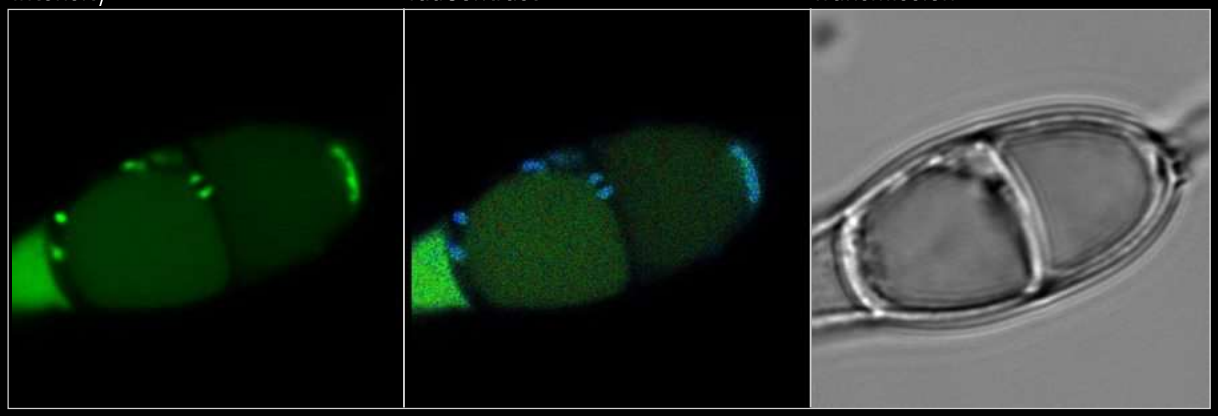


NIR790 membrane labeling in living HeLa cells. The pH response of the internalized probe in lysosomes is monitored using TauContrast.
Scale bar: 2µm. Rainbow LUT (TauContrast): 0-4 ns.

Application Example

Mitophagy in Rice Blast Infection Monitored with TauSense

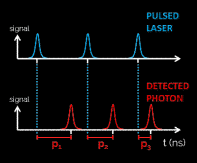
Intensity | TauContrast | Transmission

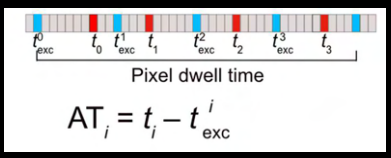


Mito-GFP in *Magnaporthe oryzae* (Rice blast fungus that causes blast disease, a devastating infection in rice crops). As a new cell forms (to the right), the older cells die and the GFP starts to be seen in the lysosome (left). TauContrast changes confirm the localization of GFP in the vacuole (AAT longer compared to the mitochondria). Image and sample courtesy of Shen Qing, Naweed Naqvi lab, Temasek Lifescience Laboratory, Singapore.

The Technology Behind TauContrast

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





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

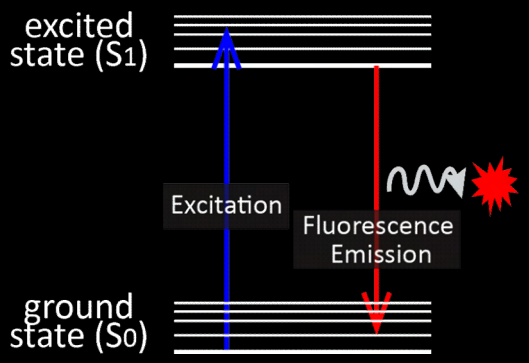
N = number of photons detected during the pixel dwell time

$$AAT = \frac{\sum_{i=1}^{N} AT_i}{N}$$

TauContrast Image

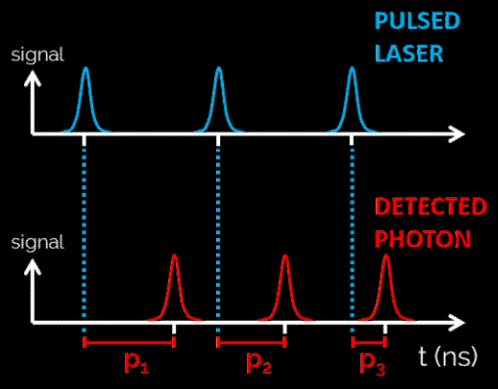
- Fluorescence Intensity ($N_{photons}$)
- Average Photon Arrival Times (AAT, ns)

The Technology Behind TauContrast



- Fluorescence Intensity ($N_{photons}$)
- Fluorescence Lifetime (ns)

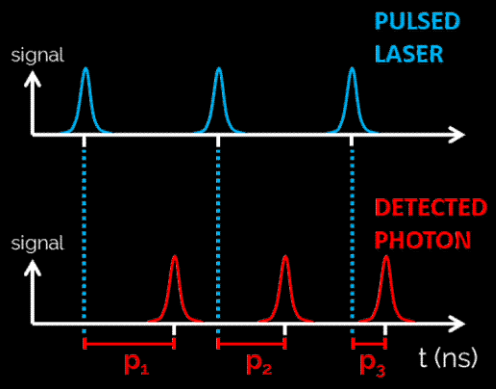
The Technology Behind TauContrast



- Fluorescence Intensity ($N_{photons}$)
- Fluorescence Lifetime (ns)

Leica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

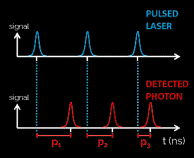
The Technology Behind TauContrast



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

The Technology Behind TauContrast

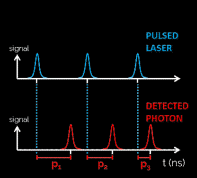
- Fluorescence Intensity ($N_{photons}$)
- Photon Arrival Time (ns)



ica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

The Technology Behind TauContrast

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

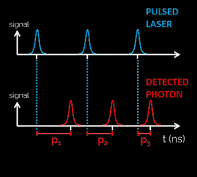


1) FPGA
2) Pixel-by-pixel
3) On the fly

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

The Technology Behind TauContrast

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



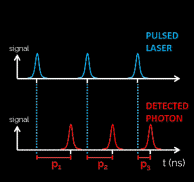
1) FPGA
2) Pixel-by-pixel
3) On the fly

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

Leica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

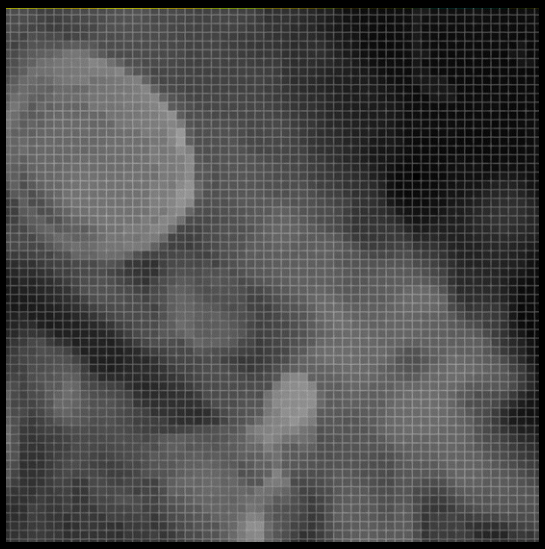
The Technology Behind TauContrast

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



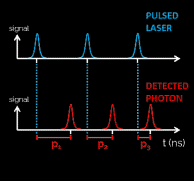
1) FPGA
2) Pixel-by-pixel
3) On the fly

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



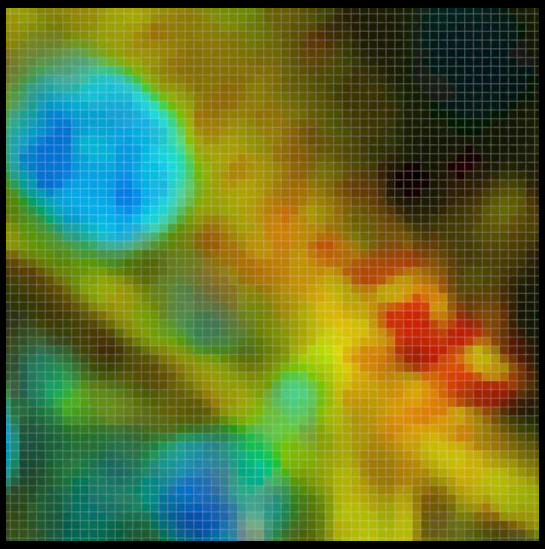
The Technology Behind TauContrast

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



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

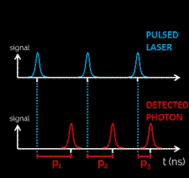
- Fluorescence Intensity ($N_{photons}$)
- **Average** Photon Arrival Times (AAT, ns)



Leica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

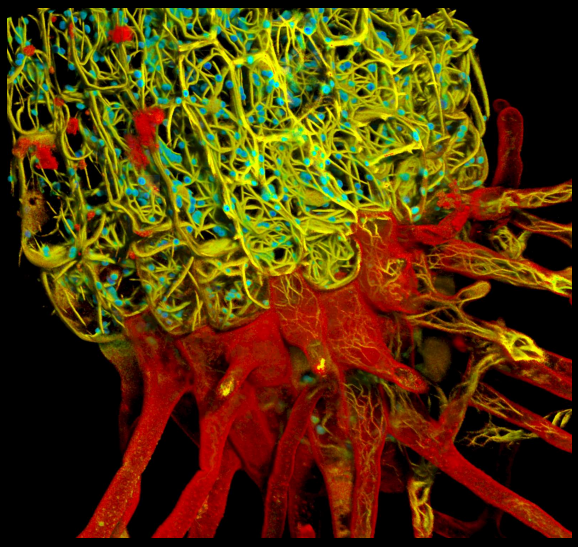
The Technology Behind TauContrast

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



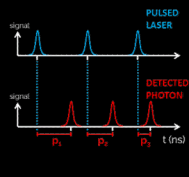
1) FPGA
2) Pixel-by-pixel
3) On the fly

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



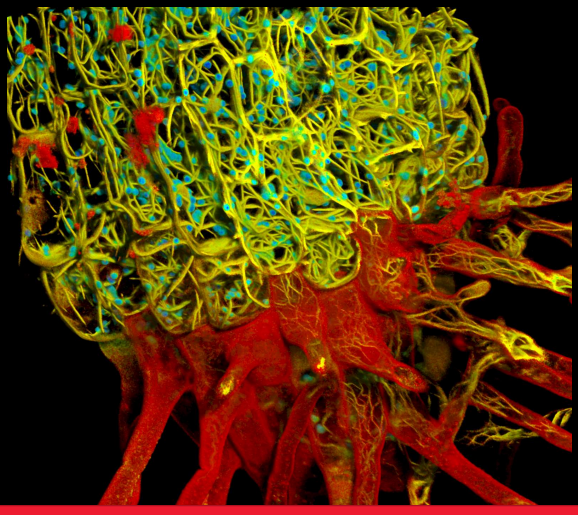
The Technology Behind TauContrast

- 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)

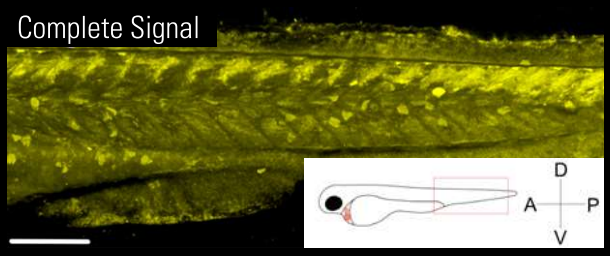


TauContrast Gives Instant, Pixel-by-Pixel AAT, With Every Image (Live/Acquired)

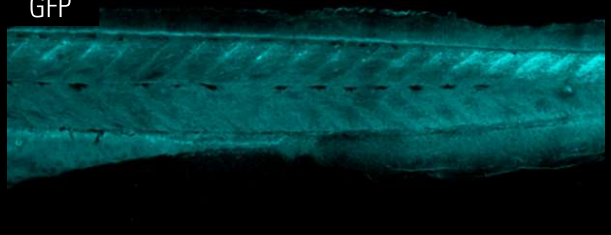
Leica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

Improve Image Quality

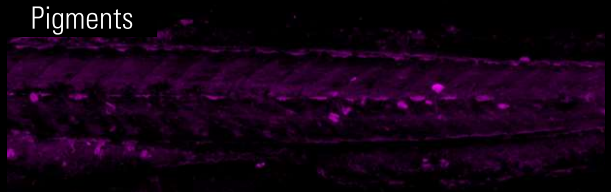
Complete Signal



GFP



Pigments



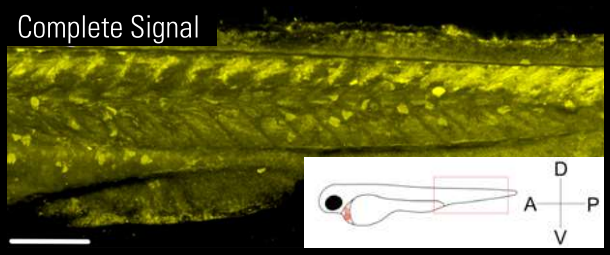
TauSense Tool:
TauGating

Zebrafish 4xGTIIC:d2GFP line exhibiting native pigments. GFP fluorescence provides a readout of Yap1/Taz-Tead activity and is used here to visualize the striated muscle of the trunk at 55 hpf. The signal of interest (cyan, long ATs) is singled-out from endogenous pigment contributions (magenta, short ATs). Scale bar: 200 µm.
Sample courtesy: Julien Vermot, IGBMC, Strasbourg.

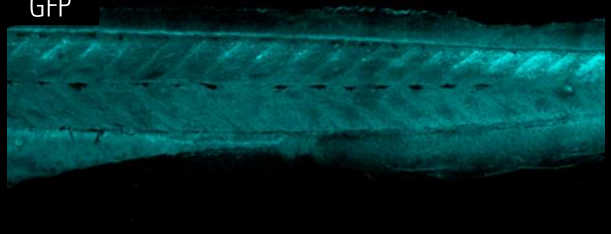
Application Example: TauGating

Isolate Signal Of Interest From Endogenous Contributions Using TauSense

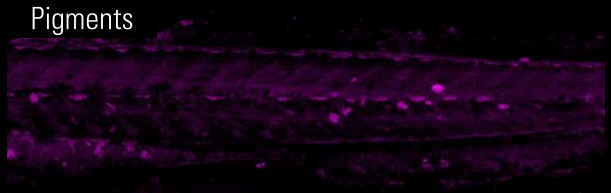
Complete Signal



GFP



Pigments

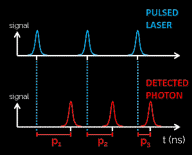


Zebrafish 4xGTIIC:d2GFP line exhibiting native pigments. GFP fluorescence provides a readout of Yap1/Taz-Tead activity and is used here to visualize the striated muscle of the trunk at 55 hpf. The signal of interest (cyan, long ATs) is singled-out from endogenous pigment contributions (magenta, short ATs). Scale bar: 200 µm.

Sample courtesy: Julien Vermot, IGBMC, Strasbourg.

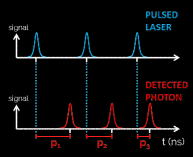
The Technology Behind TauGating

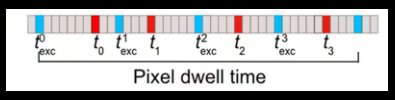
- Fluorescence Intensity ($N_{photons}$)
- Photon Arrival Time (ns)



1) FPGA
2) Digital gates (2/16)
3) On the fly

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



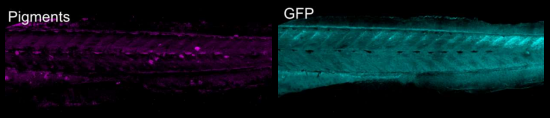


The Technology Behind TauGating

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

1) FPGA
2) Digital gates (2/16)
3) On the fly

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



Gate Channel 1

Gate Channel 2

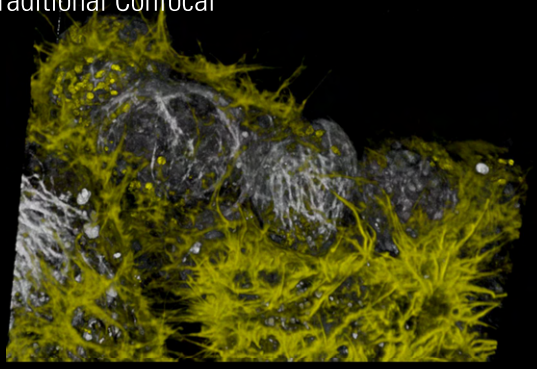
TauGating Gives Digital Gate Channels With Every Image (Live/Acquired)

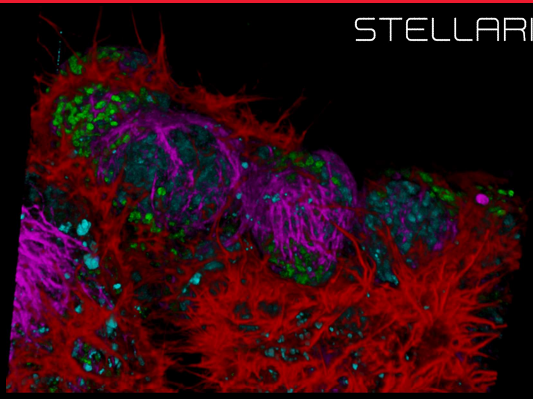
Leica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

Multiplex Beyond The Spectral Options

Traditional Confocal

STELLARIS





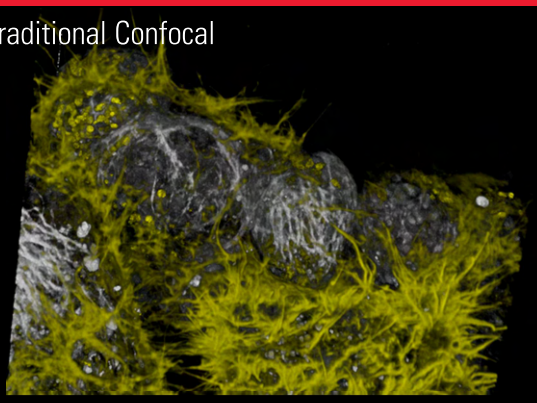
TauSense Tool:

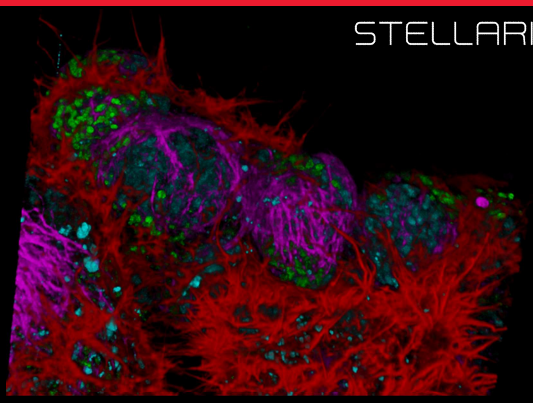
TauSeparation

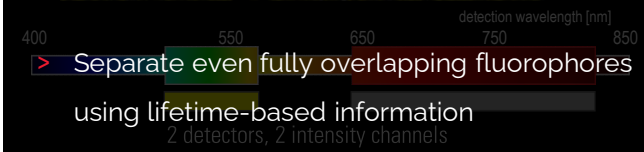
Multiplex Beyond The Spectral Options

Traditional Confocal

STELLARIS







> Separate even fully overlapping fluorophores

using lifetime-based information

2 detectors, 2 intensity channels

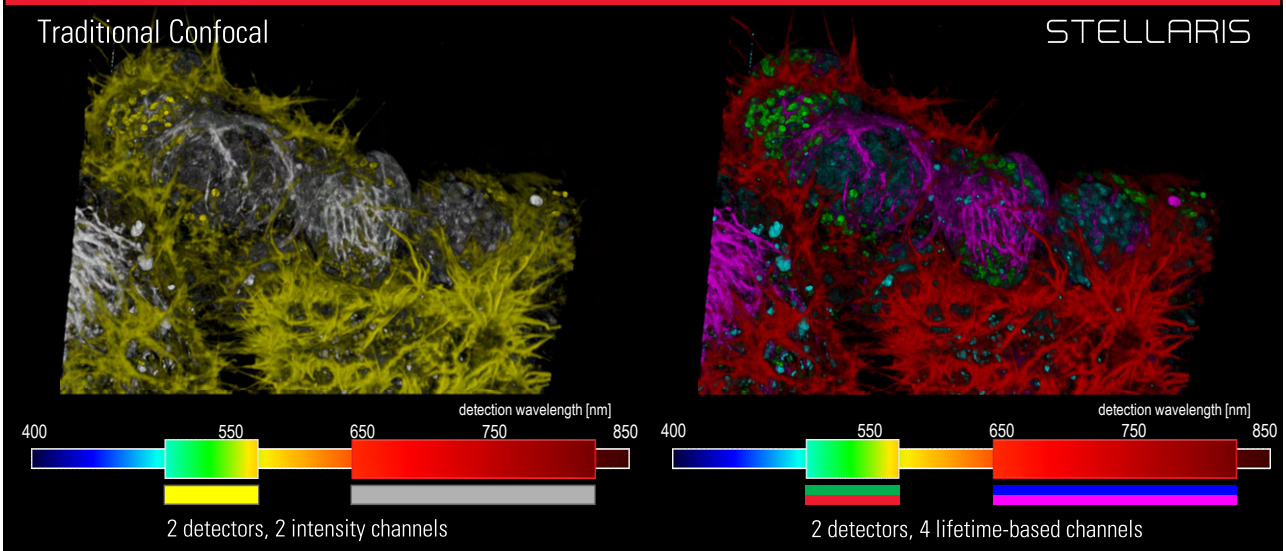
NE-115 cells. LifeAct-mNeonGreen (left: yellow, right: red), MitoTracker Green (left: yellow, right: green) NUC Red (left: gray, right: blue), and SiR-tubulin (left: gray, right: magenta).
Courtesy: Max Heydasch, University of Bern and Spirochrome

ca Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

Multiplex Beyond The Spectral Options

Traditional Confocal

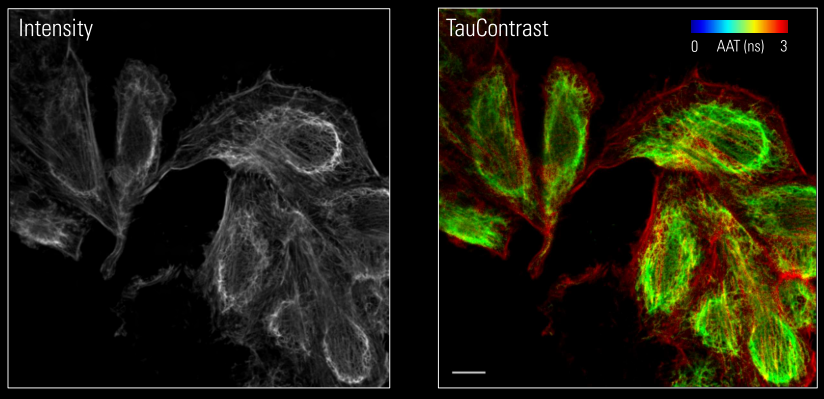
STELLARIS



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

Application Example

Species Separation using TauSense



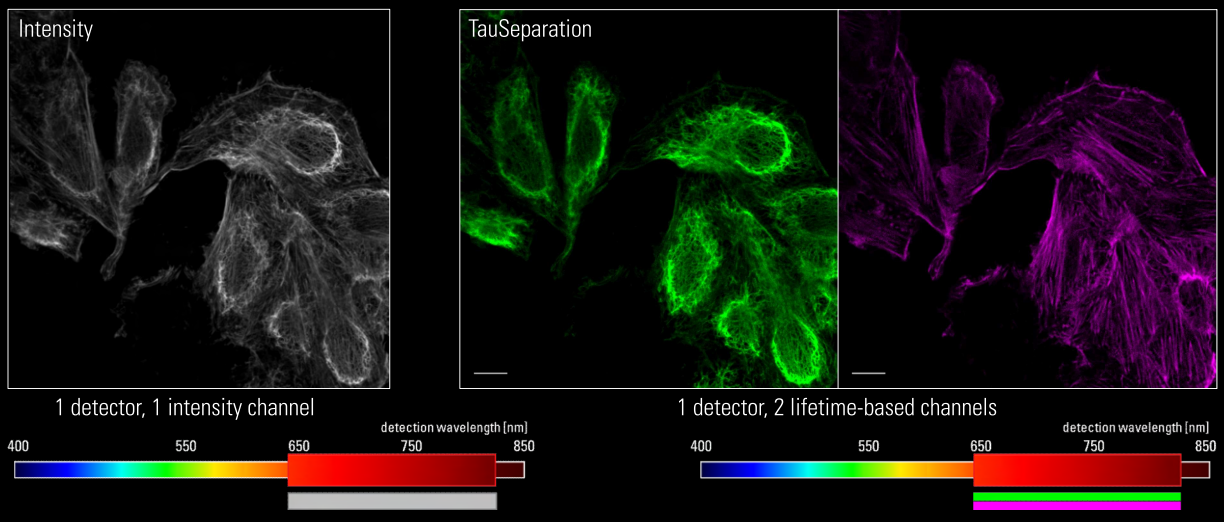
The TauContrast information, complementary to the intensity (photon counts), reveals the two species labeled with AF647 and ATTO647N, with highly overlapping emission spectra.

HEK cells. Vimentin (left: gray, Alexa 647 IF), actin (left: gray, ATTO647N-phalloidin). TauContrast describing average photon arrival times (right, spectrum LUT). Scale bar 10µm.
Sample Courtesy: Sebastian Hänsch, Stephanie Weidtkamp-Peters, CAI, Düsseldorf.

#CONFOCALREIMAGINED

Application Example

Species Separation using TauSense

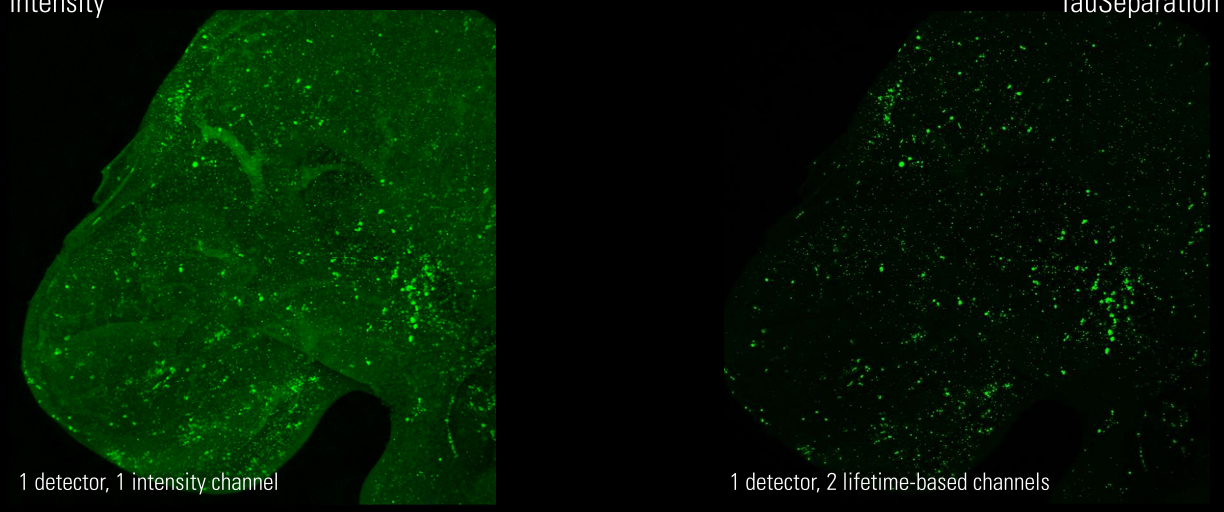


HEK cells. Vimentin (left: gray, Alexa 647 IF), actin (left: gray, ATTO647N-phalloidin). TauSeparation separates the signals coming to the detector according to the lifetime components distribution generated online at the FPGA level (right: green, Vimentin; right: magenta, Actin). Scale bar 10µm.
Sample Courtesy: Sebastian Hänsch, Stephanie Weidtkamp-Peters, CAI, Düsseldorf.

#CONFOCALREIMAGINED

Application Example

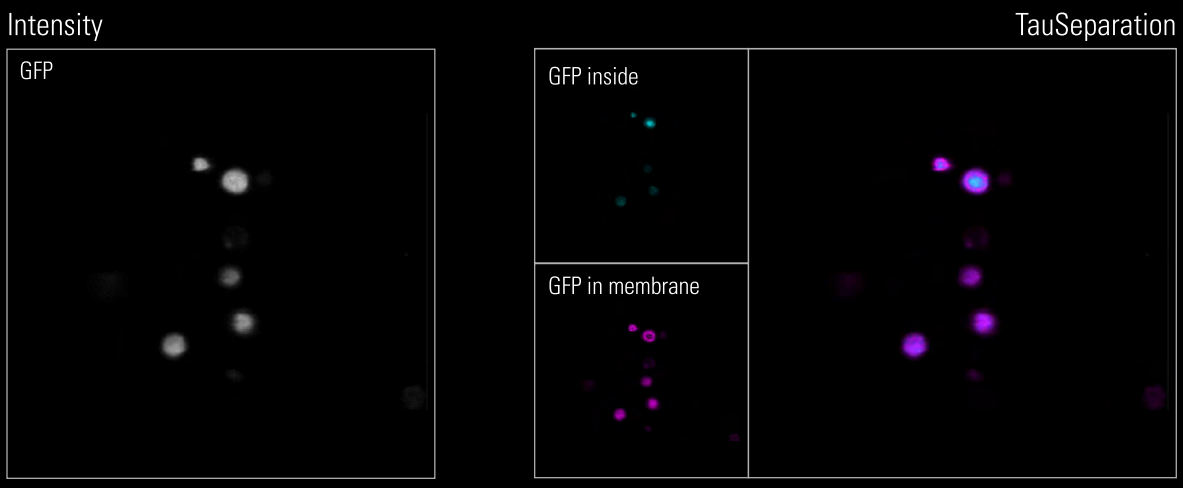
Species Separation using TauSense



Mouse embryo (E9.5) section. The unspecific signal contribution from the PFA fixative ("background autofluorescence") interferes with the observation of the cleaved Caspase-3 signal (left). The fluorescence signal from the apoptosis marker in the green can be extracted from the unwanted autofluorescence signal using the TauSeparation tool in TauSense (right). Sample courtesy: Corinna Cozzitorto, UCSF.

Application Example

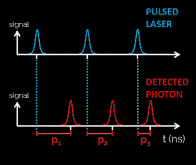
Species Separation using TauSense



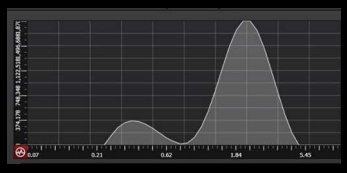
Kita mycelia with GFP expressed at the membrane and in the interior of the bacteria. GFP in the interior can be separated from the membrane contribution using the TauSeparation tool in TauSense, as the environment is different. Sample courtesy: University of Leiden.

The Technology Behind Separation

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



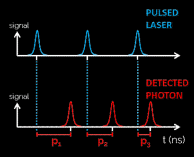
1) FPGA
2) Gate-based multi-component fit
3) Diagram lifetime components
4) On the fly



- Lifetime-based Separated Channels (Intensity, $N_{photons}$)

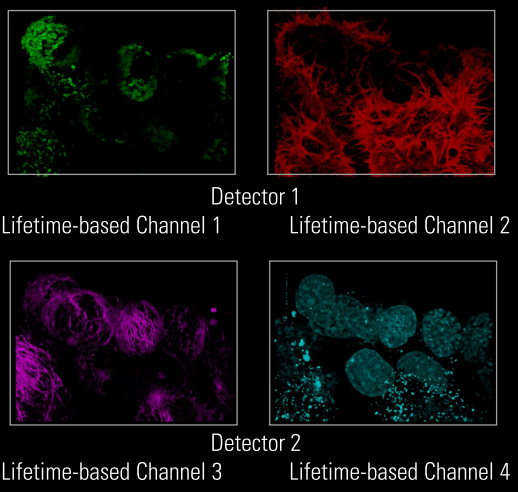
The Technology Behind Separation

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



1) FPGA
2) Gate-based multi-component fit
3) Diagram lifetime components
4) On the fly

- Lifetime-based Separated Channels (Intensity, $N_{photons}$)



Detector 1
Lifetime-based Channel 1 | Lifetime-based Channel 2

Detector 2
Lifetime-based Channel 3 | Lifetime-based Channel 4

The Technology Behind Separation

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

1) FPGA
2) Gate-based multi-component fit
3) Diagram lifetime components
4) On the fly

- Lifetime-based Separated Channels (Intensity, $N_{photons}$)

Detector 1
Lifetime-based Channel 1 | Lifetime-based Channel 2

Detector 2
Lifetime-based Channel 3 | Lifetime-based Channel 4

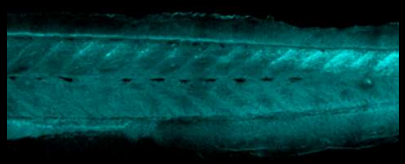
TauSeparation Identifies Species With Every Image (Live/Acquired)

Leica Microsystems | STELLARIS Presentation | June 2021
Leica Microsystems GmbH. Registered Office: Wetzlar. All rights reserved, also regarding any disposal, exploitation, reproduction, editing, distribution, as well as in the event of applications for industrial property rights.
Public

What is TauSense Good For?

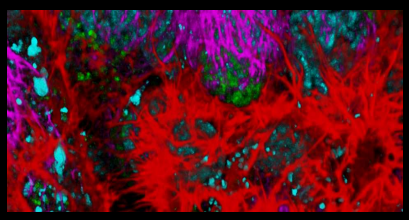
TauContrast

- 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



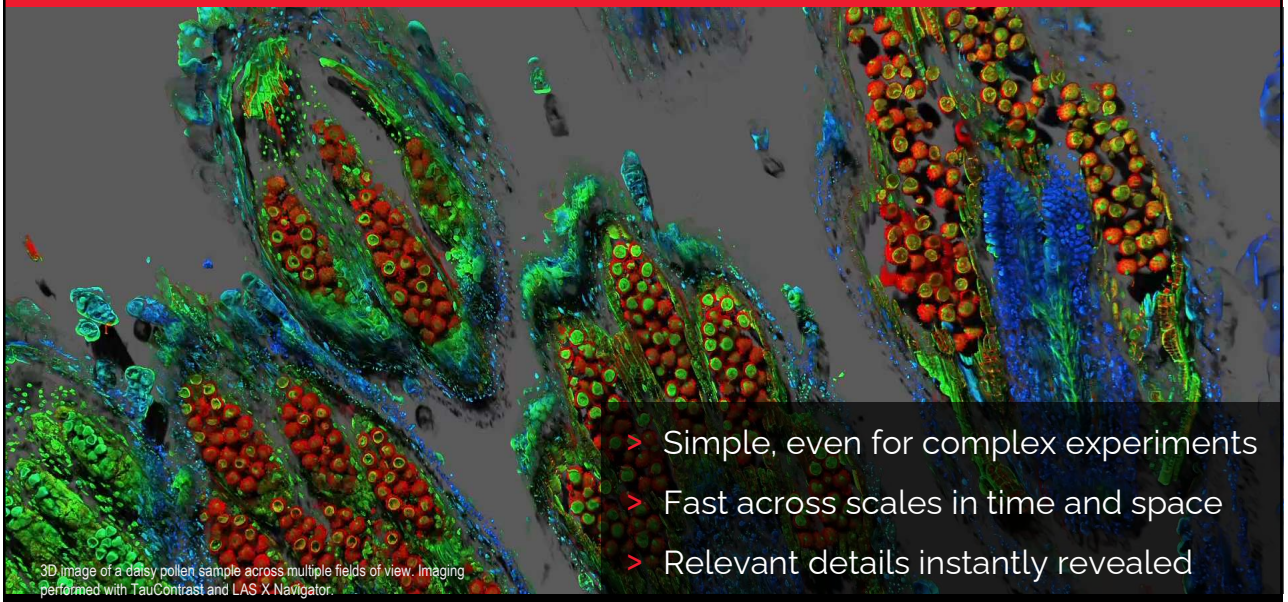
TauSeparation

- Separate species with different lifetimes

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

#CONFOCALREIMAGINED

STELLARIS Gives You The Productivity To Do More



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

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

#CONFOCALREIMAGINED

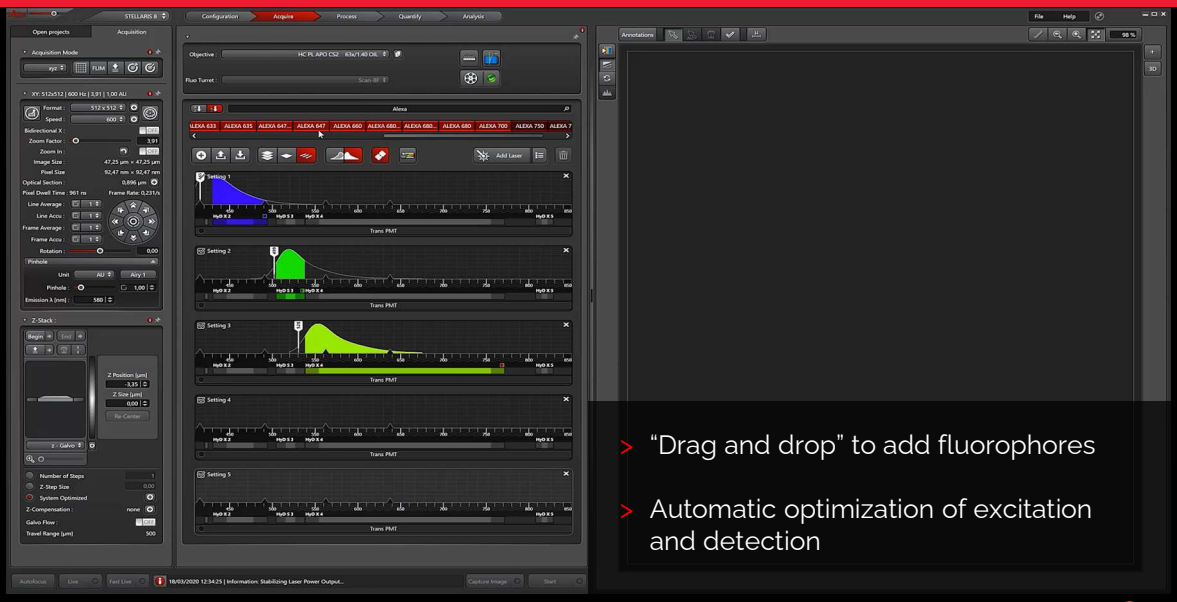
What are the guiding principles behind ImageCompass?

- Put the **sample** in the center of any experimental set-up
- Provide **Cockpit View** of full experiment
- **Reduced complexity** to allow addition of **new functionality**

Simple, Even For Complex Experiments

#CONFOCALREIMAGINED

Simple, Even For Complex Experiments

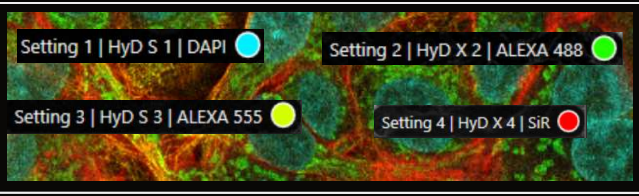


- "Drag and drop" to add fluorophores
- Automatic optimization of excitation and detection

#CONFOCALREIMAGINED

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

- Complete experimental setting at a glance
- No more switching between single settings (formally known as sequences)
- Reduce cognitive load to active elements only

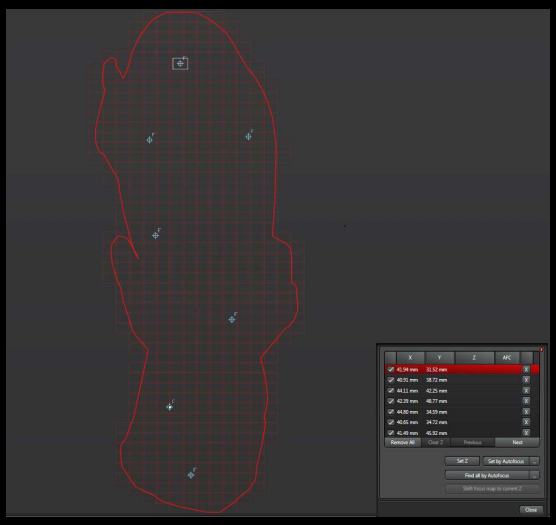


- Image channel information
- Unambiguous attribution channel <-> detector
- Clear assignment also with Quick LUTs and in case of more than 1 channel per detector

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

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



#CONFOCALREIMAGINED

Microscopy Dos and Don'ts

- Initialize the stage – Yes/No?
- Sample prep optimization

  coverslip #1.5; dye selection etc.;
- Lens selection

  10x/0.40; 20x/0.75 IMM; 63x/1.40 oil;
- Save settings – Laser/Gain etc
- User manuals and YT channel

- When finishing your imaging:
- Clean off the liquid / oil
- Save raw data
- Offline image review (LAS X vs FIJI)
- Cover when not in use
- Errors – Leica Support Center
- Practice 866-830-0735 x3
- PM Annually