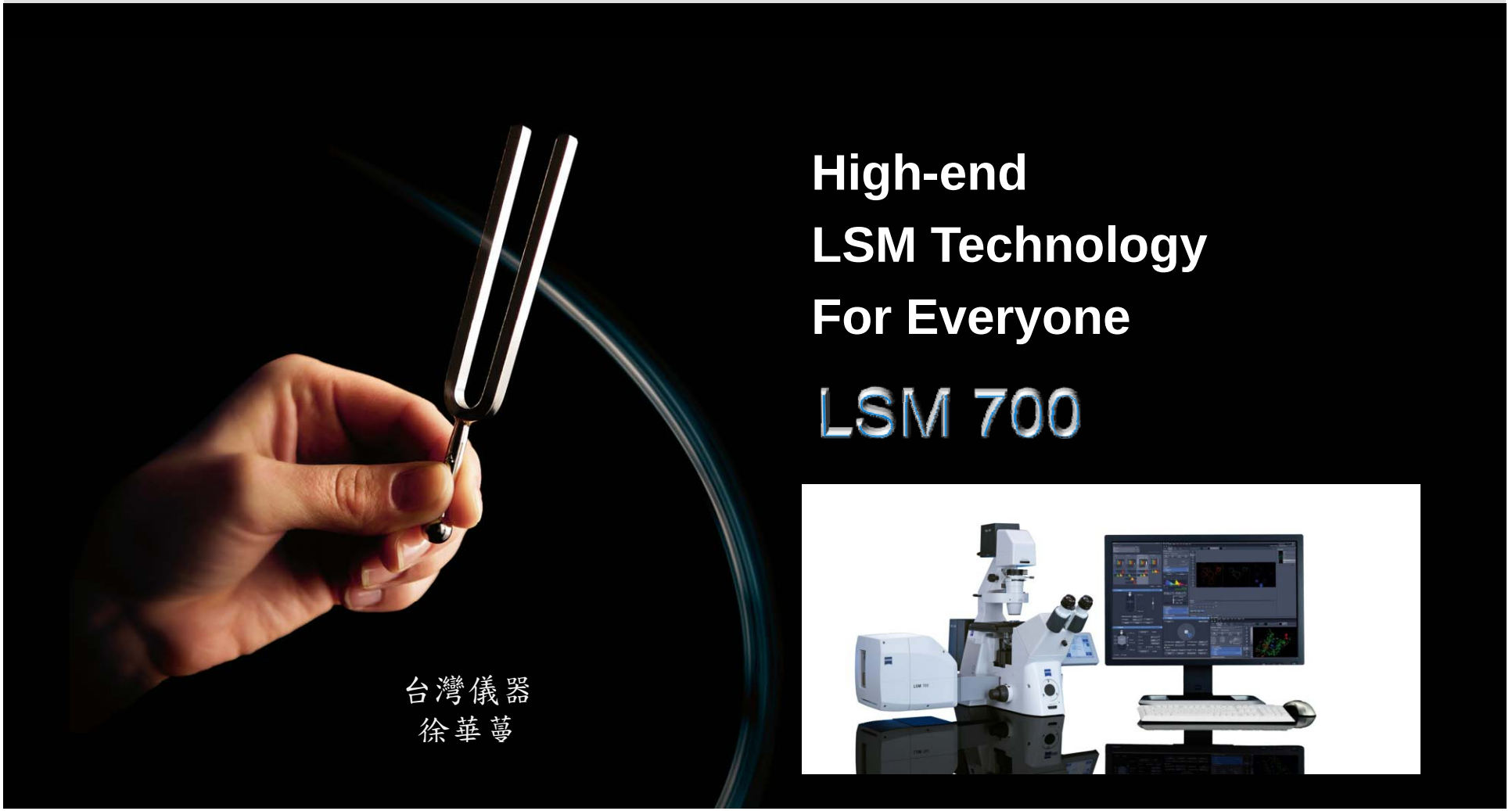
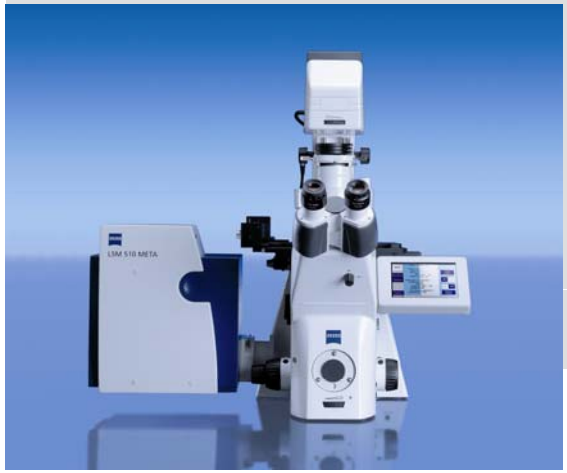
# 雷射共軛焦顯微鏡介紹



# Evolution of High End Confocal Systems

# LSM 5 Family

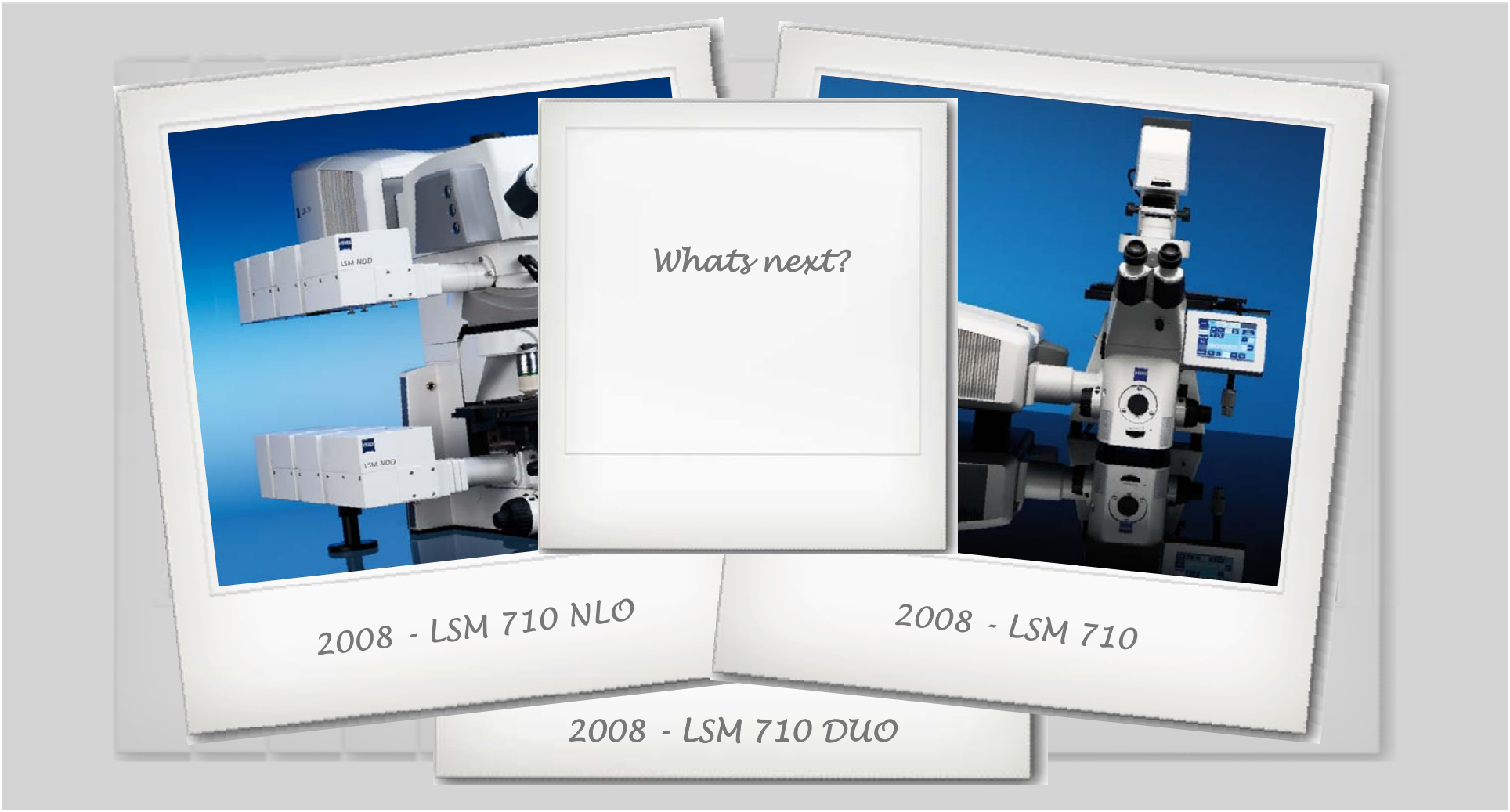


| 使用單位 |
| --- |
| 中研院分生所共儀 x2 |
| 中研院植物所共儀x2 |
| 中研院分生所簡正鼎博士 |
| 中研院生醫所共儀 |
| 中研院農生中心共儀 |
| 清大腦科學中心 x2 |
| 台大醫研部第二共研 |
| 長庚大學x2 |
| 中山醫學大學 |
| 中原大學醫工所 |
| 台灣大學物理系 |
| 慈濟醫學中心合心實驗室 |
| 中研院分生所共儀 |
| 國醫中心生解科 |
| 清華大學生科系 |
| 台北榮總教研部 |
| 高雄榮總教研部 |
| 國泰醫院汐止院區 |

| 使用單位 |
| --- |
| 中研院分生所 |
| 中研院生化所 |
| 清華大學生科系 |
| 台大醫院心臟內科 |
| 國醫中心生解所 |
| 國醫中心航醫所 |
| 中興大學生科所 |
| 中興大學組織醫學幹細胞中心 |
| 東海大學生物系 |
| 清大腦科學中心 |
| 中正大學生科系 |
| 國立海洋博物館 |
| 亞東醫院 |
| 中研院植物所 |
| 台大醫研部第一共研 |
| 中山醫學大學 |
| 中研院分生所 |

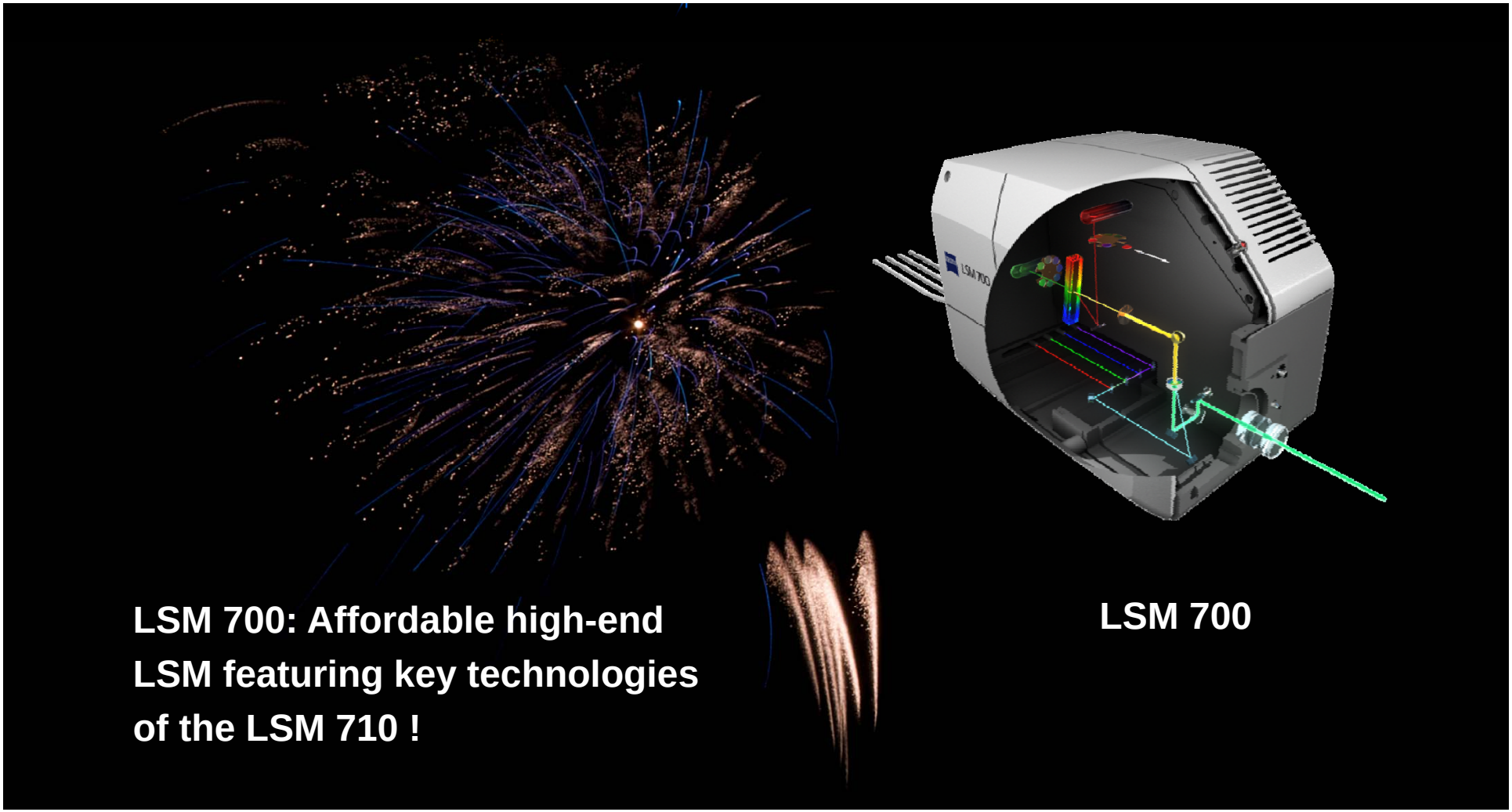
# Evolution of High End Confocal Systems

# Developments in 2008 at Carl Zeiss



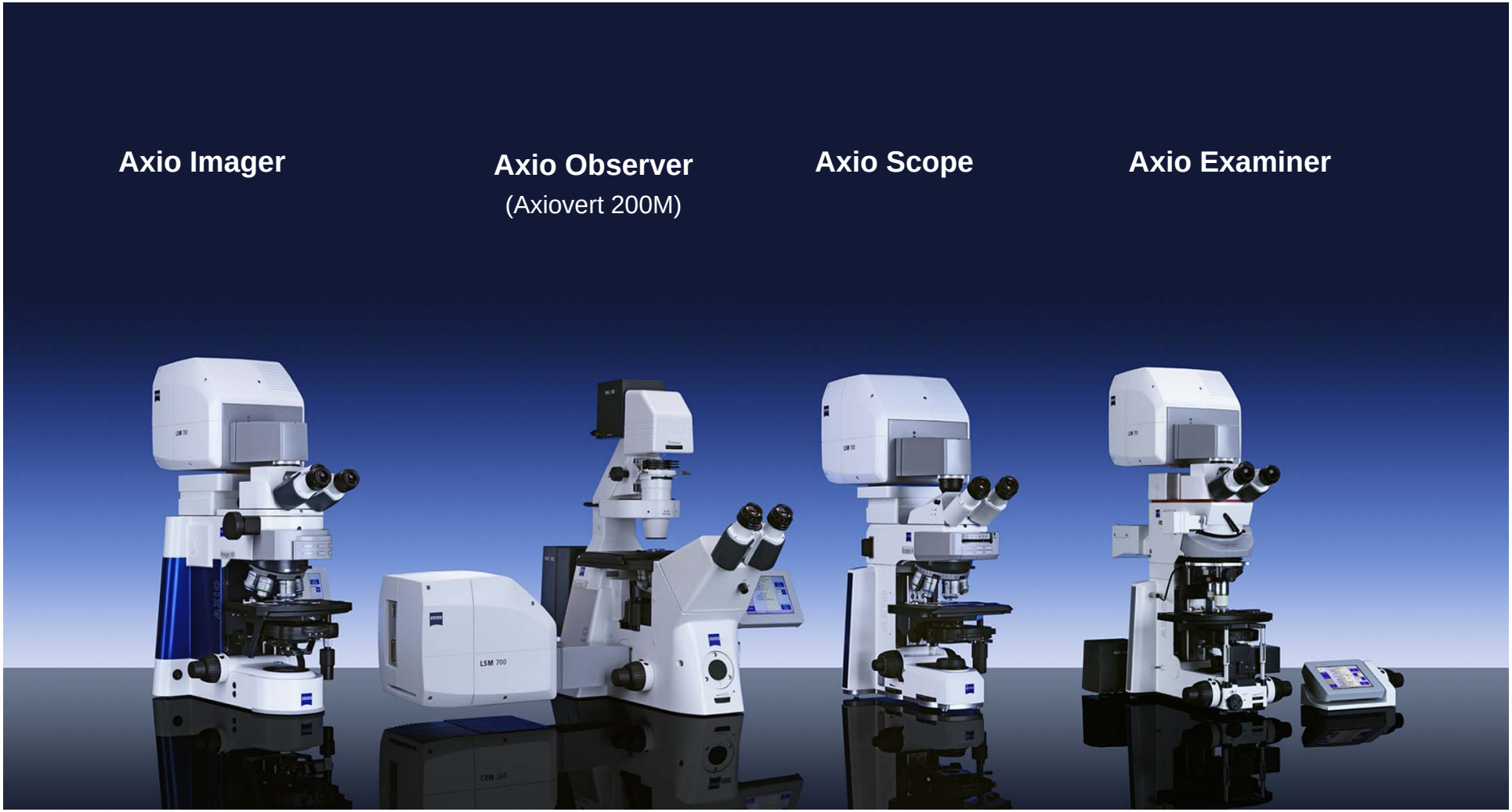
# LSM 700

2009: Introducing high-end LSM technology for everybody!



# LSM 700

Upright and inverted microscopes for the LSM 700



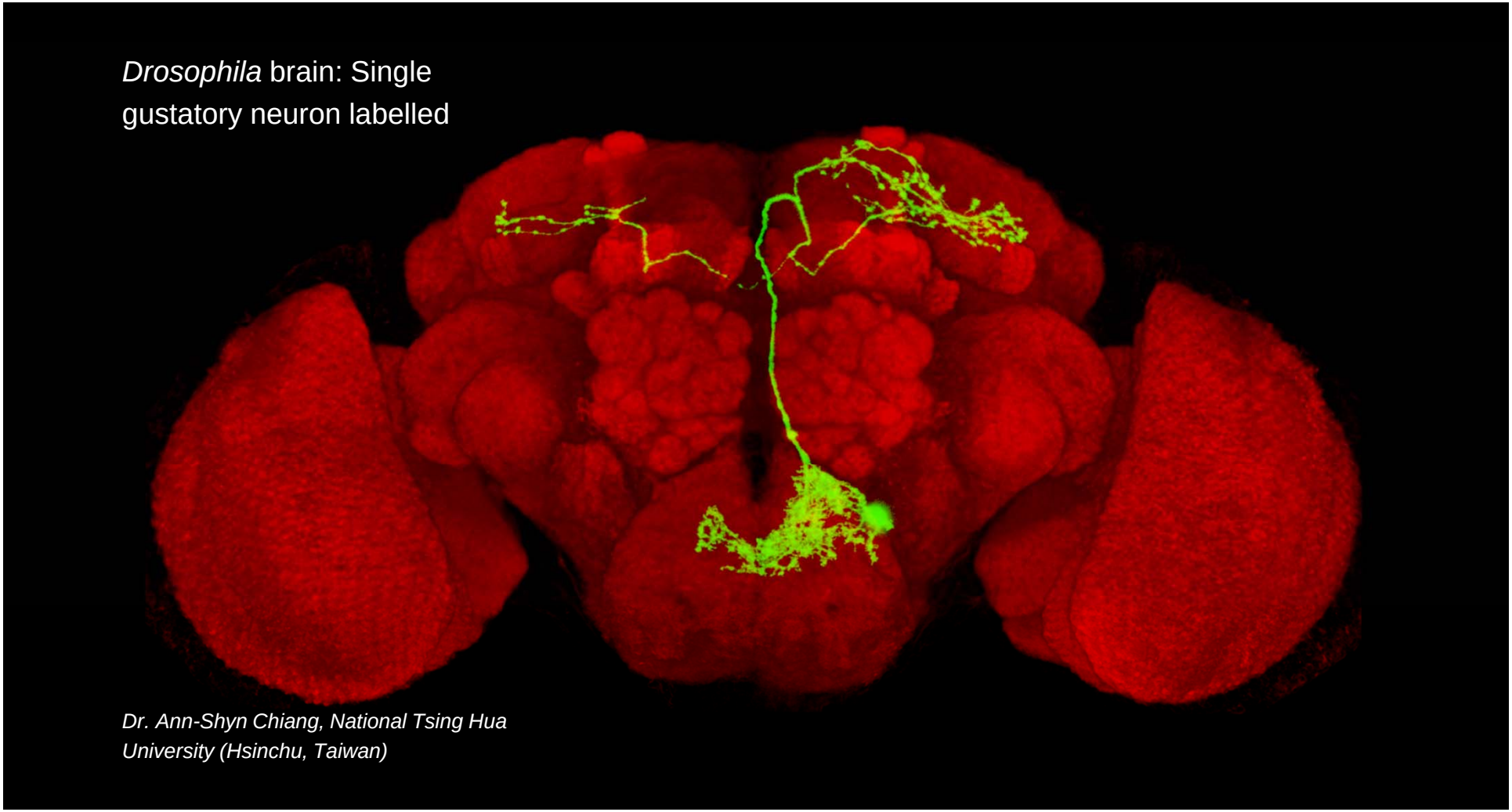
# LSM 700

High-end LSM technology for everybody!

# Confocal Laser Scanning Microscopy

## Examining three-dimensional structures

*Drosophila* brain: Single gustatory neuron labelled



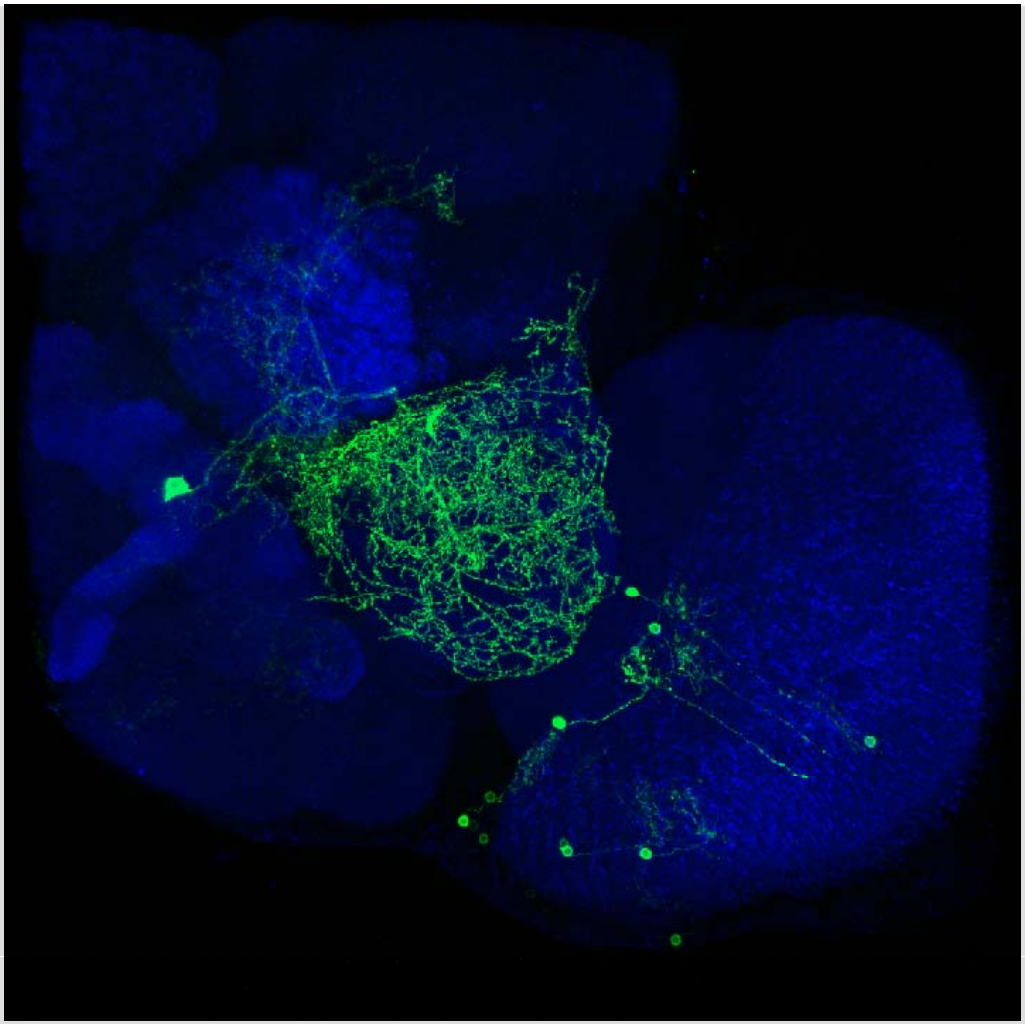
*Dr. Ann-Shyn Chiang, National Tsing Hua University (Hsinchu, Taiwan)*

# Confocal Laser Scanning Microscopy

Examining three-dimensional structures

**Sample**

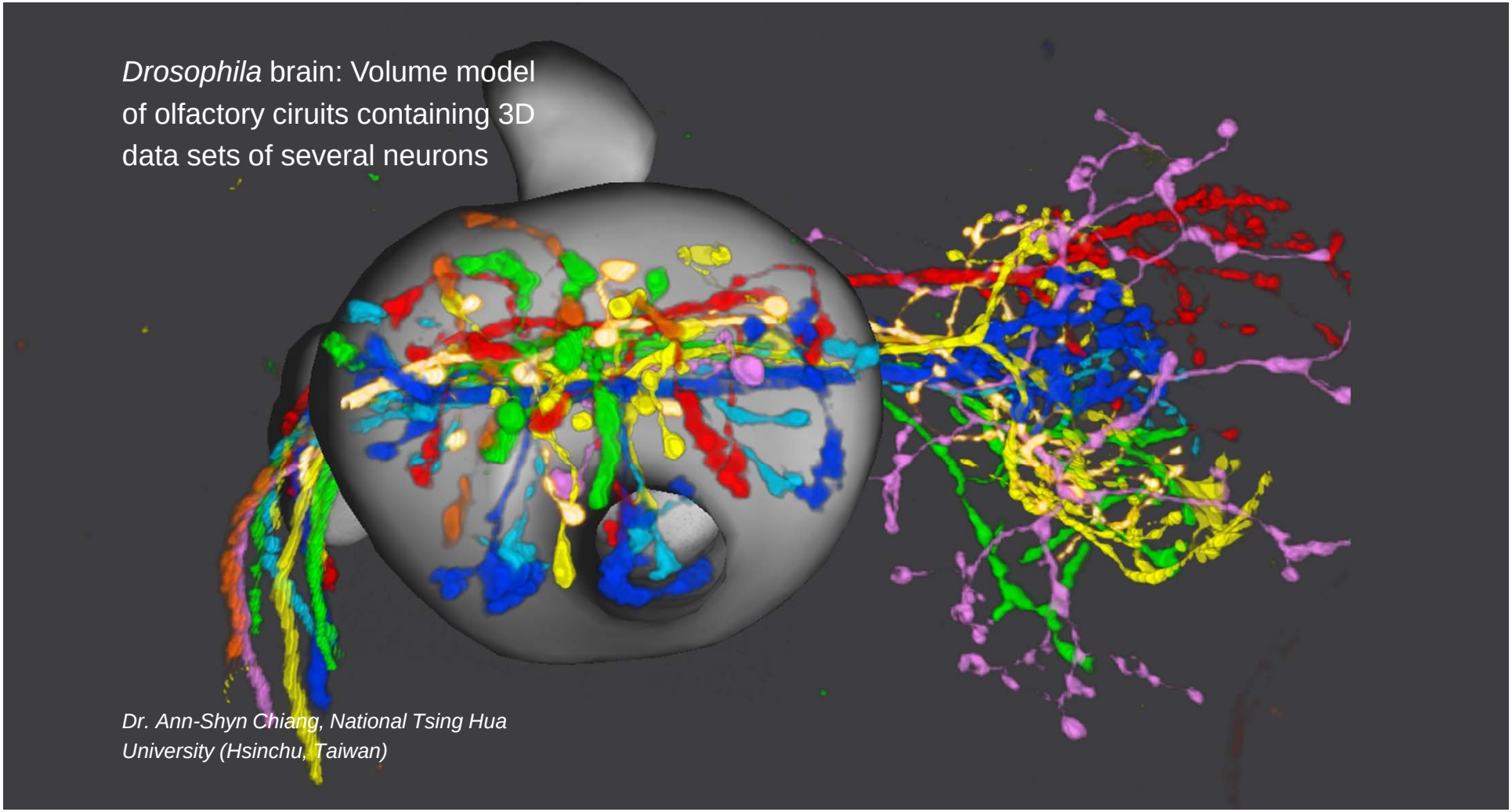
*Drosophila* brain, expression of GFP restricted to very few individual neurons due to genetic mosaic generation.



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

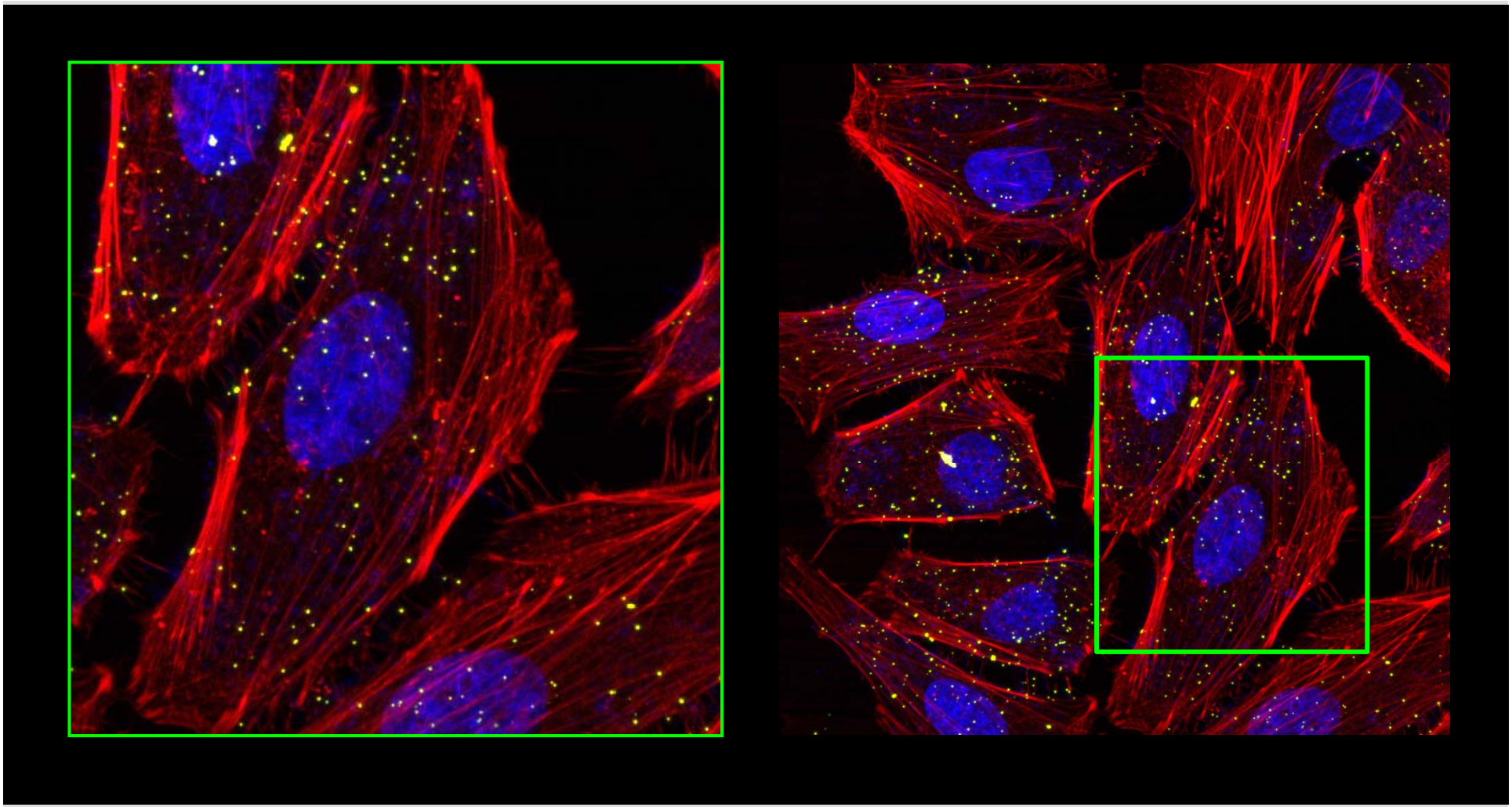
# Confocal Laser Scanning Microscopy

Examining three-dimensional structures



# Confocal Laser Scanning Microscopy

Examining three-dimensional structures

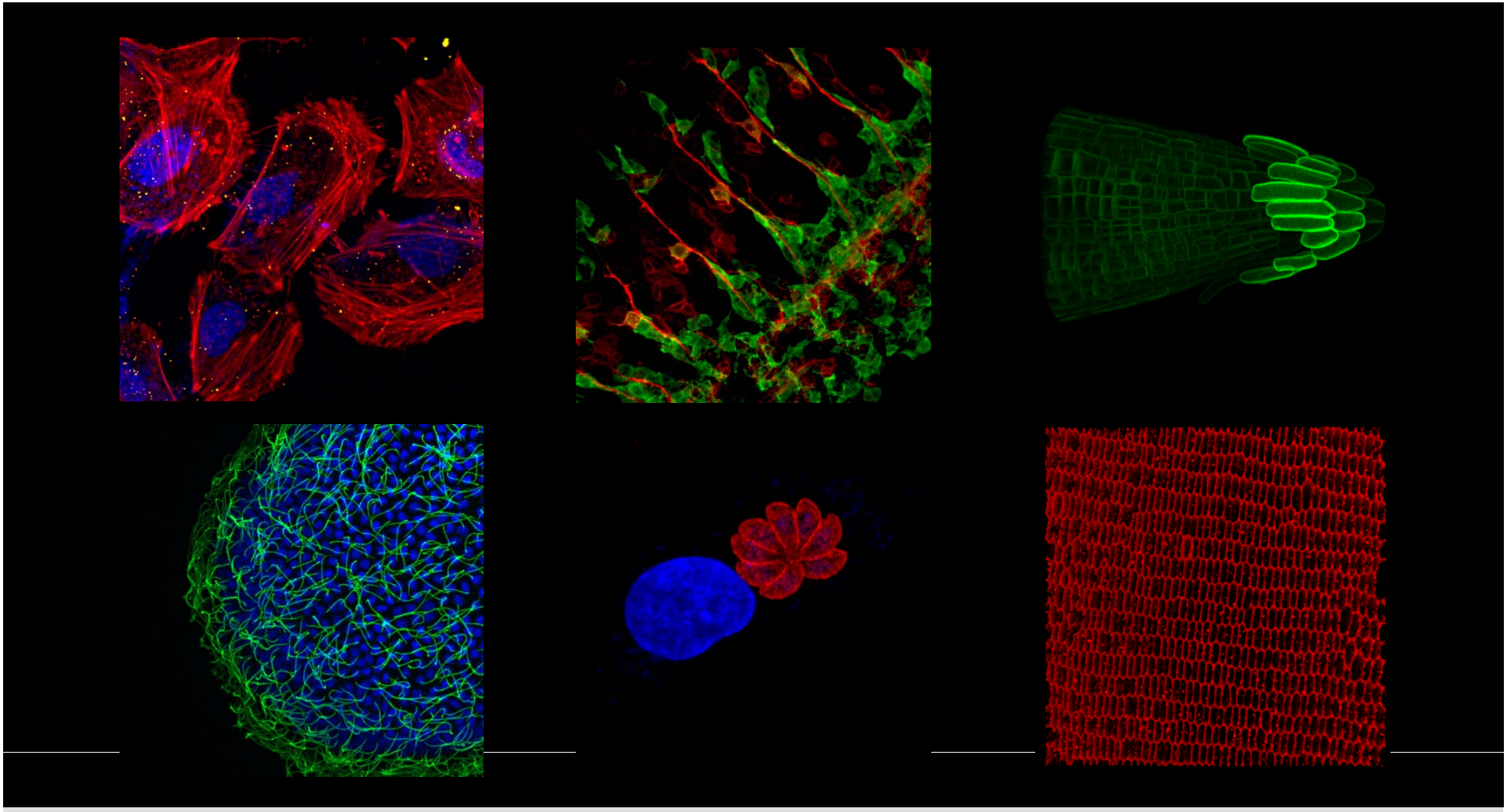


# LSM 700

High-end LSM technology for everybody!

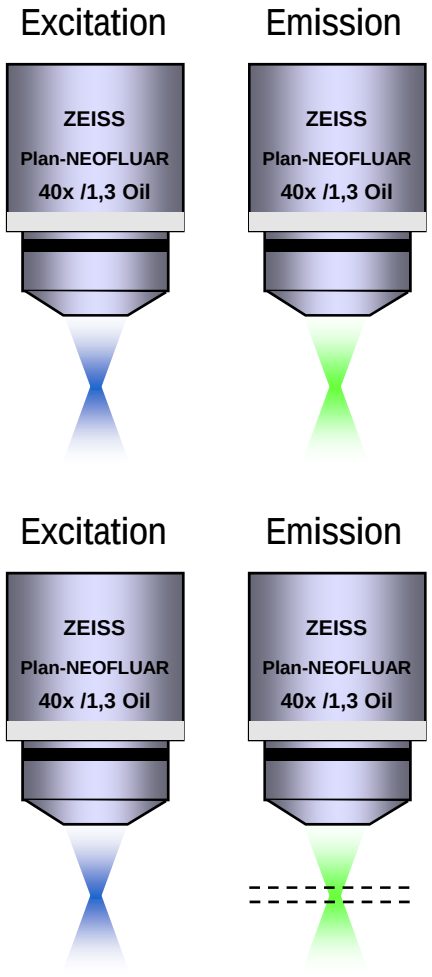
# Laser Scanning Microscopy

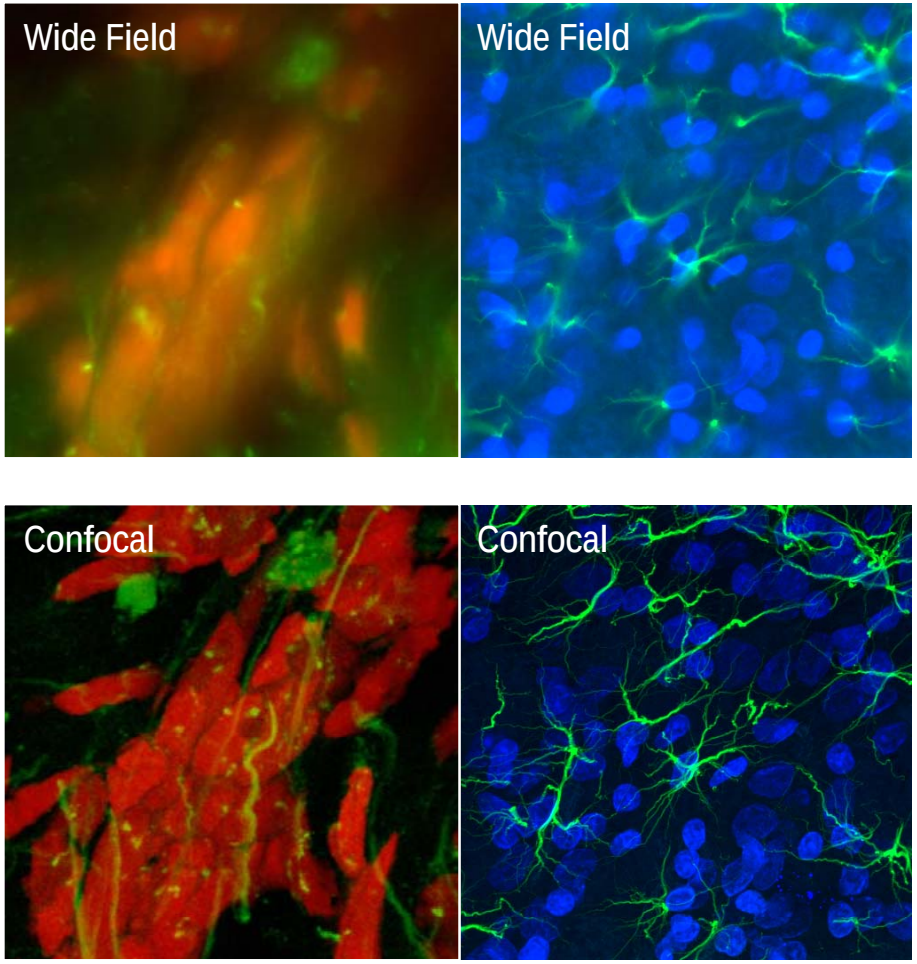
## The power of optical sectioning



# Laser Scanning Microscopy

## The power of optical sectioning



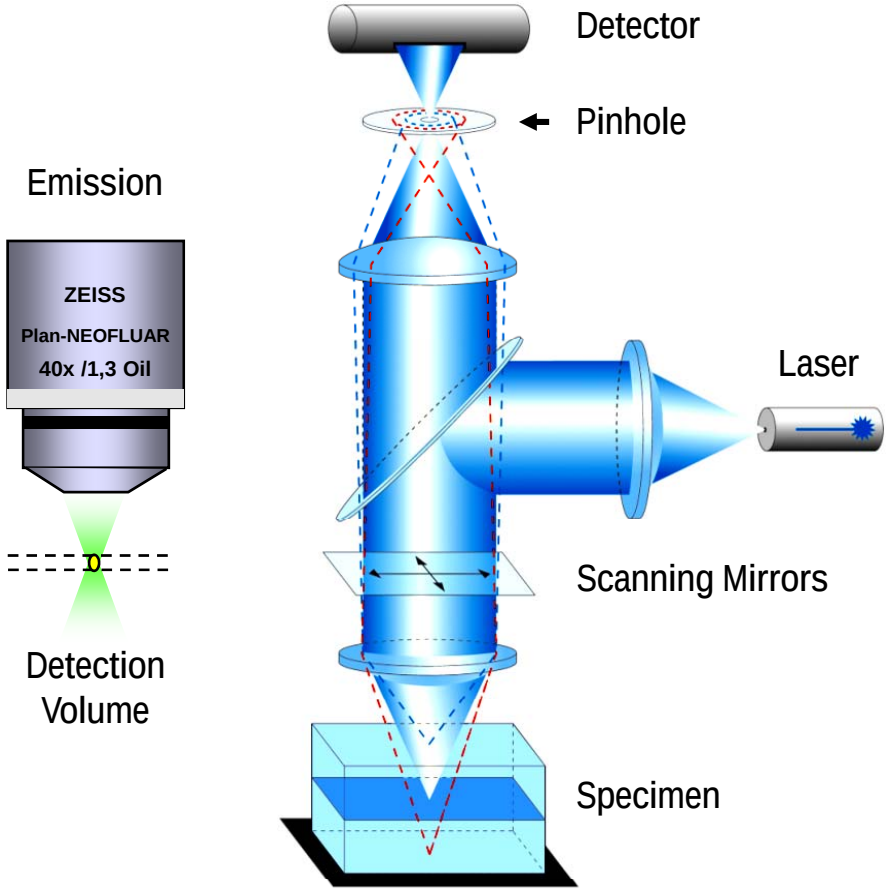
# Laser Scanning Microscopy

## The power of optical sectioning

**Confocal imaging: optical sectioning through rejection of out-of-focus light**

A minute diaphragm, situated in a conjugated focal plane, prevents the detection of out-of-focus light.

The detection of fluorescence signals is restricted to a tiny volume within the focal plane only!

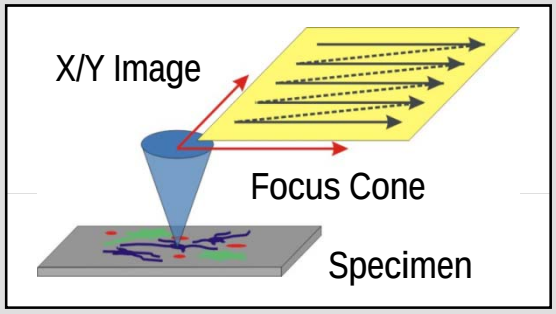


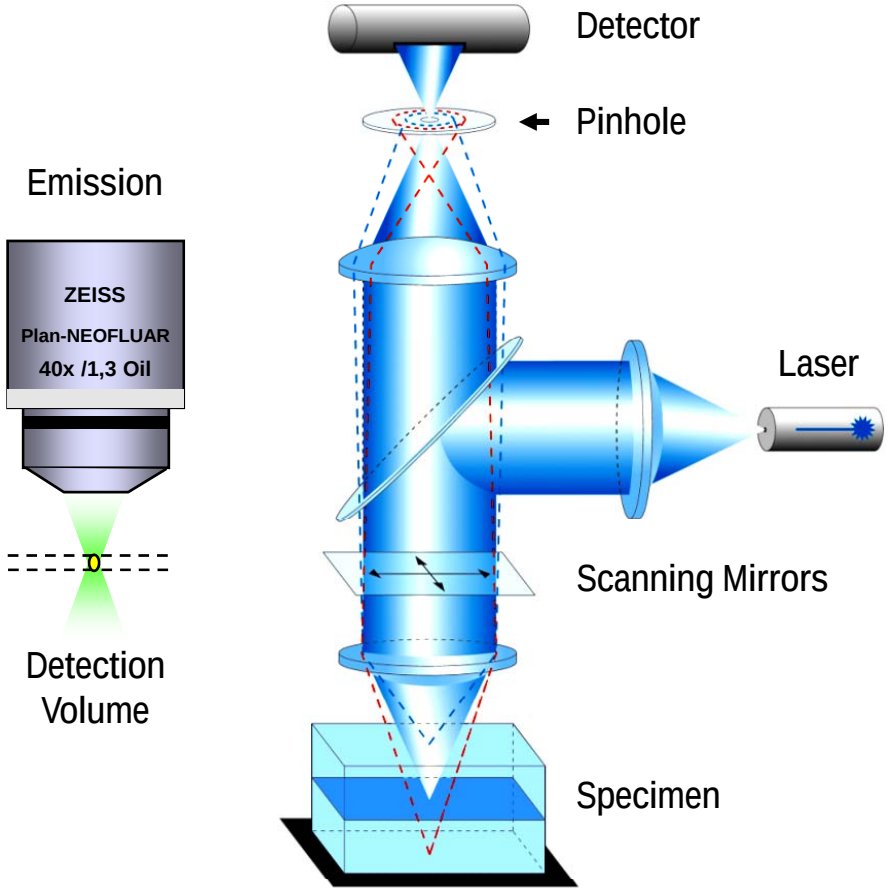
# Laser Scanning Microscopy

## The power of optical sectioning

### Acquisition of optical sections

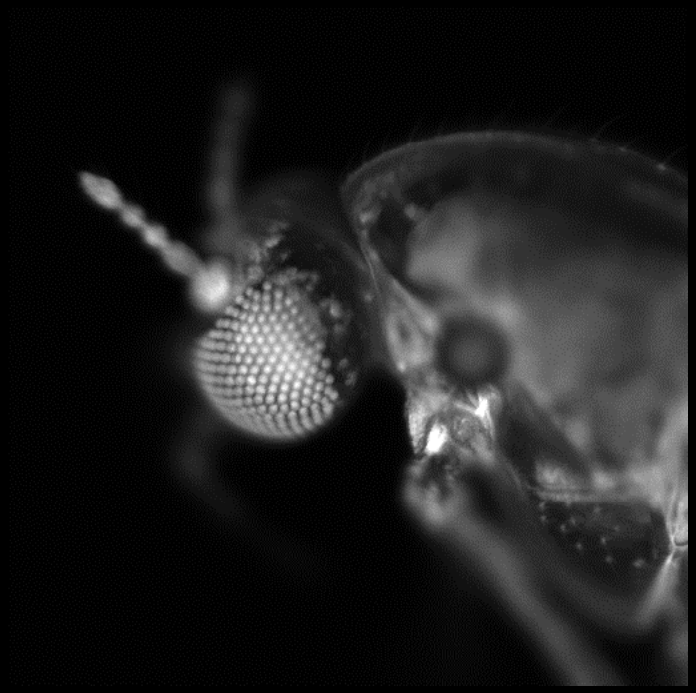
Two-dimensional images are acquired by scanning the excitation spot in a line-wise fashion across the specimen.



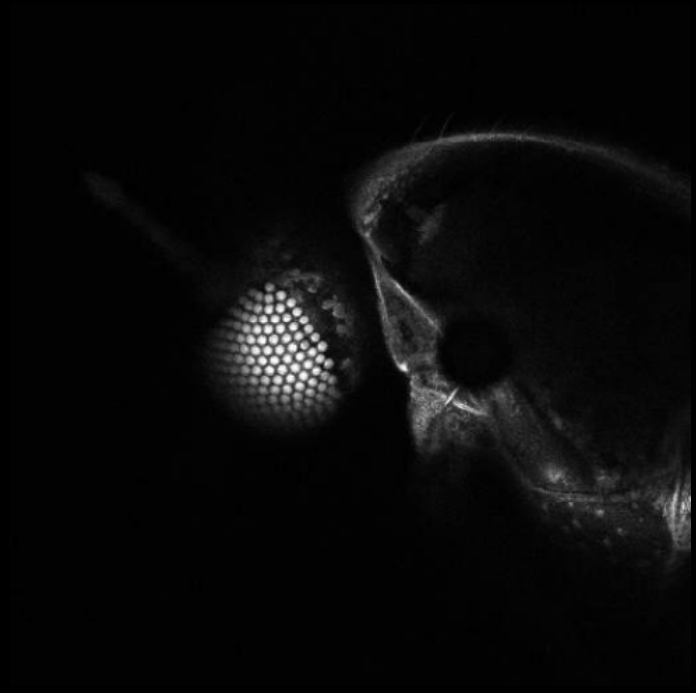
# Laser Scanning Microscopy

The power of optical sectioning



**Wide Field**

(out-of-focus light blurs the image)



**Confocal**

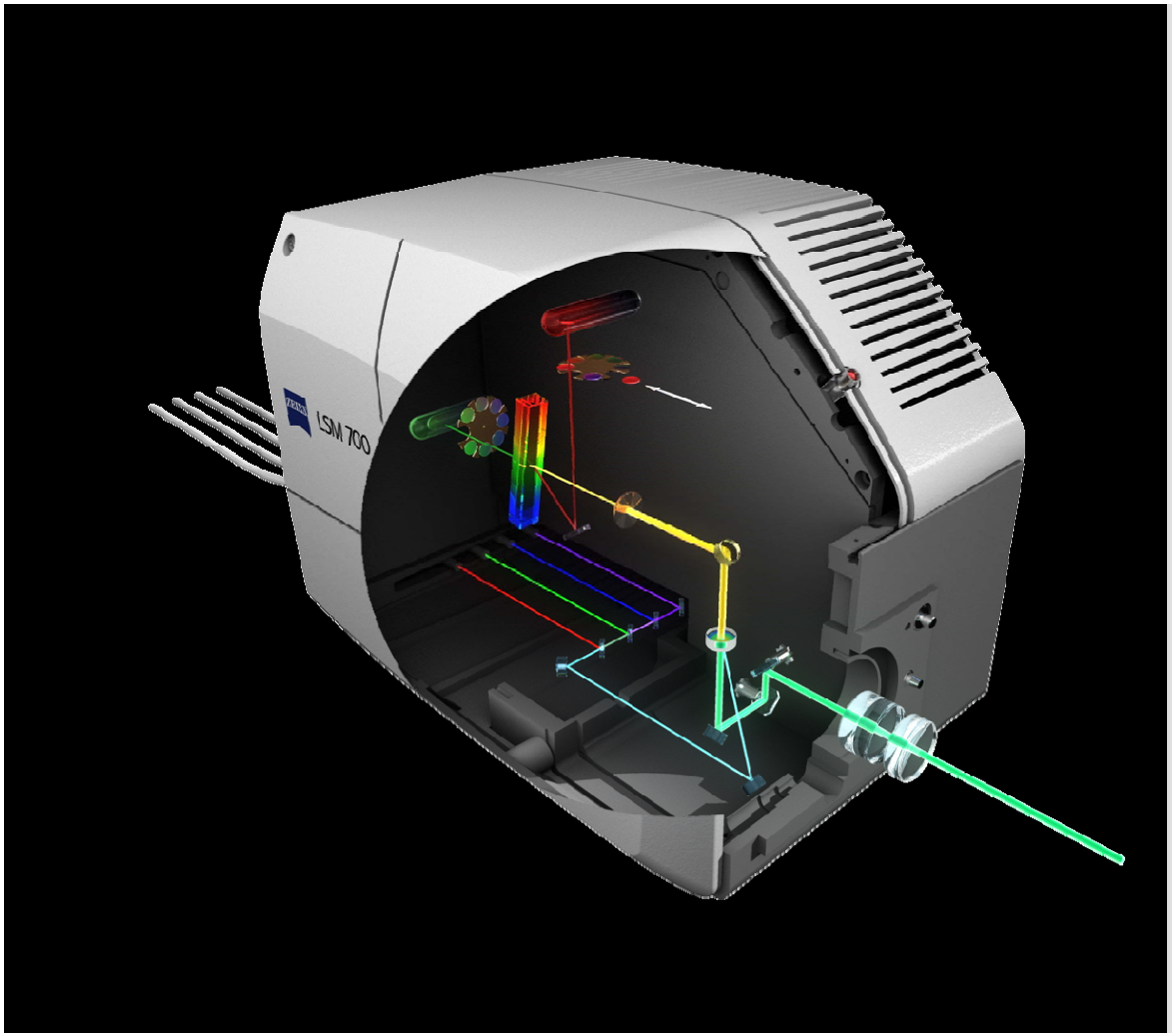
(optical sectioning rejects out-of-focus light)

# LSM 700

High-end LSM technology for everybody!

# LSM 700

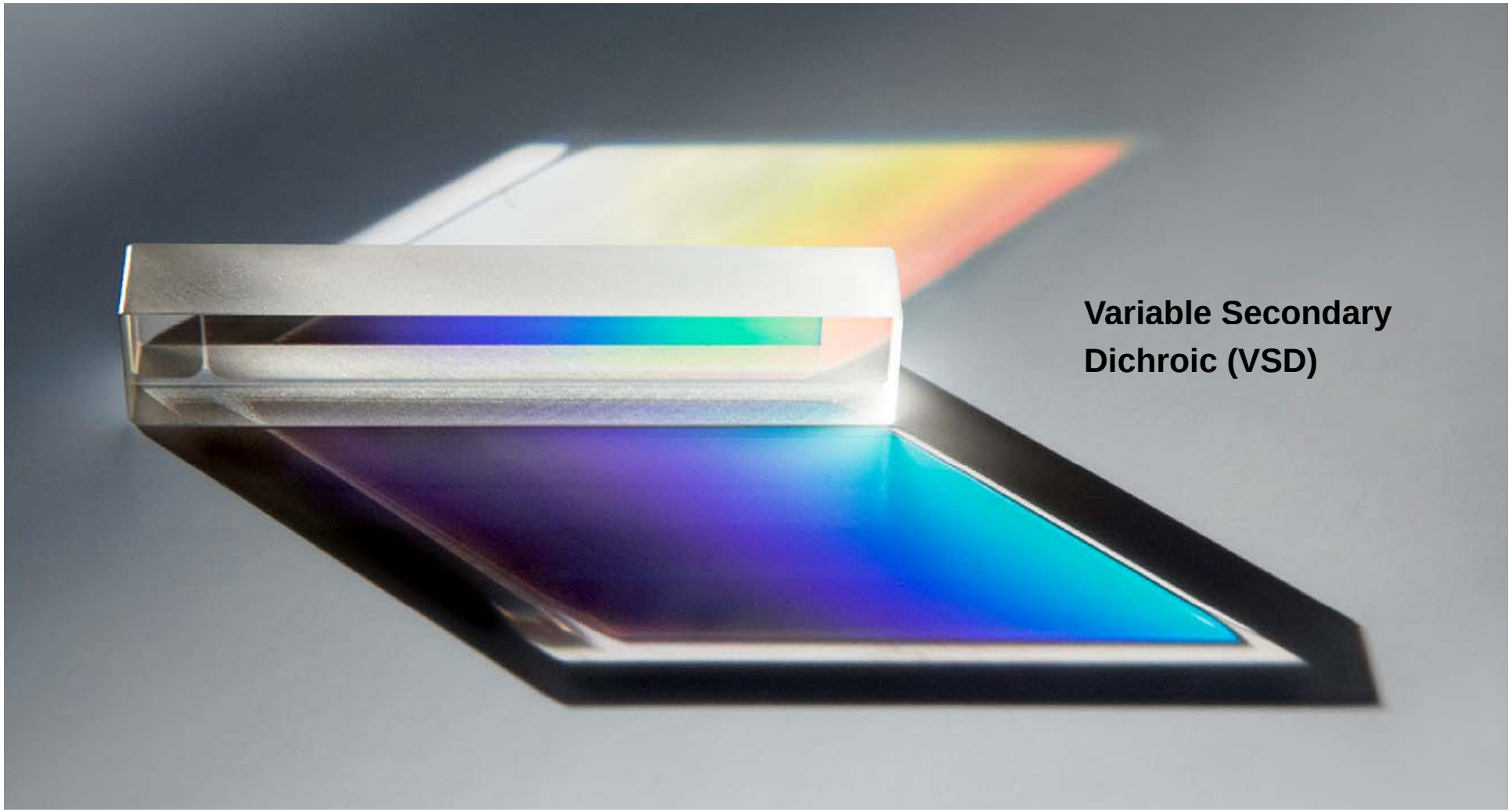
## High-end LSM technology for everybody!




- Highly light-efficient beam path design
- Features key technologies of the LSM 710 for enhanced sensitivity and optimal performance
- Up to two calibrated PMT detectors
- Innovative variable secondary dichroic (VSD); optional emission filters changeable by user
- Up to four diode lasers (405 or 445, 488, 555, 635 nm)

# LSM 700

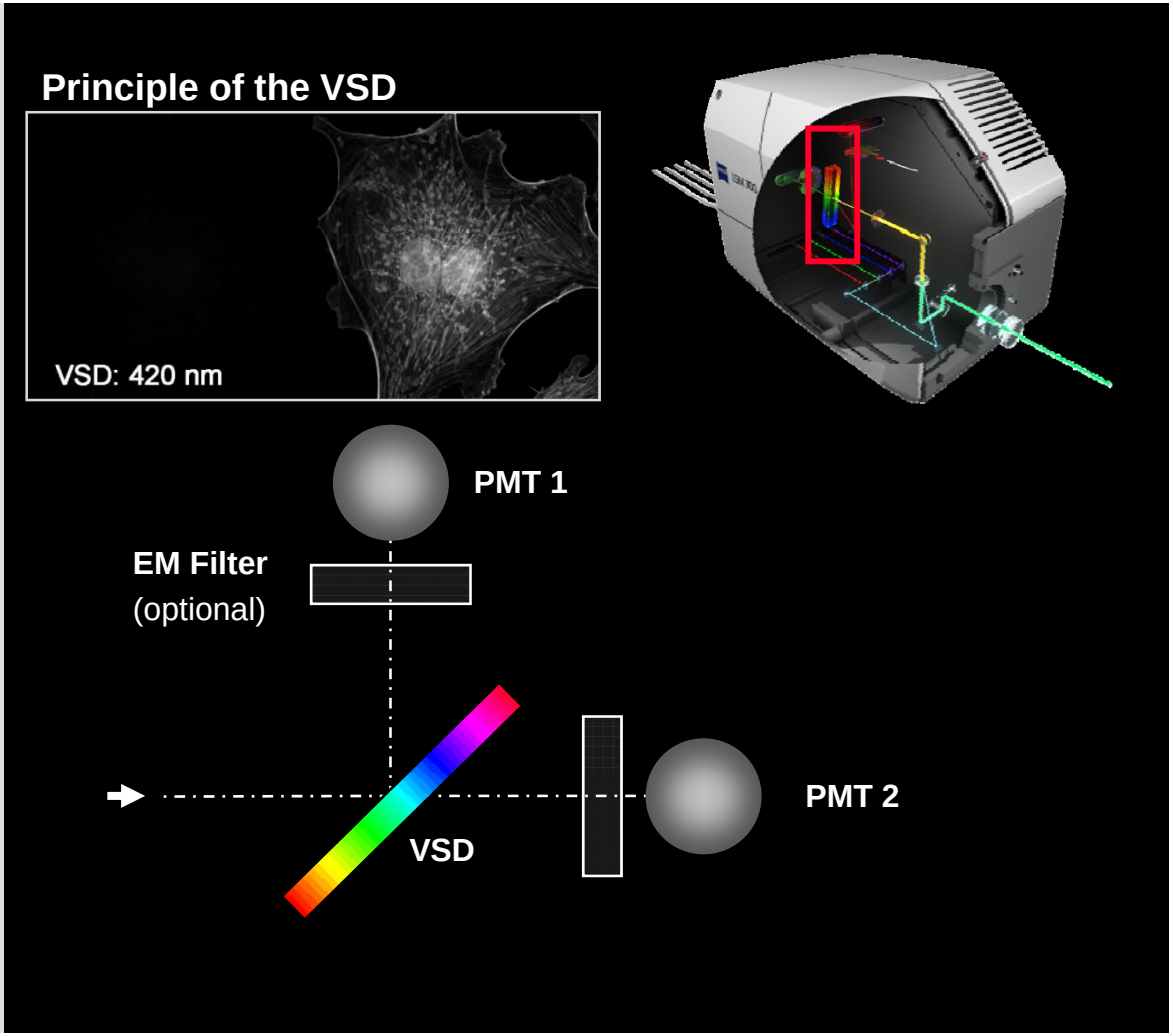
## VSD – Variable Secondary Dichroic



# LSM 700

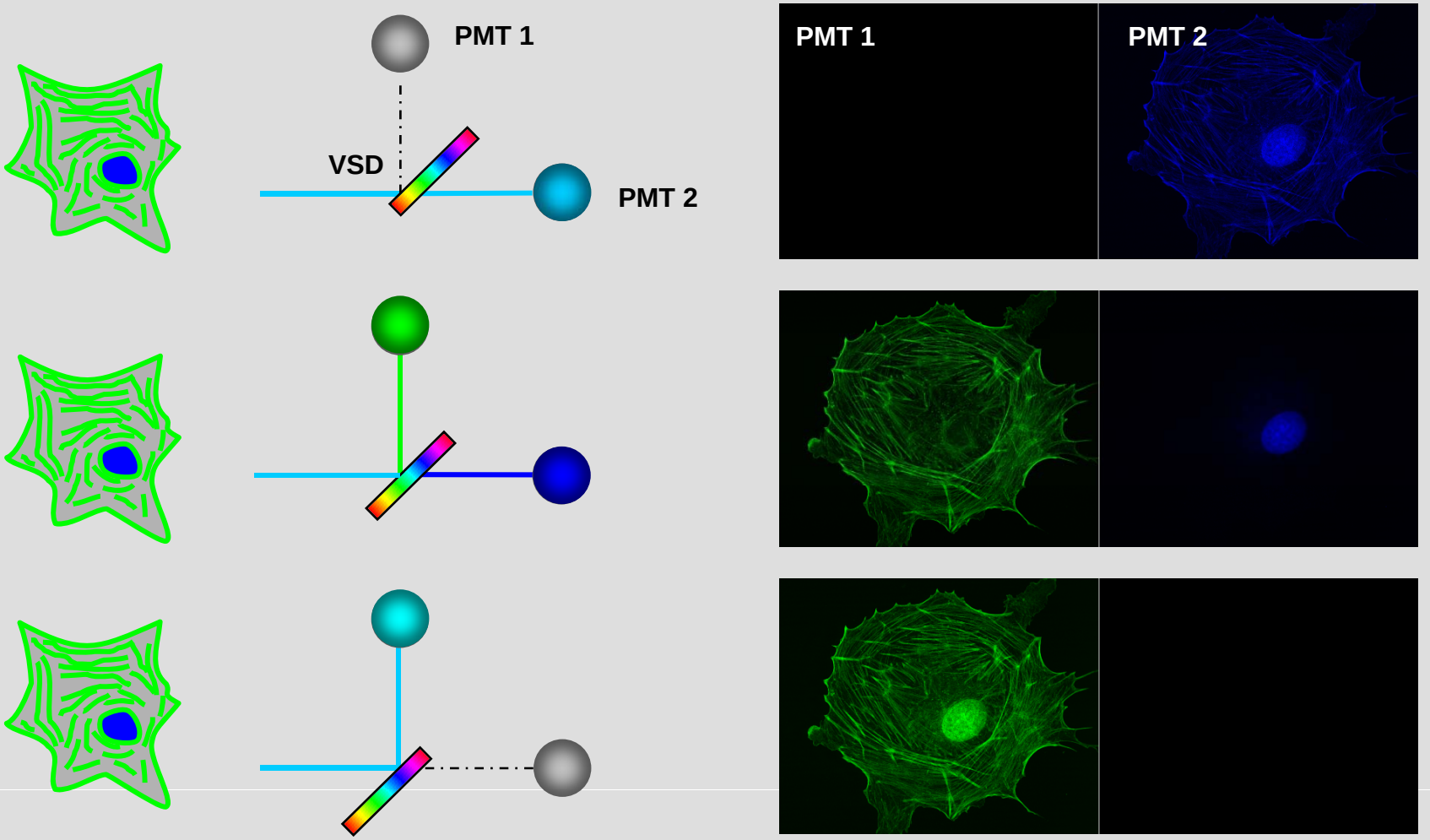
## VSD – Variable Secondary Dichroic

- VSD is a variable short pass beam splitter for splitting signals between detectors
- Positioning of VSD allows precise tuning of wavelenth at which signals are split (splitting possible between 420 and 630 nm, min. step: 1 nm)
- Enables highly light-efficient detection strategies and spectral imaging (lambda stack acquisition)
- Patented Zeiss innovation

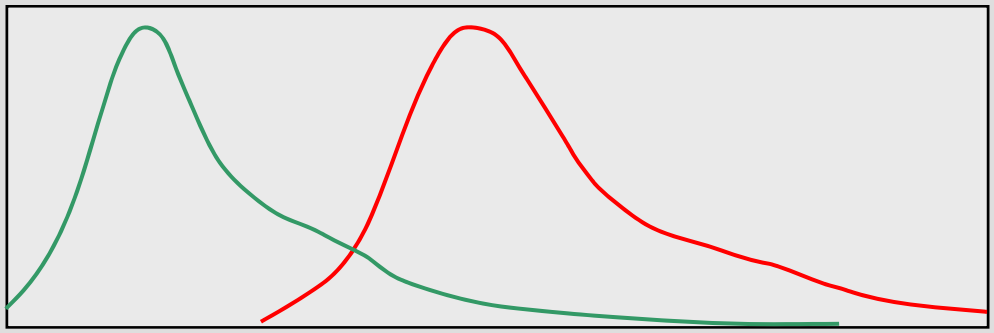


# LSM 700

## VSD – Variable Secondary Dichroic

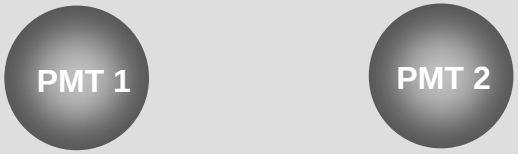


# LSM 700



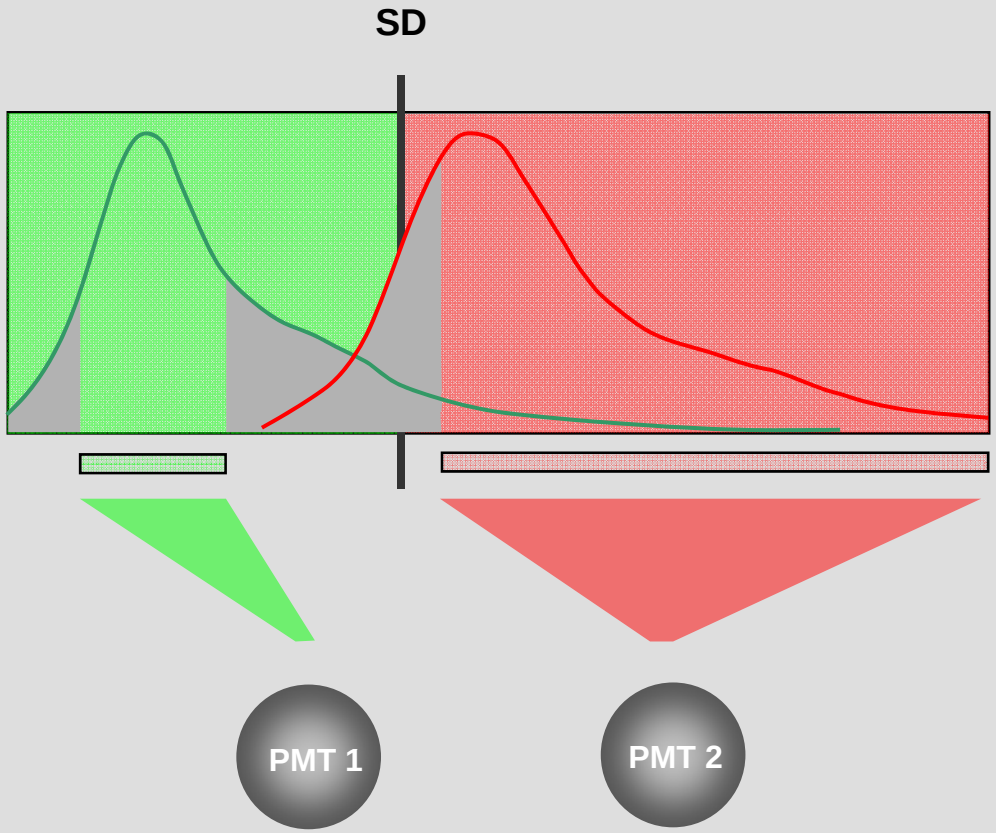
**Example:**

**Dual-color Detection of two dyes, green and red**



# Multi-Colour Detection with LSMs

The classical way…




**Example: Dual-color Detection of GFP and MitoTracker Orange**

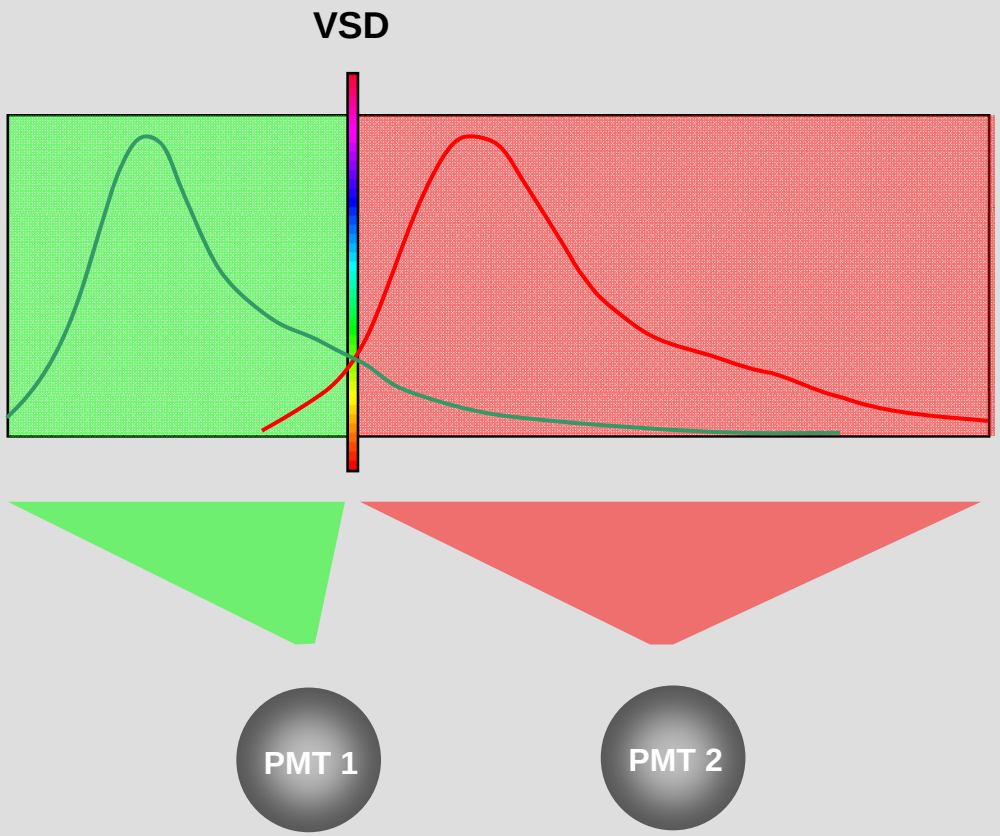
**Approach: “The Classical Way”**

Beam path for dual-color detection contains a secondary dichroic mirror (SD) and emission filters.

**Problem: Fixed characteristics of dichroic mirror and emission filters compromise light-efficiency of signal detection**

# LSM 700

VSD – the new flexible way




**Example: Dual-color Detection of GFP and MitoTracker Orange**

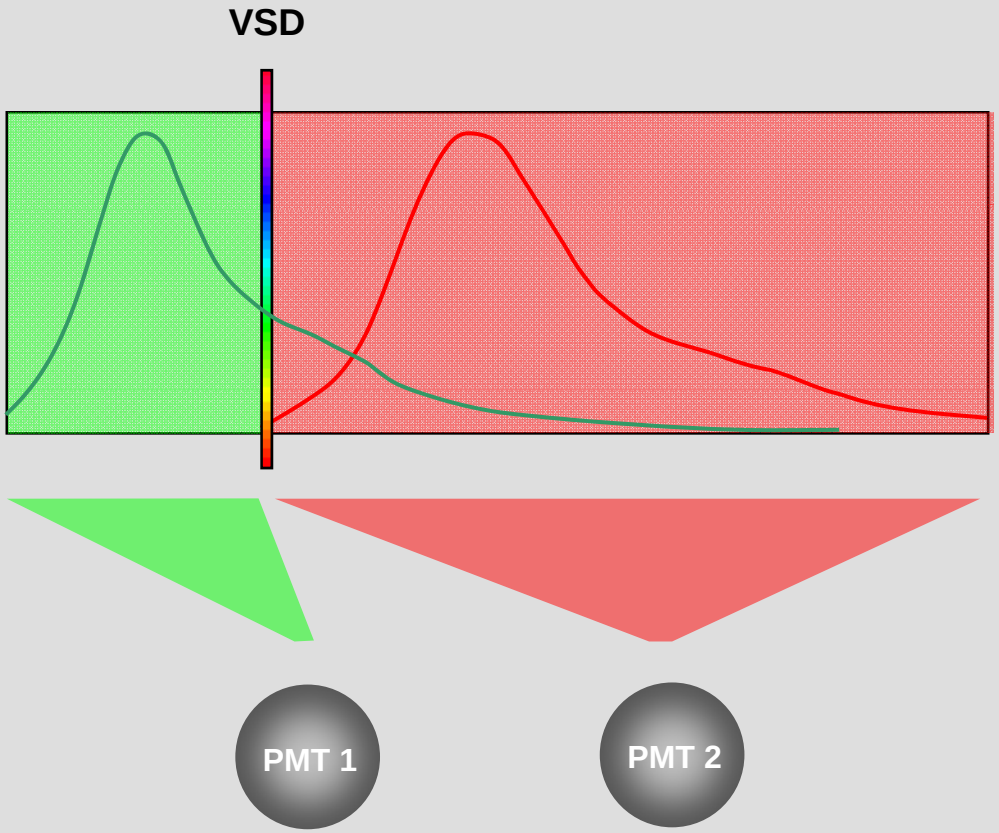
**Approach: “New Flexible Way”**

Flexible dual-color detection enabled by the new variable secondary dichroic (VSD) of the LSM 700.

**Improvement: Enhanced light efficiency because no portion of the signal is excluded from the detection process.**

# LSM 700

VSD – the new flexible way




**Example: Dual-color Detection of GFP and MitoTracker Orange**

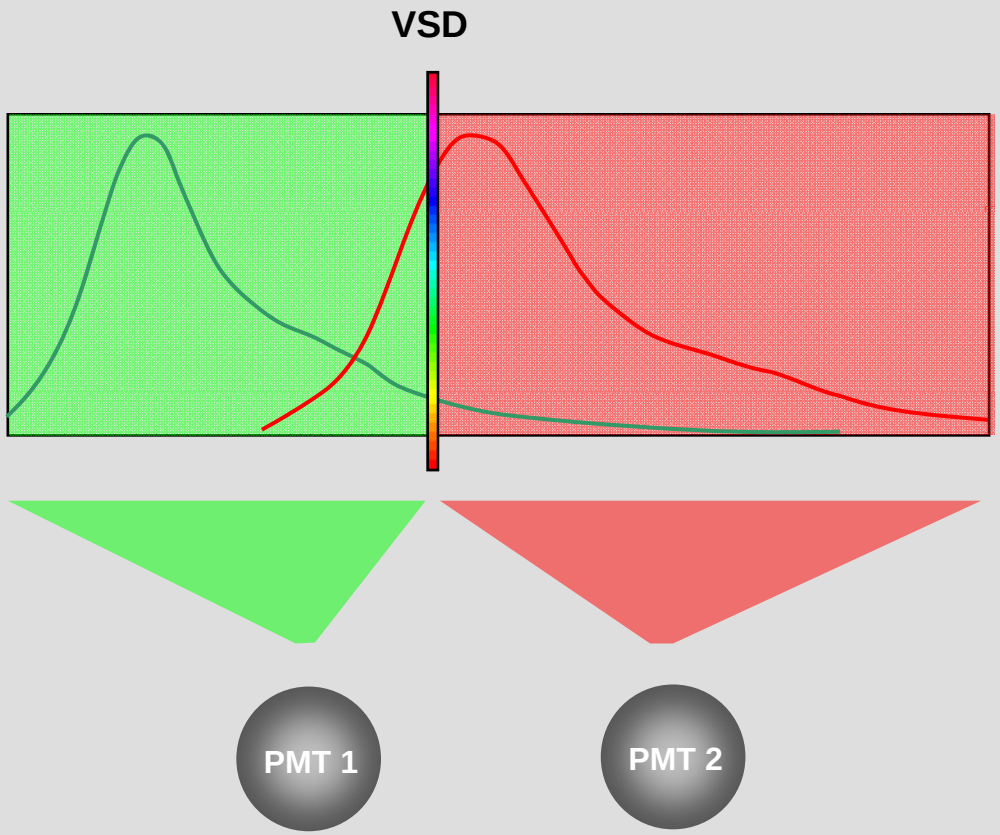
**Approach: “New Flexible Way”**

Flexible dual-color detection enabled by the new variable secondary dichroic (VSD) of the LSM 700.

**Improvement: Enhanced light efficiency because no portion of the signal is excluded from the detection process.**

# LSM 700

VSD – the new flexible way




**Example: Dual-color Detection of GFP and MitoTracker Orange**

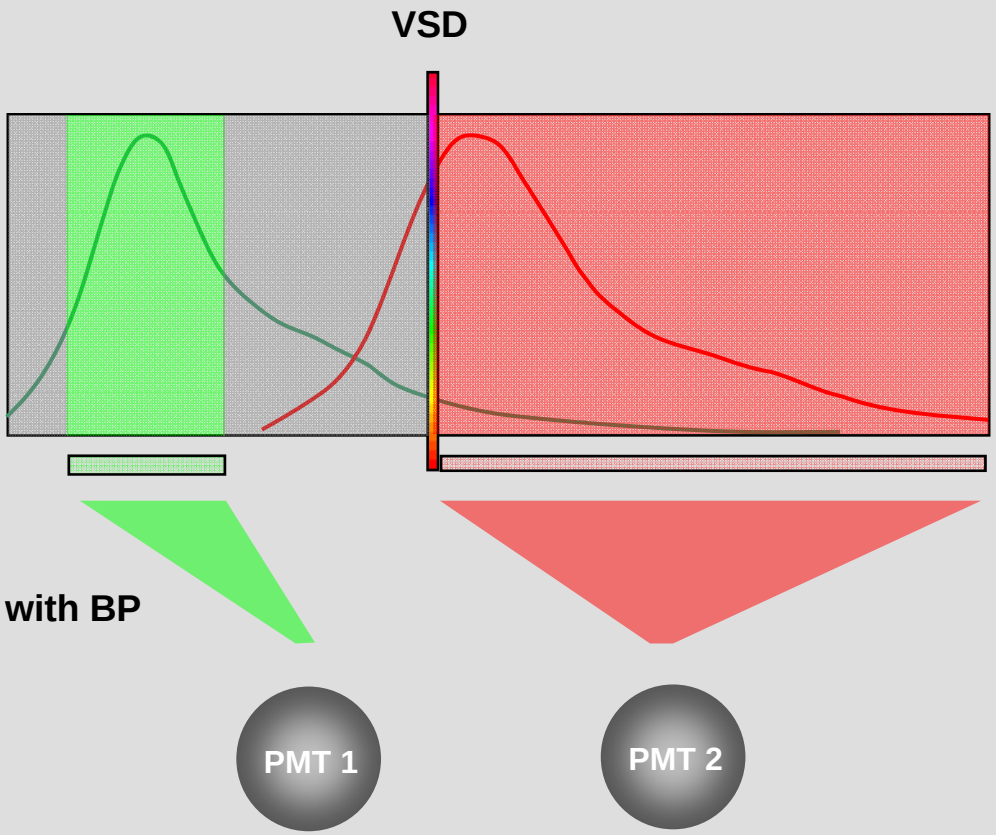
**Approach: “New Flexible Way”**

Flexible dual-color detection enabled by the new variable secondary dichroic (VSD) of the LSM 700.

**Improvement: Enhanced light efficiency because no portion of the signal is excluded from the detection process.**

# LSM 700

## VSD – the new flexible way




**Example: Dual-color Detection of GFP and MitoTracker Orange**

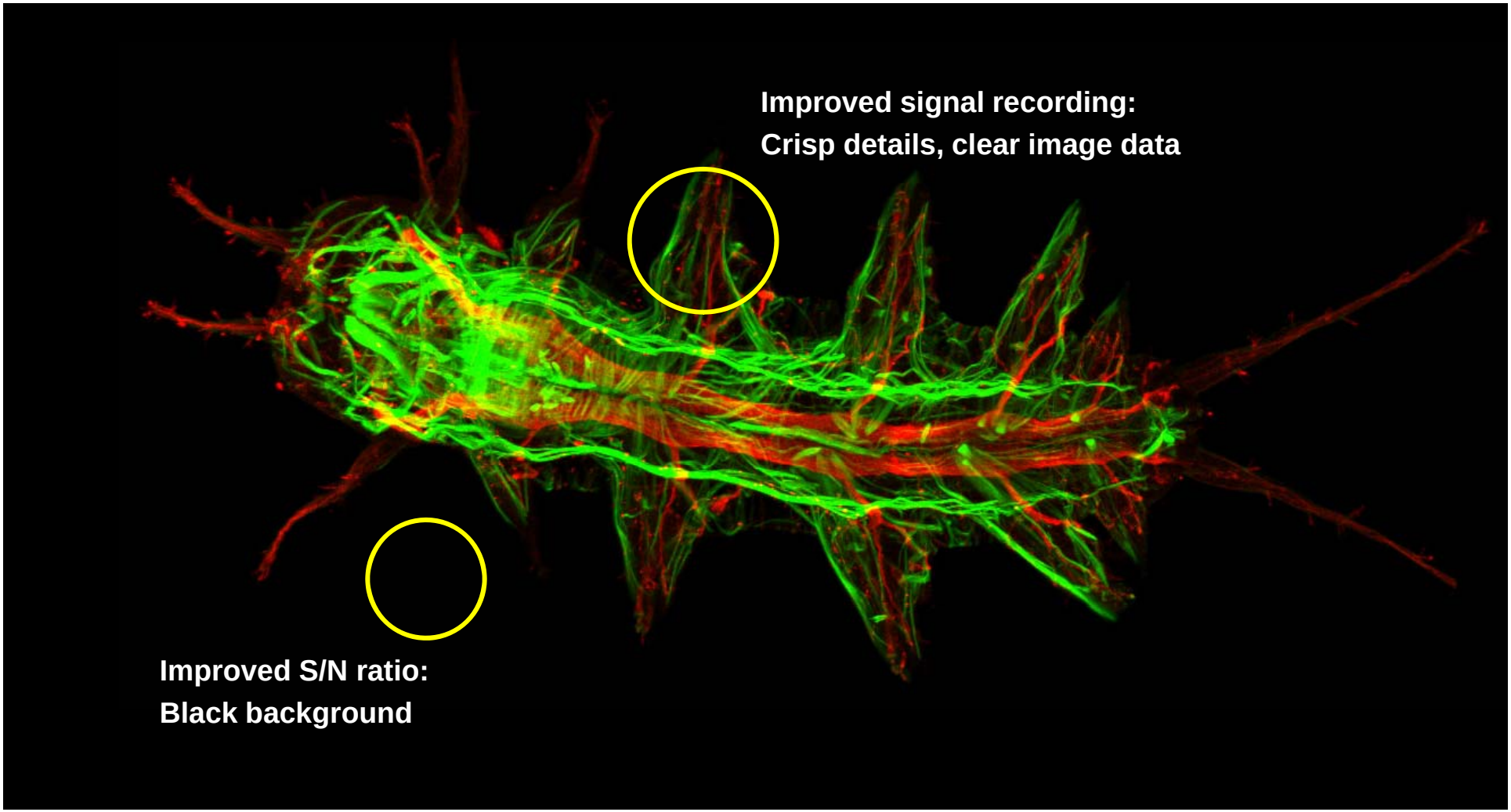
**Approach: “New Flexible Way”**

Flexible dual-color detection enabled by the new variable secondary dichroic (VSD) of the LSM 700.

**Also possible: use of emission filters (optional) for additional specificity.**

# LSM 700 in Standard Applications

- Uncompromized Image quality



# LSM 700 in Standard Applications

- Uncompromized Image quality

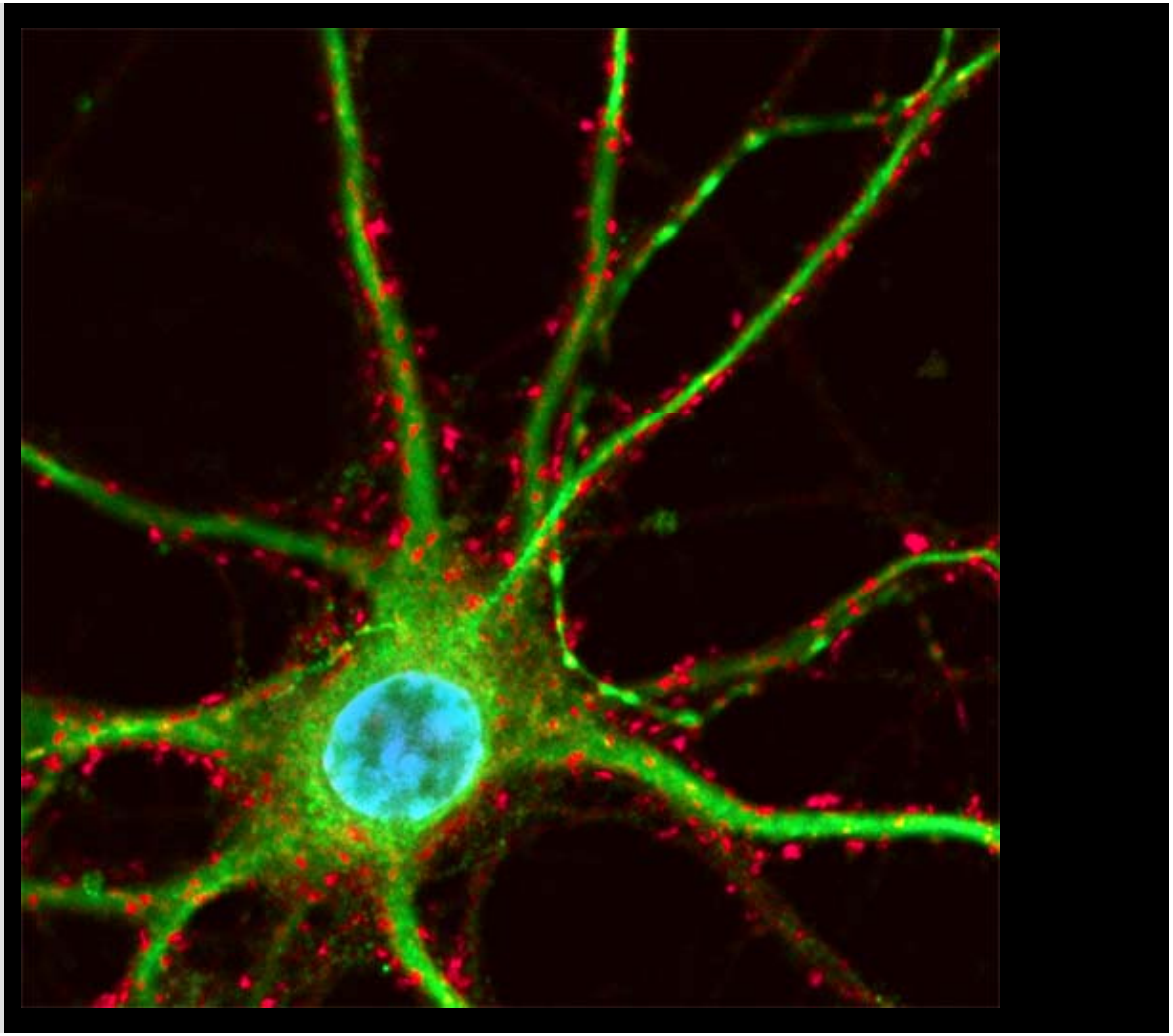
**Sample**

Rat Neuron

Red: Spines

Green: Dendrites

Maximum Projection



# LSM 700 in Standard Applications

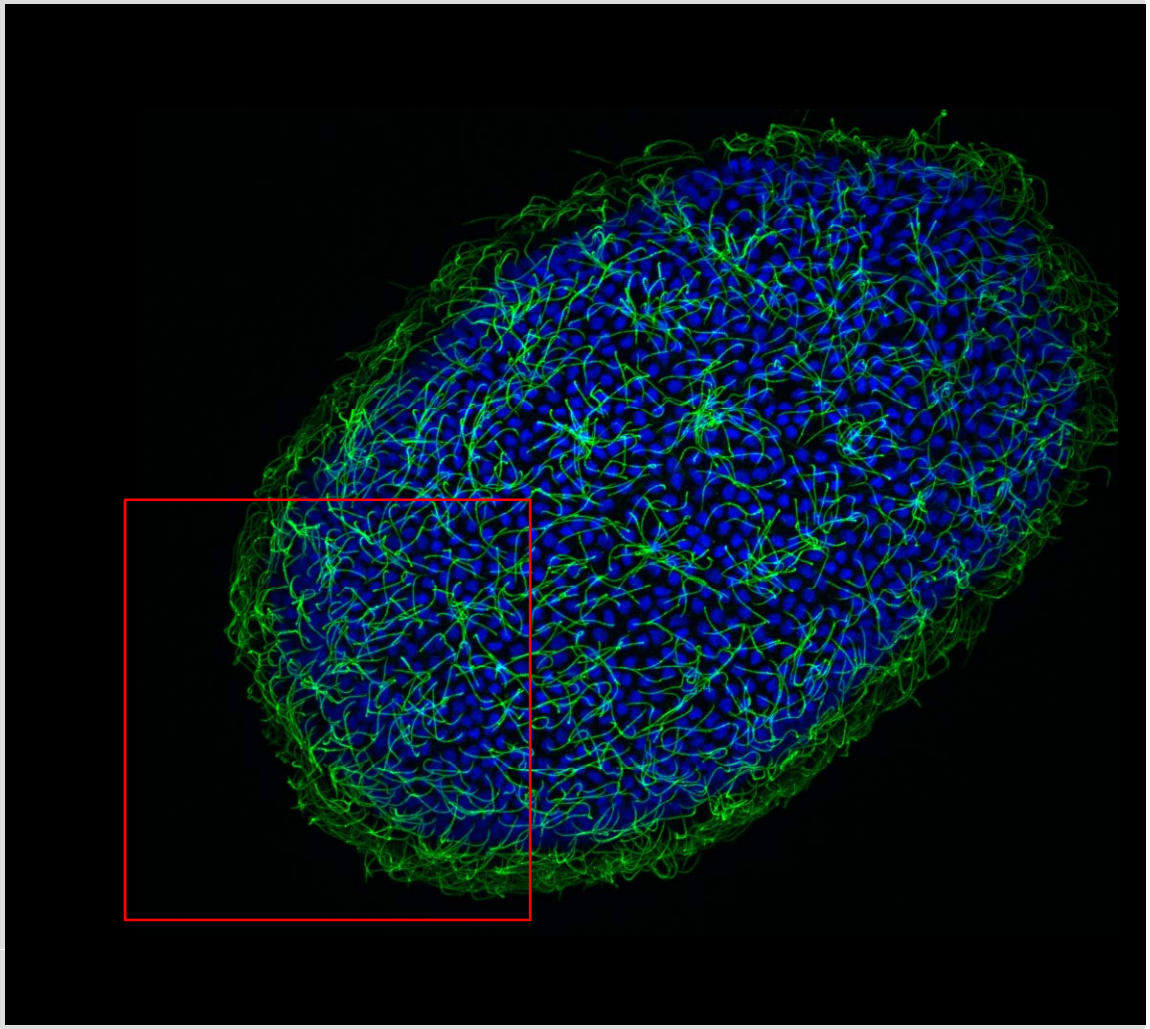
- Uncompromized Image quality for 3D rendering

**Sample**

amphioxus embryo
a-tubulin (in green) shows the complex ciliary structure on the body surface of an early neurula stage amphioxus embryo.
DAPI (in blue) was used to counterstain nuclei.

**Projection**

Maximum intensity projection



# LSM 700 in Standard Applications

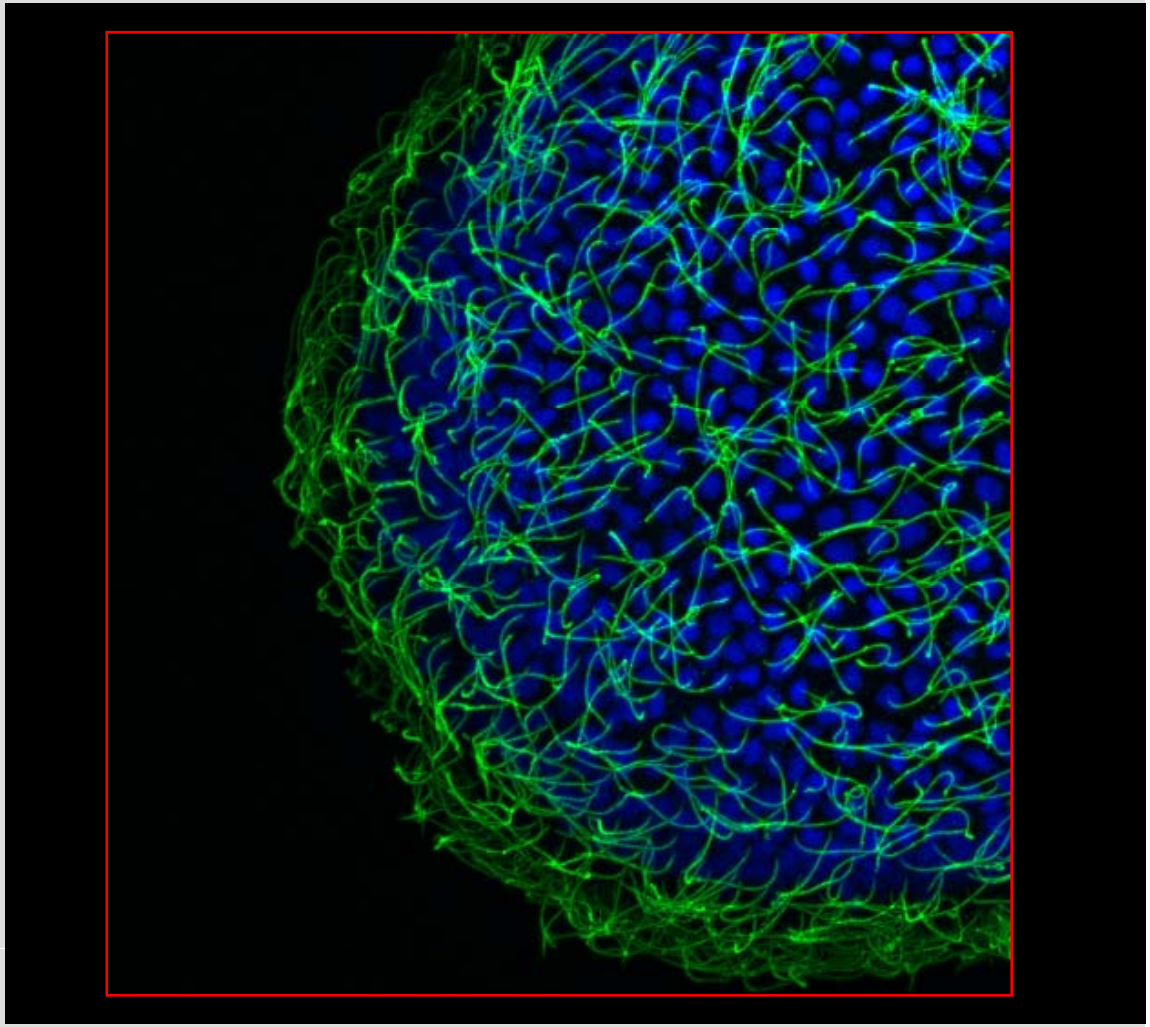
- Uncompromized Image quality for 3D rendering

**Sample**

amphioxus embryo
a-tubulin (in green) shows the complex ciliary structure on the body surface of an early neurula stage amphioxus embryo.
DAPI (in blue) was used to counterstain nuclei.

**Projection**

Maximum intensity projection



# LSM 700

Spectral imaging and linear unmixing

# LSM 700

## Spectral imaging and linear unmixing

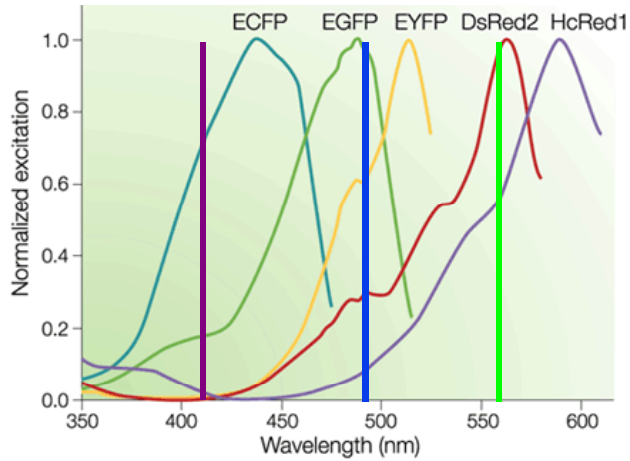
**Challenge:**

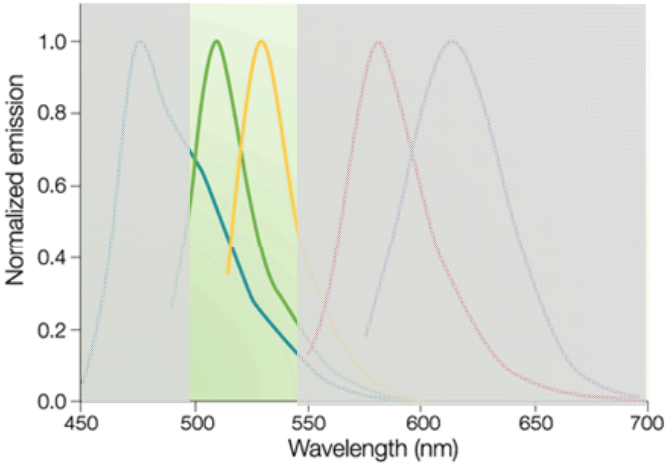
Conditions of excitation and emission cross-talk (e.g. multiple fluorescent proteins)

**Solution:**

***Emission Fingerprinting***

Spectral detection and linear unmixing used to separate similar fluorescent signals.



# LSM 700

## Spectral imaging and linear unmixing

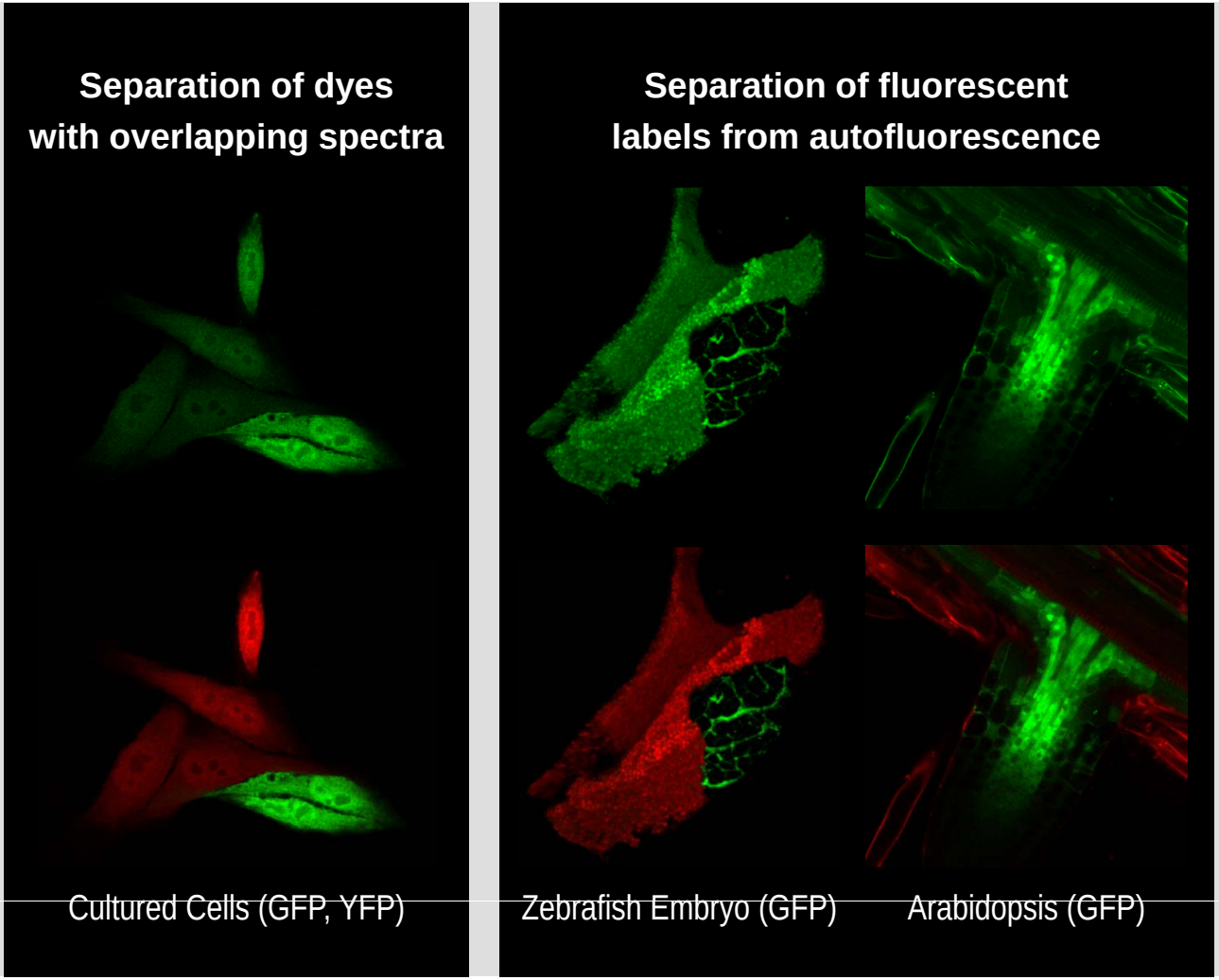
**Imaging Task:**

**Separation of dyes with overlapping spectra**

**Separation of fluorescent labels from autofluorescence**

**without linear unmixing**

**results of linear unmixing**



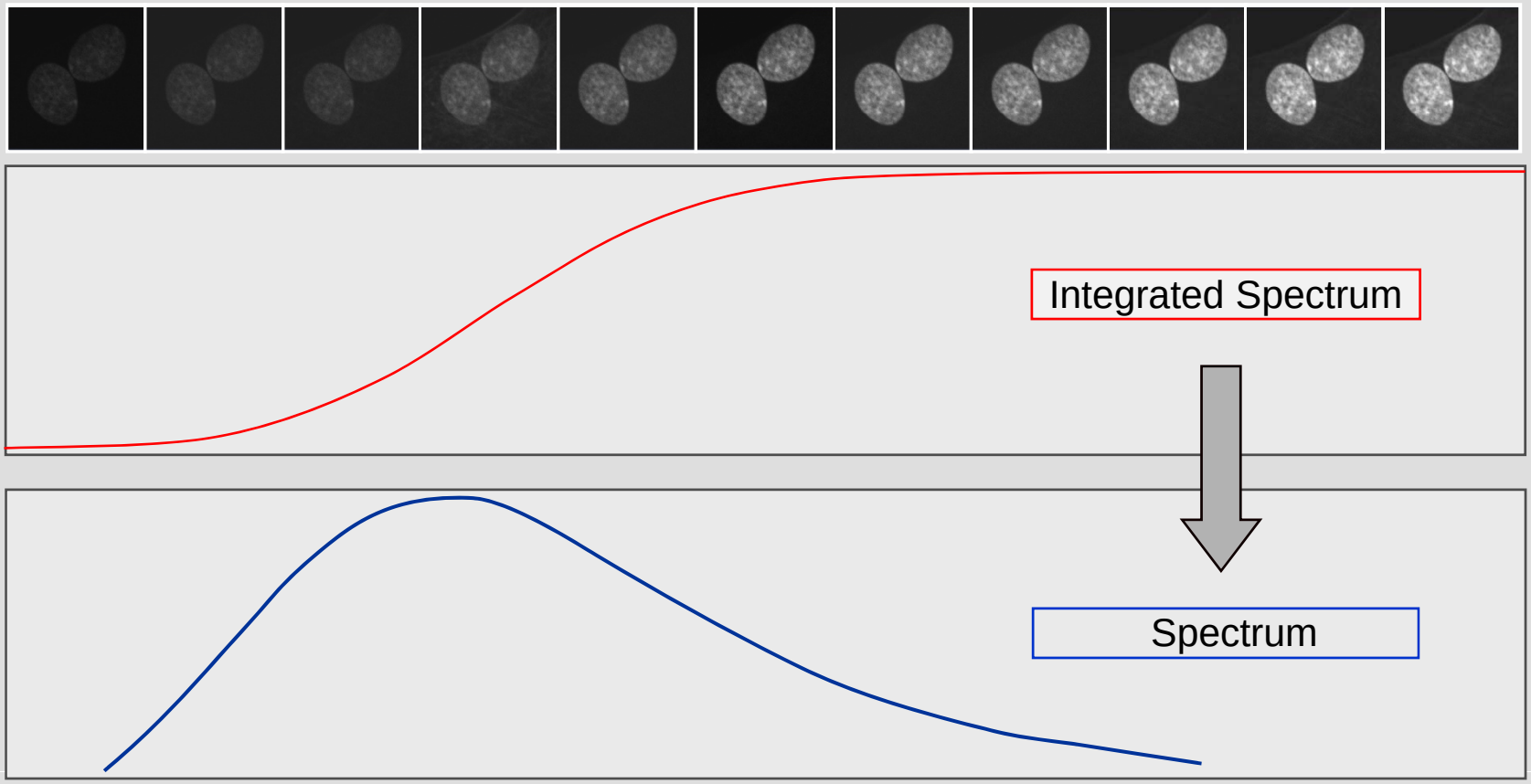
Cultured Cells (GFP, YFP)

Zebrafish Embryo (GFP)

Arabidopsis (GFP)

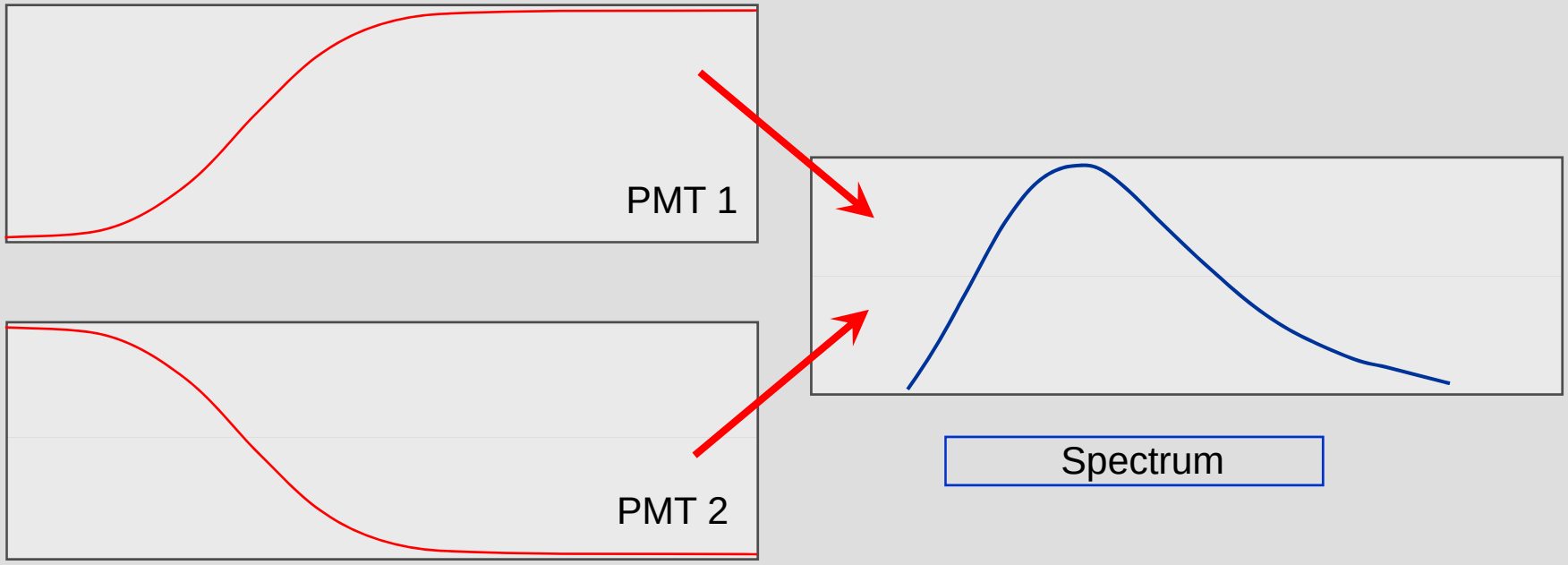
# LSM 700

## Spectral imaging and linear unmixing – Spectra Generation



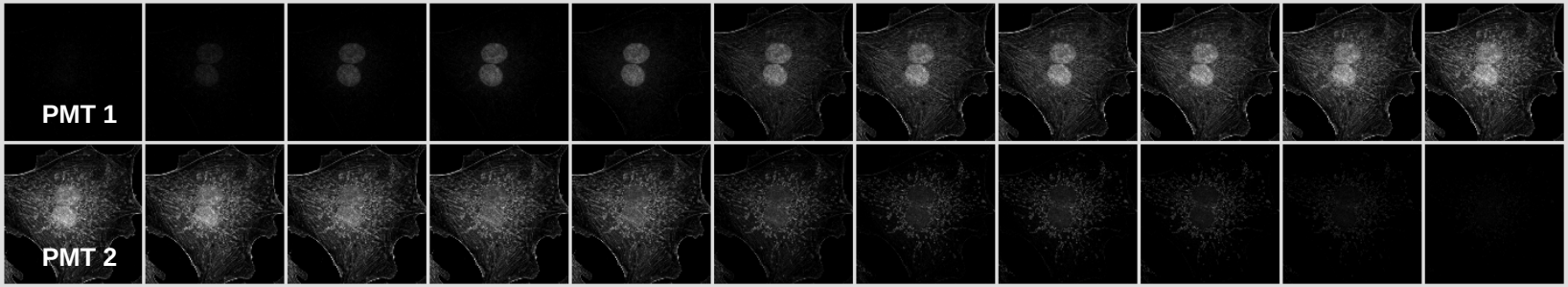
# LSM 700

## Spectral imaging and linear unmixing



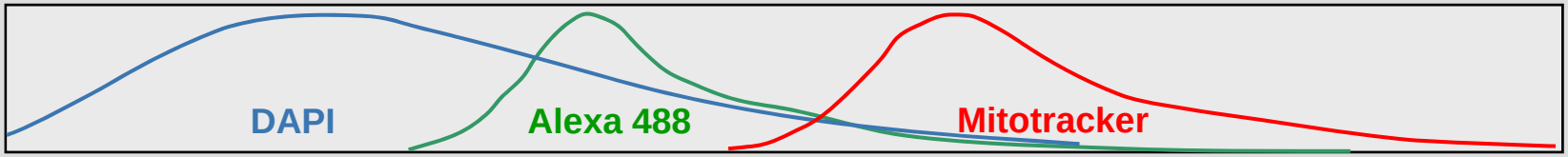
# LSM 700

## Spectral imaging using the new VSD



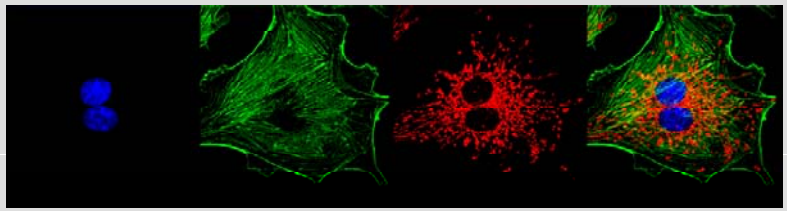

**Raw dual-channel input**

calculated from dual-channel input




**Unmixing**

**Result**

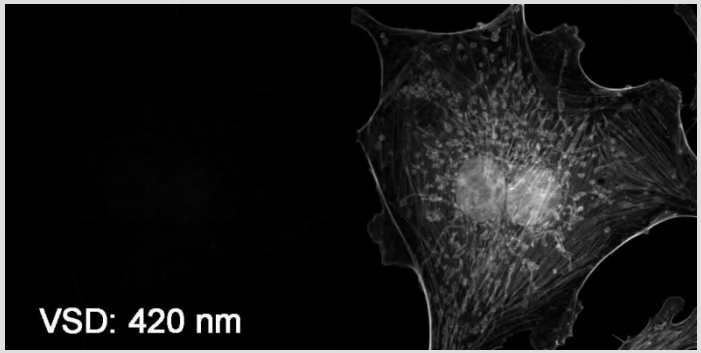


# LSM 700

## Spectral imaging and linear unmixing

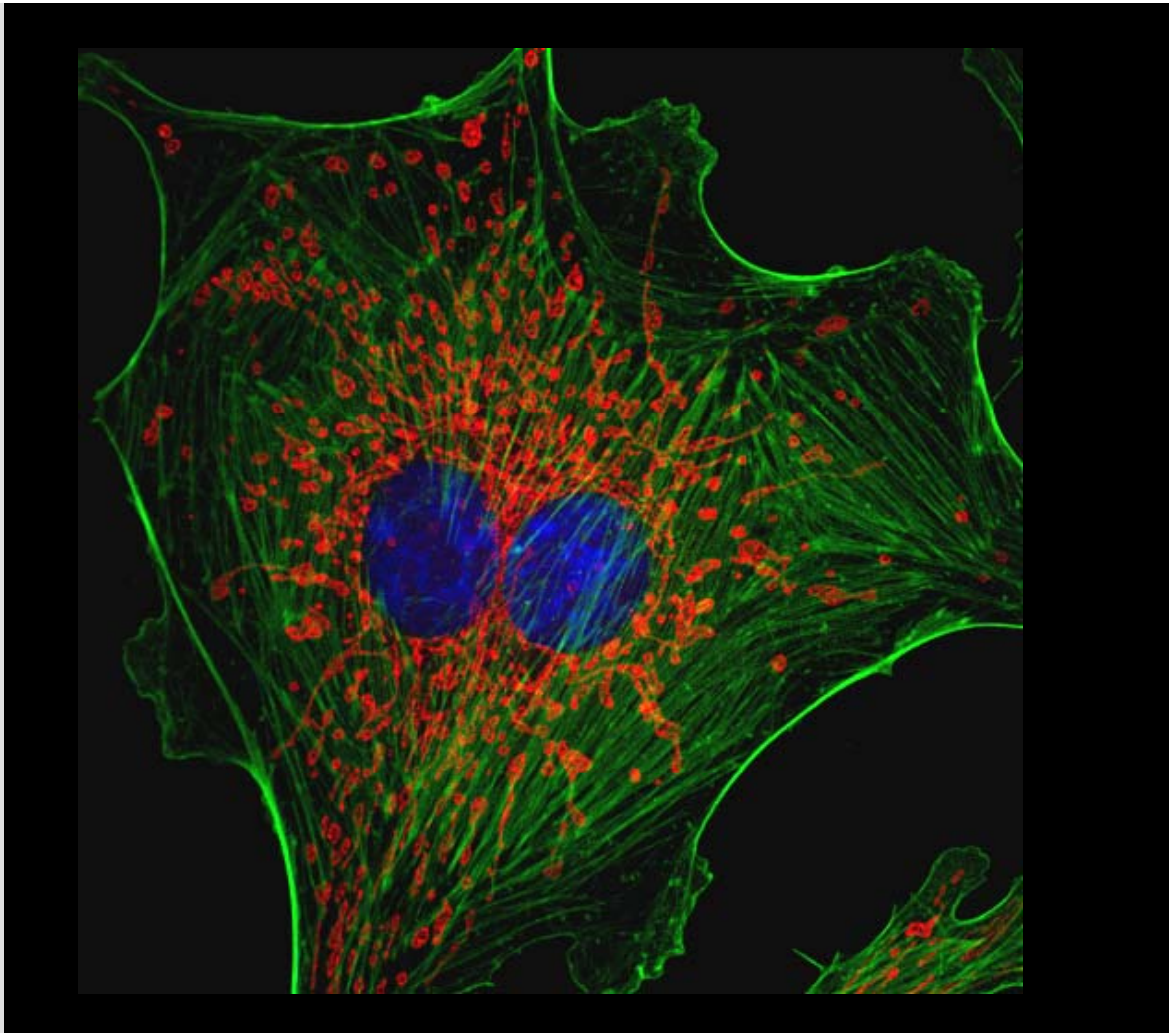
### Principle of the VSD

Dual-channel imaging with both PMTs at different VSD positions




### Fluorochromes

DAPI (chromating), Alexa488 (actin), MitoTracker (mitochondria)

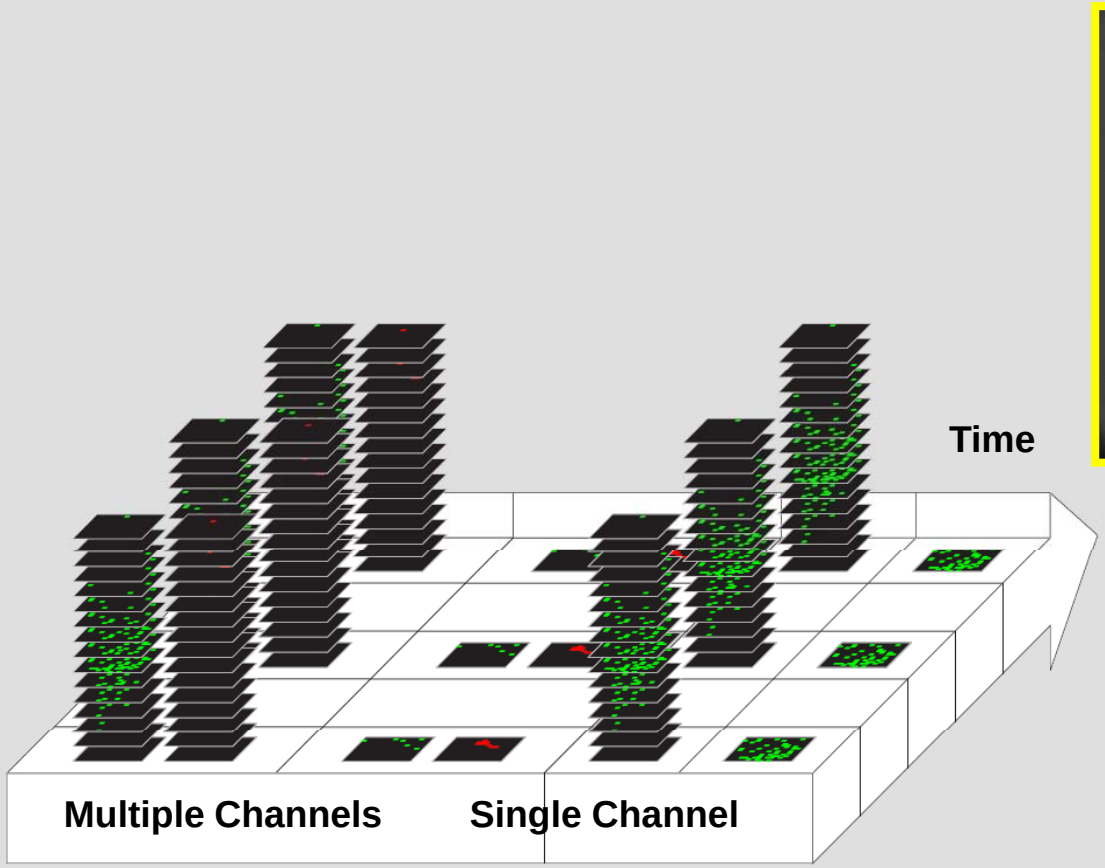


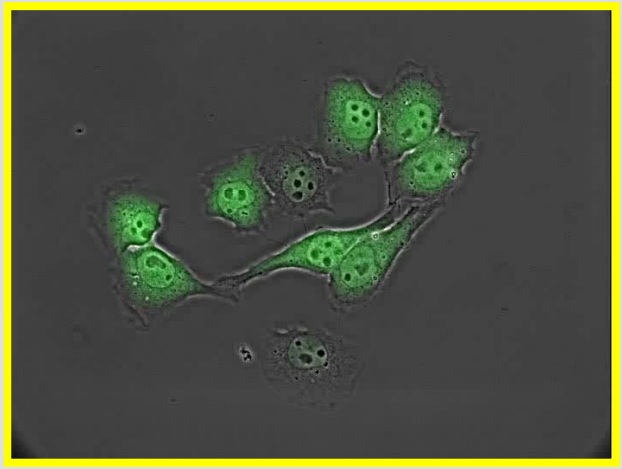
# LSM 700

Fast and sensitive Live Cell Imaging

# Confocal Laser Scanning Microscopy

## Typical imaging modes



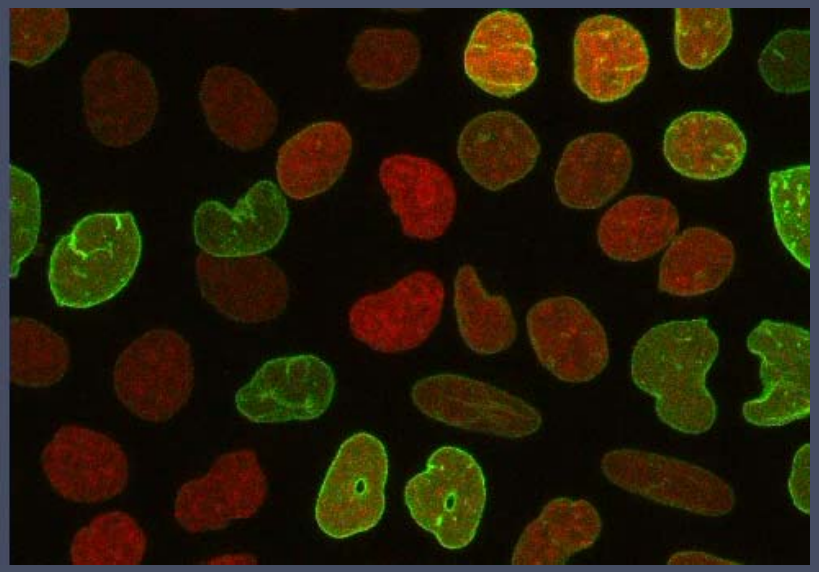



# Definite Focus

Ensures stable focus in long-term imaging

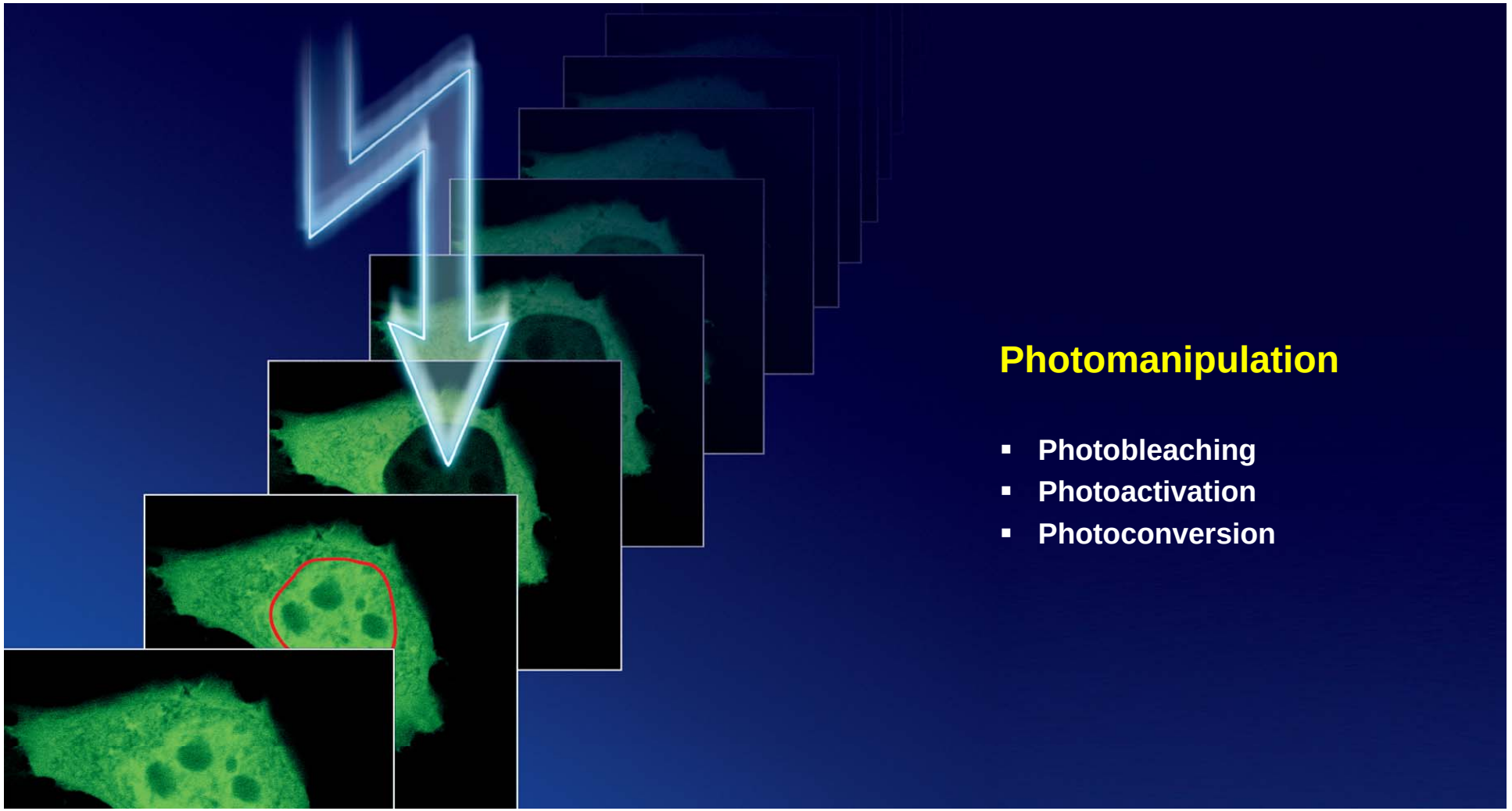
**Stable imaging conditions in long-term live cell imaging**

**Control of the focus plane using the Definite Focus attachment for the Axio Observer**



# Confocal Laser Scanning Microscopy

Disturbing equilibria for studying dynamic processes

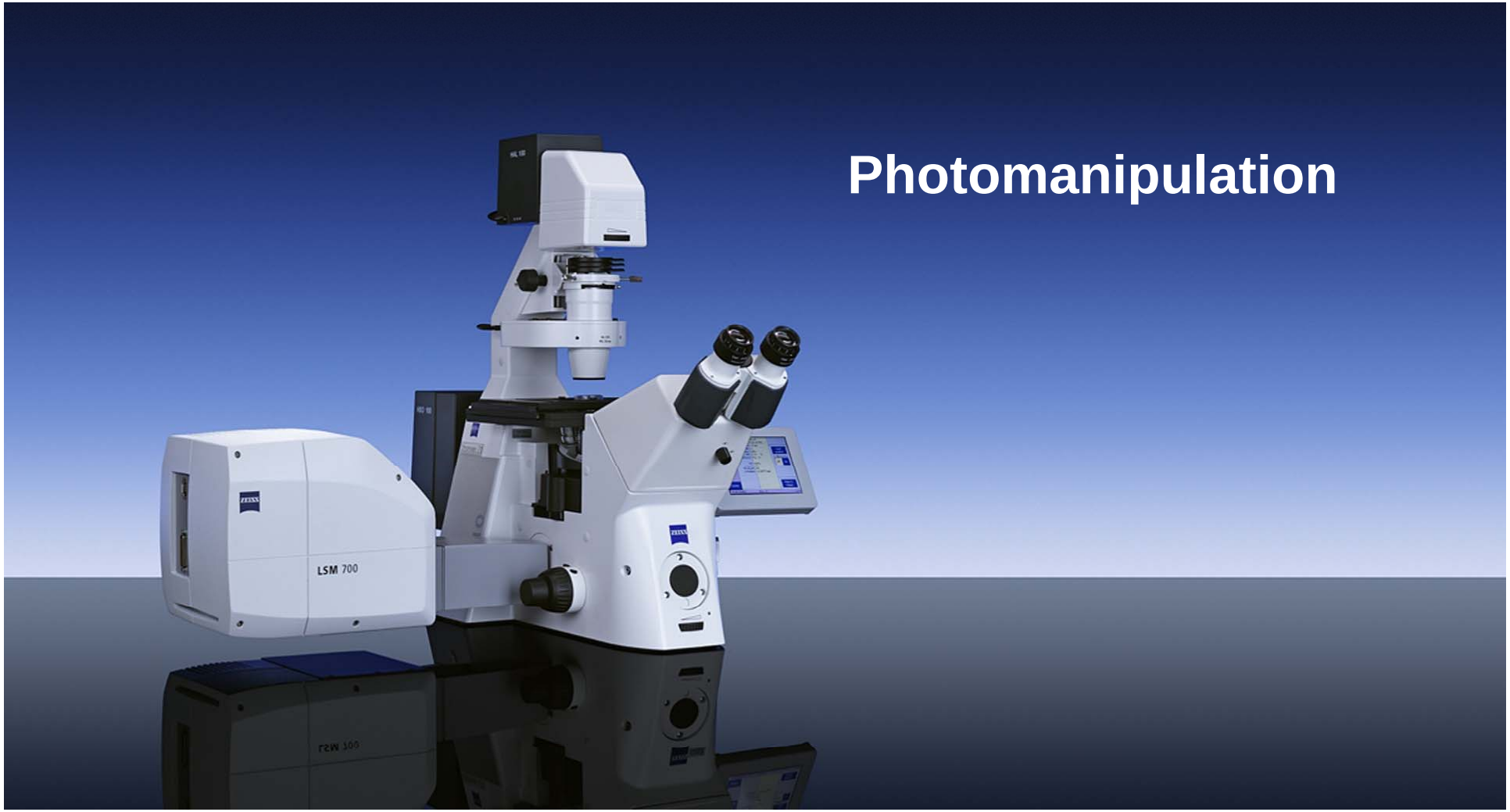


## Photomanipulation

- Photobleaching
- Photoactivation
- Photoconversion

# LSM 700

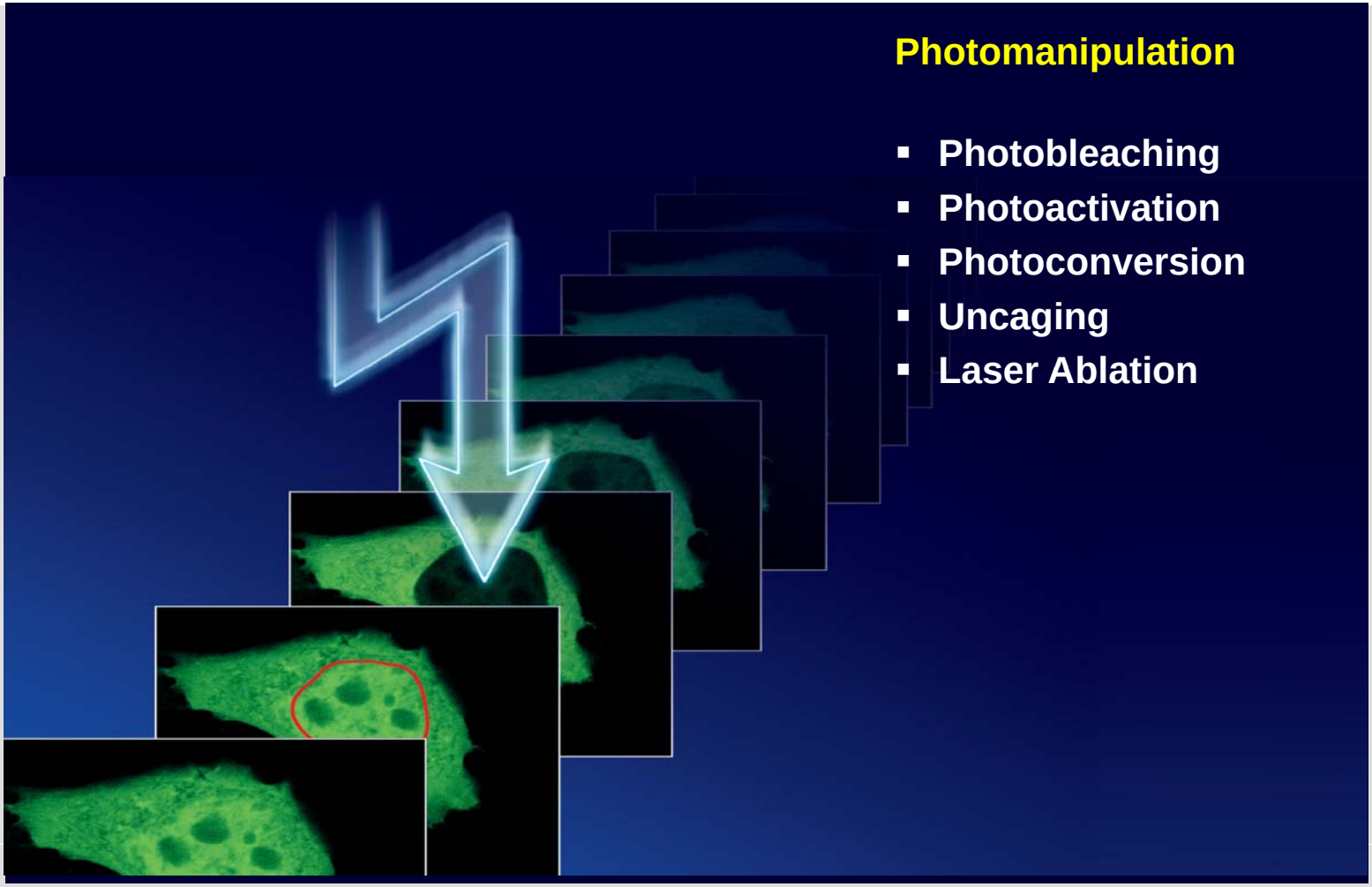
F-techniques in live cell imaging



# Major tasks of a LSM

## Laser and scanning mirror control

Photomanipulation for studying cellular dynamics



# LSM 700 for interaction & measurement –

## FRAP: Fluorescence Recovery after Photobleaching

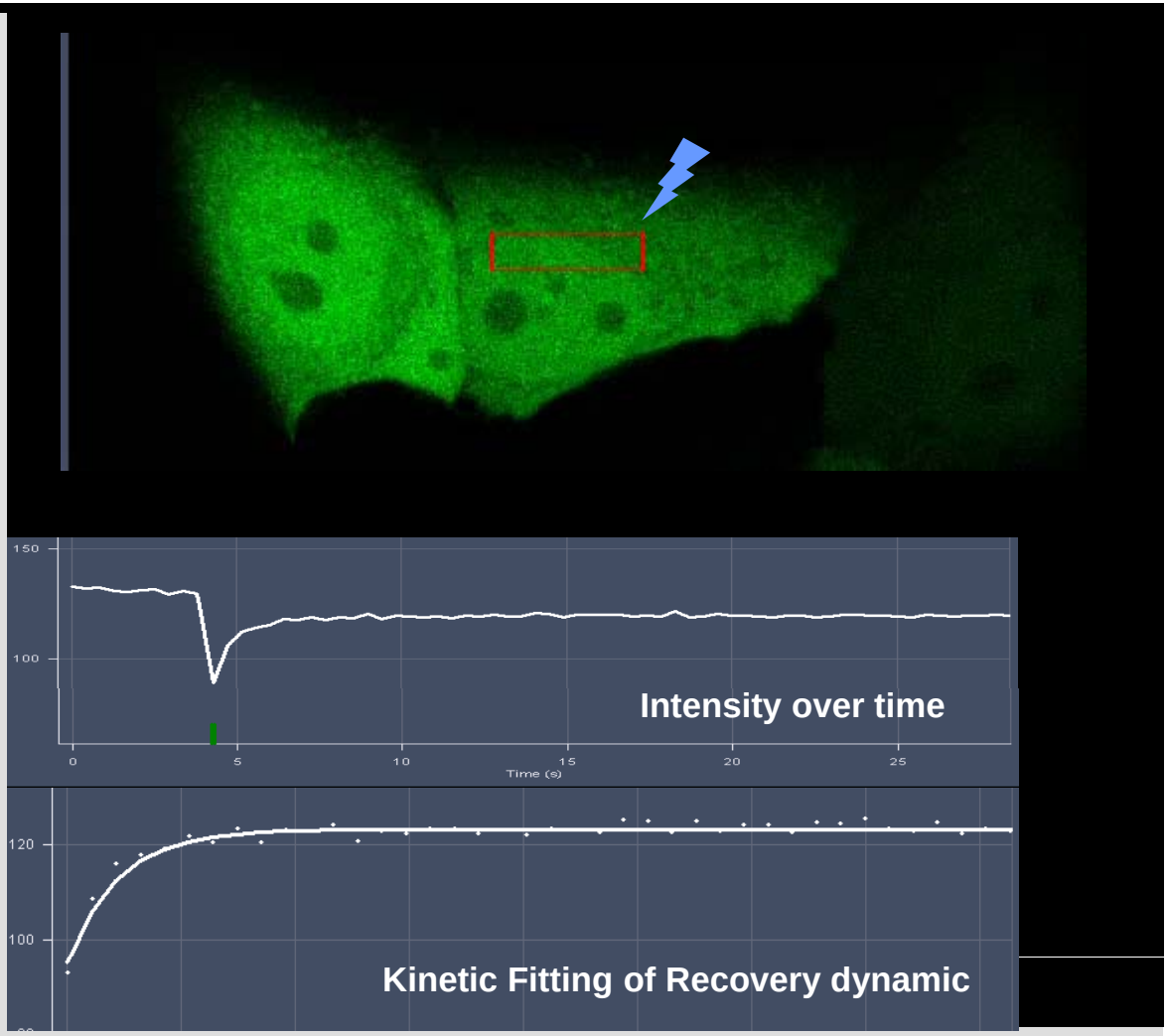
**Sample**

Cell culture stable expressing cytoplasmatic GFP

Imaging with 488 Laser (0,5%)
Bleaching with 488 Laser (100%)

Recovery at ½ time of 600ms

Imaging rate 400ms / frame



# LSM 700 for interaction –

Photoconversion from green to red

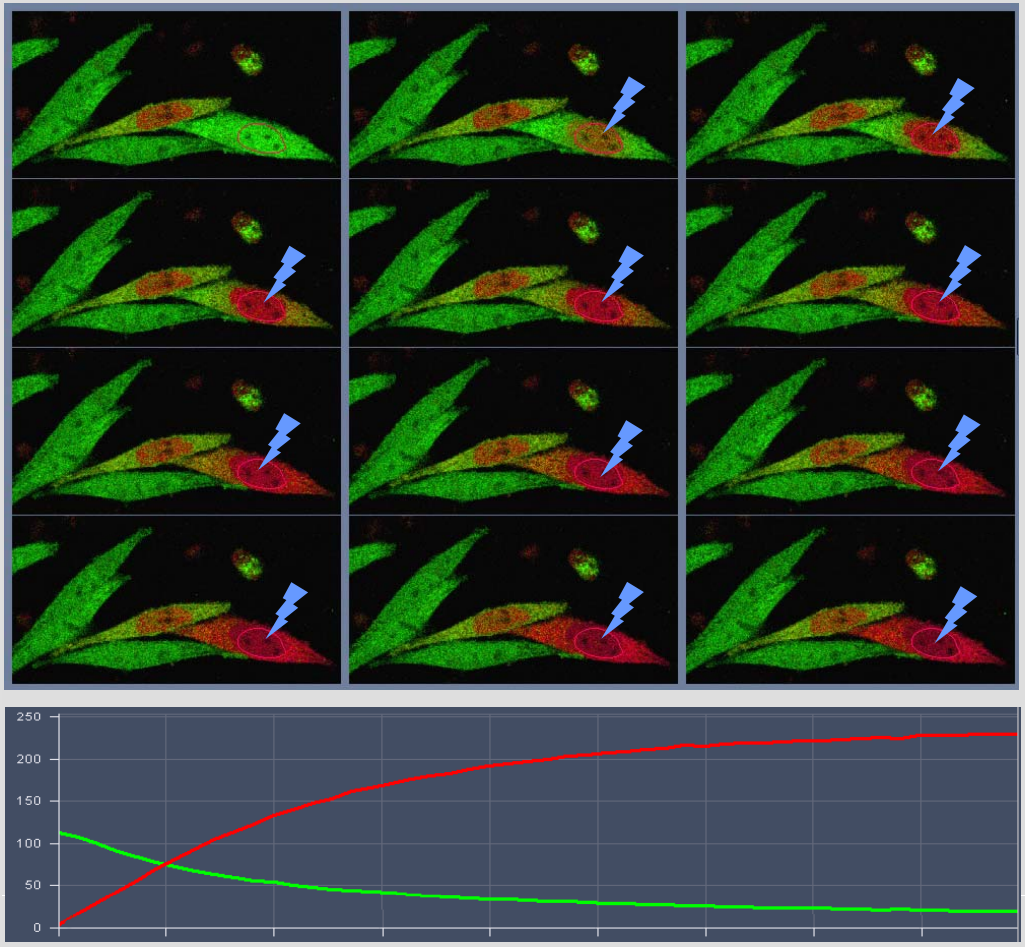
**Sample**

Cell culture stable expressing cytoplasmatic KAEDE

Convertible Fluorescent Protein (from green to red)

Recorded time: 80 sec (480ms/f)

Conversion: 405 Laser



# LSM 700 for interaction –

## Photoconversion from green to red

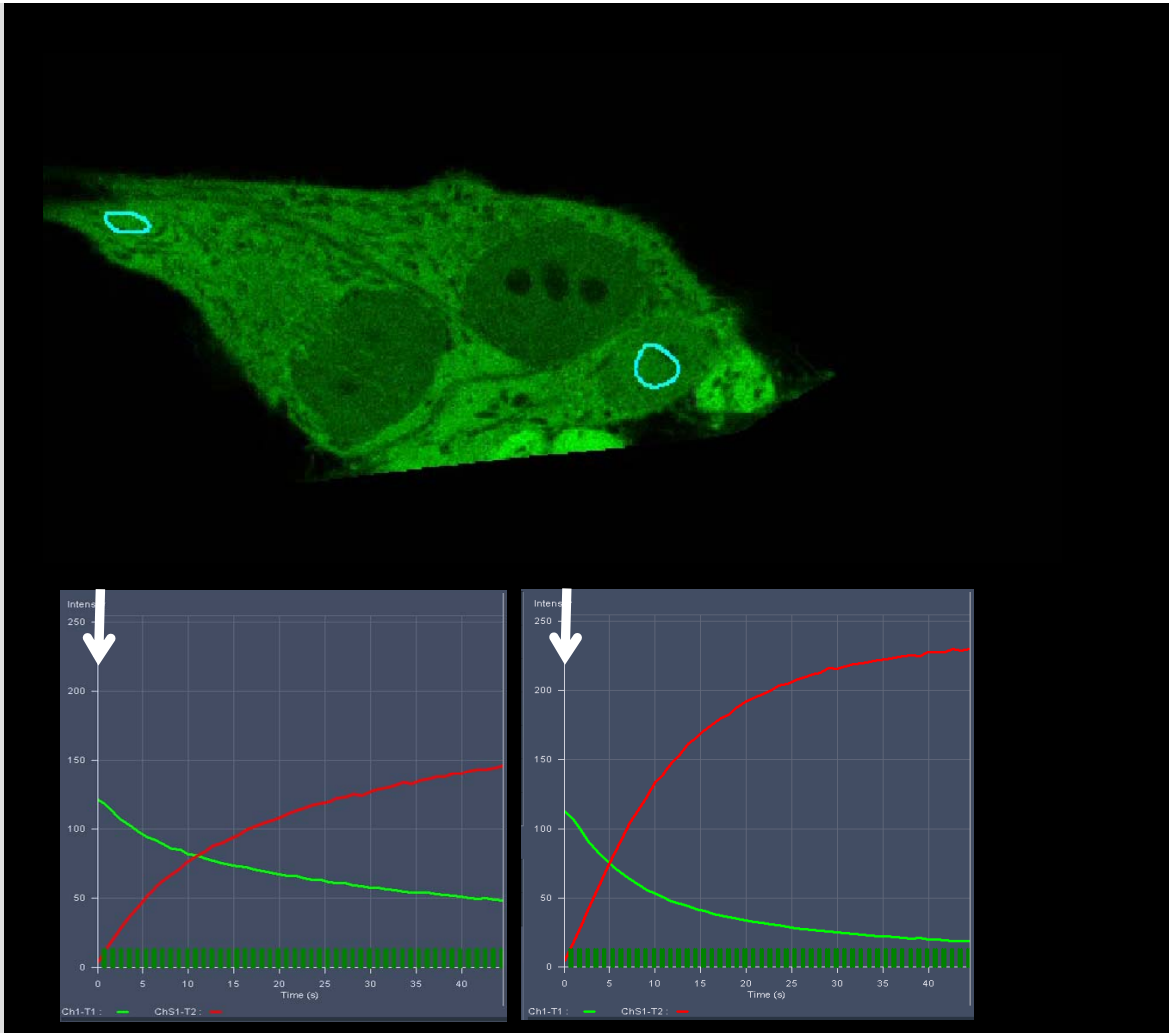
**Sample**

Cell culture stable expressing cytoplasmatic KAEDE

Convertible Fluorescent Protein (from green to red)

Recorded time: 45 sec

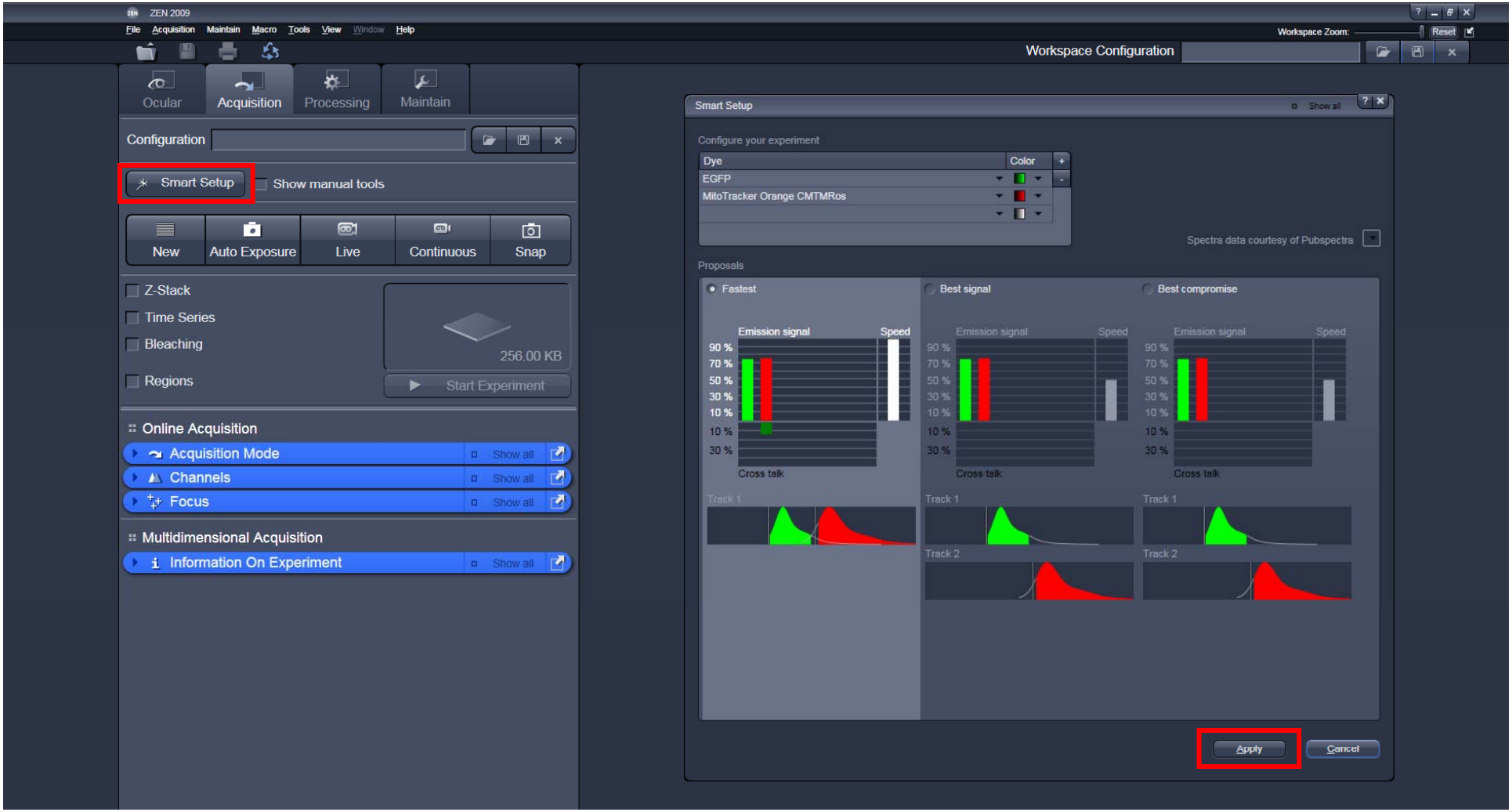
Conversion: 405 Laser
Left region: 50%
Right region: 100%



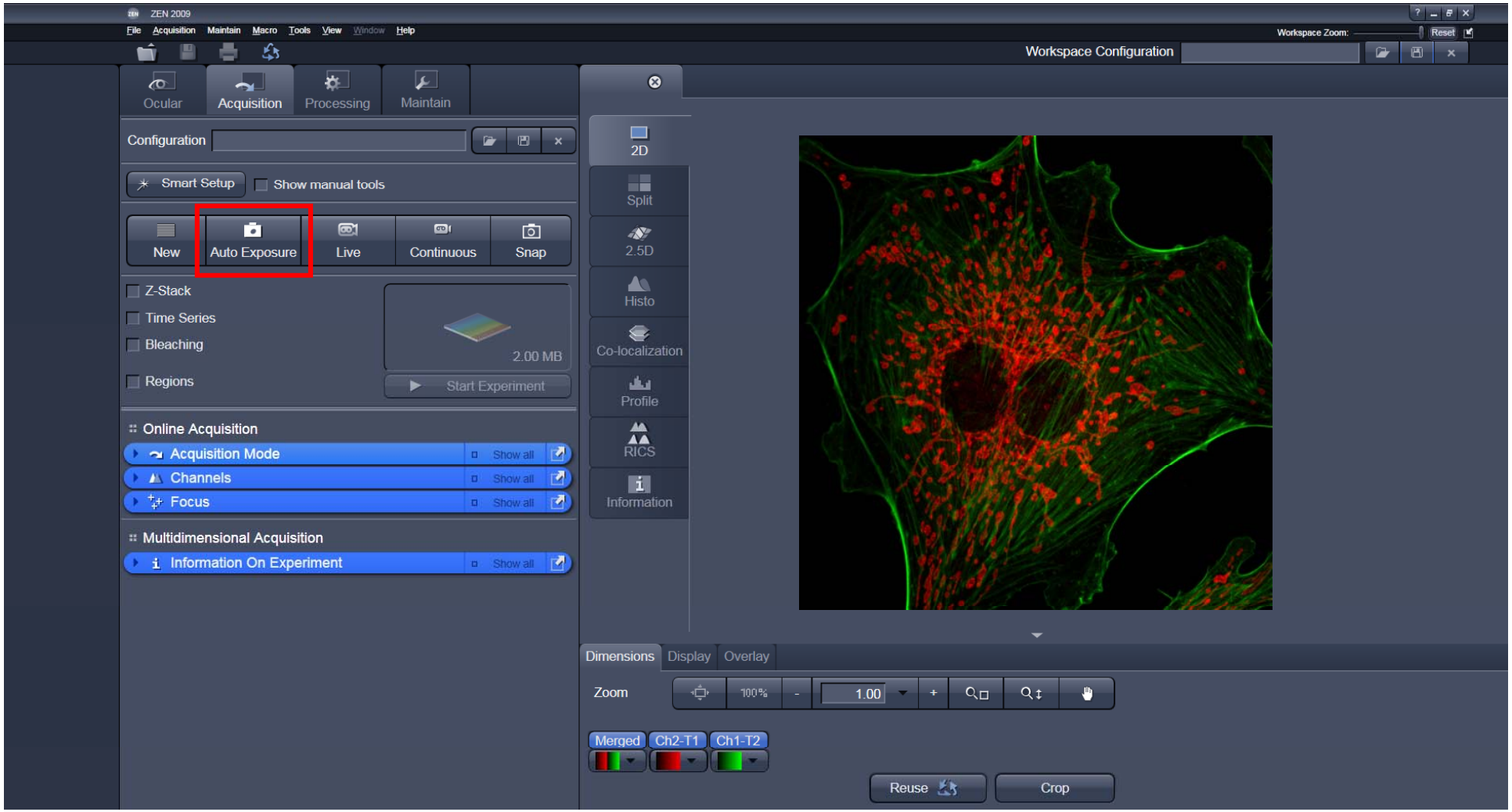
# LSM 700

Intuitive software for the LSM 700: ZEN 2009

# LSM 700 – ZEN 2009
## Smart Setup

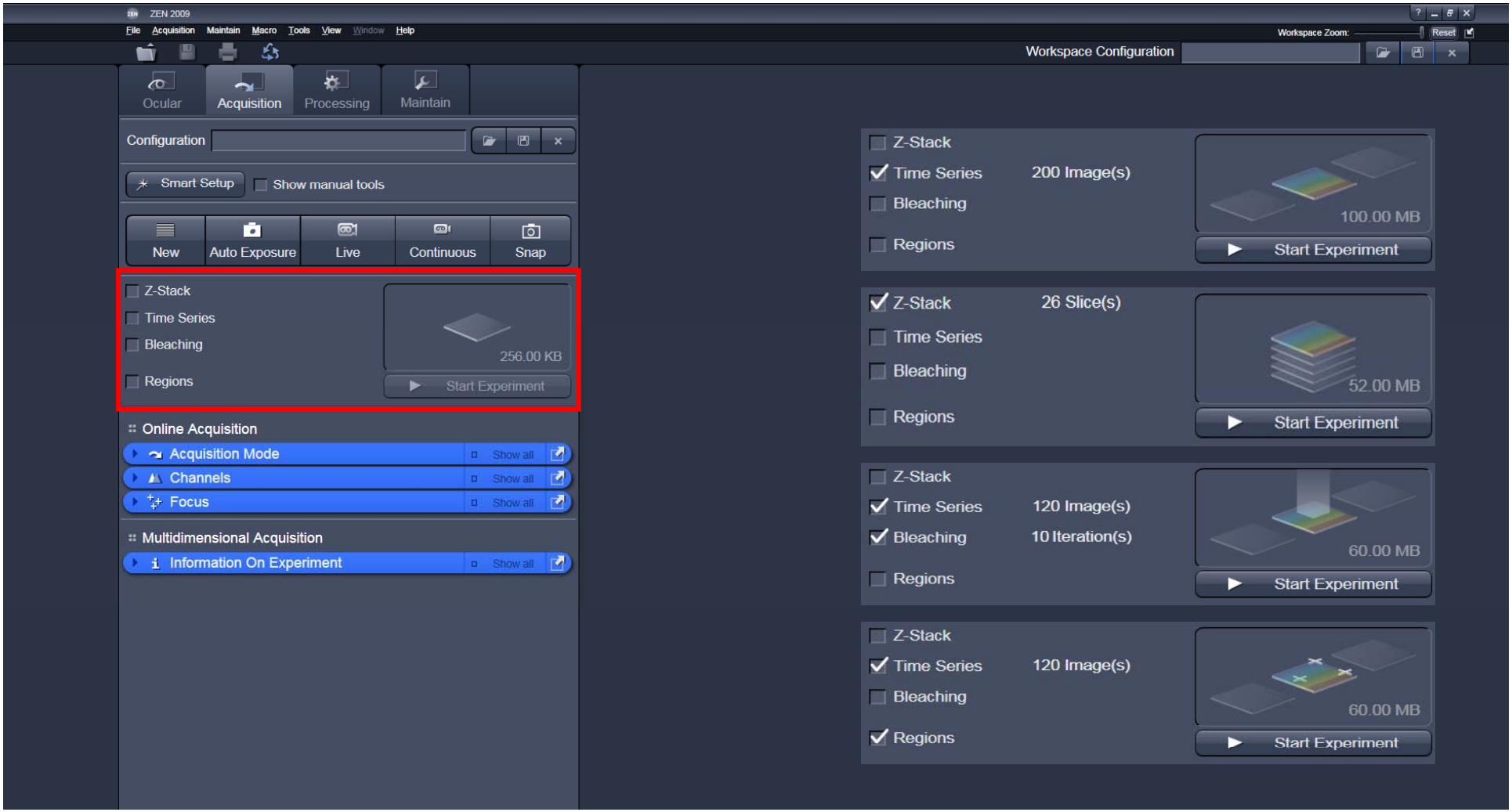


# LSM 700 – ZEN 2009



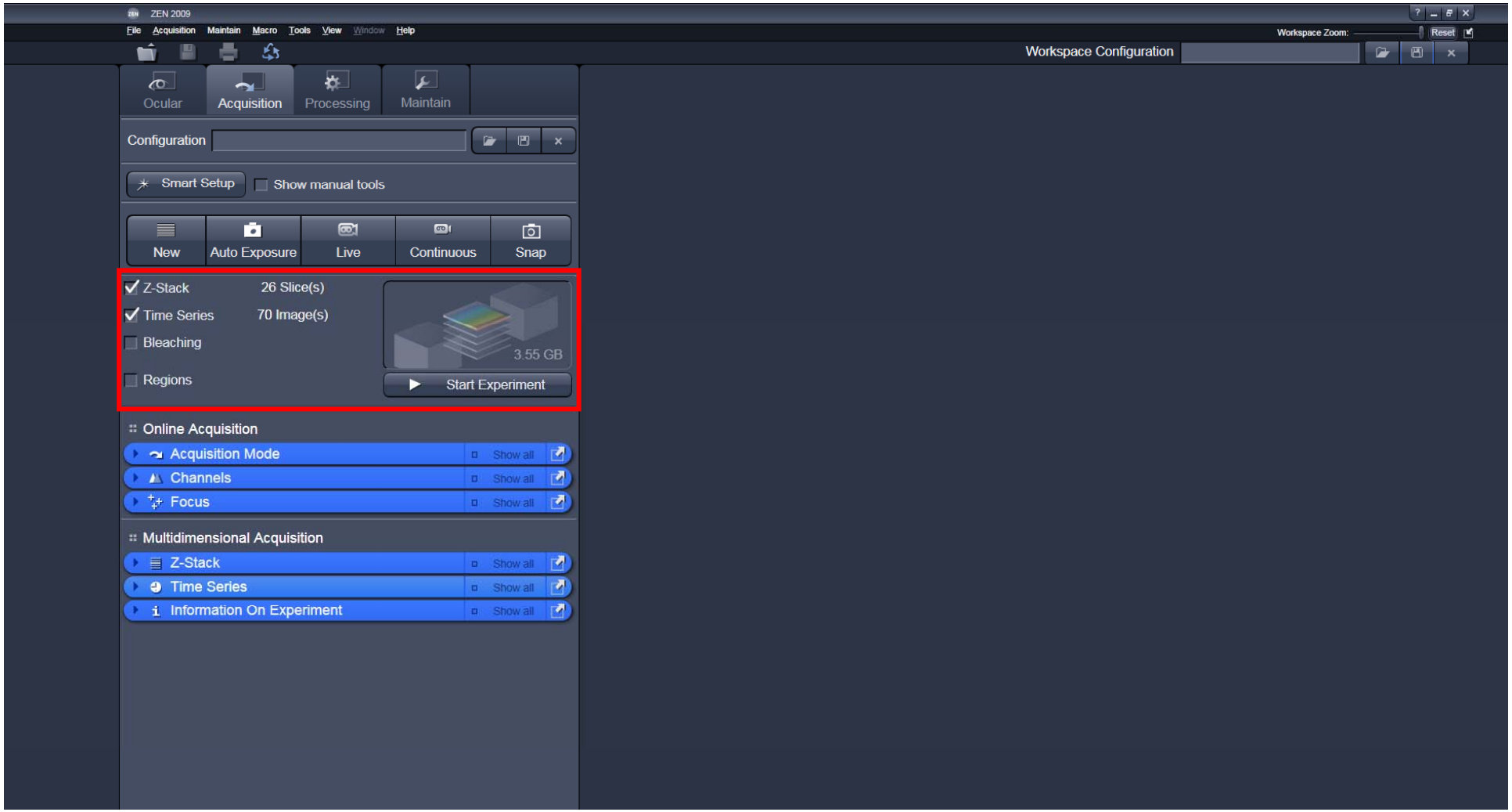
# LSM 700

## ZEN 2009: Multi-dimensional imaging made easy



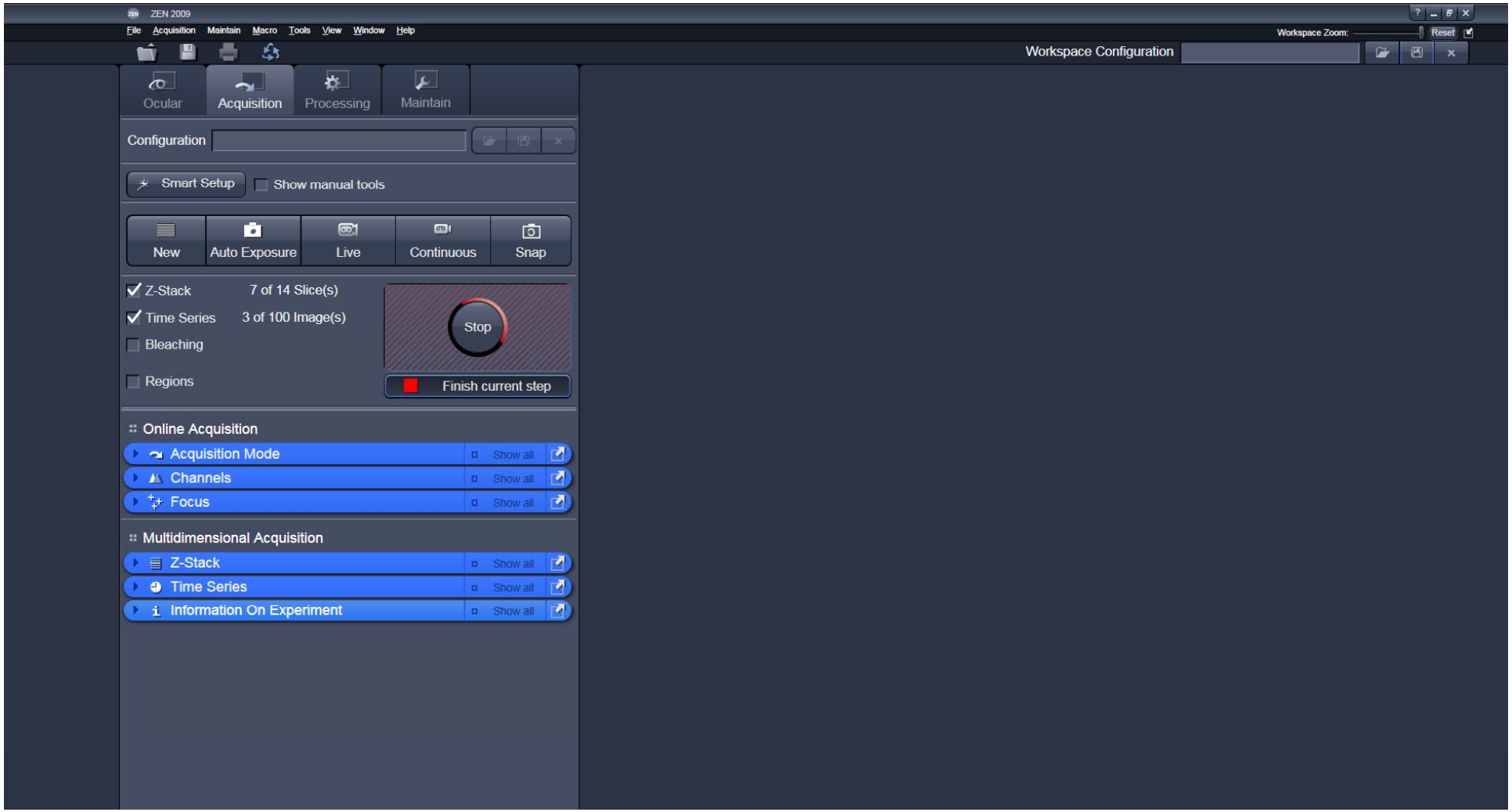
# LSM 700

## ZEN 2009: Multi-dimensional imaging made easy



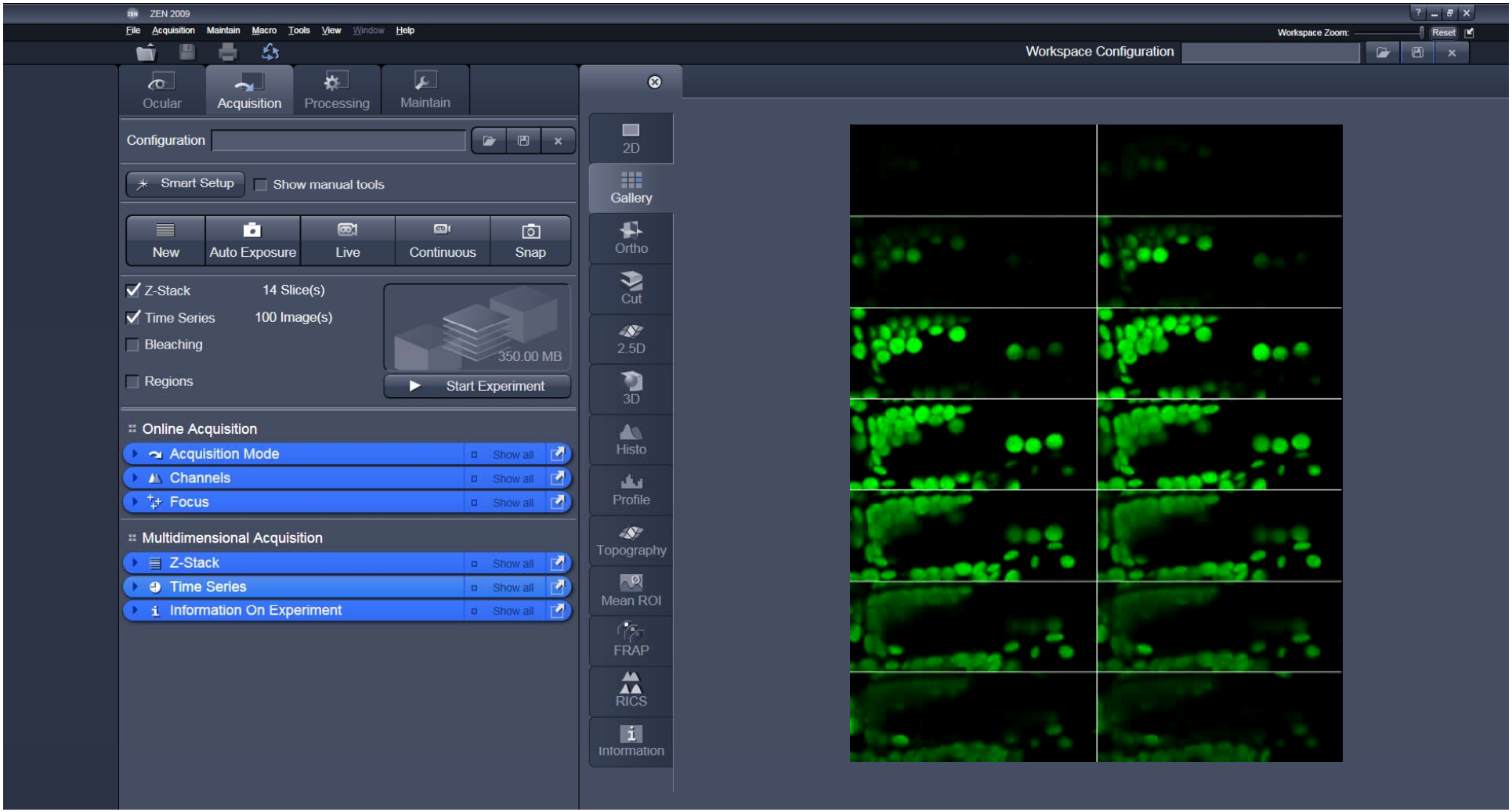
# LSM 700

## ZEN 2009: Multi-dimensional imaging made easy



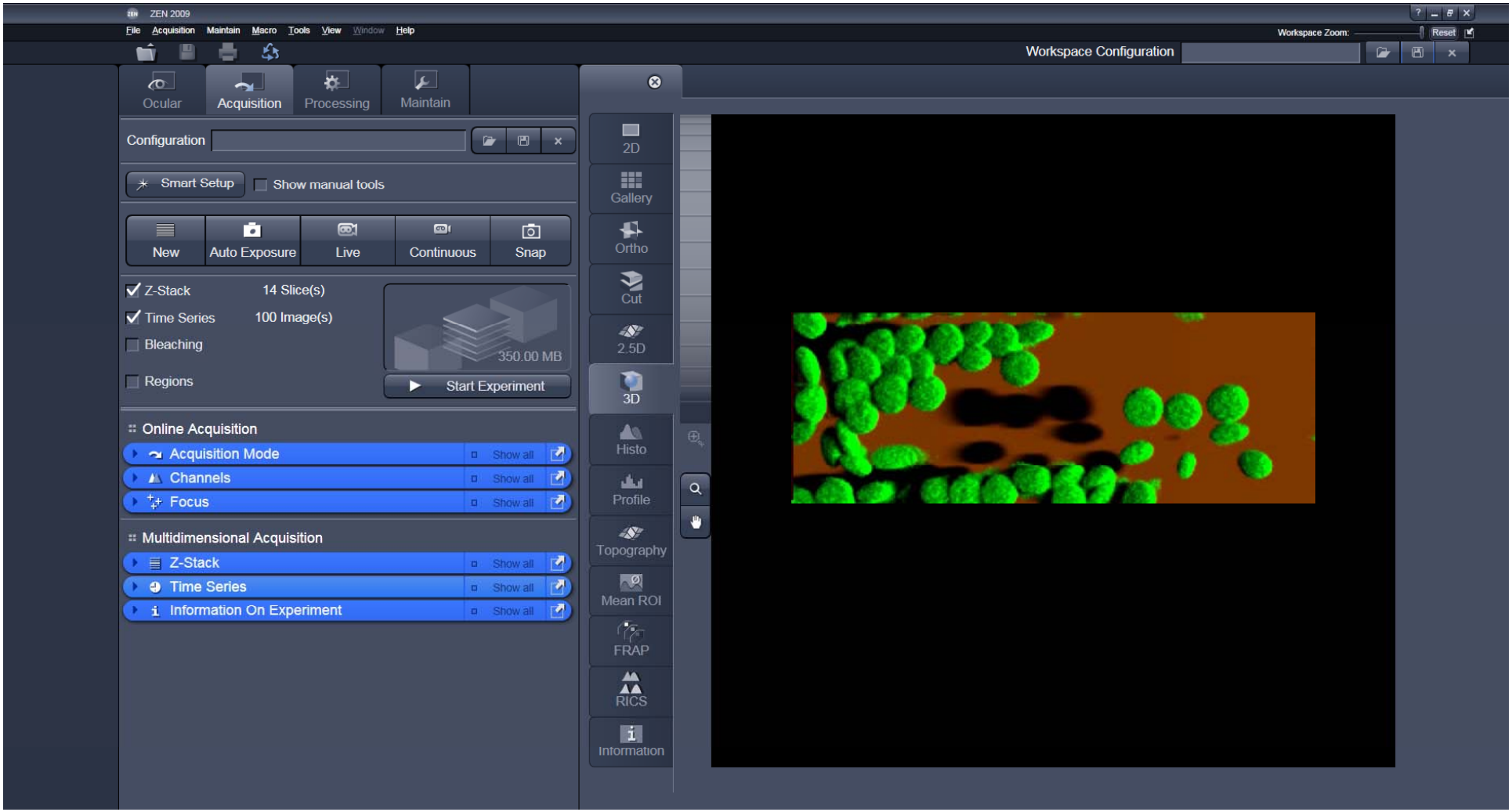
# LSM 700

## ZEN 2009: Multi-dimensional imaging made easy



# LSM 700

## ZEN 2009: Multi-dimensional imaging made easy

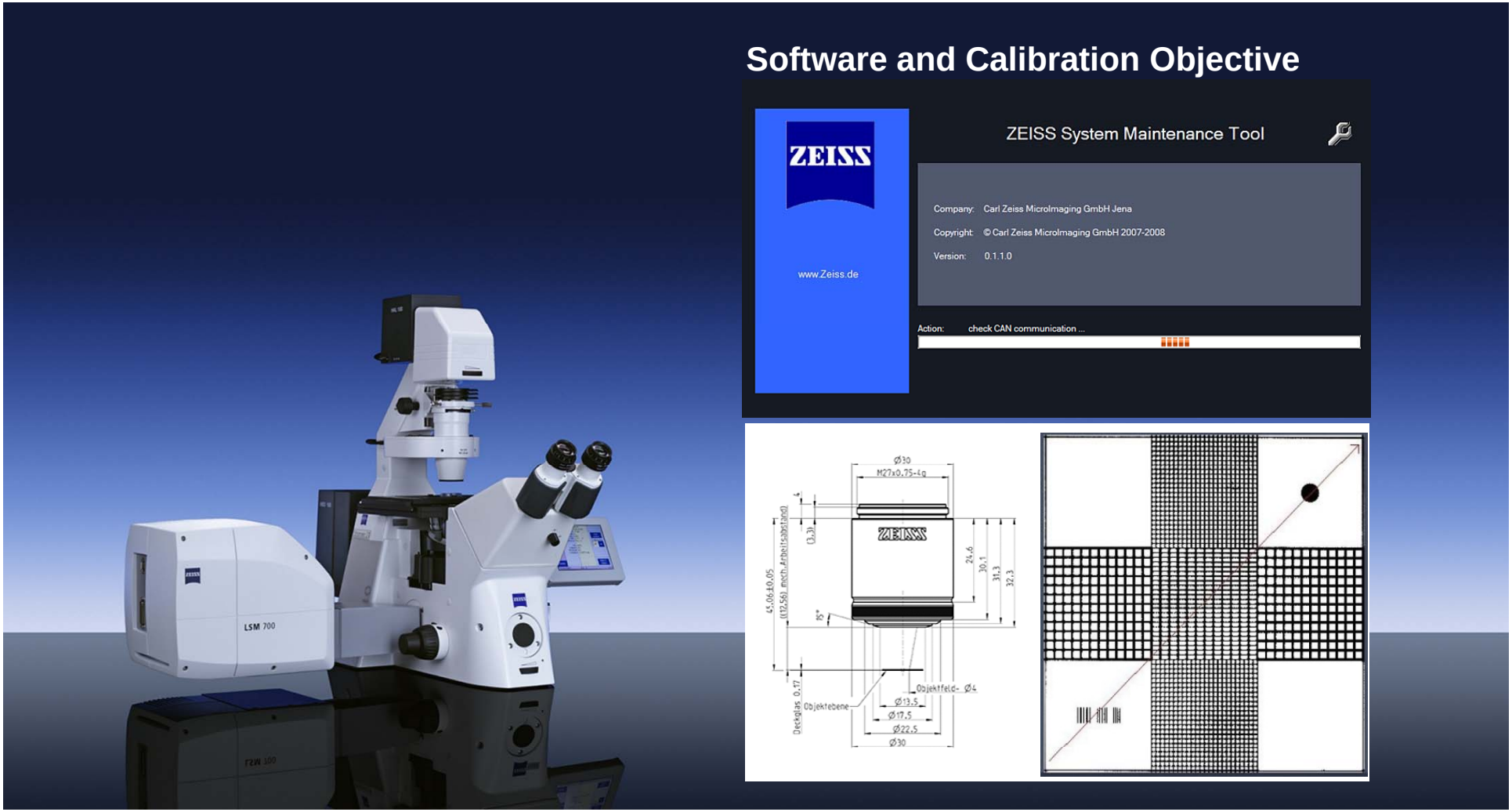


# LSM 700

Affordable high-end for everybody!

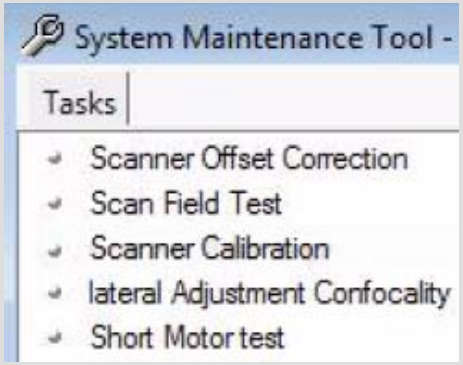
# LSM 700

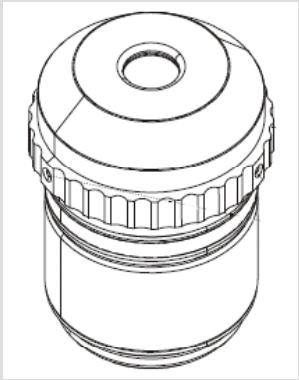
## Concerted self-calibration tools



# LSM 700

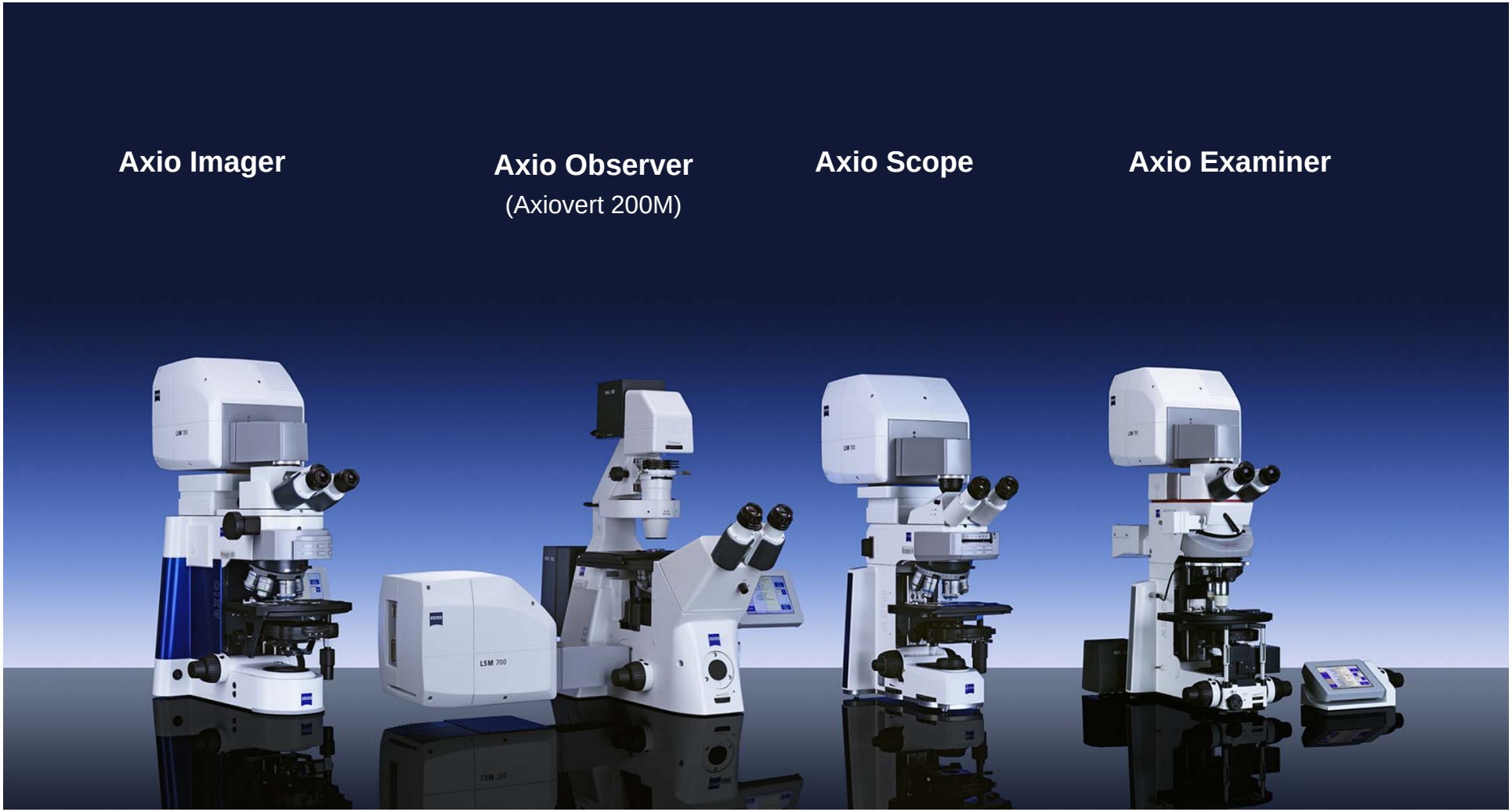
## Concerted self-calibration tools



# LSM 700

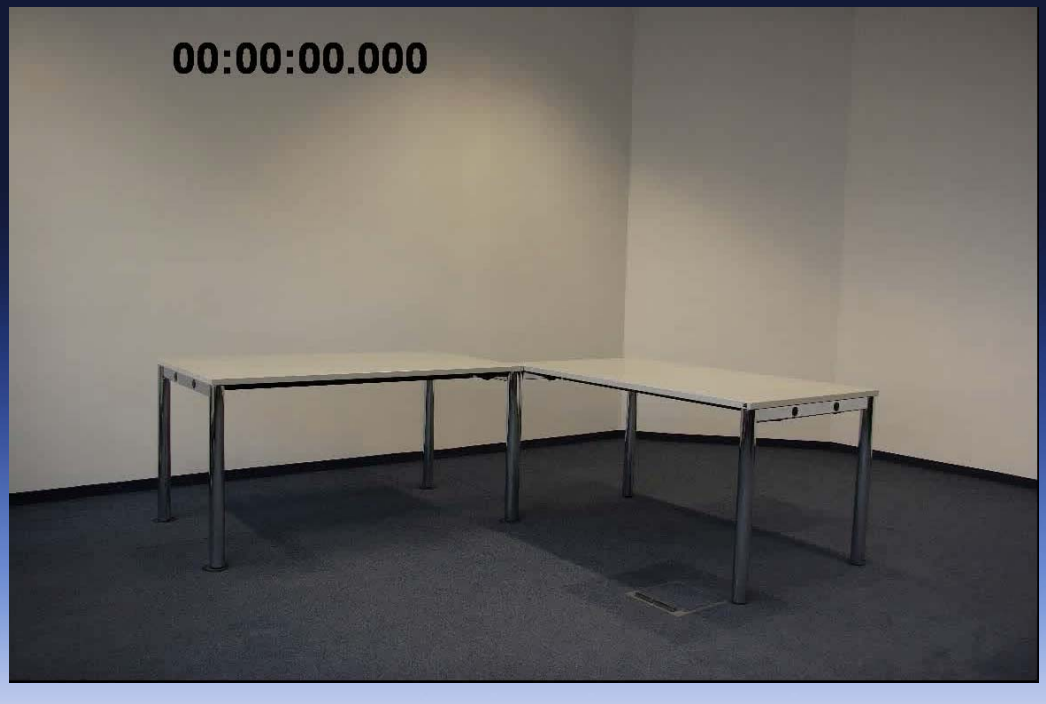
Upright and inverted microscopes for the LSM 700



# LSM 700

Out of the box …

**Ready for imaging in less than one hour!**

# LSM 700

Summary: Everything it does – it does it very well


- Affordable high-end LSM
- Ideal as a dedicated instrument for small workgroups or “personal LSM”
- Features key technologies introduced with the LSM 710 for optimal performance, outstanding sensitivity and superb image quality
- Innovative VSD concept for efficient and flexible signal recording
- Broad range of common applications


We make it visible.