Leica Mica – 全同步螢光澄清影像擷取儀

迎接智慧影像新紀元

2024.10.21
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www.major.com.tw

MICA -- 全同步螢光澄清影像擷取儀

Dimensions of Mica:
(W x D x H)

Without incubator
69cm x 79cm x 140cm + work table

With incubator
69cm x 85cm x 140cm + work table

WHY Mica ?

1	Microhub
2	Anti-vibration table
3	Supply Unit
4	Workstation
5	Monitor
6	Mouse, keyboard, Smart Move
7	Work table (optional)
8	Incubator

Leica

Light Microscopy

光學顯微鏡
Widefield Microscopy

優點：
- 價格相對較低
- 操作相對簡便
- 無需專人管理
- 後續維護成本較低
- 影像擷取速度快
- 光毒害與光漂白低

缺點：
- 影像解析度，對比差
- 3D重組影像品質不佳
- 不適合多維多色螢光使用
- 高階應用受限

MICA

- 高性價比價格
- AI智慧，操作簡便
- 專人管理程度低
- 後續維護成本較低
- 更快的擷取速度

- 良好的影像解析度
- 3D重組影像佳
- 適合多維多色螢光影像
- AI後續影像管理

雷射掃描共軛焦顯微鏡
Confocal Microscopy

缺點：
- 價格高昂
- 學習操作時程長
- 需專人管理
- 後續維護成本極高
- 影像擷取速度慢
- 光毒害與光漂白高

優點：
- 影像解析度，對比高
- 3D重組影像佳
- 適合多維多色螢光影像
- 多種高階影像應用

Meet Mica
The world's first Microhub

Access for all

- 非光學顯微技術經驗者亦可快速使用
- 減少使用者的教育訓練時程

No constraints

- 依據需求，Widefield，Confocal，Live cell all in one
- 分擔共軛焦螢光顯微鏡的使用

Radically simplified workflows

- 智慧化設計減少60%螢光影像處理步驟
- 簡化流程降低影像擷取的出錯率
- 確保影像參數的重複性與再現性

Mica - Experience the future

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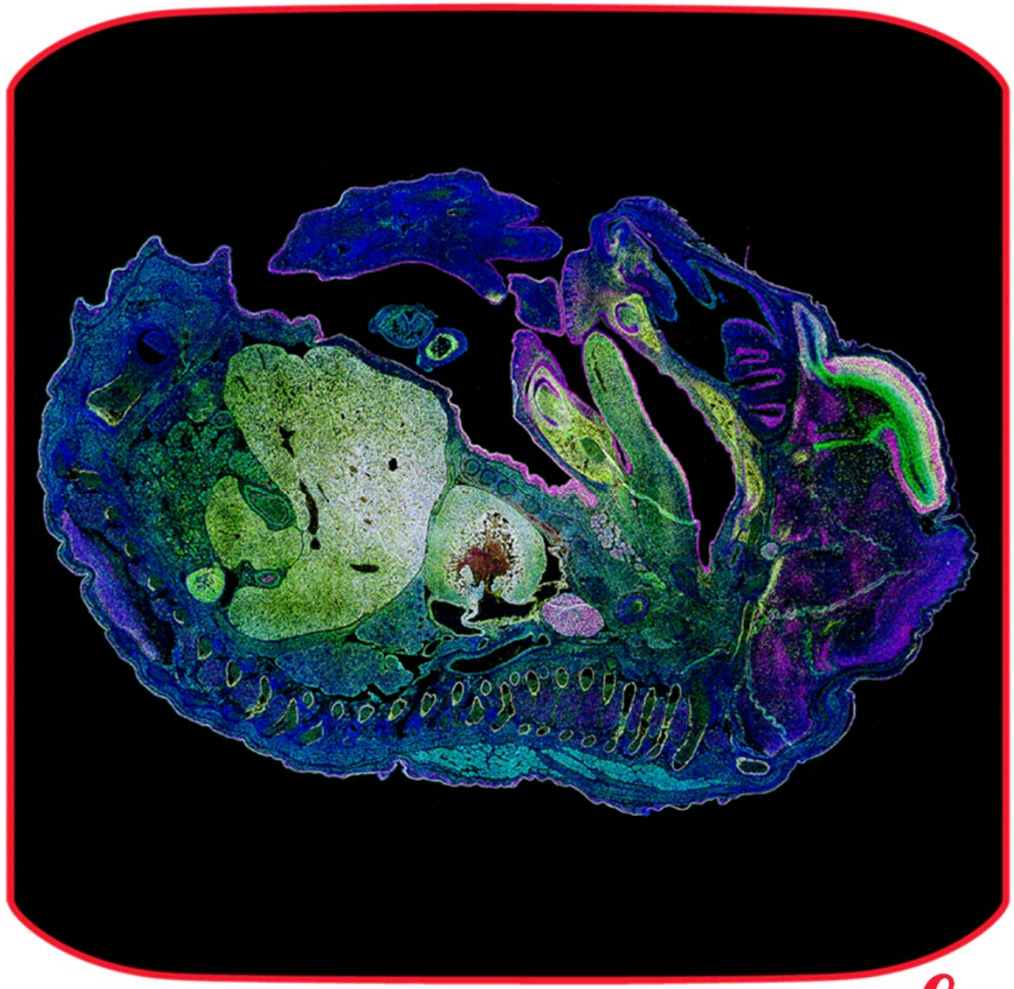
Mica – Access for all

Everyone can now leverage microscopy to make more discoveries

- **85% fewer steps** to the first image
- **33% less time** to the first image
- **50%** of the **training time**



Mouse embryo (E15.5) cryosection captured with the ***PL APO 20x/0.75 CS2*** objective. Section shows Tbr2 cells labeled with CF488A, Satb2 cells labeled with CF555 and Ctip2 cells with CF633 plus nuclei counterstaining with DAPI. Sample and images are courtesy of Giulia Di Muzio at the lab of Dr. Pei-Chi Wei at the DKFZ, Heidelberg, Germany.

The acquisition of two sections took **less than 5 minutes**, while previously it took **2 hours on the lab's comparison device.**

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Reduced number of steps from setup to acquisition to analysis

Conventional Microscopes

18 STEPS

Sample Setup → Mount sample → Observe through ocular → Focus → Set illumination → Set Exposure /Gain → Confirm conditions → Create Overview → Define Acquisition → Define positions → Run acquisiton → Screen dataset → Assess suitability for analysis → Pre-process data to run analysis → Setup analysis → Run Analysis → Extract information → Create figure

VS.

Mica

1. **Mount sample & Define characteristics**
2. **Define positions & Run acquisiton**
3. **Create Analysis & Export Figure**

Powered by: Sample Finder | OneTouch- Auto-Illumination | AI-based Analysis

6 STEPS

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Mica - Experience the future

Faster acquisition Shorter training time Less mistake

1. Sample loading

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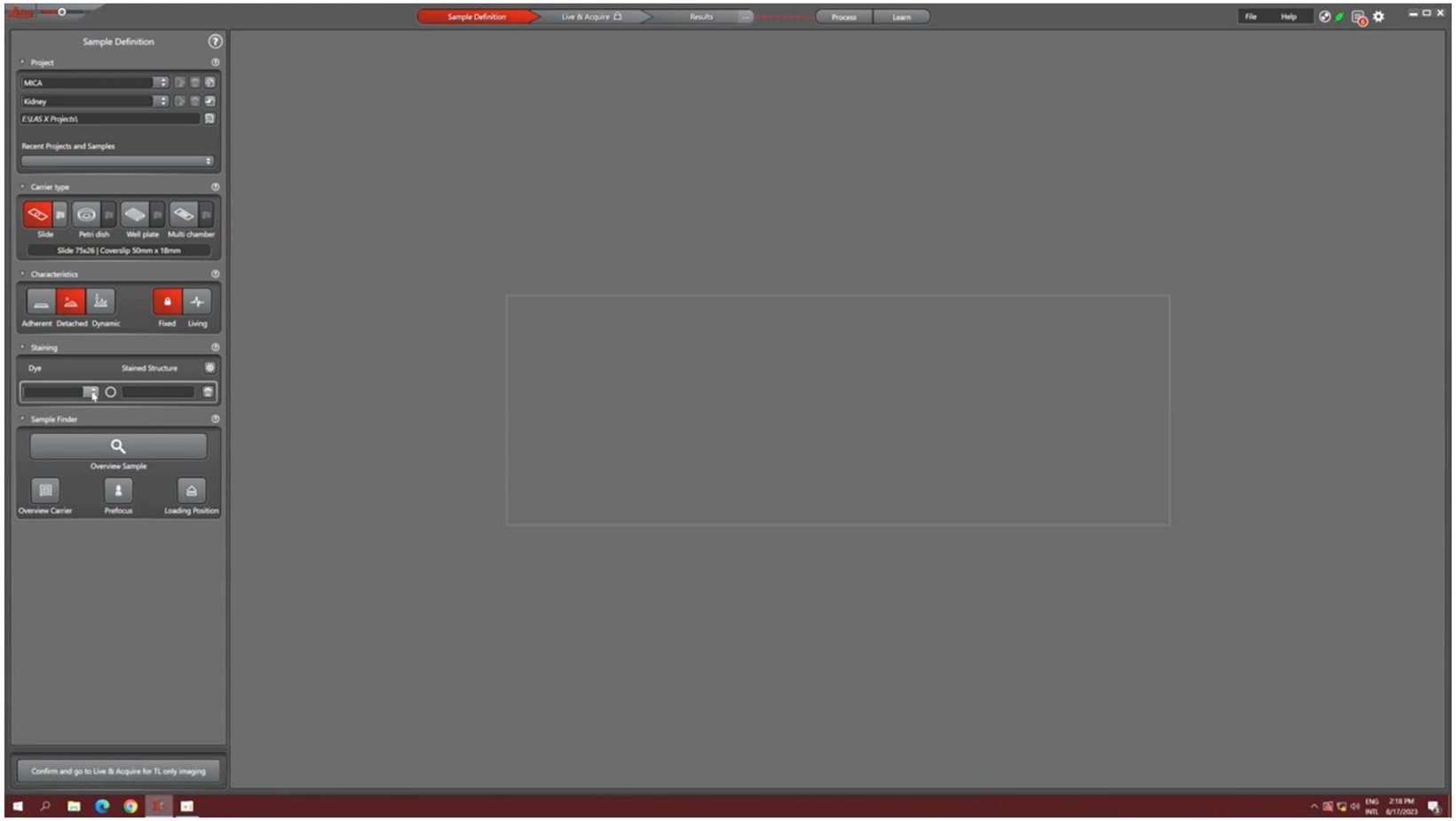
Mica - Experience the future

Faster acquisition Shorter training time Less mistake

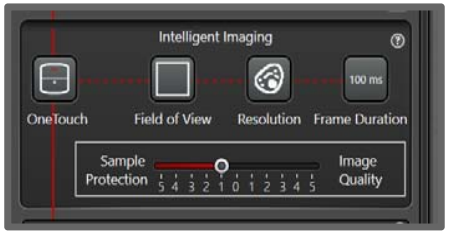
2. Auto Sample finder (by built-in HC PL FL 1.6x objective)



Mica - Experience the future

Faster acquisition Shorter training time Less mistake

3. Screening (by built-in HC PL FL 10x objective)
4. Acquisition: 2D, 3D, 4D… (by optional HC PL APO 20x, APO 63x W objective)



Objectives for Mica

- **PL FLUOTAR 1.6x/0.05** (built-in, for sample finder, IMC trans. Image)
- **PL FLUOTAR 10x/0.32** (built-in, for FL screening, acquisition)
- **PL APO 20x/0.75 CS2** (option, for FL acquisition, WF or confocal)
- * **PL APO 63x/1.2 W CS2** (option, for FL acquisition, WF or confocal)
  UVIS Smart CORR, Intelligent auto-immersion

  ** PL APO 40x/1.1 W CS2 mot. CORR, Intelligent auto-immersion*
  PL APO 40x/1.30 oil CS2
  PL APO 63x/1.40 oil CS2

Mica – No constraints

Intelligent auto water immersion

Smart mot. CORR

Mica - No constraints

Environmental control

Mica Widefield

Fully automated
Simultaneous Multichannel
FluoSync 4 ch Widefield
Built-in objectives 1.6x, 10x, optional 20x, 40x or 63x

Mica Widefocal (Widefield + Confocal module)

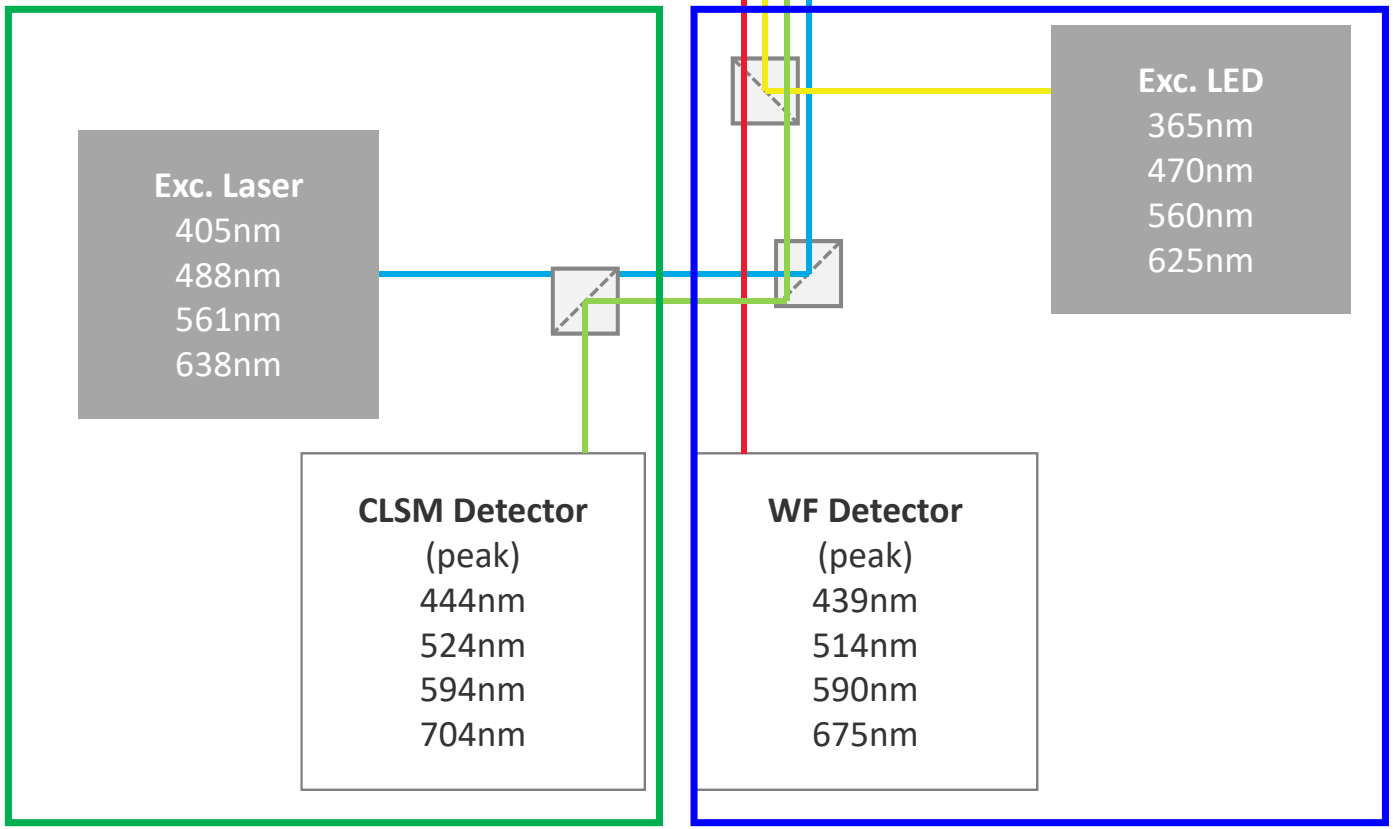
Fully automated
Simultaneous Multichannel
FluoSync 4 ch Widefield + 4 ch Confocal
Built-in objectives 1.6x, 10x, optional 20x, 40x or 63x

Mica allows simultaneous 4 color fluorescence assay!

•**Point scan confocal** :
4 lasers & 4 HyD FS detectors

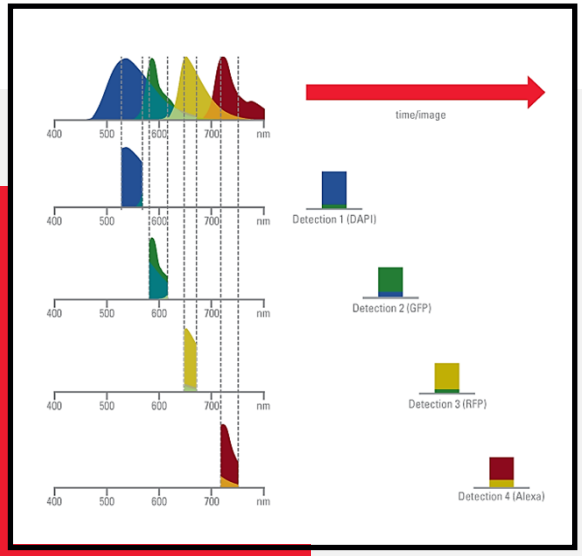
Sample

•**Widefield** :
4 LEDs & 4 CMOS detectors



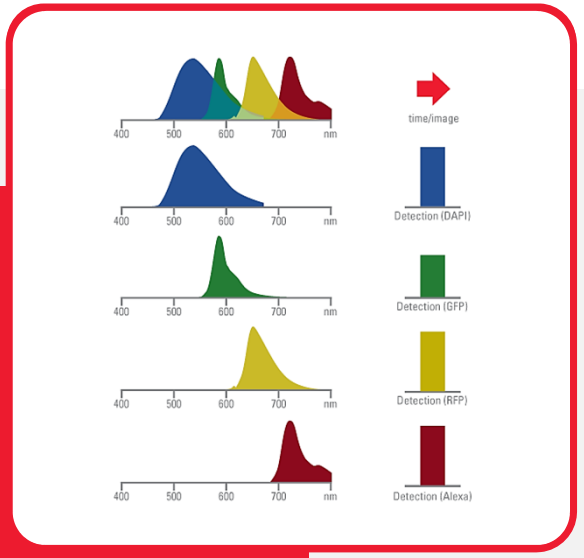
FluoSync -- simultaneous 4 Label Imaging

Simultaneous 4 Label Imaging, Broad Spectrum Detection and Hybrid Unmixing



Conventional 4 color fluorescence imaging

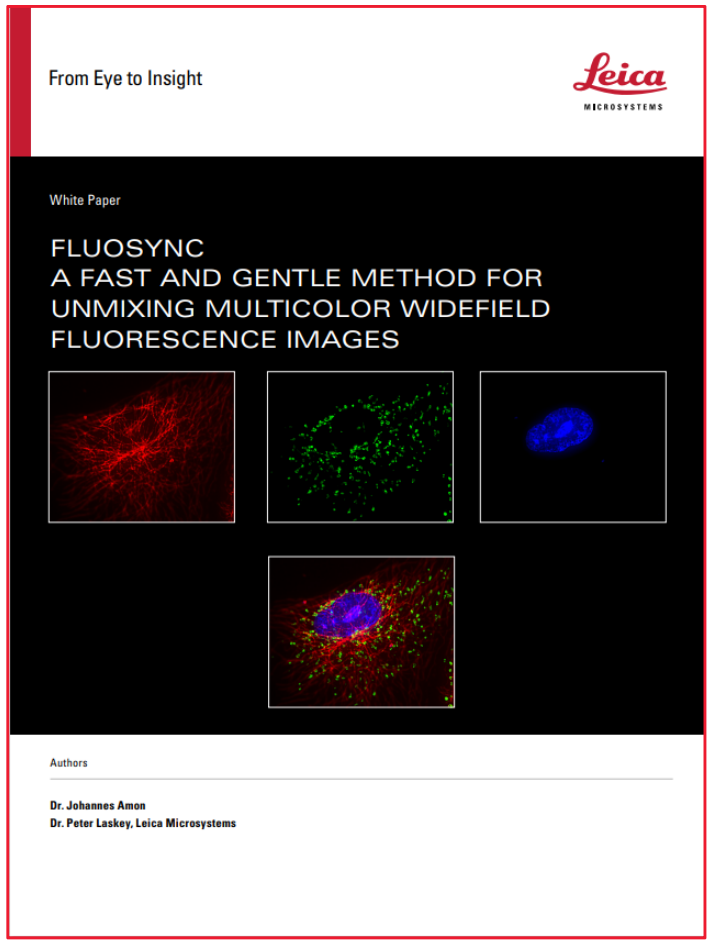
- Poor dye separation results in low localization accuracy
- Cut away signals to reduce cross talk
- Slow sequential imaging



FluoSync – Simultaneous true 4 label imaging

- Broad spectrum detection
- True dye separation
- 4 times faster imaging simultaneously

Mica FluoSync Hybrid Unmixing


From Eye to Insight

Leica MICROSYSTEMS

White Paper

FLUOSYNC
A FAST AND GENTLE METHOD FOR UNMIXING MULTICOLOR WIDEFIELD FLUORESCENCE IMAGES

Authors

Dr. Johannes Amon
Dr. Peter Laskey, Leica Microsystems

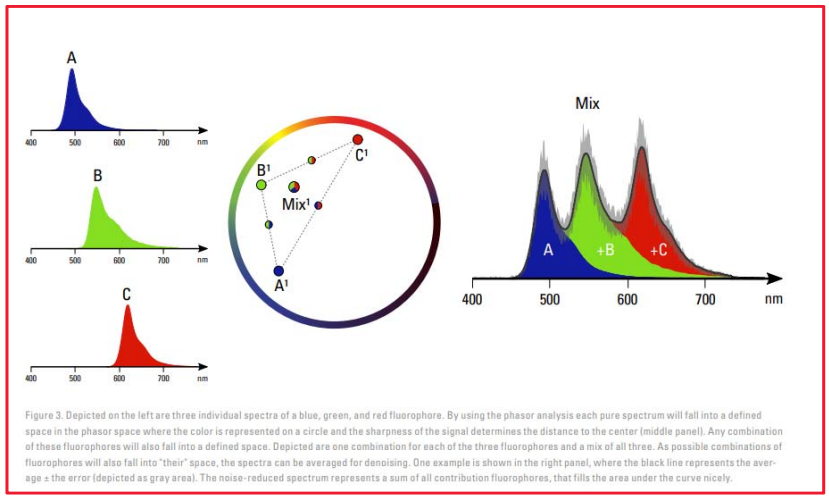
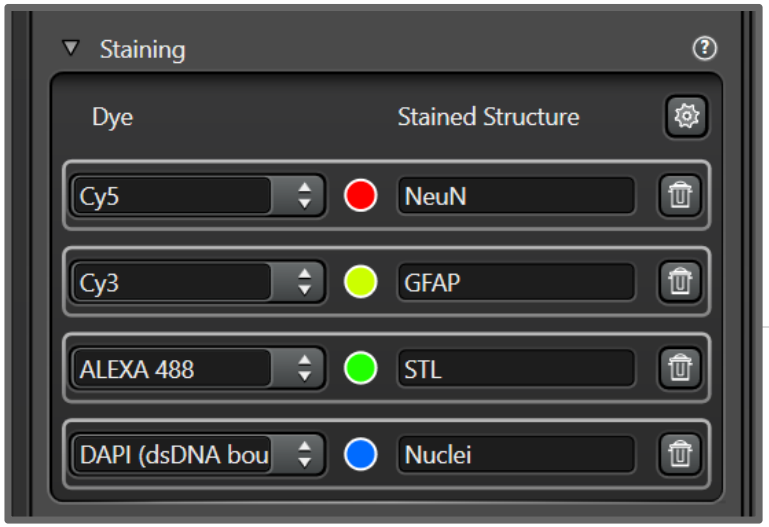
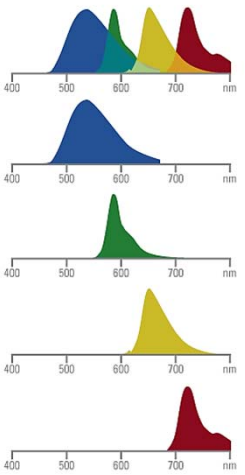


Figure 3. Depicted on the left are three individual spectra of a blue, green, and red fluorophore. By using the phasor analysis each pure spectrum will fall into a defined space in the phasor space where the color is represented on a circle and the sharpness of the signal determines the distance to the center (middle panel). Any combination of these fluorophores will also fall into a defined space. Depicted are one combination for each of the three fluorophores and a mix of all three. As possible combinations of fluorophores will also fall into "their" space, the spectra can be averaged for denoising. One example is shown in the right panel, where the black line represents the average ± the error (depicted as gray area). The noise-reduced spectrum represents a sum of all contribution fluorophores, that fills the area under the curve nicely.

References: Digman MA, Caiolfa VR, Zamai M, Gratton E. The phasor approach to fluorescence lifetime imaging analysis. Biophys J. 2008 Jan 15;94(2): L14-6.
F. Fereidouni, A. N. Bader, H. C. Gerritsen, Opt. Express 2012, 20, 12729.
Francesco Cutrale, Vikas Trivedi, Le A Trinh, Chi-Li Chiu, John M Choi, Marcela S Artiga & Scott E Fraser. Nature Methods 14, 149–152 (2017).





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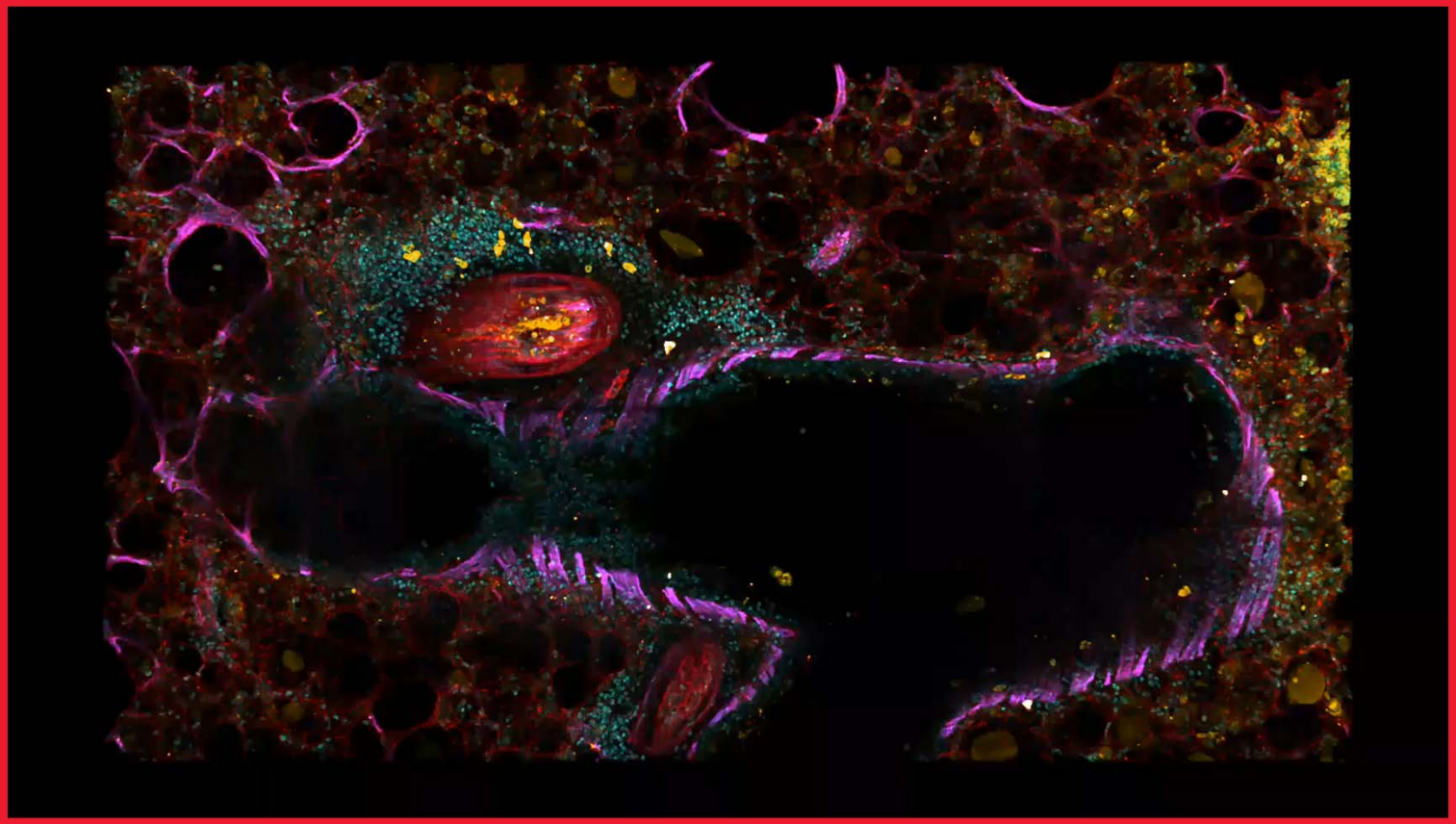
3D Large Volume Tissue Imaging

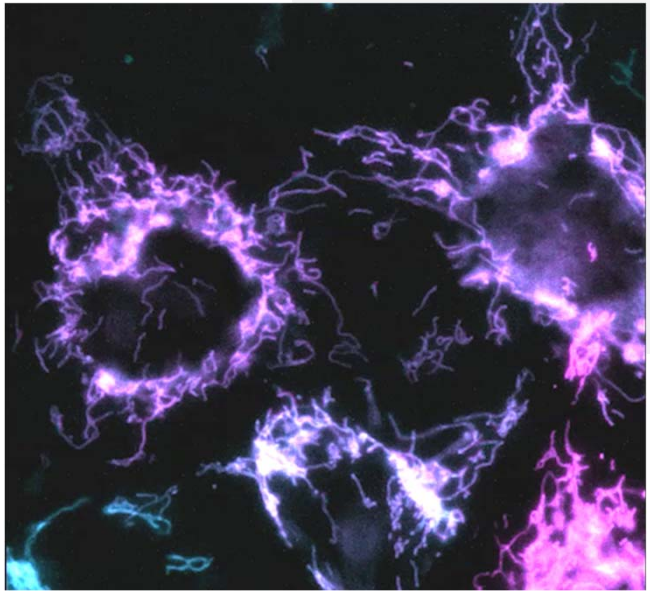


The adult mouse lung tissue was cleared with BABB. The nuclei are labeled with DAPI (cyan), the smooth muscle (Acta2) with Alexa 488 (magenta), the b-cells (b220, yellow), and endothelial cells (cd31, red). Sample courtesy of Adam Andruska, Stanford University (USA)

No Constraints

Absolute spatiotemporal correlation

Conventional Microscope
Sequential Acquisition



Mica
Simultaneous Acquisition

Mica delivers absolute correlated labels without spatiotemporal mismatch

U2OS cells stained with MitoTracker green (mitochondria structure, cyan) and TMRE (active mitochondria, magenta). **Sequential acquisition** of the two channels over 2 minutes 100 frames using the 63x/1.20 CS2 Water MotCORR objective.

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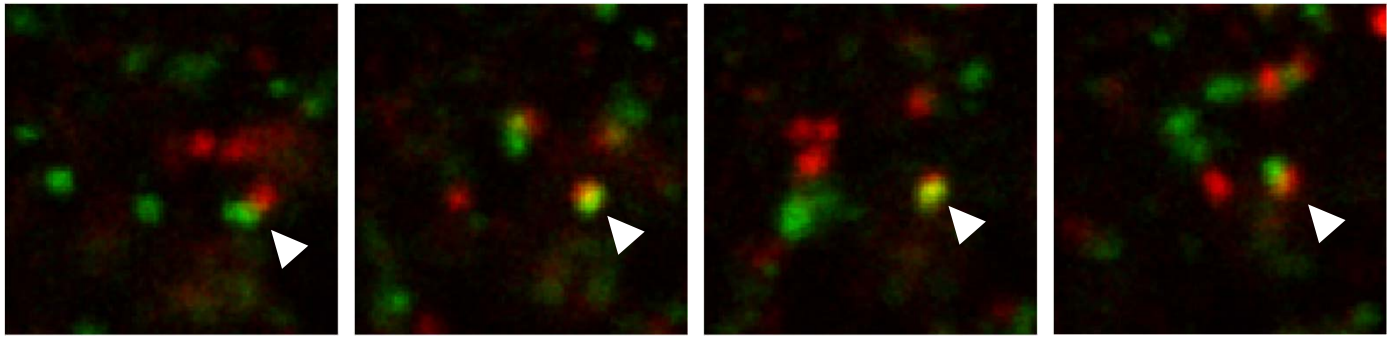
Mica FluoSync delivers absolute correlated labels without spatiotemporal mismatch

…wouldn´t it be great to follow stained structures (e.g., XFPs) in living cells SIMULTANEOUSLY

Conventional Microscope

Sequential Acquisition

CLSM Sequential

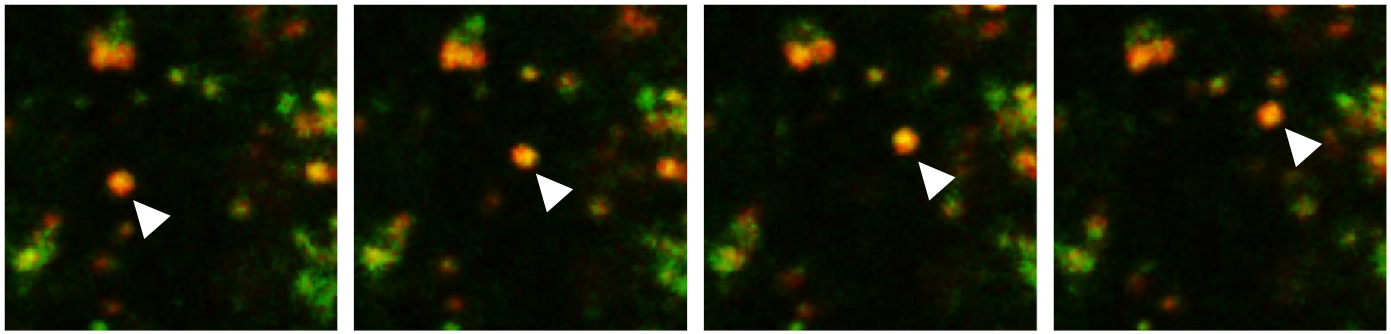


Separate transport vesicles?

Mica

Simultaneous Acquisition

CLSM Simultaneous



Reality:

Double labeled vesicle!

Vesicle staining **WGA-A488** + **WGA-A555**

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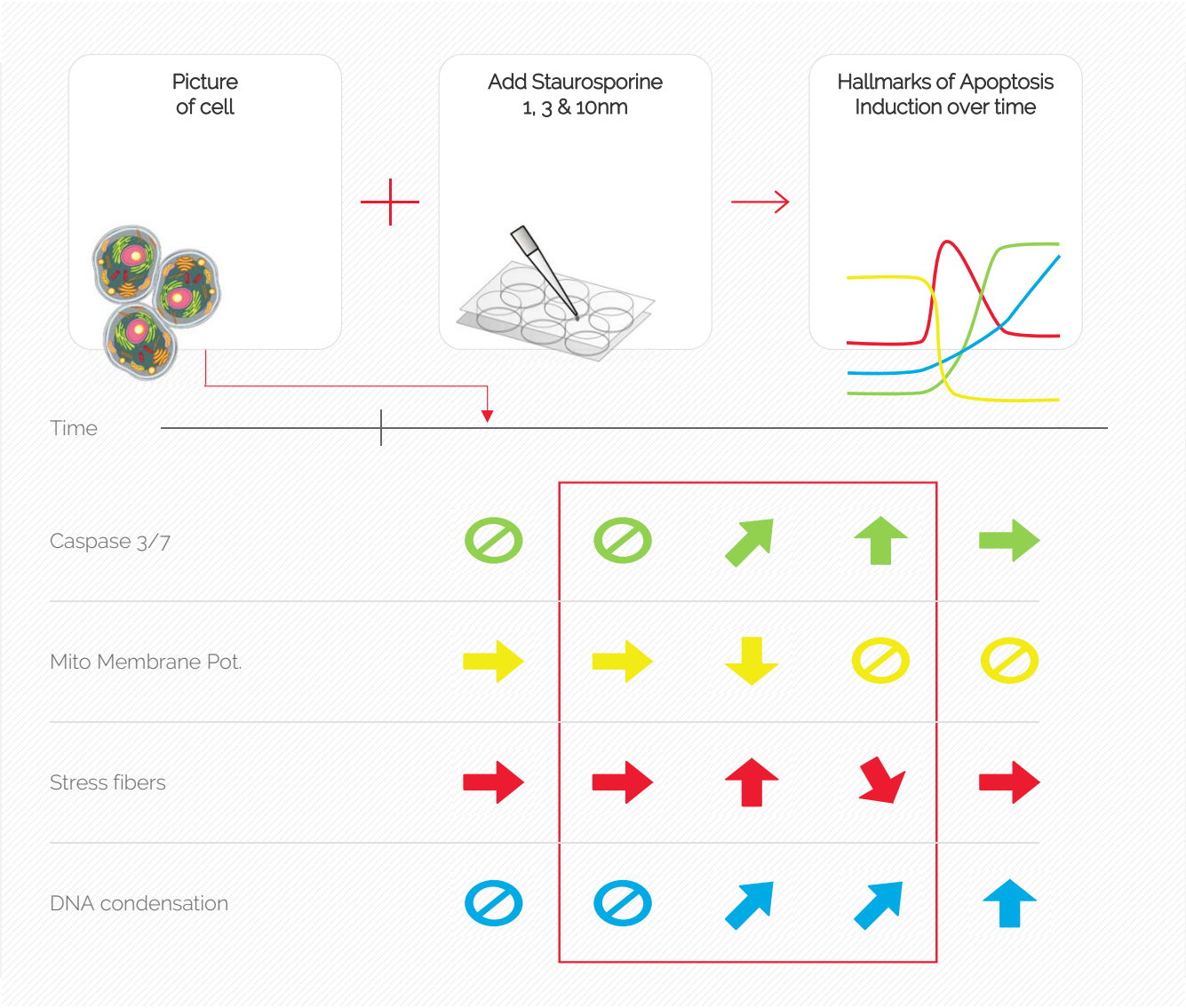
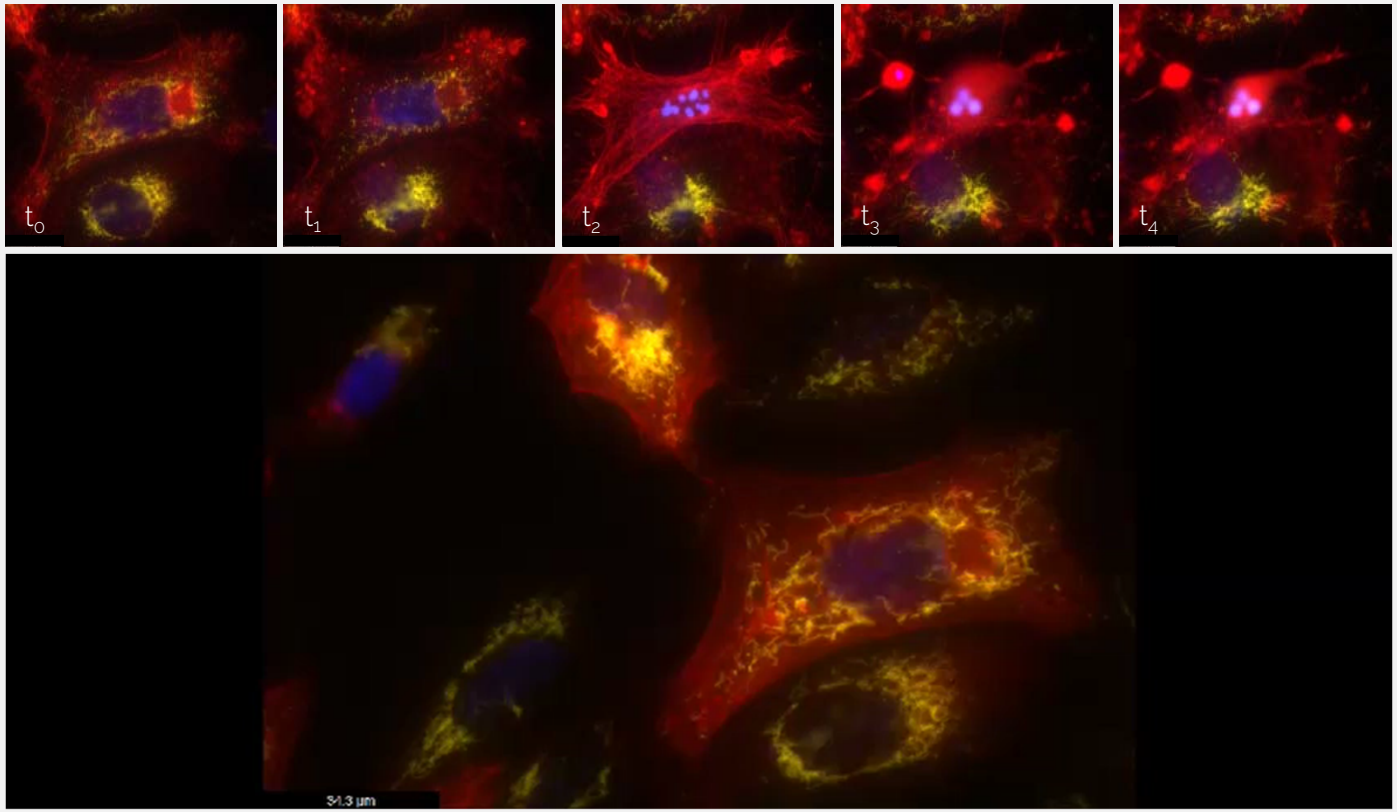
Fluorescence Caspase Assay



Fluorescence Caspase Assay

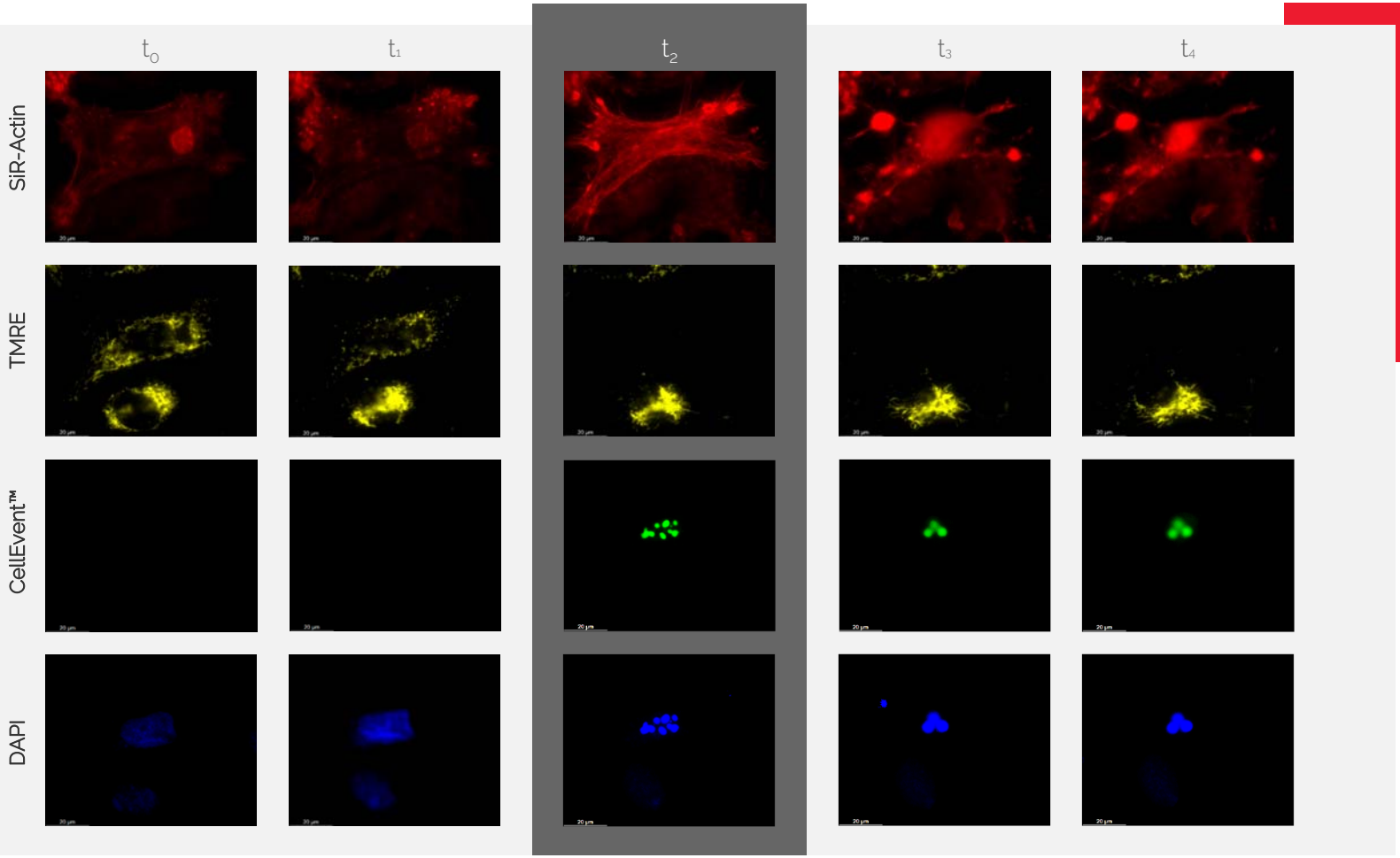


U2OS cells were labelled with SiR-Actin, TMRE (63x magnification, mitochondria activity), CellEvent™ (caspase activity), and DAPI (nuclei). Apoptosis inducer staurosporine was added at time-point 0. widefield mode. 13 hours time-lapse.

Absolute spatiotemporal correlation of 4 markers monitoring the hallmark of early apoptosis induction.

We can observe the formation of stress fibers coinciding with the loss of mitochondrial membrane potential at the beginning of Caspase 3/7 activation. DNA condensation is directly following the caspase activation.

Fluorescence Caspase Assay



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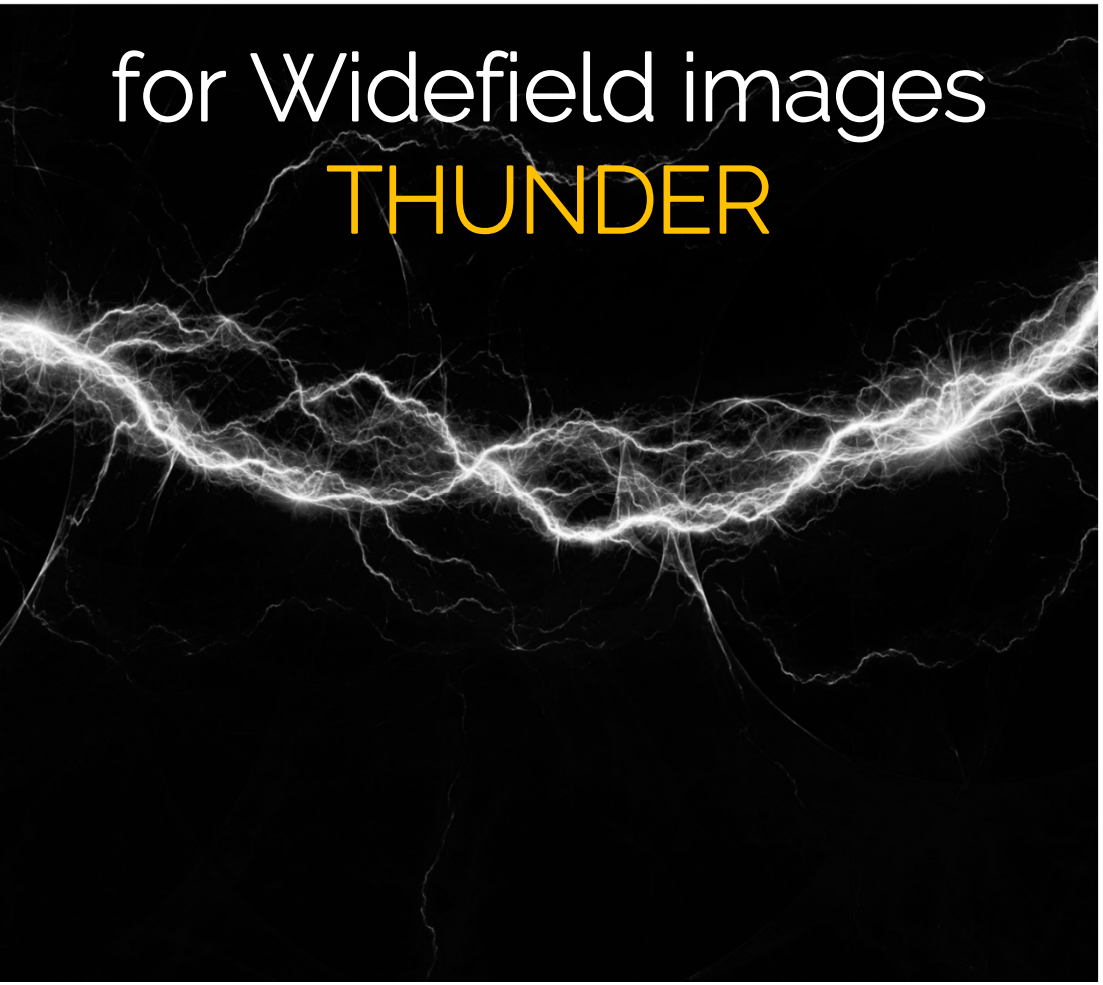
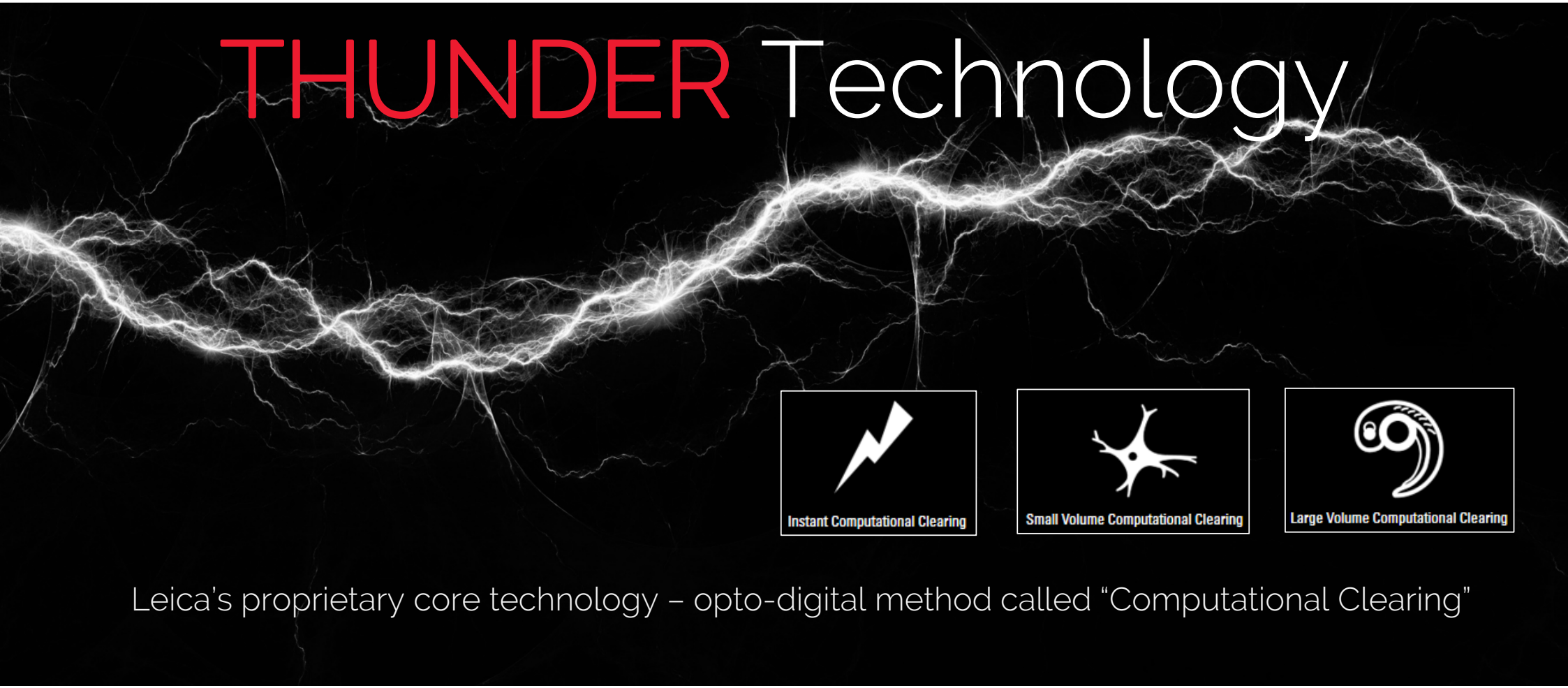
Mica - Opto-digital method “Computational Clearing”



Mica - Experience the future

Faster acquisition Shorter training time Less mistake

5. Intelligent image processing



THUNDER Imager : Computational Clearing Technology

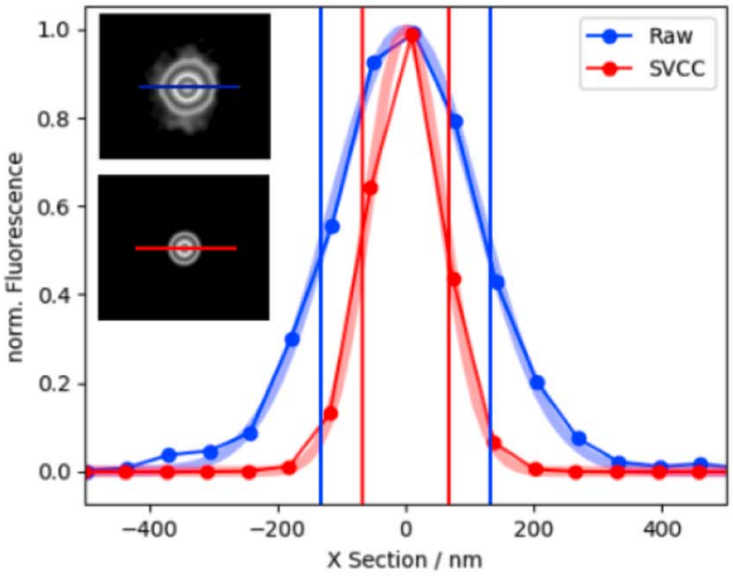
Only background is removed

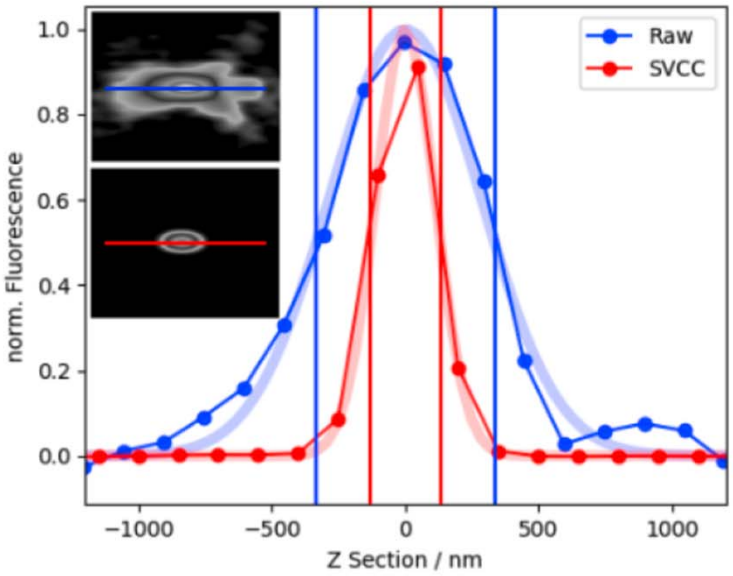
Leica

Resolution improvement with THUNDER

single bead of 40 nm diameter was imaged(100x, 1.4 NA objective)

2 times laterally (ratio FWHMX SVCC/Raw = 0.51)
more than 2.5 times axially (ratio FWHMZ SVCC/Raw = 0.39).

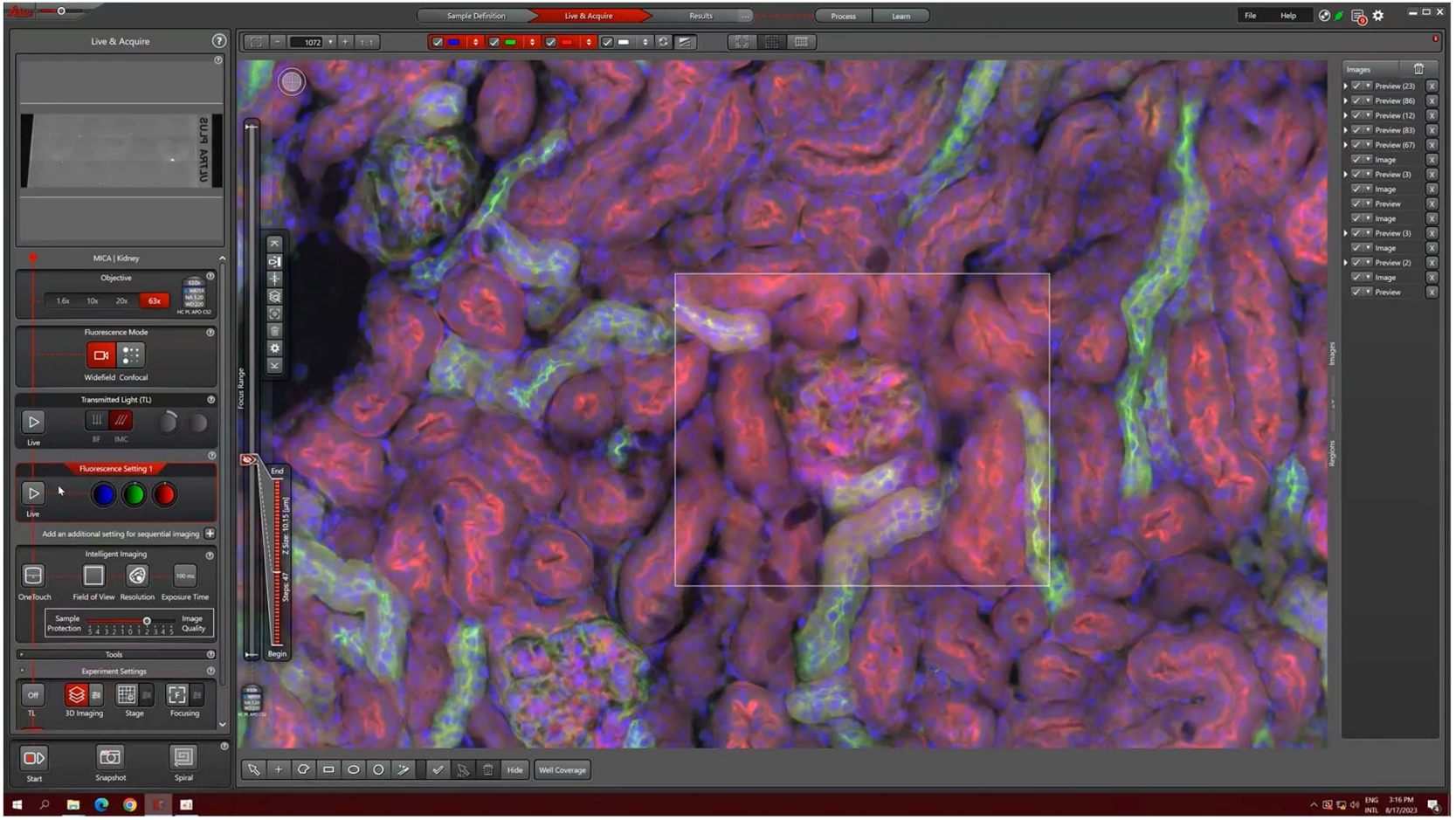




Mica - Experience the future

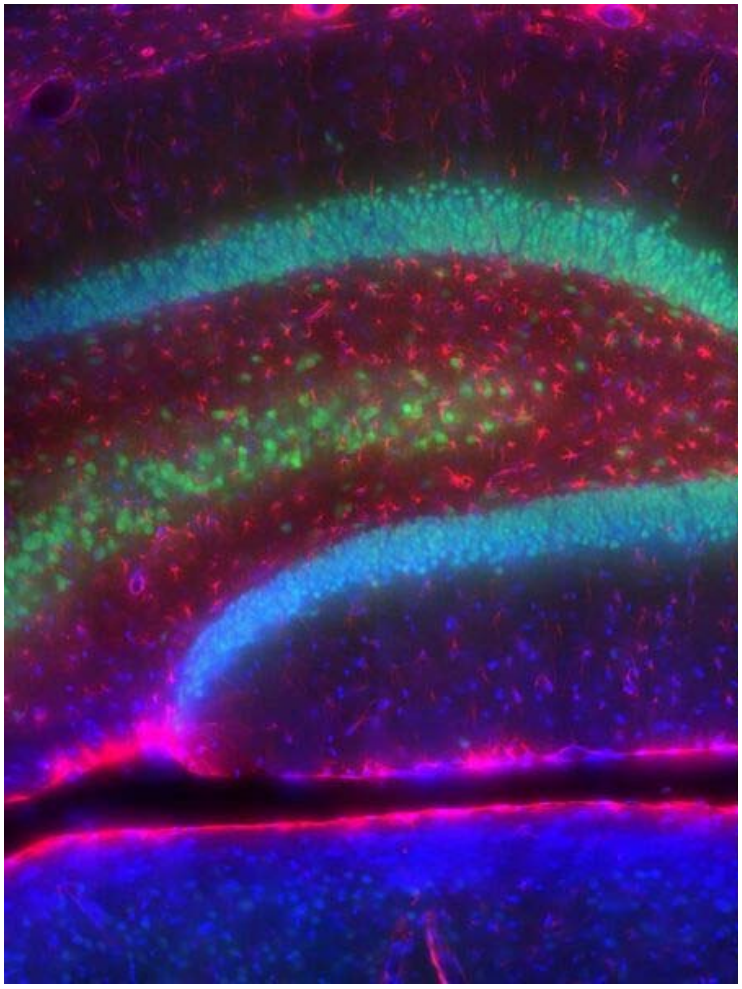
Faster acquisition Shorter training time Less mistake

Thunder image processing

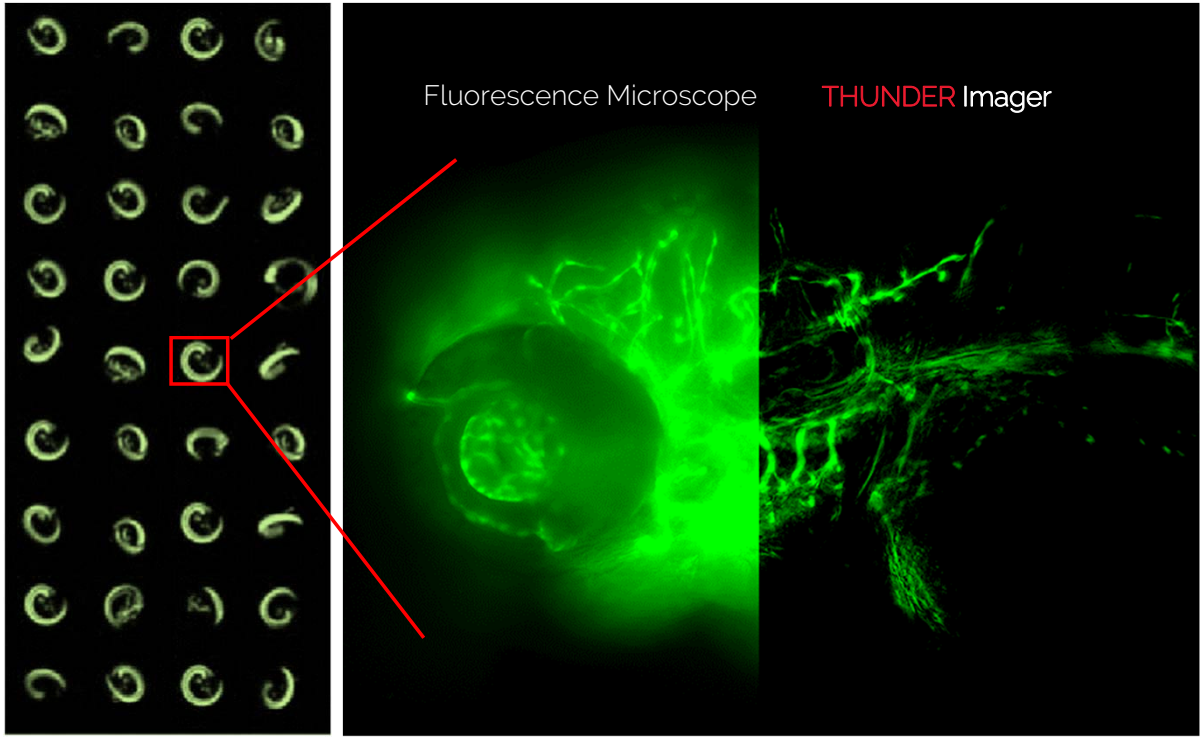


Mica - Experience the future

Faster acquisition Shorter training time Less mistake



Zebrafish



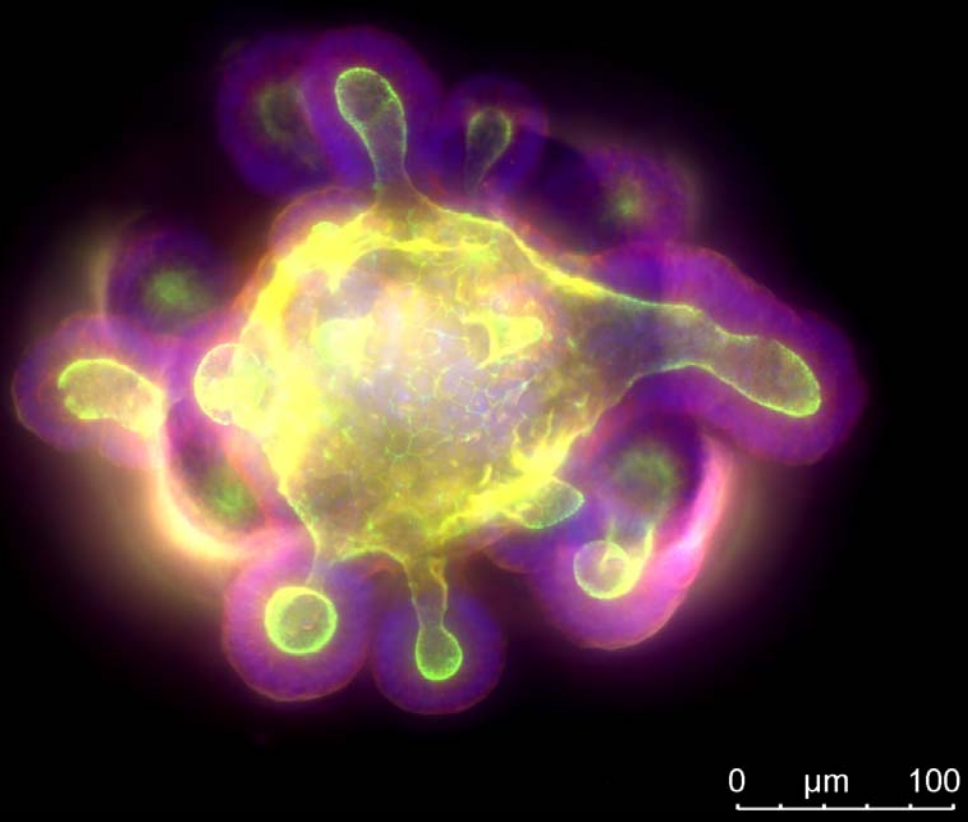
Zebrafish larvae (72 hours post fertilization).
Blood vessels (green)

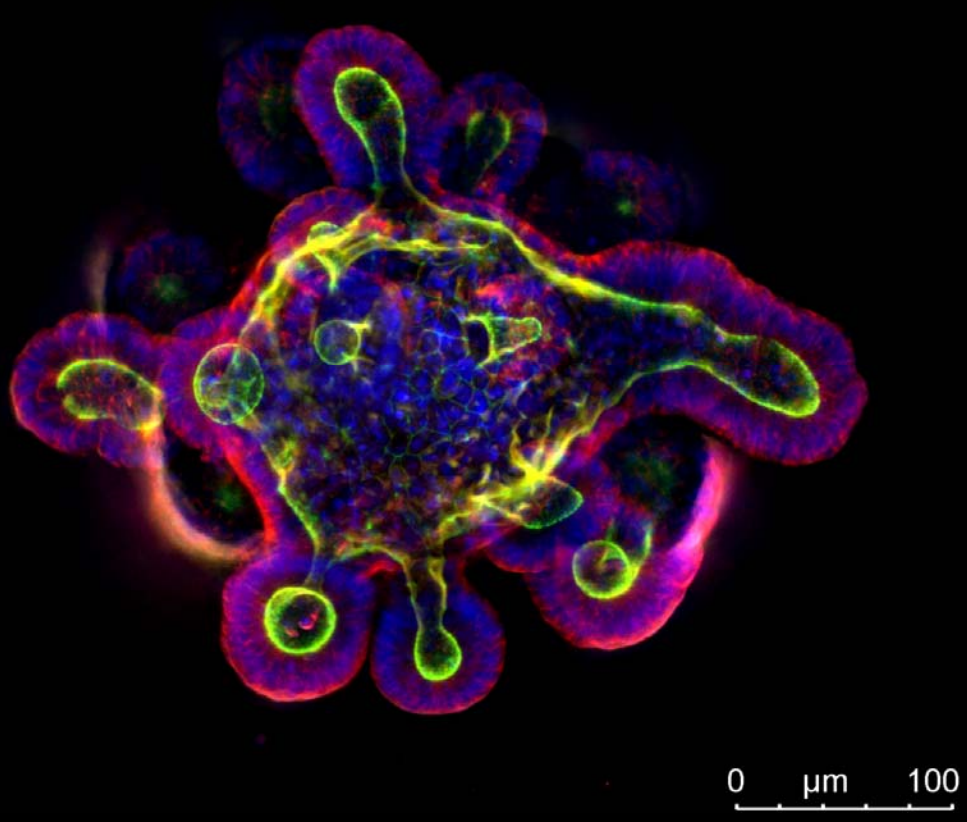
Sample courtesy Dr. Almary Guerra & Dr. Didier Stainier
Max Planck Institute for Heart and Lung Research, Bad Nauheim (Germany)

Leica

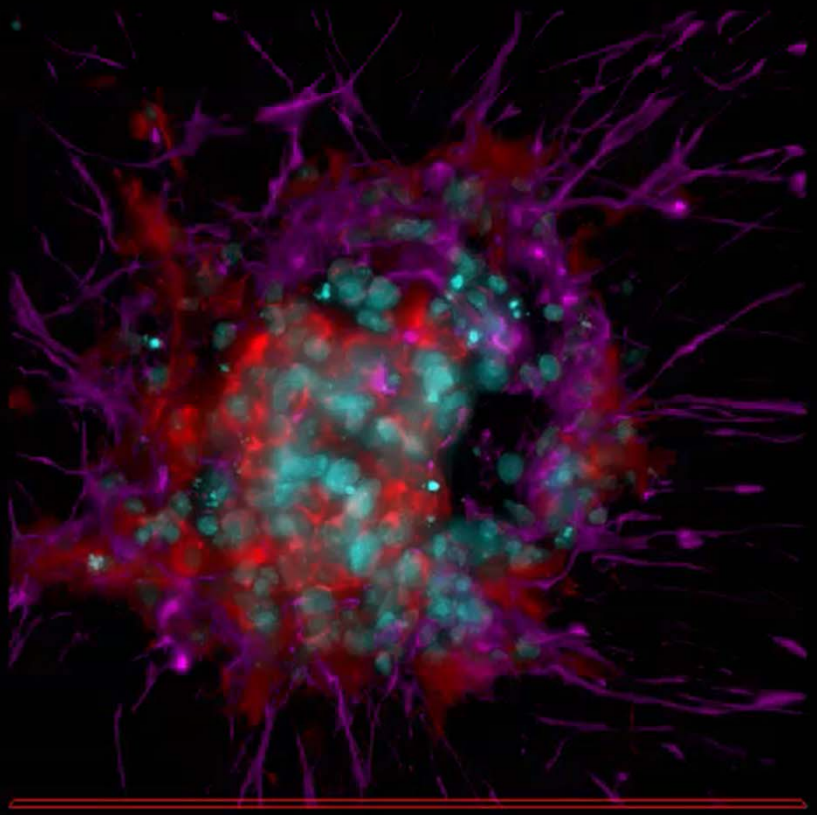
MICA 4c intestine Organoid WF&THUNDER

HC PL APO CS2 20x/0.75
DAPI-AF488-AF555-AF647

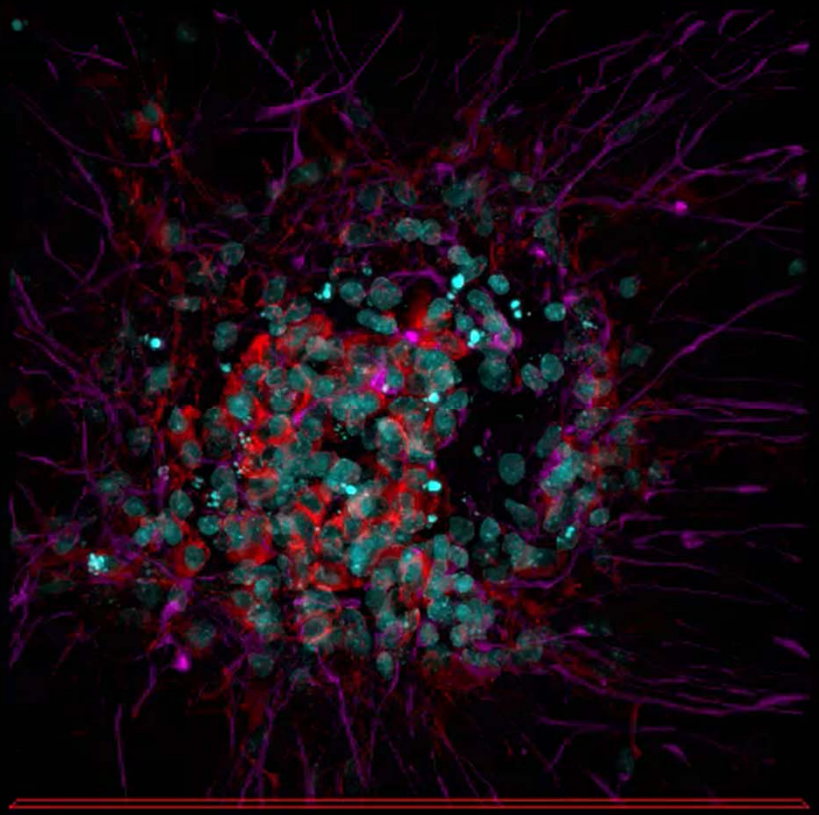




State-of-the art Fluorescence Microscope



THUNDER Imager

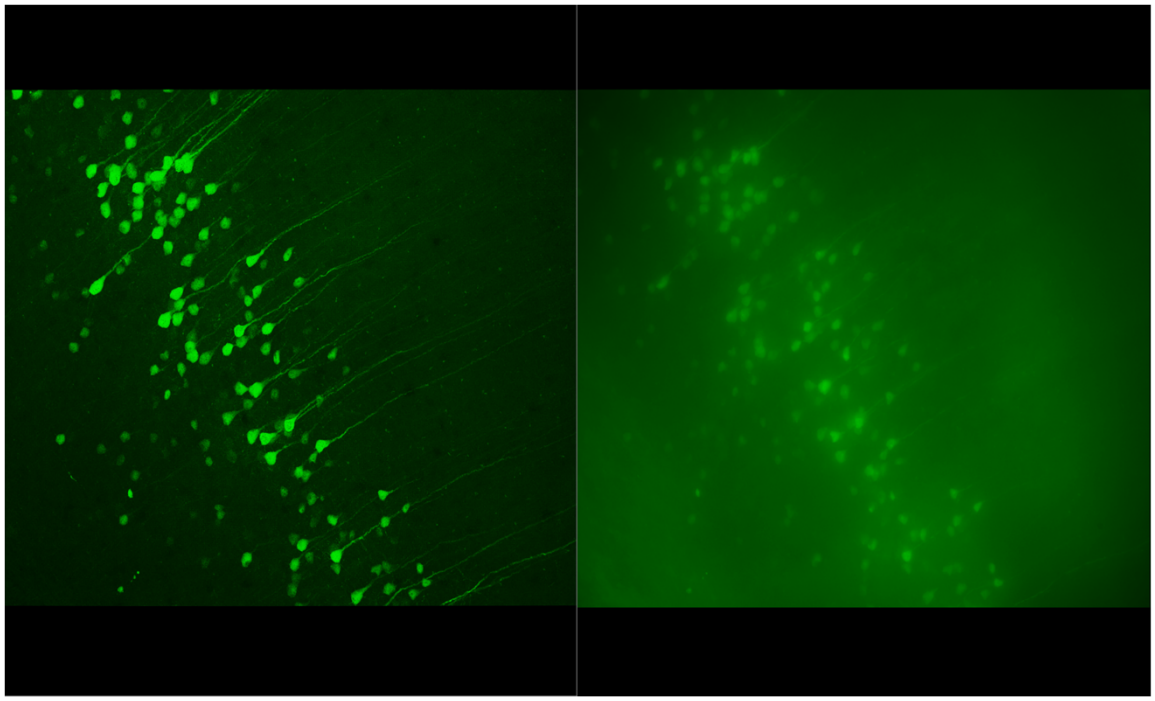


Cultured cortical neurons. Z-stack of 59 planes (thickness: 21µm). Sample courtesy FAN GmbH Magdeburg (Germany).

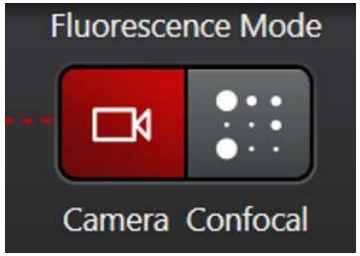
Neuronal 3D Cell Culture

No Constraints

Widefield & Point scan confocal @ Mica



Mica confocal | Mica widefield



Maximum Image Projection of a Z-Stack over 150 z slices using Confocal (left, 2448x2048, 600Hz, 1 AU) and Widefield (right,2448x2048, 100ms exposure time).

Dye:

neurons expressing cytoplasmic GFP

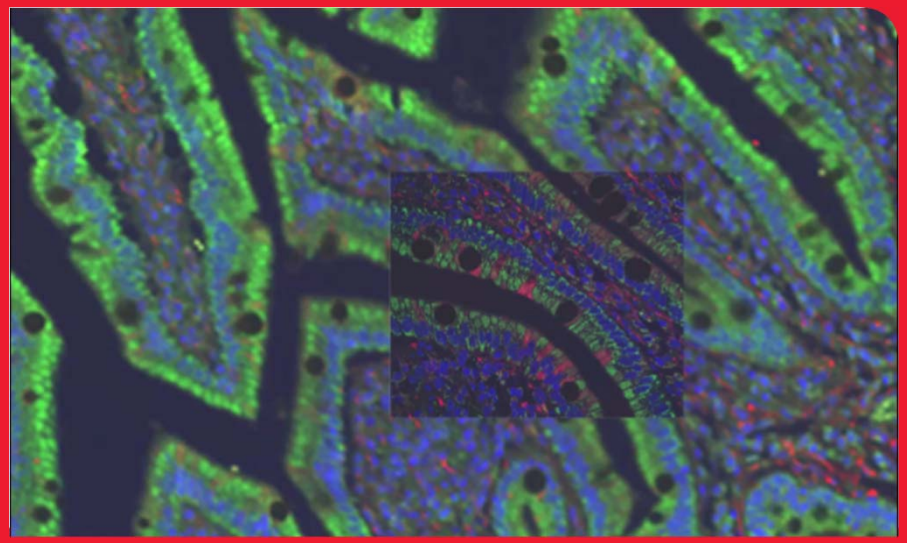
Objective:

HC PL APO 20x/0.75 CS2

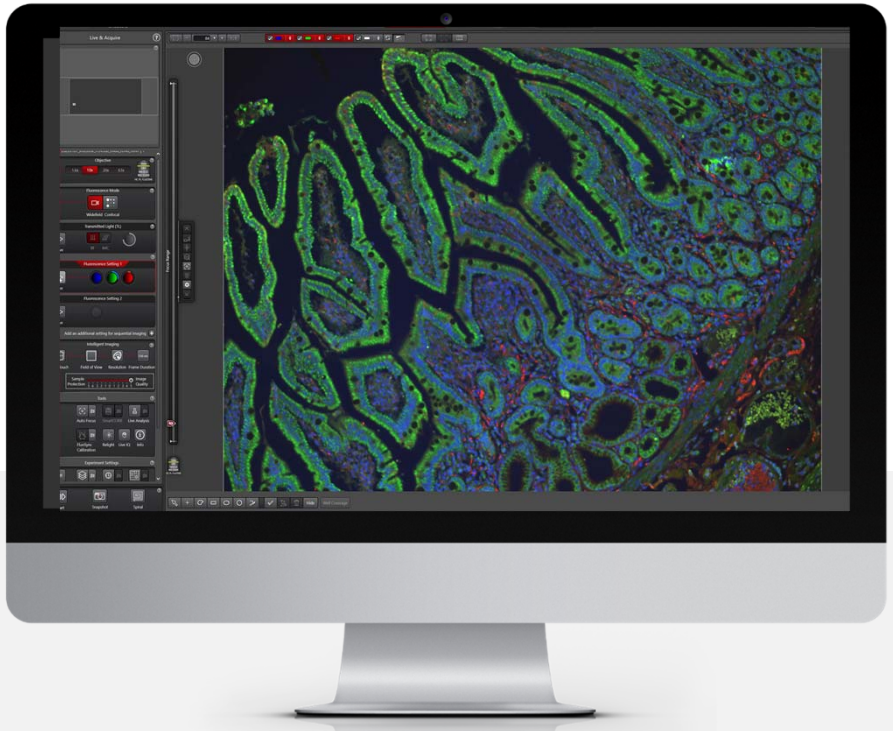
Leica

No Constraints

Seamlessly connecting modalities



Traditional Microscope



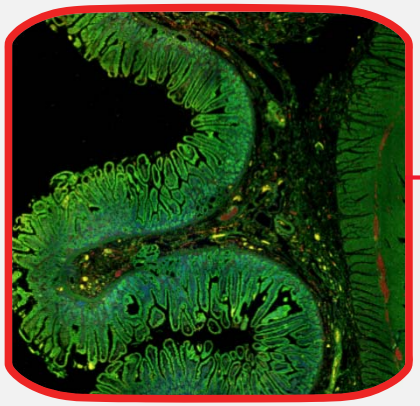
MICA

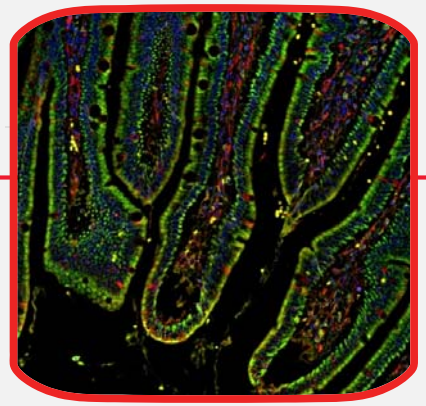
Leica

No Constraints

Seamlessly move from fast overview to high resolution

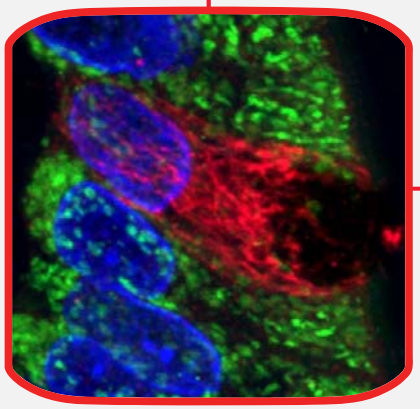
1.6 X Widefield
Create Overview

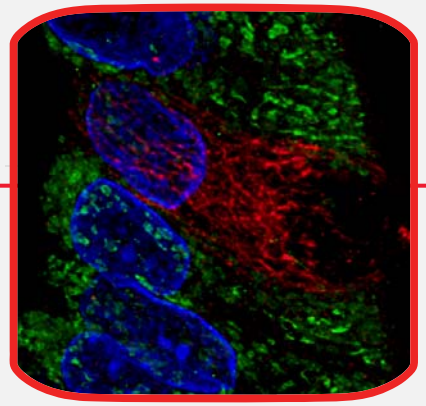




20 X THUNDER
Select the cell of interest

63X Confocal
Get the subcellular information





63X LIGHTNING
Get even more of the subcellular information

Intestine tissue section acquired with different objectives ranging from low to high magnification (1.6x, 10x, 20x, 63x), using widefield and confocal imaging. 20x widefield images are processed with THUNDER and 63x confocal images with LIGHTNING. Nuclei are labeled in blue, mitochondria in green, and detyrosynated tubulin in red.

Powered by:

Unified Imaging Modalities

Point Scanning Confocal

Mica is an incubator

Leica

Widefield – THUNDER - Point scan confocal- Lightning

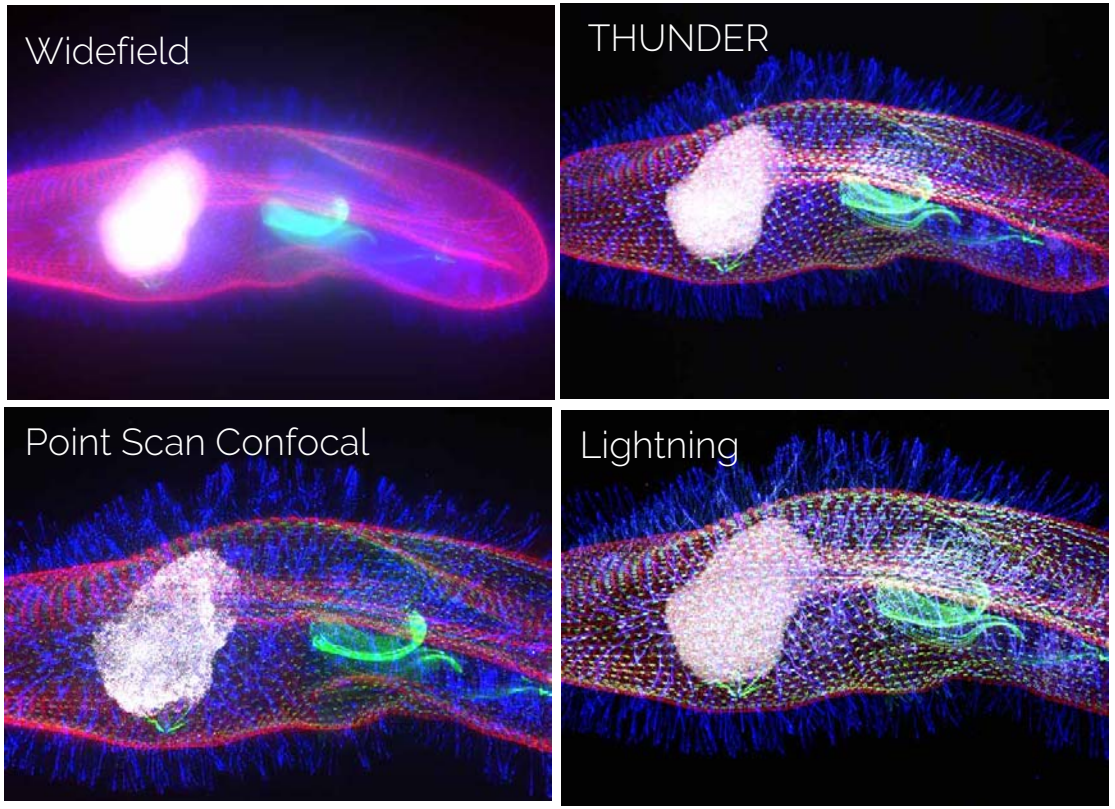


Image shows a protist Paramecium (Paramecium tetraurelia) stained to show the nucleus (Hoechst, white), the basal body, a protein ring found at the base of a cilium (AF488, green), the epiplasm, a thin dense layer at the base of a cilia where basal bodies are inserted (AF568, red) and the cilia (Star635P, blue). Images were acquired on Mica with HC PL APO CS2 63x/1.20 water objective using widefield (plus THUNDER ICC and LVCC) and confocal imaging (LIGHTNING grade and processing with +5 sample protection) without moving the sample. Sample courtesy: A. Aubusson-Fleury, CNRS, GIF sur Yvette, France.

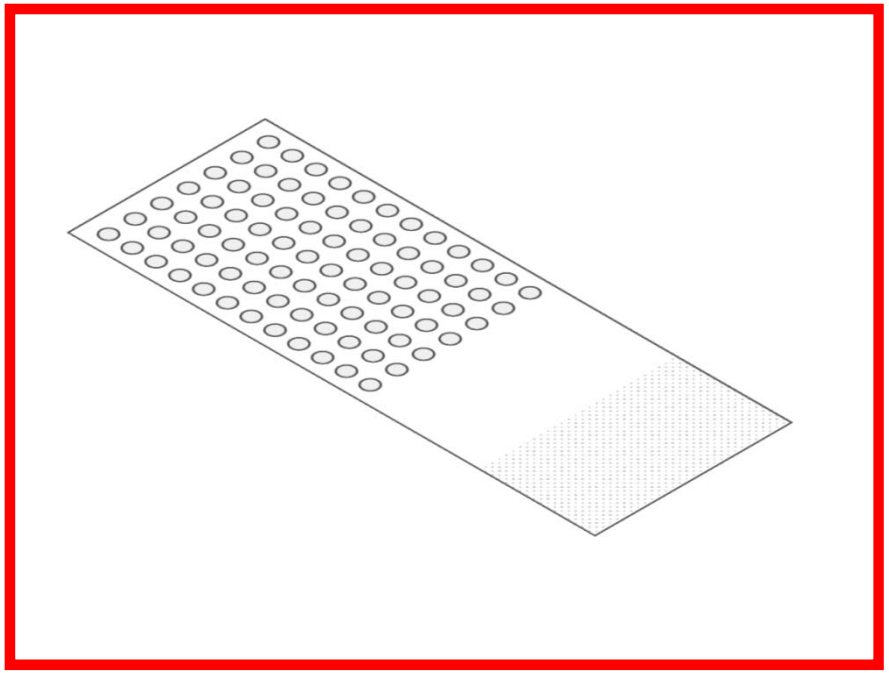
Tissue Microarray

Experiment description:

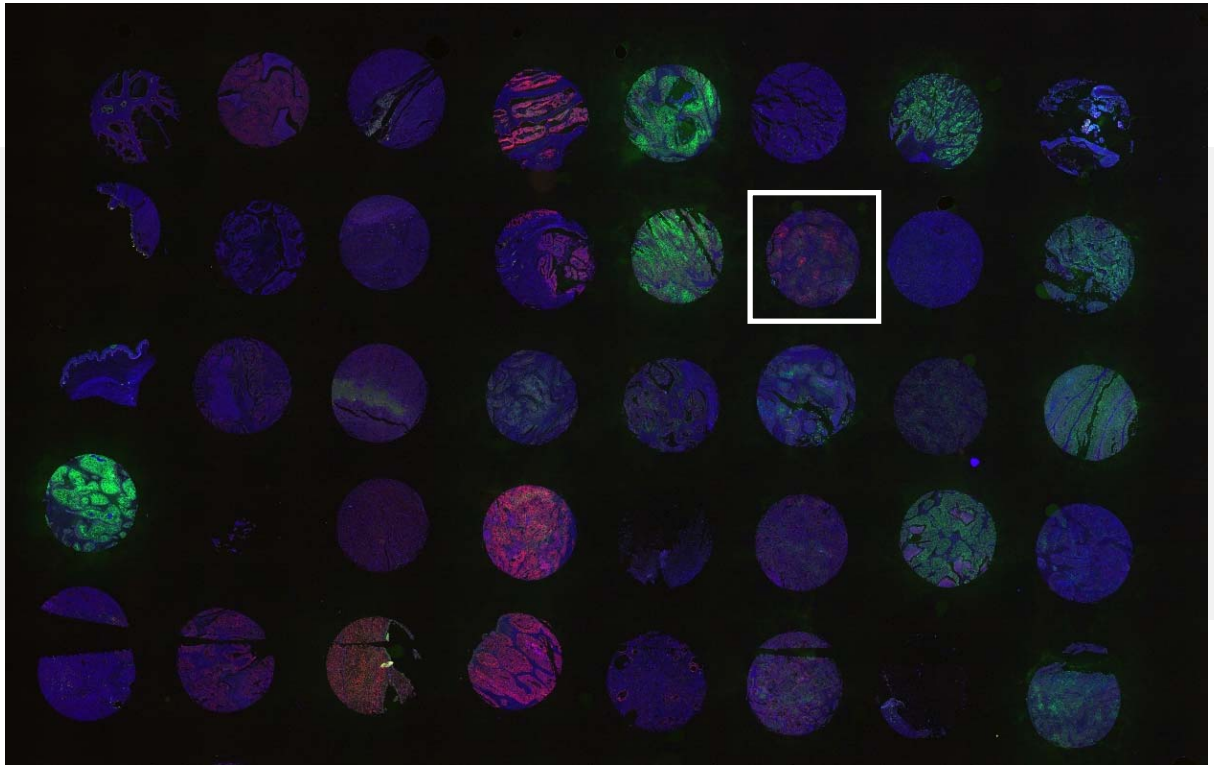
In this experiment we use a variety tissue samples arranged in an array. The samples can be stained with e.g., immunohistochemistry or fluorescent in situ hybridization (FISH).

Experiment Challenges:

- With increasing magnification, it is difficult to keep the overview.
- With increasing magnification finding the same location is challenging.
- Keeping consistent focus over the whole slide.



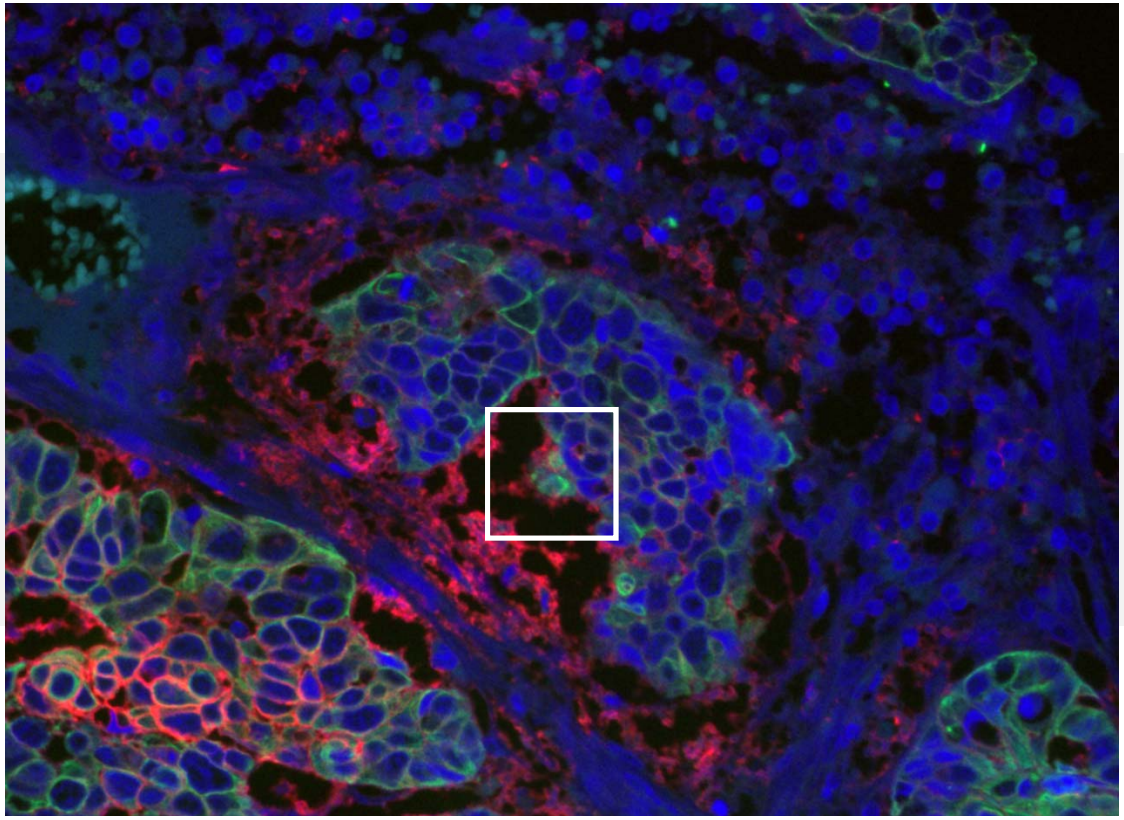
Tissue Microarray



Seamlessly move from fast overview to high resolution when required by your experiment.

TMA MTU481, commercially available. Nuclei are labeled in blue, p-Cytokerasin in green, and NaKATPase in red.
The fluorescent overview was acquired with 10x in widefield.

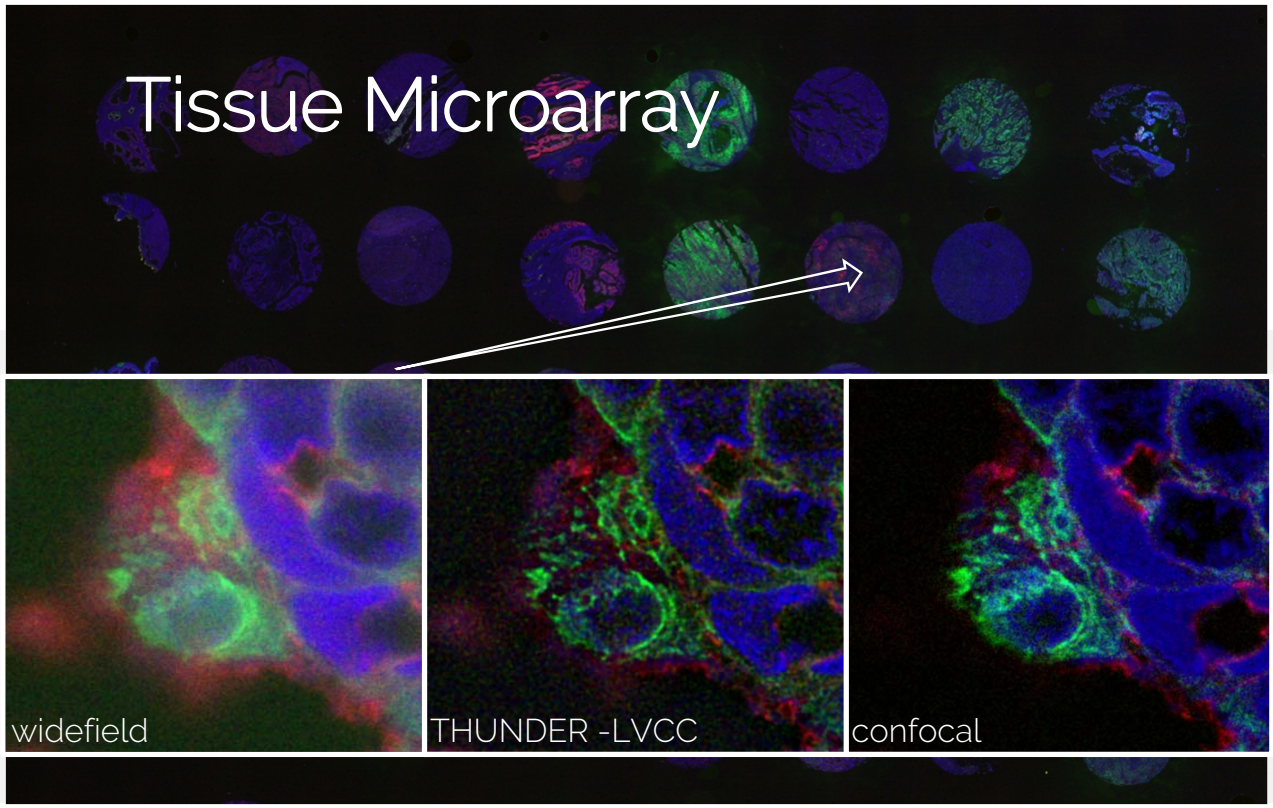
Tissue Microarray



TMA MTU481, commercially available. Nuclei are labeled in blue, p-Cytokerasin in green, and NaKATPase in red.
Individual tissue sections were acquired with the 20x/0.75 CS2 DRY objective.

Seamlessly move from fast overview to high resolution when required by your experiment.

Leica



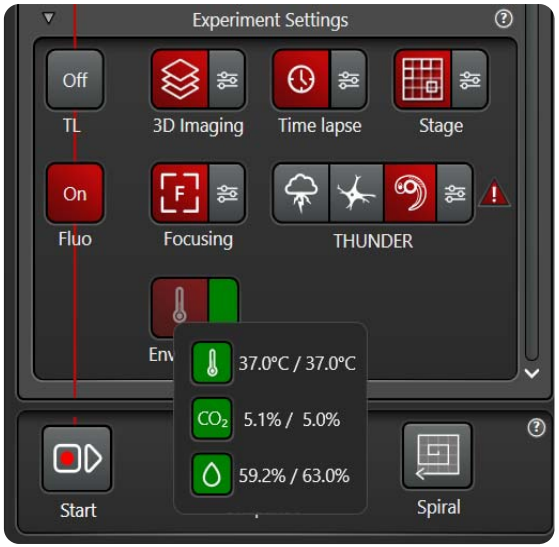
Seamlessly move from fast overview to high resolution when required by your experiment.

Choose from widefield, THUNDER, confocal, and LIGHTNING.

TMA MTU481, commercially available. Nuclei are labeled in blue, p-Cytokerasin in green, and NaKATPase in red.
The high detail inspection of the sample was done with the 63x/1.20 CS2 Water MotCORR.

Leica

„Mica is an incubator“



- Temperature
  Humidity CO2 (O2)

- Software integrated
  control & feedback

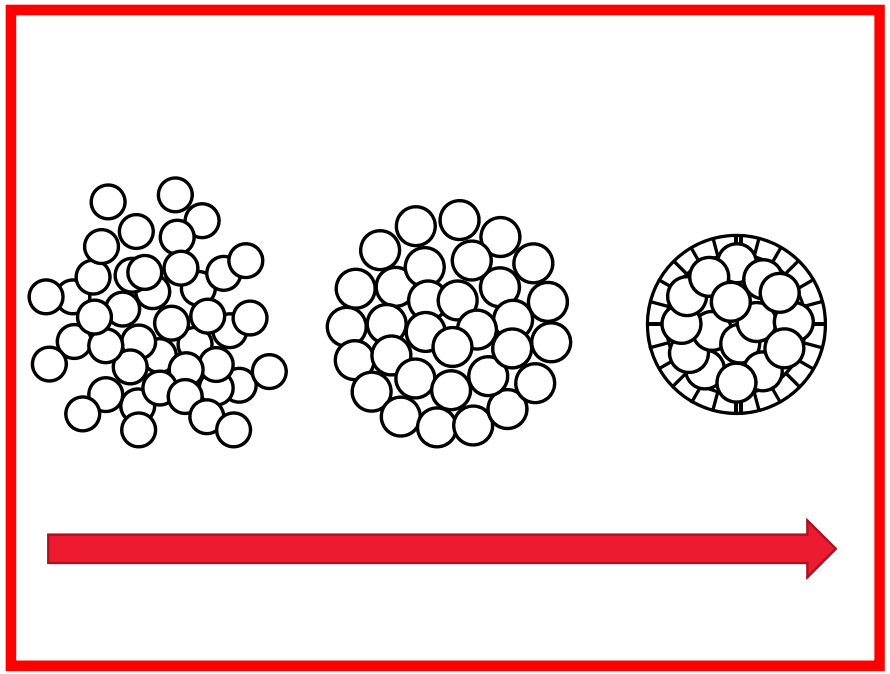
Long-term time-lapse experiment

Experiment description:

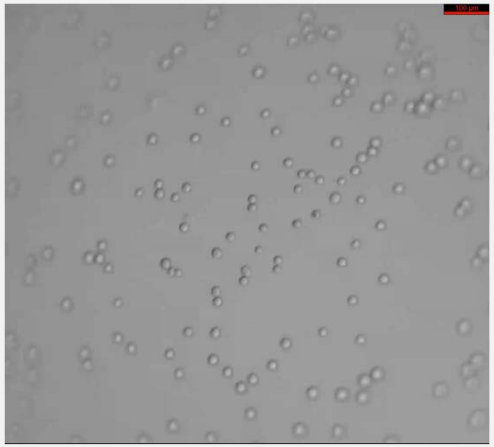
- Formation of spheroids starting from a mono-cell layer.

Experiment Challenges:

- **Prolonged sample survival**,
  --ensuring physiological conditions.
- **Low expression levels of markers**,
  --endogenous levels need to be kept to not impair cell homeostasis.
- **Stable supply of nutrients** and unchanged concentration in the medium – impaired by evaporation.
- **Staying in focus**.

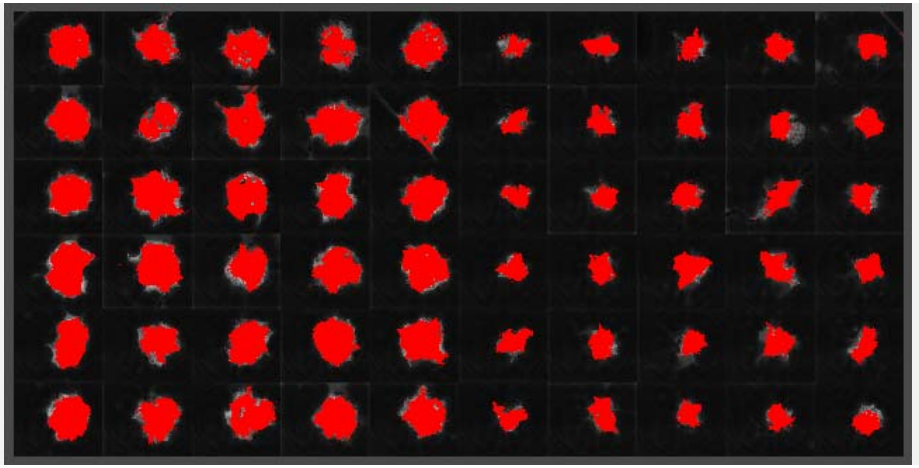


Multiple Spheroids Growing over 2.5 days



Formation of 3D spheroids from 1000 stably transfected MDCK MX1-GFP cells per well (left half) and 1000 U2OS cells per well (right half).
Timelapse acquisition over 72 hours with 30 minutes interval.
Green, GFP. Gray, integrated modulation contrast.

Long-term time-lapse experiment



Long-term time-lapse experiment

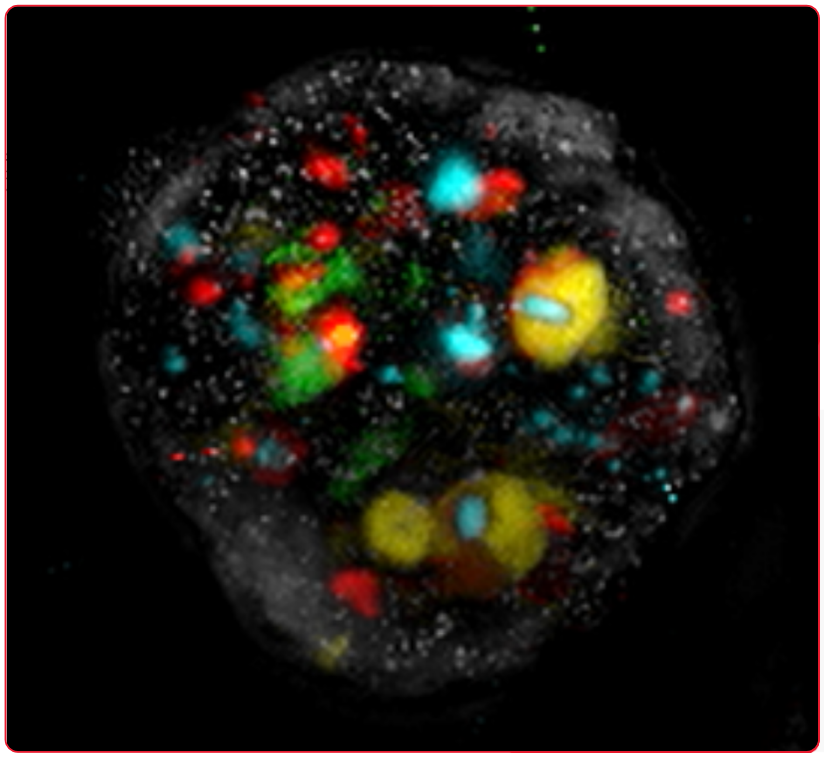
Supplement: spheroid growth

- MX1-GFP stably transfected cells (left half)
- U2OS cells (right half)
- Formation of 3D Spheroids
- 1000 cells per well
- Timelapse over 72 hrs. every 30 minutes
- Green, GFP
- Black and white, integrated modulation contrast

Long-term! time-lapse - MDCK cells – cyst formation in matrigel

Day 1 | Day 10 | Day 17

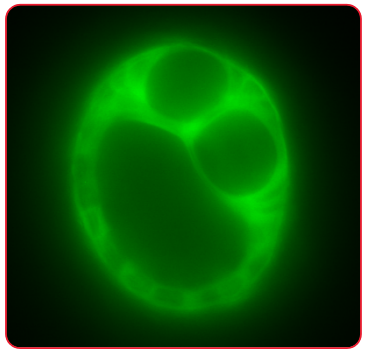
20 x | 63 x



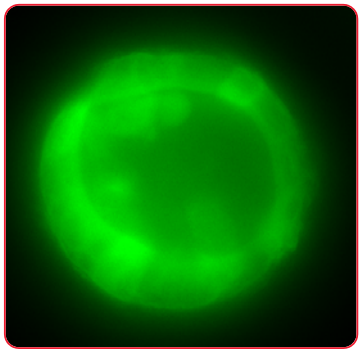
- Day 9: Some of the cysts were labeled with DAPI (blue), SiR-Actin (red), and Tubulin-SPY555 (yellow), in addition to EB1-GFP.
- Z-stack acquisition allows to get a 3D impression of the cysts.

63 x | 63 x

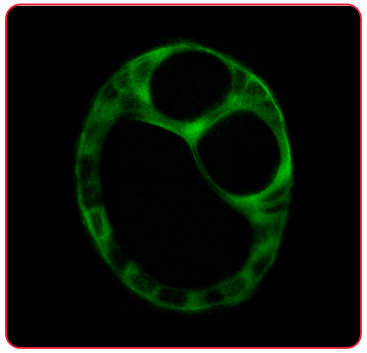
WF



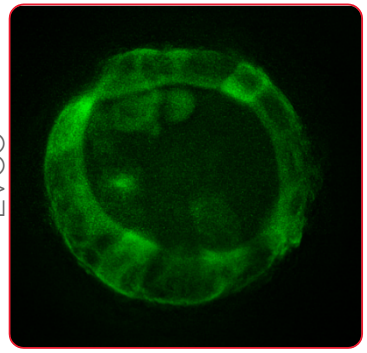
WF



LVCC



LVCC



Mica - The world's first Microhub

Specifications

			Mica Widefield	Mica Widefield Live-Cell	Mica WideFocal	Mica WideFocal Live-Cell
TRANSMITTED LIGHT CONTRAST	Integrated modulation contrast (IMC), automatically adjusted and brightfield contrast in RGB or gray scale mode		x	x	x	x
INCIDENT FLUORESCENCE ILLUMINATION	LED	365 nm, 470 nm, 555 nm, 625 nm	x	x	x	x
FluoSync WIDEFIELD DETECTION	Simultaneous detection channels	4 with FluoSync fluorophore separation	x	x	x	x
	Detector type	5 MP CMOS	x	x	x	x
CONFOCAL ILLUMINATION	Laser diode	405 nm, 488 nm, 561 nm, 638 nm			x	x
FluoSync CONFOCAL DETECTION	Detector type	HyD FS			x	x
	Simultaneous detection channels	4 with FluoSync fluorophore separation			x	x
ENVIRONMENTAL CONTROL	Live Cell Package	Temperature (room temperature +3 °C to 45 °C), CO_2 (0 - 10 %), humidity		x		x
IMMERSION DISPENSION	Closed loop water dispenser. Water immersion for one objective is feedback controlled and does not require any interaction		opt.	x	opt.	x
THUNDER	Methods	Instant Computational Clearing (ICC), Small Volume Computational Clearing (SVCC), Large Volume Computational Clearing (LVCC)	x	x	x	x
LIGHTNING	Methods	Basic, upgradeable to LIGHTNING Expert			x	x

Experience the future

Meet Mica

Leica